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Development of long-acting polymer-single domain antibody conjugates to inhibit neutrophil elastase in chronic obstructive pulmonary disease

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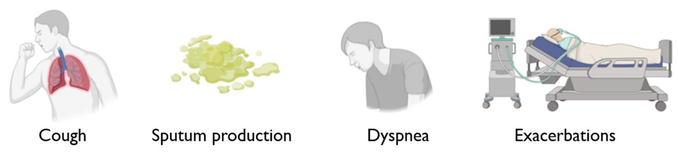
Introduction

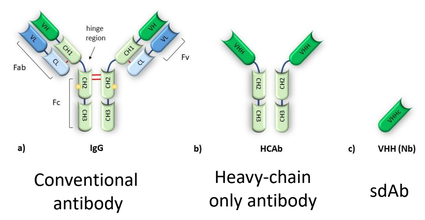
COPD

is a muco-obstructive lung disease associated with chronic inflammation due to the accumulation of neutrophils in the lungs and the excessive secretion of neutrophil elastase, which leads to the progressive destruction of the lung tissue and loss of pulmonary function[1,2].



NbE201

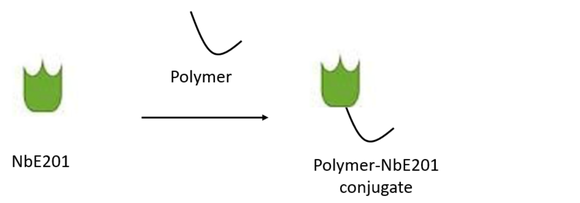
is a variable domain of camelid heavy-chain only antibody, called single-domain antibody (sdAb). It is able to fully inhibit human neutrophil elastase (hNE)[3].



Its rapid clearance from the lungs limits its therapeutic efficacy → need to deliver it several times a day.

PAS/XTEN

are two polypeptide polymers fused with NbE201 to increase its residence time in the lung[4]. They do not have the disadvantages of PEG (non-biodegradability)[5,6,7].



Frequent lung administration
Poor patient compliance

Protection against aggregation
Increased half-life due to
1) low absorption/low elimination
2) protection against proteases

Objectives

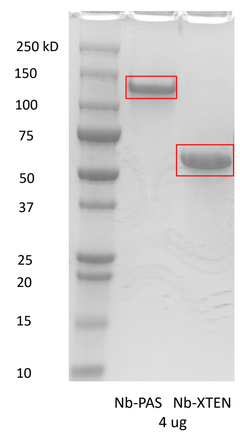
The purpose of this work is to recombinantly produce, purify and characterize long-acting polymer-VHH conjugates able to inhibit hNE: XTENylated and PASylated NbE201.

Methodology and results

Similar molecular weight of approximately 30kDa were chosen for XTEN (27.6kDa) and PAS (27.3kDa) and linear 30kDa PEG used as a well-characterized comparator. The amino acid composition of PAS is Pro, Ala and Ser, and that of XTEN is Pro, Ala, Ser, Gly, Glu and Thr.

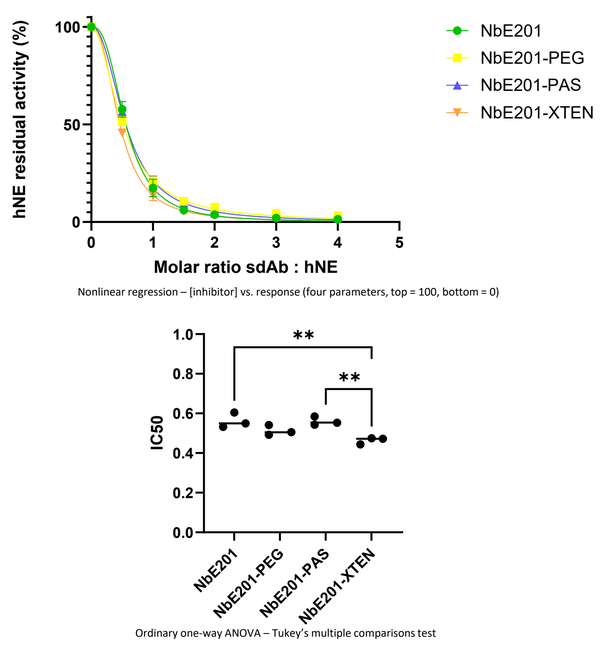
NbE201-PAS and NbE201-XTEN have been produced, extracted and purified successfully

6 mg of NbE201-PAS and **30 mg** of NbE201-XTEN were produced per litre of bacterial culture

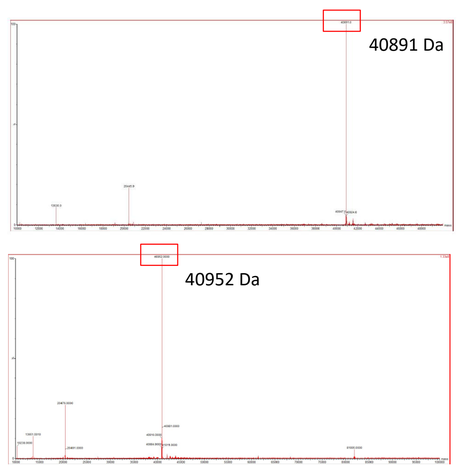


XTENylated and PASylated NbE201 were produced in *Escherichia coli* BL21(DE3) by bacterial expression. The proteins were recovered by periplasmic extraction and purified through a two-step procedure: immobilized metal affinity chromatography (IMAC), exploiting the C-terminal His-tag, followed by size-exclusion chromatography (SEC).

The addition of a polymer to NbE201 does not adversely affect its inhibitory activity



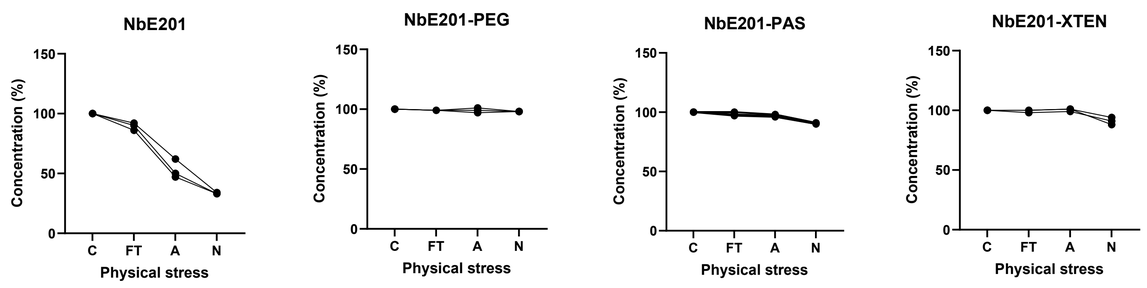
The exact molecular weights are consistent with the theoretical values



Mass spectra of NbE201-XTEN and NbE201-PAS after deconvolution

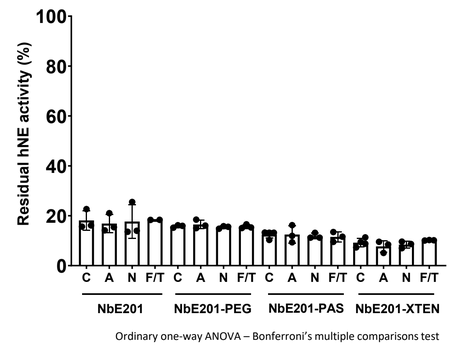
Hybrid quadrupole/orthogonal acceleration, time-of-flight mass spectrometer with nano-electrospray ionization source

The addition of a polymer to the NbE201 is shown to stabilise it against aggregation at air-water interfaces during agitation, nebulisation and freeze/thaw cycles



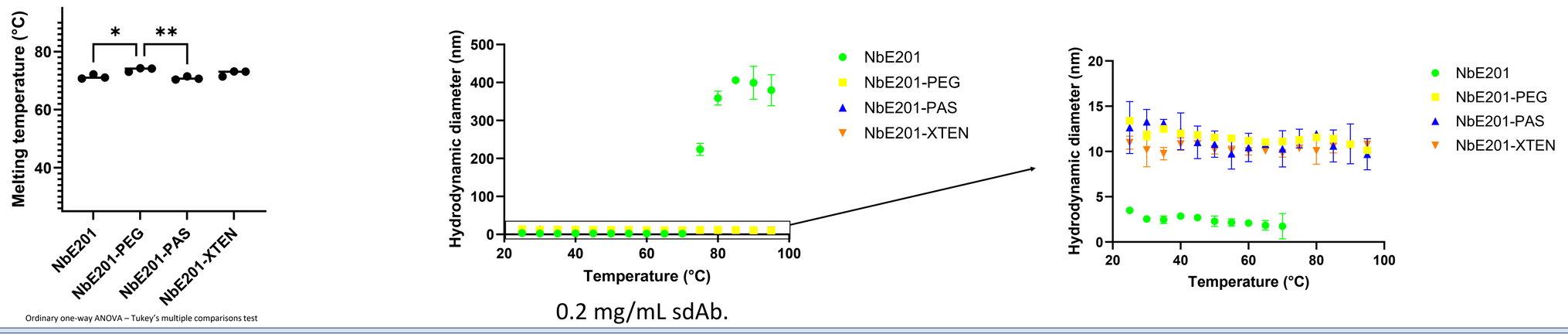
Concentration after removal of insoluble aggregates following physical stress

Anti-hNE activity after the removal of insoluble aggregates following physical stress



C = no stress, **FT** = 10 **freeze/thaw cycles** at -20°C, **A** = magnetic stirrer **agitation** for 4 hours at 220 rpm and **N** = mesh **nebulization** with InnoSpire Go nebulizer. 0.5 mg/mL sdAb.

The addition of a polymer to the NbE201 is shown to stabilise it against heat-induced aggregation



0.2 mg/mL sdAb.

Conclusions

- We produced significant quantities of purified polymer-NbE201 conjugates.
- We characterized the polymer-NbE201 conjugates for molecular weight, biological activity and melting temperature.
- We showed the stabilization of NbE201 against physical stresses and heat-induced aggregation when conjugated with a polymer.
- In the future, we would like to perform *ex vivo* characterization using human COPD sputa and *in vivo* pharmacokinetic experiments.

References

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Images were created on PowerPoint and BioRender.