DEVELOPMENT OF A STABLE SPRAY DRIED FORMULATION OF AN IL-17A HUMABODY VH DOMAIN

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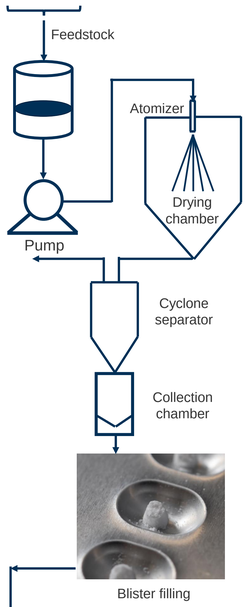
INTRODUCTION

- Interleukin 17A is a pro-inflammatory cytokine. IL-17A in the lung has been postulated to be involved in neutrophilic inflammation & airway remodelling of chronic respiratory conditions [1].
- Created using a proprietary transgenic mouse, Humabody VHs are small (12 kDa), robust, *in vivo* matured, fully human antibody VH domains [2]. A Humabody with high affinity and specificity to IL-17A was prepared.
- One of the biggest challenges for biologics is the maintenance of potency and integrity during manufacture, storage and drug delivery [3].
- The goal of this study was to evaluate the stability and aerodynamic properties of a spray dried therapeutic targeting IL-17A for pulmonary delivery.

METHODS

Table 1 - Dry product constituent components of three selected formulations

API / Excipients	Humabody (% w/w)	Buffers (% w/w)	Trehalose (% w/w)	Leucine (% w/w)	Trileucine (% w/w)	Ammonium bicarbonate (% w/w)
Formulation 1	10	1.5	67.5	20	-	-
Formulation 2	10	1.5	77.5	-	10	-
Formulation 3	10	1.5	77.5	10	-	60



- A pre-formulation buffer compatibility screen was carried out.
- Humabody was mixed with sterile water containing dissolved excipients as stated in Table 1.
- Laboratory scale, customised co-current spray dryer (Vectura Ltd.) with a two fluid nozzle at a nominal 1g batch size was used.
- The liquid feedstock was atomized into droplets (inlet temperature > 100°C); the droplets were rapidly dried to form composite dry powder particles of a controlled particle size distribution [4,5].
- The dry powder formulations were collected under controlled humidity conditions (<20% RH).
- Spray dried formulations were filled and sealed into aluminium unit dose blisters.

- Biological Stability: Potency and integrity
- Physical Characterization: Moisture content, particle size distribution, glass transition temperature (Tg) and particle morphology by scanning electron microscopy (SEM).
- Aerosol Performance – Blister evacuation (Emitted dose, ED) and fine particle fraction (FPF; % ≤5µm) from Vectura's medium resistance unit dose device were determined by gravimetric fast screening impactor (FSI) at 30 and 60 L/min flow rate.

RESULTS

Biological stability

- Analysis by size exclusion chromatography (SEC) showed that the Humabody was unaffected by spray drying and storage at the selected condition of 40˚C/75% RH for 3 months. Initial aggregate levels were not elevated above the 3% found in the standard (starting material).
- Also, the percentage monomer of the three formulations over all time points remained the same as the reference standard by SDS-PAGE, between 98-100 %.
- ELISA results showed that the bioactivity of the protein did not drop below that of the reference standard activity of the API (Table 2).

Table 2. SEC/SDS-PAGE/ELISA results from the three formulations over three months.

Test		Formulation 1		Formulation 2		Formulation 3	
		T=0	T=12W	T=0	T=12W	T=0	T=12W
SEC %	Monomer	95.6	96.5	96.8	97.3	95.6	96.3
	HMWS	4.4	3.5	3.2	2.7	4.4	3.7
SDS-PAGE	% main band	98.1	100	98.3	100	98.2	100
Binding - ELISA	IC50 (nM)	3.2	4.3	3.6	2.8	3.6	3.8
	Control IC50	2.7	4.1	3.1	2.6	3.0	3.2

AN IL-17A HUMABODY WAS SUCCESSFULLY SPRAY DRIED WITH EXCELLENT STABILITY AND AEROSOL PROPERTIES

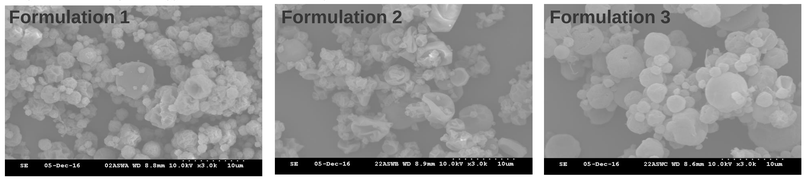
Physical Characterization

- Particle size was unchanged for all formulations over the stability period. Formulation 3 showed larger particles sizes at D50 and D90 which is attributable to the use of ammonium bicarbonate [5] (Table 3).
- The moisture content was consistent across all formulations after an initial uptake of moisture between 0-4 weeks, which is typical for spray dried powders. (data not shown)
- Glass transition was detected in all formulations indicating substantially amorphous powder with no re-crystallisation on storage
- SEM of F1 & F2 showed shrivelled collapsed spheres which correspond to the presence of surface active agents. F3 showed smooth spherical particles with some venting holes characteristic of the use of ammonium bicarbonate blowing agent

Table 3. Physical Characterization summary

Test		Formulation 1		Formulation 2		Formulation 3	
		T=0	T=12W	T=0	T=12W	T=0	T=12W
Particle size by Laser Diffraction	D_{10} µm	1.0	1.0	1.0	1.0	1.2	1.7
	D_{50} µm	2.3	2.3	2.9	2.8	4.1	4.4
	D_{90} µm	4.7	4.7	5.9	5.8	7.5	7.8
Moisture content	%	1.1	2.5	1.0	2.5	1.0	2.4
Tg	°C	87	74	74	77	72	75

Figure 1. Morphology of formulations by SEM

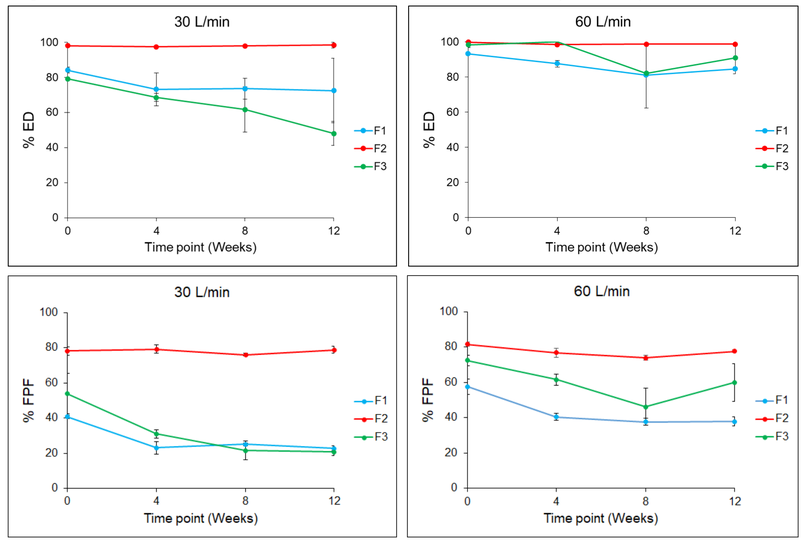


RESULTS

Aerosol performance

- F2 had superior FPF and blister evacuation that was not affected by time on storage or flow rate, indicating an optimal performance for inhaled drug delivery (Figure 2)
- F1 and 3 exhibit a drop in blister evacuation on storage which is exacerbated by aerosolization at lower flow rates. A concurrent drop in FPF is also observed for both formulations

Figure 2. ED and FPF of formulations over time at 2 two flow rates (n=3 ± SD)



CONCLUSIONS

- A spray dried formulation of an IL-17A-specific Humabody VH was developed that demonstrated both excellent biological stability and consistent aerosol performance. This was achieved by formulating with a combination of trehalose and trileucine.
- The aerosol performance (ED and FPF) and bio-stability has been demonstrated over a 12 week storage period at accelerated conditions.
- This demonstrates the feasibility of formulating a spray dried Humabody therapeutic intended to address key drivers of pulmonary inflammation by inhalation.

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