Short Course treatment of Subcutaneous Peptide Hydrolysate from Lolium Perenne - gp-ASIT+™ - suppresses Basophil Responses and induces IgG-associated Blocking Antibodies: A RDPCT

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Conventional Allergen Immunotherapy

Subcutaneous

Sublingual
Year 1  Year 2  Year 3

SCIT  Subcutaneous immunotherapy

SLIT  Sublingual immunotherapy

Daily administration 180 to 365 days/year

SCIT 40-60 doctor visits

<25%

<12.5%

Kiel et al., J Allergy Clin Immunol 2013;132:353-60

Novel Approach AIT

4-5 doctor visits

3 weeks prior each pollen season
Purified *Lolium Perenne* peptides for Seasonal Allergic Rhinitis

ASIT Biotech has developed a new AIT preparations based on highly purified allergen fragments from natural source.

**Broad epitope composition**

- Hydrolysis of a highly purified allergen extract which results in highly purified linear peptides.
- No need of epitope screening to target all allergic patients (1,000 Da < MW < 10,000 Da).
Characterisation of Peptide Hydrolysate from *Lolium Perenne* (gpASIT+™) and its ability to bind to IgE compared to Grass Pollen extract
Characterisation of Peptide Hydrolysate from *Lolium Perenne* (gpASIT+™) and its ability to bind to IgE compared to Grass Pollen extract

Reproducibility

Shamji et al., JACI (under revision)
Characterisation of Peptide Hydrolysate from *Lolium Perenne* (gpASIT+\textsuperscript{TM}) and its ability to bind to IgE compared to Grass Pollen extract

Reproducibility

Baseline activation of CD63+ basophils

$\text{IgE binding (\%)}$

Concentration [\(\mu g/ml\)]

Concentration [\(ng/mL\)]

*\(p \leq 0.05\)

**\(p \leq 0.05\)

***\(p \leq 0.05\)

Graph#8

4-P Fit: $y = \frac{(A - D)}{1 + (x/C)^B} + D$

Plot#2 (PROT 08J20 (1/3): Concentration vs %INHIB)

$A = -0.72$

$B = 0.973$

$C = 0.0861$

$D = 95.7$

$R^2 = 0.999$

Plot#4 (PROT 00597 (1/3): Concentration vs %INHIB)

$A = 4.86$

$B = 0.835$

$C = 0.0905$

$D = 97.9$

$R^2 = 0.999$

Plot#6 (PEP 00597 (1/3): Concentration vs %INHIB)

$A = -8.18$

$B = 0.269$

$C = 136$

$D = 143$

$R^2 = 0.999$

Plot#10 (PEP 08K05 (1/3): Concentration vs %INHIB)

$A = -12.8$

$B = 0.227$

$C = 497$

$D = 170$

$R^2 = 0.998$

Shamji et al., JACI (under revision)

Shamji et al., JACI (under revision)
Hypotheses

- 3-week treatment with subcutaneous peptide hydrolysates from *Lolium perenne* (LPP, gpASIT+™) is associated with reduction in CSMS and RTSS during the peak and throughout the entire pollen season.

- gpASIT+™ immunotherapy but not placebo blunts the seasonal increases of sIgE

- gpASIT+™ immunotherapy but not placebo treatment suppresses grass pollen-induced basophil hyperresponsiveness and basophil reactivity.

- A short-course of gpASIT+™ immunotherapy induces IgG4-associated blocking antibodies that conferred clinical benefit during the pollen season.
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Study design – RDBCT

Immune mechanisms analyses on participant from a single site - (Ghent, Belgium).
3-week treatment with subcutaneous peptide hydrolysates from *Lolium perenne* (LPP, gpASIT+™) suppresses CSMS and RTSS

CSMS reduction in Belgium
Peak period: **-35.1%; \( P=0.03 \).**
Entire pollen season: **-53.7%; \( P=0.03 \).**

RTSS reduction in Belgium
Peak period: **-27.4%, \( P=0.04 \).**
Entire pollen season: **-56.9%, \( P=0.01 \).**
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- A short-course of gpASIT+™ immunotherapy induces IgG4-associated blocking antibodies that conferred clinical benefit during the pollen season.
Effect of LPP immunotherapy on sIgE levels

V2 = Before treatment
V6 = After treatment
V8 = After the grass pollen season
Effect of LPP (gpASIT+™) immunotherapy on sIgE levels

V2 = Before treatment
V6 = After treatment
V8 = After the grass pollen season
Hypotheses

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Effect of LPP (gpASIT+<sup>TM</sup>) immunotherapy on CD203c<sup>bright</sup>CRTh2<sup>+</sup> Basophils

- **V2** = Before treatment
- **V6** = After treatment
- **V8** = After the grass pollen season

33 ng/mL of grass pollen allergen
Effect of LPP (gpASIT+™) immunotherapy on CD63⁺CRTh2⁺ Basophils

V2 = Before treatment
V6 = After treatment
V8 = After the grass pollen season
Effect of LPP (gpASIT+™) immunotherapy on CD63⁺CRTh2⁺ Basophils

V2 = Before treatment
V6 = After treatment
V8 = After the grass pollen season
Effect of LPP (gpASIT+™) immunotherapy on Basophil Reactivity

V2 = Before treatment
V6 = After treatment
V8 = After the grass pollen season

CD203c^bright CRTH2^+ Basophils [%]

- Anti-IgE (1 µg/mL)

CD63^+ Basophils [%]

- Anti-IgE (1 µg/mL)

LPP  PL
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- A short-course of gpASIT+™ immunotherapy induces IgG4-associated blocking antibodies that conferred clinical benefit during the pollen season.
Effect of LPP (gpASIT+™) immunotherapy on serum IgG4 and allergen neutralising blocking antibodies

V2 = Before treatment
V6 = After treatment
V8 = After the grass pollen season

** = p < 0.01
* = p < 0.05
Summary/Conclusions

- 3-week treatment with subcutaneous peptide hydrolysates from *Lolium perenne* (LPP, gpASIT+™) is associated with reduction in CSMS and RTSS during the peak and throughout the entire pollen season.

- gpASIT+™ immunotherapy but not placebo blunts the seasonal increases of sIgE.

- gpASIT+™ immunotherapy but not placebo treatment suppresses grass pollen-induced basophil hyperresponsiveness and basophil reactivity.

- A short-course of gpASIT+™ immunotherapy induces IgG4-associated blocking antibodies that conferred clinical benefit during the pollen season.
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