Anakinra repurposing in Cystic Fibrosis through inhalation drug delivery technology

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Introduction
Cystic Fibrosis (CF) is one of the most common genetic inherited diseases. Lung infections are responsible for death in 80% of people with CF, which has a significant impact on innovation of anti-infectious therapies. Commercially available recombinant non-glycosylated form of the endogenous IL-1 receptor antagonist (Anakinra) demonstrated an inhibition of inflammasome-dependent inflammation in CF without impairing antimicrobial resistance (1). In this work we have performed a preclinical study to evaluate the therapeutic efficacy of an inhalable formulation of anakinra.

Results
SEM analysis showed that obtained microparticles are wrinkled and porous with evident voids inside with no aggregation (Figure 1). Aerodynamic assessment of anakinra formulations showed good performances in terms of respirable fraction (Tab. 1). DSF and in-vitro activity experiments showed preservation of protein structure and inhibitory activity on IL-1β mRNA expression (Figure 2). The formulation effectively restrained lung infection of mice infected with live A. fumigatus, particularly in terms of inflammatory cytokine reduction, while reducing peripheral neutropenia (Figure 3).

Conclusions
The results obtained show that a promising anakinra inhalable form was developed with a perspective application in CF therapy management, as well as for other inflammatory diseases such as COVID-19 disease, other viral or bacterial infections, rheumatoid arthritis and chronic obstructive pulmonary disease. In this work, we demonstrated the possibility of producing potentially scalable formulations of the recombinant protein suitable for pulmonary administration using spray-drying technique.

Materials and Methods
Anakinra was obtained from MedChemExpress, USA as an aqueous solution. Exipients used were mannitol (Roquette Pharma, France) and D-leucine (Sigma-Aldrich, Milan, Italy). All water was Milli Q grade (Milli-Q System, Millipore, Milan, Italy). Spray-dried formulation of anakinra with different excipients was prepared by Mini Spray-dryer model B-290 (BÜCH, Switzerland) (2). Particle morphology, surface characteristics and particle aggregation of spray-dried microparticles were assessed by scanning electron microscopy (SEM). A PSS Accusizer C770 equipped with an autodilution system was employed to measure particle size distributions. Protein integrity was assessed by differential scanning fluorimetry (DSF). The aerodynamic behavior was evaluated using a Twin-Stage Glass Impinger (TSGI) (Disa, Milan, Italy) with DPI low resistance RS01 model T from Plastitec (Lecco, Italy). Conditions were set following the European Pharmacopeia (apparatus A) guidelines. The amount of protein deposited on the capsule, the inhaler, throat, and each stage of the TSGI, was recovered and analyzed by HPLC. The functional activity was assessed in vitro on THP-1 cells and in vivo in a murine Aspergillus fumigatus lung infection (2) using a dry powder inhalator model DPI-4M (Penn-Century Inc., Wyndmoor, PA, USA).

References

Table 1: Aerodynamic assessment of Anakinra dry powders.

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<tr>
<th>Emitted Fraction (%)</th>
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<td>47.28</td>
<td>29.17</td>
<td>61.69</td>
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Figure 1: Morphology of Anakinra microparticles observed with SEM. Scale bar: 20 µm (A) and 5 µm (B). The image shows the absence of aggregates and irregular and wrinkled surface of recombinant protein MPs.

Figure 2: THP-1 cells were treated with 10 µg/ml Standard reference protein or formulated protein before the stimulation with 50 ng/ml recombinant cytokine. Cells were harvested at 2, 6 and 24 hours after treatments and evaluated for cytokine gene expression by RT-PCR. Graph shows the mean ± s.d. and is representative of one experiment. ***p-value < 0.0001. One-way-ANOVA, Bonferroni’s post hoc test.