Characterization of a new antiasthmatic therapeutic target: synergy between EP$_2$ agonism and anti-IgE through a biosimilar candidate with omalizumab

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Common chronic inflammatory disease in the airways
  - The most frequent in childhood and adolescence
  - Characterized by:
    - Spasmodic contraction of bronquial muscles
      - Triggered by several factors
    - Shortness of breath (Airflow obstruction)
      - Decreased caliber of the bronchi
        » Airway hyperresponsiveness (AHR)
        » Mucus hyperproduction
        » Airway wall remodeling

Asthma is a global health problem affecting around de 10% of the global population
  - Allergic asthma (80%):
    - Immunologically responses to nonspecific stimuli
There is no effective curative or preventive treatment

- Inhaled corticosteroids (ICS)
- Long acting $\beta_2$-agonist (LABA)
- Glucocorticoides (GC)

Identification of a new anti-asthmatic target:

- $\text{EP}_2$ receptor

INTRODUCTION

Need for new anti-asthmatic targets

Treatment & Therapeutic target candidate

- Protector effect on asthmatic response

$\text{PGE}_2$-$\text{EP}_2$-$\text{MC}$
Need for new anti-asthmatic targets

Omalizumab

- **Omalizumab** (Xolair®)
  - Humanized monoclonal antibody with anti-IgE function
  - It binds selectively to free IgE (Fc region)
  - Prevents mast cell activation (release of inflammatory mediators)
Biosimilars

What is a biosimilar?

Unlike typical drugs that are made from synthetic chemicals, biologics are produced from living organisms.

**Increasing Complexity**

Biosimilars are biological products that have been proven to be as safe as originator biologic drugs.

- Are designed from scratch to match a reference original biological.
- Bear in essence the same:
  - Active substance
  - Comes in the same pharmaceutical form
  - Administered via the same route and dose
  - Indications

**Comparability exercise**

- Quality
- Preclinical
- Clinical studies

**Biosimilars deliver comparable efficacy, safety and quality results as originator biologics**
“The mast cell EP$_2$ activation in combination with the IgE neutralization by a biosimilar candidate of omalizumab, exerts a synergic potentiating anti-asthmatic effect”
**Thesis objective**

In order to verify the hypothesis the main objective is:

Develop an Omalizumab biosimilar candidate and study its effect beside an EP$_2$ agonist in a *in vivo* and *in vitro* model

<table>
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<th>Sub-objective 1</th>
<th>Sub-objective 2</th>
<th>Sub-objective 3</th>
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| • Asses in *in vivo* models  
  • EP$_2$ activity  
  • anti-IgE function | • Design and execute a *predictive quality program* to assess potential molecules to be biosimilar candidates at early stages. | • Assess the *synergic effect* of an EP$_2$ agonist and an anti-hIgE biosimilar candidate.  
  • *In vitro* model  
  • *In vivo* model |
Results
Study 1 (Objective 1)
Assess the activity of $\text{EP}_2$ and anti-IgE

A) Validate the protector effect of $\text{EP}_2$ in a transgenic model with this receptor overexpressed

B) Assess the anti-IgE activity in a transgenic model expressing hFcεRI
**Study 1 (Objective 1)**

**Assessment of MC EP₂ receptor role**

**A.** \( R_L \) – Lung resistance

**B.** mMCP1 - Mast cell activity

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**Fig. 1.** A) Airway hyperresponsiveness to methacholine was assessed in BALB/c mice (wildtype and transgenic with EP₂ overexpressed) by measuring lung resistance \( (R_L) \) in HDM-sensitized mice. B) Determination of the local lung production of the specific mast cell protein, mMCP-1, in sensitized and non-sensitized, transgenic and wildtype mice by an ELISA assay (*p-value<0.05, **p-value<0.01, ***p-value<0.001 and ****p-value<0.0001). HDM (House Dust Mice), SF (Physiological Serum), TG (Transgenic mice), WT (Wildtype mice), mMCP-1 (mouse mast cell protease 1), PBS (Phosphate Buffered Saline).
Study 1 (Objective 1)

Assessment of MC EP$_2$ receptor role

A. Differential inflammatory cell count

- **Fig. 2.** A) Differential inflammatory cell count per mL determined from counting at least 300 cells. B) Airway MC activity assessed in mice by measuring the local production of mMCP1. (*p-value<0.05, **p-value<0.01, ***p-value<0.001 and ****p-value<0.0001). HDM (House Dust Mice), SF (Physiological Serum), TG (Transgenic mice), WT (Wildtype mice), mMCP-1 (mouse mast cell protease 1), PBS (Phosphate Buffered Saline).
Study 1 (Objective 1)

Assessment of the anti-IgE activity (PRA protocol)

A. \( R_L \) – Lung resistance

\[ \begin{array}{c|c|c}
\text{Grupamento} & \text{Grupamento} & \text{P valor} \\
\hline
\text{IgE+/NP- / OMA -} & \text{IgE+/NP+ / OMA -} & ** \\
\text{IgE+/NP- / OMA -} & \text{IgE+/NP+/ OMA+ (90µg)} & *** \\
\text{IgE+/NP- / OMA -} & \text{IgE+/NP+/ OMA+ (30µg)} & 0.1 \\
\text{IgE+/NP-/ OMA -} & \text{IgE+/NP+/ OMA+ (90µg)} & \text{ns} \\
\text{IgE+/NP+/ OMA -} & \text{IgE+/NP+/ OMA+ (30µg)} & *** \\
\text{IgE+/NP+/ OMA+ (90µg)} & \text{IgE+/NP+/ OMA+ (30µg)} & ** \\
\end{array} \]

**Fig. 3. A)** Airway response, bronchospasm, to nebulized NP-BSA through the lung in FcεRI transgenic mice sensitized with NP-BSA and treated with omalizumab following a protocol of passive respiratory anaphylaxis (PRA). **B)** Airway MC activity assessed by measuring the local production of mMCP1. (*p-value<0.05, **p-value<0.01, ***p-value<0.001 and ****p-value<0.0001). IgE (human immunoglobulin E), NP (Nitrophenyl), OMA (omalizumab), mMCP-1 (mouse mast cell protease 1).
Study 1 (Objective 1)

Discussion

• The protective effect of EP$_2$ has been validated
  – Having a high number of EP$_2$ allows a lower airway response in HDM-sensitized mice due to a decrease in MC activity.
  – Blockade the activity of EP$_2$ receptor worsens asthmatic parameter

• PRA model is useful to study the anti-IgE function in transgenic hFcεRI mice.
  – This protocol can be use to compare the omalizumab *in vivo* activity versus a biosimilar candidate.
Study 2 (Objective 2)

Predictive Quality Study for a BC with omalizumab

Predictive Quality Study

Physicochemical Characterization
- Composition and physical properties
- Primary structure assess
- PTMs
  - Glycosylation

Biological Characterization
- Biologic activity
- Immunochemical properties
Study 2 (Objective 2)

Physicochemical characterization

- **IDENTITY** (Composition and physical properties)
  - **Primary structure assessment** (Aminoacid composition)
  - **Molecular size & Purity**
    - CE-SDS (Capillar Electrophoresis)
  
  - **Isoform pattern** (Charge heterogeneity)
    - IEF (Isoelectric Focusing)

- **Glycosylation**

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<th>Xolair</th>
<th>Biosimilar Candidate</th>
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<tr>
<td><strong>pI</strong></td>
<td>7.8</td>
<td>7.6</td>
</tr>
<tr>
<td>7.7(^1)</td>
<td>7.5(^1)</td>
<td></td>
</tr>
<tr>
<td>7.6</td>
<td>7.4</td>
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\(^1\)Species more predominant

Fig. 4. CE-SDS electropherograms of (A) Non-reduced and (B) Reduced antibodies.

Fig. 5. IEF gel image of Xolair and the biosimilar candidate.
Study 2 (Objective 2)

Biological characterization

- **Biological activity**
  - *In vitro*
  - *In vivo*

- **Immunological activity**
  - Target affinity
    » **SPR**
    (Surface Plasmon Resonance)

Omalizumab

IgE
Study 2 (Objective 2)

Biological activity

*In vitro*

**β-hexosaminidase release assay**

*In vivo*

PCA (Passive Cutaneous Anaphylaxis)

**Fig. 6.** Comparison between Xolair® and biosimilar candidate activity. (A) **β-hexosaminidase release assay.** (B) Passive Cutaneous Anaphylaxis dosing 2mg of anti-IgE antibody for 300ng of chimeric hlgE. (*p-value*<0.05, **p-value*<0.01, ***p-value*<0.001 and ****p-value*<0.0001).
**Study 2 (Objective 2)**

**Immunological activity**

- **Target affinity**
  - **SPR** (Surface Plasmon Resonance)

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<thead>
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<th>Ligand</th>
<th>$K_a$ (1/Ms)</th>
<th>$K_d$ (1/s)</th>
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<tbody>
<tr>
<td>Xolair®</td>
<td>$2.12 \times 10^6$</td>
<td>0.0024</td>
</tr>
<tr>
<td>Biosimilar Candidate</td>
<td>$1.28 \times 10^6$</td>
<td>0.0044</td>
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**Fig. 7.** Kinetic Analysis of (A) Xolair® and (B) biosimilar candidate using BiaEval 1.1. Coloured lines correspond to observed results at different analyte concentration and black lines correspond to theoretical adjustment.
Study 2 (Objective 2)

Discussion

• The **Biosimilar Candidate:**
  
  – Have **physicochemical differences** compared with the reference product
    • Due to the post-traductional modifications (PTMs)
      – Glycosylation
    – Recognize the desired antigen (**hIgE**)
      • With a **lower affinity** than the reference product
      • Has **biological activity** in **in vitro** and **in vivo** models
        – More studies are needed to be done.
Study 3 (Objective 3)

Evaluation of the synergic activity *in vivo* and *in vitro*

— *In vivo*
  - PRA model

— *In vitro*
  - β-hexosaminidase release test
Thanks for your attention!