Development of a decision-making biomarker for CRTH2 antagonism in clinical studies

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ABSTRACT

Biomarkers have shown to improve success rates in the development of novel drugs, providing essential information in the early phases of clinical development for decision-making. Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) is pursued as a drug target for a number of inflammatory diseases. CRTH2 antagonists block the activation and migration of key inflammatory cells such as eosinophils, basophils, and Th2 cells. The mechanism of action of CRTH2 antagonists was established in cells isolated from human blood. Biomarkers derived from these experiments were included in clinical studies to investigate the mechanism of action and potency of CRTH2 antagonists in human. For clinical phase I studies with the CRTH2 antagonist ACT-453859, a follow-up molecule of setipiprant, inclusion of the most precise and robust pharmacodynamic (PD) biomarker with a clinically relevant target effect was desired to aid phase II dose selection.

Candidate biomarkers such as IL-13 secretion from Th2 cells and CRTH2, CD11b and CD203 modulation on basophils and eosinophils in whole blood were compared in terms of signal intensity and variability. Blockade of CRTH2 receptor internalization was finally chosen as PD biomarker and rigorously tested in a feasibility study. The assay showed excellent robustness, an intra-assay precision of 5% and inter-subject variability smaller than 15%. Based on phase II clinical study results with setipiprant, 90% CRTH2 receptor blockade was defined as clinically relevant PD effect. This target PD effect provides the means to take decisions based on the data generated in the phase I clinical studies with ACT-453859.

Focal points:

• Bedside
  Biomarkers offer a great potential to influence decisions taken during early clinical development. For clinical phase I studies with the CRTH2 antagonist ACT-453859, a follow-up molecule of setipiprant, inclusion of a biomarker was desired to aid phase II dose selection. In order to facilitate decision-making, we developed a biomarker that delivers high quality data under clinical circumstance and defined a relevant target biomarker effect.

• Benchside
  In-vitro experiments with human whole blood identified CRTH2 receptor internalization on basophils and eosinophils as the most precise and robust biomarker. Clinical results obtained with setipiprant in a seasonal allergic rhinitis study were used to define the clinically relevant target biomarker effect of 90% CRTH2 receptor blockade. Proof for the chosen target biomarker effect remains to be demonstrated in phase II clinical studies with ACT-453859.

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1. Introduction

The Prostaglandin D2 receptor 2 (PTGDR2) formerly described as GPR44, DP2 or CRTH2 is a G protein-coupled receptor expressed on inflammatory cells such as eosinophils, basophils, and Th2 cells...
Expression of CRTH2 has also been described on epithelial and tissue resident inflammatory cells such as mast cells, but its role has not been fully elucidated so far [23,25,28]. CRTH2 is activated by Prostaglandin D$_2$ (PGD$_2$) and metabolites thereof [5,18]. PGD$_2$ is released transiently upon activation of mast cells in the tissue and is rapidly metabolized in the circulation [18,21]. Beside mast cells also dendritic cells, Th2 cells, and eosinophils were described to express the hematopoietic PGD synthase and release PGD$_2$ [9,21,32].

The expression in key inflammatory cells (eosinophils, basophils, Th2, and mast cells) and the production of Th2 cytokines after stimulation strongly suggested CRTH2 as a suitable drug target to treat diseases such as allergic rhinitis, asthma, atopic dermatitis, eosinophilic esophagitis, chronic urticaria and also chronic obstructive pulmonary disease [2,6,7,20,27,31]. Therefore, CRTH2 antagonists are being developed by several pharmaceutical companies [30].

Because of the limited understanding of CRTH2 in these diseases, biomarkers have played an important role during the discovery and development. Biomarkers were postulated from the mechanism of action of CRTH2 which was established in in vitro experiments using eosinophils, basophils and Th2 cells isolated from human blood. Eosinophils react to CRTH2 activation with shape-change and degranulation [4], basophils show upregulation of the activation marker CD203c on the cell surface [13] and Th2 cells secrete cytokines such as interleukin-4, 5 and 13 [19,33]. Common to all three cell types is the upregulation of the adhesion molecule CD11b, the internalization of the CRTH2 receptor and chemotaxis along a PGD$_2$ gradient [5,12,13,22,34].

To investigate an antagonistic effect in healthy subjects in a phase I clinical study, stimulation of CRTH2 is required. Ex vivo stimulation of circulating basophils, eosinophils and Th2 cells can easily be carried out in human whole blood. As a matter of fact, cell surface markers in ex vivo stimulated blood from treated healthy subjects have been investigated in most phase I clinical studies done with CRTH2 antagonists. Eosinophils were monitored by shape change [18,24], CRTH2 receptor internalization [1,29], and CD11b upregulation [11]. To our knowledge basophils and Th2 cells were so far not deployed as PD biomarkers.

In order to use the PD biomarker data generated in clinical phase I studies for decision-making, setting of a target PD effect is needed which will be assay specific and, in case of competitive antagonists, agonist concentration-dependent. Biomarker data from a relevant animal model or phase II clinical study data would allow identifying such a target PD effect but so far no such decision-making PD biomarker assay has been published.

Here we present the selection of CRTH2 receptor internalization as PD biomarker. We show that receptor internalization was the most precise biomarker and sufficiently robust for implementation in a phase I clinical study. We describe the definition of a target PD effect based on results from a phase II clinical study in seasonal allergic rhinitis patients obtained with setipiprant. This decision-making PD biomarker was successfully implemented in the phase I clinical study with the follow-up CRTH2 antagonist ACT-453859 [3].

2. Methods

2.1. Collection of human blood

Blood samples were collected from healthy human subjects either by the Swiss Red Cross Basel or Actelion Pharmaceuticals Ltd. under the approval of the ethics committee Nordwest und Zentralschweiz (EKNZ).

2.2. Whole blood IL-13 secretion

Blood samples from 26 healthy donors were collected using heparin as anti-coagulant. The heparin blood was divided into four aliquots and incubated for 10 min with CRTH2 antagonist (1 μM) or the corresponding vehicle (1% v/v of DMSO in PBS). Afterwards, 1 μM dk-PGD$_2$ (12610, Cayman, Brunschwig Chemie, Switzerland) or the corresponding vehicle (10% v/v of ethanol in PBS) was added and the samples were incubated for 4 h at 37 °C. Samples were centrifuged for 10 min with 3000 rpm at 20 °C and the resulting plasma was collected and frozen at −80 °C. After thawing the plasma sample at room temperature the IL-13 concentration was quantified using the Enrena® system from Singulex following the manufacturers' instruction (Singulex, 03-0069-00, USA). Friedman test including Dunn’s multiple comparison test was used to test for statistical significance between the different treatment groups.

2.3. Whole blood eosinophil and basophil surface marker regulation

Blood samples from five healthy donors were collected using EDTA as anti-coagulant. The EDTA blood was divided into aliquots and incubated for 10 min with CRTH2 antagonist (1 μM) or the corresponding vehicle (0.01% v/v of DMSO in PBS). Afterwards, 300 nM dk-PGD$_2$ or the corresponding vehicle (10% ethanol in ddH$_2$O) was added to the samples and stimulated for 15 min at 37 °C. The cells were stained with anti–CCR3–PE (B-CCR-SR, Bühlmann Laboratories, Switzerland), anti-CRTH2-Alexa Fluor 647 (658042, BD Biosciences, Switzerland), anti-CD11b–APC-Cy7 (557754, BD Biosciences, Switzerland) and anti-CD203c–PE-Cy7 (A66906, Beckmann Coulter, Switzerland). Samples were lysed (B-CCR-LYR, Bühlmann Laboratories, Switzerland) for 5–10 min and centrifuged for 5 min at 500 g. The supernatant was discarded and pelleted cells were re-suspended in wash buffer (B-CCR-WB, Bühlmann Laboratories, Switzerland). The cells were analyzed on a flow cytometer (FACSComp™ II, BD Biosciences). Basophils and eosinophils were gated based on high CCR3 expression level and the side scatter (SSC) profile (low basophils, high eosinophils). The mean fluorescence intensity (MFI) levels were recorded for each surface marker, expressed with mean and percent CV. The ratio of mean stimulation signal to background was calculated. Statistical significance was tested using a one-way ANOVA including Dunnett’s multiple comparison test.

2.4. CRTH2 receptor internalization on eosinophils and basophils

Blood sample from six healthy human donors was collected using EDTA as anti-coagulant. The CRTH2 receptor was stimulated using 9 concentrations of dk-PGD$_2$. The cells were stained with anti–CCR3–PE and anti–CRTH2–Alexa Fluor 647. Samples were lysed (349202, BD Biosciences, Switzerland) for 5–10 min and centrifuged at 500 g. The supernatant was discarded and pelleted cells were re-suspended in wash buffer (B-CCR-WB, Bühlmann Laboratories, Switzerland). The cells were analyzed on a flow cytometer (FACSComp™ II, BD Biosciences). Basophils and eosinophils were gated based on high CCR3 expression level and SSC profile (low basophils, high eosinophils). The MFI for CRTH2 was acquired and analyzed using a GraphPad Prism v6.0 (La Jolla, USA) applying a four parameter fitting model.

2.5. Selection of eosinophils and basophils

The specificity of CCR3 as a basophil and eosinophil selection marker was investigated using a second selection marker; CD203c for basophils and CD49d for eosinophils. Blood samples from five human donors were collected using EDTA as anti-coagulant. Each donor was tested under two conditions (0 nM and 300 nM of dk-
PGD₂) with a 3-color staining: anti-CCR3-PE, anti-CRTH2-Alexa Fluor 647, anti-CD203c-PE-Cy7 for basophils and an anti-CCR3-PE, anti-CRTH2-Alexa Fluor 647, anti-CD49d-PE-Cy7 (304314, BD Biosciences, Switzerland) for eosinophils. The CCR3 selected cells were used as reference and the percentage recovery with the second cell marker was calculated. The percent recovery of the second cell selection marker to the reference cell selection marker CCR3 was calculated. Furthermore, the CRTH2 level was compared between the different cell selection strategies.

2.6. Selectivity of the anti-CRTH2 antibody

Selectivity of the anti-CRTH2 antibody (clone BM-16) was investigated by staining basophils and eosinophils in EDTA blood from three healthy human donors with three different methods: anti-CRTH2-Alexa Fluor 647 as positive control, a rat IgG2a, k isotype control labeled with Alexa Fluor 647 (557690, BD Biosciences, Switzerland) and a sample without CRTH2 staining reagent (fluorescence minus one) as negative control. The highest and the lowest CRTH2 receptor expression levels were investigated. The maximum control (without dk-PGD₂) provides the highest and the minimum control (50 μM dk-PGD₂) the lowest expression level of CRTH2 receptor. The MFI signal of the rat IgG2a isotype control sample needed to be equal or lower than for the lowest CRTH2 receptor expression level (50 μM dk-PGD₂) and close to the “flourescence minus one” control.

2.7. Intra-assay precision and stability of fixed sample

The EDTA blood of three different donors was spiked with 100 nM ACT-453859 and examined under baseline (no dk-PGD₂) and full internalization (50 μM dk-PGD₂) condition using five determinations per sample. The intra-assay precision was calculated in percent CV. Fixed sample stability was determined on the fixed baseline CRTH2 staining at 0, 24 and 48 h.

2.8. Feasibility study

During one single day a number of official blood donations to the Swiss Red Cross were used to collect blood of 53 healthy subjects to investigate the biomarker under “clinical study-like” conditions. All blood donations were carried out under the informed consent of the donors and the strict agreement of the Swiss Red Cross. Blood samples were collected using EDTA vacutainer tubes. Samples were collected by several nurses from the blood donation team and placed into a fridge at 4 °C. The EDTA blood of three different donors was spiked with 100 nM ACT-453859 and examined under baseline (no dk-PGD₂) and full internalization (50 μM dk-PGD₂) condition using five determinations per sample. The intra-assay precision was calculated in percent CV. Fixed sample stability was determined on the fixed baseline CRTH2 staining at 0, 24 and 48 h.

3. Results

3.1. Whole blood IL-13 secretion

Stimulation of heparinized whole blood with 1 μM dk-PGD₂ presented an increase of IL-13 (Fig. 1). The 1.85-fold increase of the mean IL-13 concentration (0.47–0.87 pg/ml) was statistically significant (p = 0.0001). The dk-PGD₂-induced increase of IL-13 was blocked by a 10 min pre-incubation with 1 μM of the CRTH2 antagonist (> IC₉₀) statistically significant (p = 0.009). The addition of 1 μM CRTH2 antagonist alone did not influence the IL-13 concentration (vehicle vs. antagonist, p = 0.82). The percent CV was 47% for the vehicle-treated samples and 82% for the dk-PGD₂ stimulated samples.

3.2. Whole blood eosinophil and basophil surface marker regulation

All cell surface markers (CD11b, CD203c, CRTH2) showed dk-PGD₂-mediated regulation which could be blocked by a 10 min pre-incubation with 1 μM (> IC₉₀) of CRTH2 antagonist (Supplementary materials). The fold change was between 1.29 for CD11b on eosinophils and 1.77 for CD203c on basophils. The CV ranged from 3.8% for CRTH2 receptor internalization on basophils to 31.5% for CD203c upregulation on basophils (Table 1).
3.3. CRTH2 receptor internalization on eosinophils and basophils

The CRTH2 receptor internalized both on basophils and eosinophils in a concentration-dependent manner displaying full internalization at concentrations above 1 μM dk-PGD2 (Fig. 2). A four-parameter fit showed an EC50 of 78 nM on basophils and 114 nM on eosinophils. The EC50 was calculated based on the EC50 and Hill-slope and was found to be 541 nM for basophils and 352 nM for eosinophils. CRTH2 baseline levels were 200 MFI higher on eosinophils compared to basophils which is caused by the fluorescence background of eosinophils. Both on eosinophils and basophils dk-PGD2 showed a maximal internalization of CRTH2 receptor by 600 MFI.

3.4. Selection of basophils and eosinophils

Basophils and eosinophils were selected based on the CCR3 high level and differentiated by the SSC profile. The selection criteria for the selection of a biomarker were a fold change greater than 1.5 and a CV smaller than 20%.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Biomarker</th>
<th>Vehicle mean</th>
<th>dk-PGD2 mean</th>
<th>Fold-change</th>
<th>Vehicle CV</th>
<th>dk-PGD2% CV</th>
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<td>IL-13 (pg/ml)</td>
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<td>1.85</td>
<td>82.0</td>
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<td>Basophils</td>
<td>CD11b (MFI)</td>
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<td>22.6</td>
<td>24.3</td>
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<tr>
<td>Basophils</td>
<td>CD203c (MFI)</td>
<td>2216</td>
<td>3921b</td>
<td>1.77</td>
<td>31.5</td>
<td>30.4</td>
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<td>422b</td>
<td>1.75</td>
<td>3.8</td>
<td>5.6</td>
</tr>
</tbody>
</table>

a 1 μM dk-PGD2, b 300 nM dk-PGD2.

3.5. Selectivity of the anti-CRTH2 antibody

The MFI signal for the CRTH2 receptor on eosinophils and basophils at baseline and after stimulation with 50 μM dk-PGD2 was higher than for isotype control and fluorescence-minus-one control staining. Furthermore, only samples stained with anti-CRTH2 antibody showed dk-PGD2-mediated CRTH2 receptor internalization (Supplementary materials).

3.6. Intra-assay precision and stability of fixed sample

The intra-assay CV was 5% for eosinophils and 4% for basophils (Supplementary materials). The stability of fixed samples was tested over a time period of 48 h. The percent CRTH2 on the cell surface increased slightly over the course of 48 h but was within the acceptance range both for eosinophils (14.6%) and basophils (5.0%).

3.7. Feasibility study

The CRTH2 receptor density is represented as MFI on the surface of basophils (Fig. 3A) and eosinophils (Fig. 3B) before intrinsic normalization displaying the maximum control, minimum control and PD sample. For all subjects an internalization of the CRTH2 receptor was observed after stimulation with dk-PGD2. The mean baseline level of CRTH2 (maximum control) was 888 MFI for basophils and 1208 MFI for eosinophils. For both cell types full internalization of the CRTH2 receptor displayed a decrease of 550 MFI. The mean PD sample value was 705 MFI for basophils and 996 MFI for eosinophils.

After normalization the percent CRTH2 receptor blocked on the surface of basophils and eosinophils was 61.7 and 58.6, respectively (Fig. 4). The CV of the normalized PD effect was 10.9% for basophils and 14.2% for eosinophils which for the basophils was slightly better than for the PD sample mean fluorescence intensity.
For eosinophils there was no difference between the percent CV of the PD sample and the normalized PD effect.

3.8. Defining the CRTH2 receptor internalization cut-off for potential clinical efficacy

A triplicate ACT-453859 and setipiprant concentration–response experiment in the presence of 300 nM dk-PGD2 were carried out with the CRTH2 receptor internalization assay using EDTA blood from 3 healthy donors (Fig. 5). The MFI from both eosinophils and basophils was acquired and the normalized percent CRTH2 blockade was calculated. The IC50 of ACT-453859 on basophils and eosinophils was 45 nM. The IC50 of setipiprant was 684 nM on eosinophils and 594 nM on basophils. The minimum effective blood concentration of setipiprant to achieve a clinically relevant effect in the clinical phase II seasonal allergic rhinitis study (4373 nM) was used to define the cut-off for the CRTH2 receptor internalization assay. The target PD effect defined on the setipiprant master curve was 90% CRTH2 receptor blockade (Fig. 5A).

4. Discussion

After CRTH2 was first described in 1999, evidence accumulated on a possible role in allergic and immunological diseases for this receptor. This sparked an interest with pharmaceutical companies to pursue CRTH2 as a drug target and several antagonists have been investigated in clinical studies in allergic rhinitis, asthma, atopic dermatitis, eosinophilic esophagitis, chronic urticaria and chronic obstructive pulmonary disease patients. The majority of clinical studies were carried out in asthma patients where ambiguous results raised the question on how much CRTH2 blockade is required to obtain a clinically relevant effect. Biomarkers provide the tools to aid in answering such a question. In particular, a clinically relevant PD biomarker incorporated in a phase I clinical study will reveal the potential of a CRTH2 antagonist and guide dose selection. Therefore a clinically relevant PD biomarker was desired for the phase I clinical study with the CRTH2 antagonist ACT-453859. Results from a previous phase II clinical study with setipiprant in allergic rhinitis patients were available to define a clinically relevant target PD effect.

The CRTH2 mechanism of action offered the possibility to test two different types of PD biomarkers: cytokine secretion and surface marker regulation. Our aim was to investigate several candidate PD biomarkers and to select the most appropriate, precise and robust biomarker for the phase I clinical study with ACT-453859. Whereas cell surface marker regulation has been utilized before, the investigation of a whole blood cytokine secretion assay has not been described to the best of our knowledge.

In both cases, blood needs to be collected and stimulated ex vivo to investigate the effect of CRTH2 antagonists. A particular difficulty of ex vivo stimulation is the agonist concentration to be used which is of particular relevance for competitive antagonists. In the case of CRTH2 the true agonist concentration of PGD2 and active metabolites at the site of action is difficult to investigate. Nevertheless, experiments with mast cells present in nasal polyps pointed towards a quick release of high levels of PGD2 after activation [16,17]. In most phase I studies a fixed agonist concentration was defined. Noteworthy is a phase I clinical study that employed two agonist concentrations with a 10-fold difference maybe accounting for this uncertainty of true agonist

Fig. 3. Performance of the CRTH2 receptor internalization assay in 53 healthy human subjects. CRTH2 receptor internalization on basophils (A) and eosinophils (B) performed with blood from 53 healthy human subjects. All donors showed internalization of the CRTH2 receptor with 50 μM dk-PGD2 (minimum vs. maximum control) and intermediate receptor blockade in the presence of 100 nM of ACT-453859 after stimulation with 300 nM dk-PGD2. Friedman test including Dunn’s multiple comparison test was used to test for statistical significance.

Fig. 4. CRTH2 receptor blockade representing the PD effect after intrinsic normalization. Representation of the PD effect for a 100 nM blood concentration of ACT-453859 after intrinsic normalization to the minimum and maximum control samples. The mean PD effect for 100 nM ACT-453859 was 61.7% for basophils and 58.6% for eosinophils.
Determination of a clinically relevant PD effect. Whole blood CRTH2 receptor blockade with setipiprant (A) and ACT-453859 (B) after stimulation with 300 nM dk-PGD2. The master curve for basophils (circle) and eosinophils (triangle) was obtained from concentration–response experiments with blood from three healthy donors. The dashed line represents the clinically relevant trough setipiprant blood concentration of 4373 nM and the dotted line represents the equivalent PD effect.

The presence of Th2 cells in whole blood allowed us to follow the production of IL-13 after stimulation of CRTH2. Due to the low concentration of IL-13 reported in healthy subjects, a high-sensitivity antibody-based system was employed [26]. In the experiment with heparin blood from 26 healthy subjects a statistically significant 1.8-fold increase in mean IL-13 concentration was observed after stimulation with dk-PGD2. The increase of IL-13 was blocked by a CRTH2 antagonist. We observed a rather high CV of 82% in the vehicle-treated samples and 47% in the dk-PGD2-stimulated samples and therefore rejected the IL-13 secretion assay as PD biomarker for phase I clinical studies.

The presence of eosinophils and basophils in whole blood allowed us to follow the regulation of specific cell activation markers such as CD11b and CD203c as well as the internalization of the CRTH2 receptor by flow cytometry. All cell surface markers reacted to stimulation of CRTH2 either by showing an increase of activation markers (CD11b, CD203c) or by internalization of the CRTH2 receptor. The CV for the activation markers (CD11b, CD203c) was in general higher than for CRTH2 receptor internalization. As an example the baseline inter-subject CV on eosinophils for CD11b was 16% compared to CRTH2 receptor internalization with only 8%. When comparing the CRTH2 receptor internalization between the different cell types, both eosinophils and basophils passed the selection criteria of signal to noise greater than 1.5 and percent CV smaller than 20%.

Taken together these results led to the selection of CRTH2 receptor internalization as the PD biomarker of choice. Due to the possibility of investigating both eosinophils and basophils using the same protocol and the acceptable performance, both cell types were carried forward. Although the whole blood IL-13 secretion assay was not selected as PD biomarker assay, it provides an interesting means to take decisions based on data obtained in the phase I clinical studies.

In summary, the precise and robust CRTH2 receptor internalization assay as PD biomarker was proved to be feasible under study-like conditions analyzing samples from 53 healthy subjects. All MFI results were normalized to obtain the percent CRTH2 receptor blockade which represents the PD effect. A 100 nM concentration of ACT-453859 led to a blockade of 61.7% on eosinophils and 58.6% on basophils. The inter-subject CV for basophils was 10.9% and for eosinophils 14.2%, which was slightly lower than for the non-normalized MFI results. This led to the inclusion of the intrinsic normalization step in the final assay protocol. In addition, the normalization step removed the dependency on the mean fluorescence signal being arbitrary and depending on flow cytometer settings and performance. The feasibility experiment provided confidence in the PD biomarker assay when processing samples within 2 h after collection. In order to make the CRTH2 internalization biomarker data more relevant for decision-making we related it to clinical efficacy. To define a target PD effect we used the results obtained in a clinical study with the CRTH2 antagonist setipiprant. The minimum effective concentration at trough for setipiprant modeled from a successful seasonal allergic rhinitis study corresponded to a PD effect of 90%. Therefore, we assume a clinically relevant target PD effect of 90% CRTH2 receptor blockade to be maintained over the intended treatment interval.

In summary, the precise and robust CRTH2 receptor internalization assay with a clinically relevant target PD effect provides the means to take decisions based on data obtained in the phase I clinical studies with ACT-453859.

5. Conclusions

The use of biomarkers has become an integral part of pharmaceutical research and development and biomarkers offer a great
potential to influence decisions taken during early clinical development. In order to facilitate decision-making, selection of an appropriate biomarker providing high quality and relevant results is critical.

For the phase I clinical study with the CRTH2 antagonist ACT-453859 we developed a decision-making PD biomarker. We compared candidate PD biomarkers representing the CRTH2 mechanism of action in eosinophils, basophils, and Th2 cells. CRTH2 receptor internalization on basophils and eosinophils was selected as the most precise and robust PD biomarker. Clinical results obtained with setipiprant in a seasonal allergic rhinitis study were used to define a clinically relevant target PD effect of 90% CRTH2 receptor blockade. Proof for the chosen target PD effect remains to be demonstrated in phase II clinical studies with ACT-453859.

Executive summary table

- Biomarkers provide essential information for decision-making in the early phases of clinical development.
- To facilitate decision-making, selection of an appropriate biomarker providing high quality and relevant results is critical.
- ACT–453859 and setipiprant are CRTH2 antagonists intended for the treatment of inflammatory diseases.
- To guide dose selection for ACT–453859, inclusion of a precise, robust and relevant pharmacometric (PD) biomarker in phase I clinical studies was needed.
- CRTH2 receptor internalization on basophils and eosinophils was selected as the most precise and robust PD biomarker.
- Phase II clinical results with setipiprant in a seasonal allergic rhinitis study were used to define a clinically relevant target PD effect of 90% CRTH2 receptor blockade.
- The target PD effect remains to be validated in phase II clinical studies with ACT-453859.

Conflicts of interest

None.

Ethical statement

The work described was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). In particular, the blood samples from healthy human subjects used in this work were either collected by the Swiss Red Cross Basel or Actelion Pharmaceuticals Ltd. under the approval of the ethics committee Nordwest und Zentralschweiz (EKNZ).

Funding source

All authors were employees of Actelion Pharmaceuticals Ltd. at the time of conduct of the work.

Authors’ contribution

DSS wrote the manuscript, developed the conceptual strategy, contributed to experimental design, interpreted the results. HF carried out the flow cytometry analysis, interpreted the results. MH developed the conceptual strategy, contributed to experimental design, interpreted the results, reviewed the manuscript. JZ developed the PKPD model, reviewed the manuscript. RR developed the conceptual strategy, reviewed the manuscript. JH carried out the immunoassay analysis. MG developed the conceptual strategy, reviewed the manuscript. PNS developed the conceptual strategy. JD developed the conceptual strategy, reviewed the manuscript. PG developed the conceptual strategy, reviewed the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.nhtm.2015.05.001.

References


