



## SELECTED OPPORTUNITIES IN IMMUNOLOGY

Targeting the Secreted IgE Poly-A Signal Allows Specific Inhibition of Allergen-Specific IgE Production (BIO19135)

# TARGETING THE SECRETED IgE POLY-A SIGNAL ALLOWS SPECIFIC INHIBITION OF ALLERGEN-SPECIFIC IgE PRODUCTION (BIO19135)

## Product factsheet

Stage: *in vivo* PoC

### ▶ **Product: antisens oligonucleotide**

### ▶ **Mechanism:**

- ◆ Immunoglobulins (Ig) are expressed either on the surface of B cells or as secreted antibodies by plasma cells
- ◆ Different polyadenylation signals across the constant region of Ig heavy chains are used
- ◆ Regulation of the production of secreted Ig is highly important for an effective immune response
- ◆ The overproduction of allergen-specific secreted IgE is one of the established features of many forms of allergies
- ◆ Inversely, regulation of the production of membrane-anchored Ig would be suitable for the treatment of B cell lymphomas by reducing the survival signaling induced by the BCR in malignant B cells

=> **Development of ASO-based strategies to modulate secreted and membrane anchored Ig production**

### ▶ **Phase of development: in vivo PoC**

- ◆ ASO targeting the secreted IgE polyadenylation signal (sec-PAS) decrease IgE production by U266 cell line
- ◆ ASO targeting the IgE sec-PAS decrease IgE production in primary B cells of a mouse model expressing humanized IgE
- ◆ ASO targeting the IgE sec-PAS decrease allergen-specific IgE expression upon treatment of InEps hybridoma cells
- ◆ IgE targeting ASO decreased IgE secretion in vivo

### ▶ **Potential applications:** Allergy / B cell lymphomas

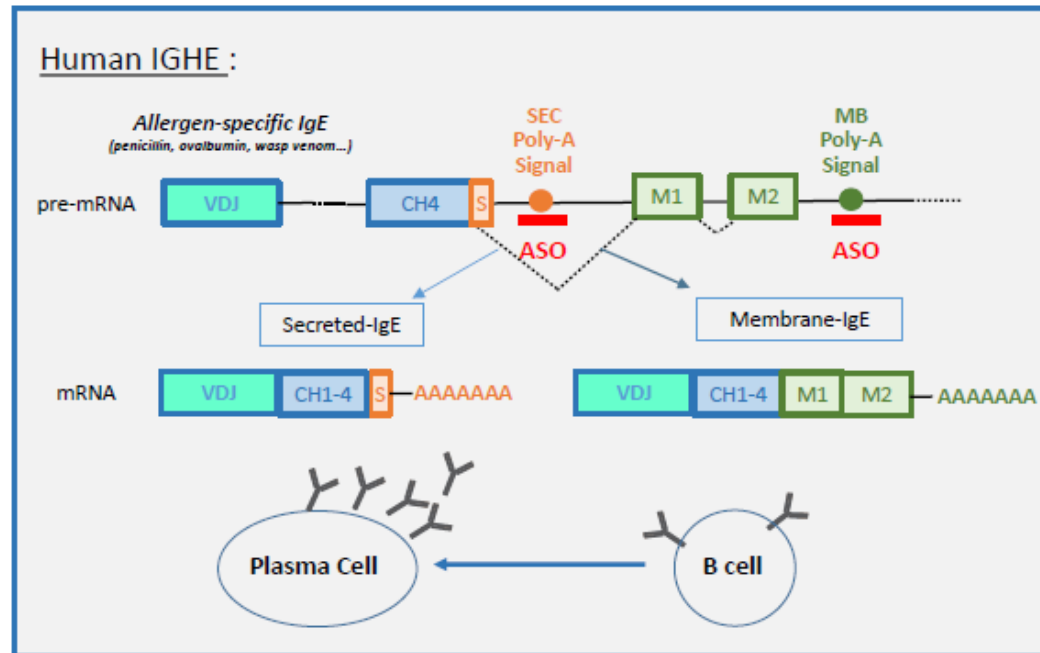
### ▶ **Publication:** Targeting IgE polyadenylation signal with antisense oligonucleotides decreases IgE secretion and plasma cell viability. Marchalot et al., The Journal of Allergy and Clinical Immunology. 2021

### ▶ **Patents :** "METHODS FOR MODULATING IMMUNOGLOBULIN EXPRESSION". EP19305716. Priority: 04 June 2019

# TARGETING THE SECRETED IGE POLY-A SIGNAL ALLOWS SPECIFIC INHIBITION OF ALLERGEN-SPECIFIC IGE PRODUCTION (BIO19135)

## Proof of Concept

### Isotype-specific inhibition of antibody production using ASO targeting the membrane or secreted immunoglobulin poly-adenylation signals



#### ASO masking Ig poly-A signals as new therapeutic weapons

(A) To decrease B-cell receptor (BCR) signaling in mature B cells with an ASO masking the membrane Ig poly-A signal  
⇒ Lymphomas (DLBCL, etc.), Leukemia (CLL)

(B) To shut-down antibody secretion in plasma cells with an ASO masking the secreted Ig poly-A signal  
⇒ Allergy (IgE), etc.

# TARGETING THE SECRETED IGE POLY-A SIGNAL ALLOWS SPECIFIC INHIBITION OF ALLERGEN-SPECIFIC IGE PRODUCTION (BIO19135)

## Proof of Concept

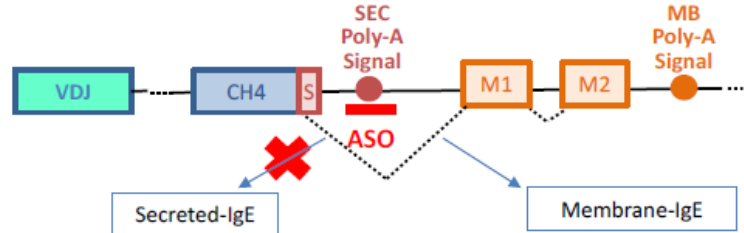
### ASO targeting the secreted IgE polyadenylation signal (sec-PAS) decrease IgE production by U266 cell line

A

Secreted IGHE poly-A signal

sec-IGHE-PAS 5' ... GACCCCAGGAAGCUACCCCCAAUAAA CUGUGCCUGCUCAGAGCCCC ... 3'

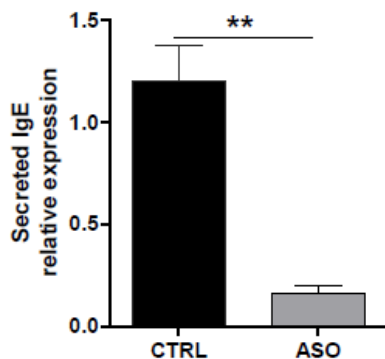
ASO-sec-IGHE-PAS 3' GTTATTTGACACGGACGAGTCTCG 5'



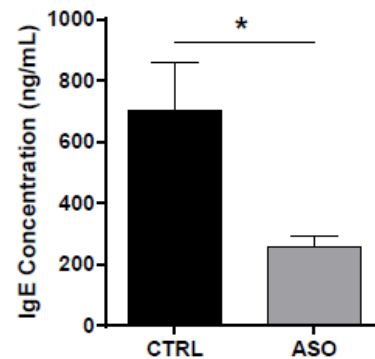
### Decreased IgE production upon administration of ASO targeting the poly-adenylation signal (sec-PAS) sequence

- U266 cells were treated with 6 $\mu$ M Vivo-Morpholino Control ASO (CTRL) or IgE-sec-PAS targeting ASO for 48 hours.
- Specific secreted IgE RT-qPCR normalized on untreated cells was performed on 48 hours total RNA.
- Total IgE ImmunoCAP assay showing IgE production in culture supernatants.

B



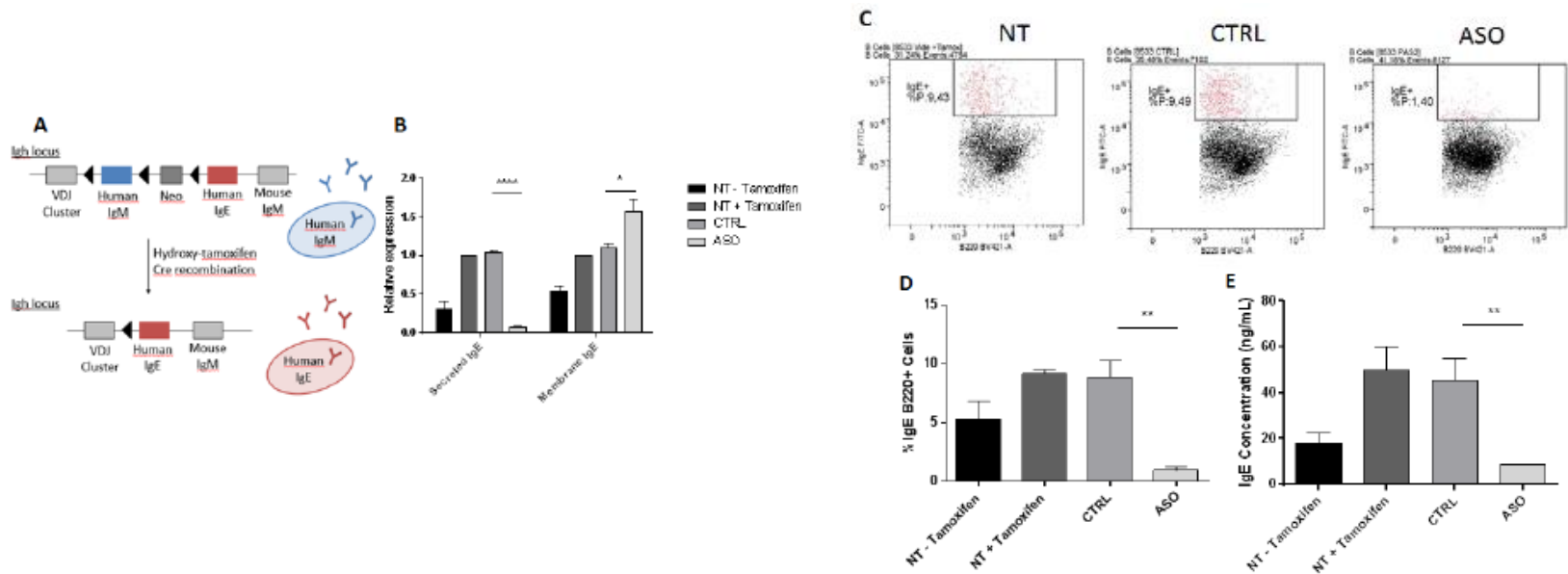
C



# TARGETING THE SECRETED IgE POLY-A SIGNAL ALLOWS SPECIFIC INHIBITION OF ALLERGEN-SPECIFIC IgE PRODUCTION (BIO19135)

## Proof of Concept

### ASO targeting the secreted IgE polyadenylation signal (sec-PAS) decrease IgE production in primary B cells of a mouse model expressing humanized IgE



### Drastic decrease of IgE production upon treatment of primary cells secreting human IgE

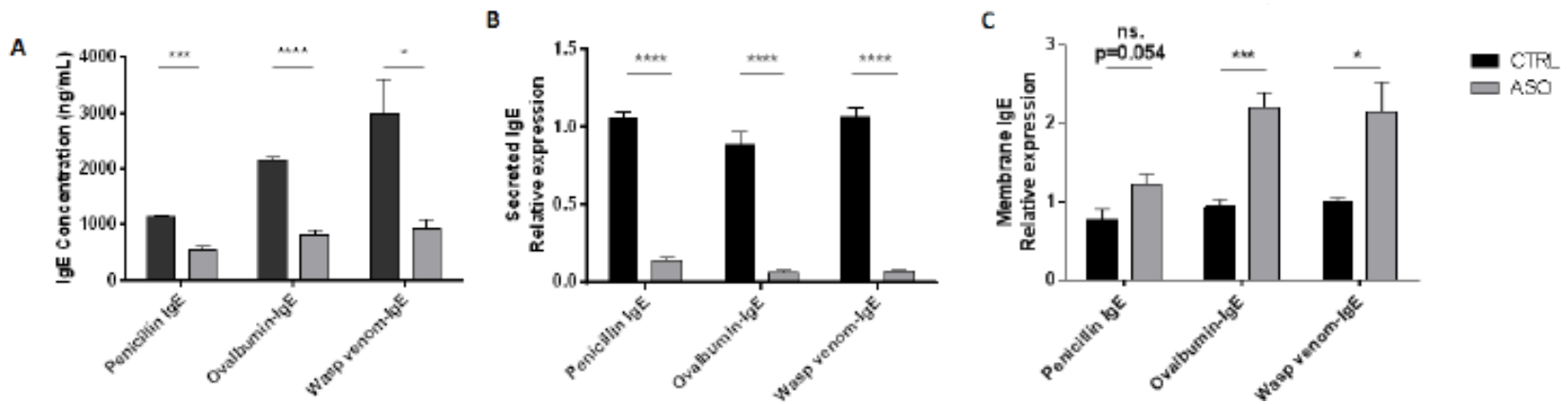
Humanized IgE-expressing spleen cells from InEps mice were treated with IgE-sec-PAS targeting ASO or Control ASO

- InEps mouse Igh locus. hydroxy-tamoxifen-induced Cre recombination allow expression of humanized IgE
- Quantification of secreted-IgE and membrane-IgE by RT-qPCR
- Flow cytometry analysis of intracellular-IgE expression in B220+ cells
- Quantification of flow cytometry analysis
- Quantification of IgE production in culture supernatants by ImmunoCAP assay

# TARGETING THE SECRETED IgE POLY-A SIGNAL ALLOWS SPECIFIC INHIBITION OF ALLERGEN-SPECIFIC IgE PRODUCTION (BIO19135)

## Proof of Concept

### ASO targeting the secreted IgE polyadenylation signal (sec-PAS) decrease allergen-specific IgE expression upon treatment of InEps hybridoma cells



### Decrease of allergen-specific IgE expression upon treatment of InEps hybridoma cells

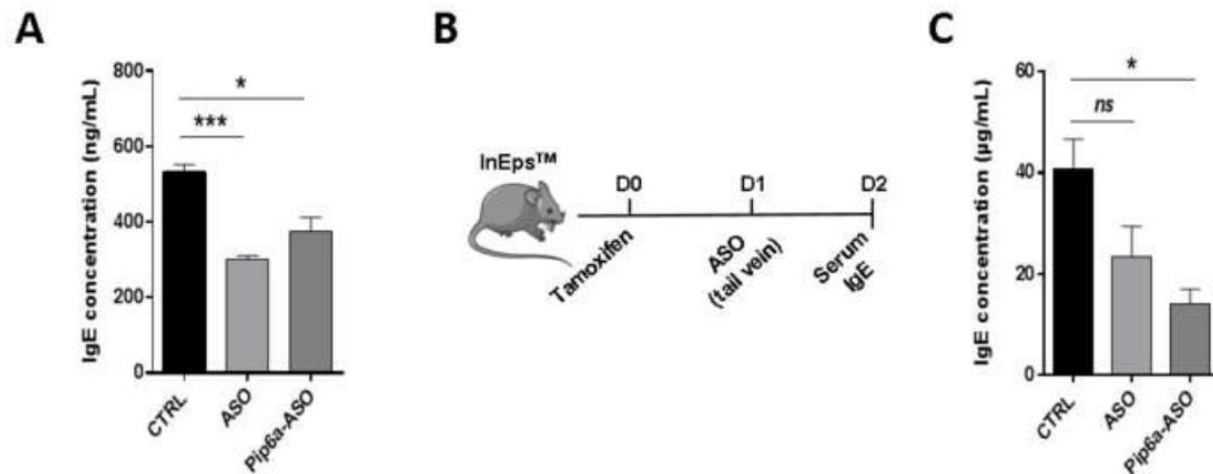
Hybridomas (allergen-specific InEps B cells merged with SP2/0 cell line) were treated with IgE-sec-PAS targeting ASO or Control ASO

- Total IgE production quantification in culture supernatants by ImmunoCAP assay
- Quantification of specific secreted IgE mRNA by RT-qPCR
- Quantification of specific membrane IgE mRNA by RT-qPCR

# TARGETING THE SECRETED IGE POLY-A SIGNAL ALLOWS SPECIFIC INHIBITION OF ALLERGEN-SPECIFIC IGE PRODUCTION (BIO19135)

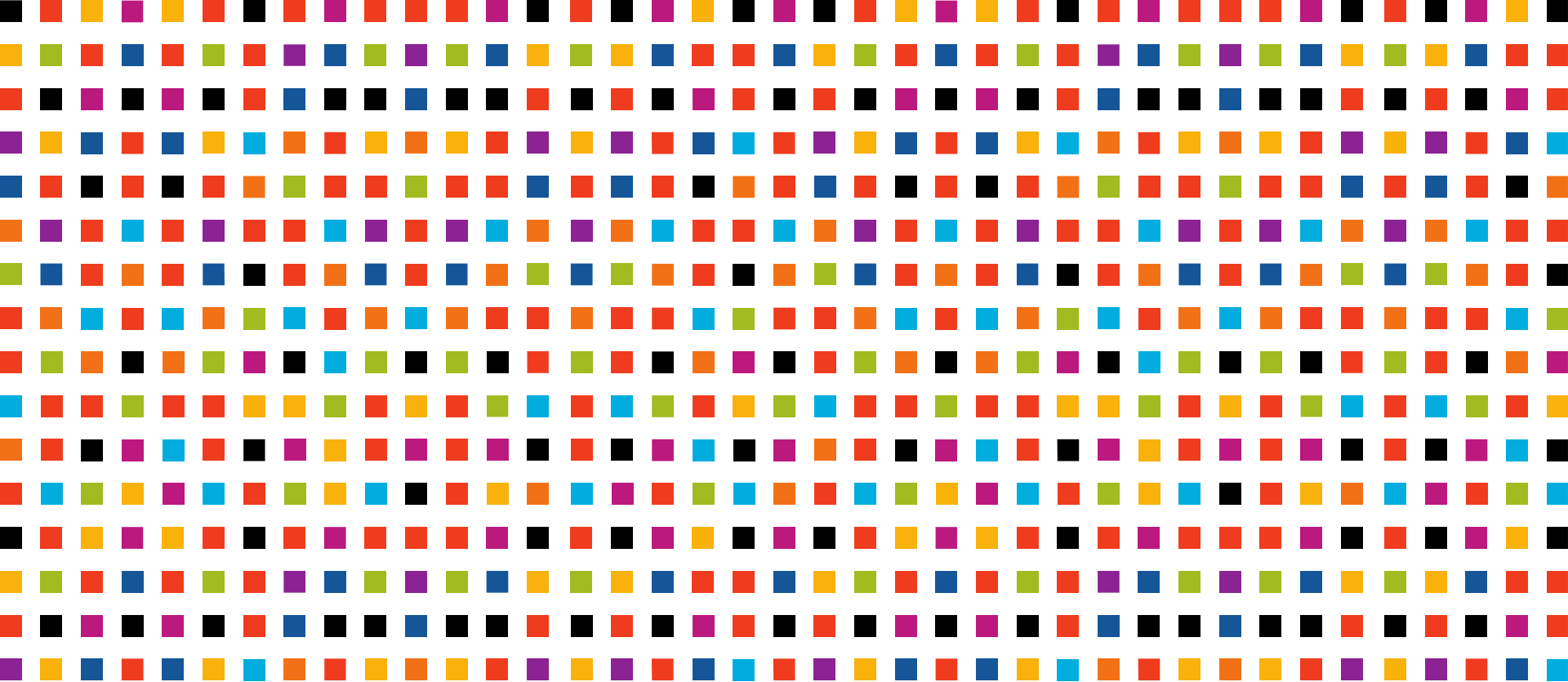
## Proof of Concept

### IgE targeting ASO decreased IgE secretion *in vivo*



### IgE targeting ASO decreased IgE secretion *in vivo*

- IgE concentrations assessed in cultured supernatants of U266 cells treated 24h with 6  $\mu$ M ASO coupled to different cell-penetrating moieties: octa-guanidine dendrimer (ASO) and arginine-rich peptide (Pip6a-ASO) (n=3).
- IgE expression was induced in Ineps™ mice (n=3/group) by administration of Tamoxifen (oral gavage) at day 0 and 24h later mice were injected iv (tail vein) with ASO (12.5 mg/kg).
- Serum IgE concentrations determined at day 2.



NATHAN.POMORSKI@INSERM-TRANSFERT.FR