

Inhaled medication and inhalation devices for lung disease in patients with cystic fibrosis: A European consensus[☆]

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Abstract

In cystic fibrosis inhalation of drugs for the treatment of CF related lung disease has been proven to be highly effective. Consequently, an increasing number of drugs and devices have been developed for CF lung disease or are currently under development. In this European consensus document we review the current status of inhaled medication in CF, including the mechanisms of action of the various drugs, their modes of administration and indications, their effects on lung function, exacerbation rates, survival and quality of life, as well as side effects. Specifically we address antibiotics, mucolytics/mucous mobilizers, anti-inflammatory drugs, bronchodilators and combinations of solutions. Additionally, we review the current knowledge on devices for inhalation therapy with regard to optimal particle sizes and characteristics of wet nebulisers, dry powder and metered dose inhalers. Finally, we address the subject of testing new devices before market introduction.

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Contents

1.	Introduction	296
2.	Inhaled medications	297
2.1.	Antibiotics	297
2.1.1.	Tobramycin	297
2.1.2.	Colistimethate sodium.	298
2.1.3.	Aztreonam lysine	298
2.1.4.	Liposomal ciprofloxacin.	299
2.1.5.	Aerosol MP-376.	299
2.1.6.	Amphotericin B	299
2.2.	Mucolytics/mucous mobilizers	299
2.2.1.	Dornase alfa.	299
2.2.2.	Hypertonic saline	299
2.2.3.	Denufosol tetrasodium	300
2.2.4.	Lancovotide (Moli1901)	300
2.3.	Inhaled anti-inflammatory therapies	300
2.3.1.	Inhaled corticosteroids	300
2.3.2.	Antiproteases	300
2.4.	Bronchodilators.	300
2.5.	Drug combinations	301
2.5.1.	Chemical stability and particle-size distribution.	301
3.	Devices for inhaled medication	302
3.1.	Physical parameters	302
3.1.1.	Particle mass, inhaled mass and respirable mass	302
3.1.2.	Lung dose.	303
3.1.3.	Output and output rate	304
3.1.4.	Residual volume	304
3.1.5.	Particle-size distribution.	304
3.1.6.	Polydisperse and monodisperse aerosols	305
3.1.7.	Administration time	305
3.1.8.	Drug waste during aerosolization	305
3.1.9.	General purpose nebuliser.	305
3.2.	Bacteriological safety and performance of nebulisers over time	305
3.2.1.	Bacteriological safety	306
3.2.2.	Performance over time	306
3.2.3.	Cleaning of nebuliser equipment	306
3.3.	Patient parameters	306
3.3.1.	Deposition pattern and breathing pattern	306
3.3.2.	The optimal particle size	306
3.3.3.	Infants and small children.	307
4.	Questions and answers	307
	References	310

1. Introduction

While the first description of the hereditary disease cystic fibrosis (CF) [1], emphasised fatal congenital steatorrhea and pancreatic destruction, lung disease has now been recognized to have the largest impact on morbidity and mortality in older people with CF [1]. Lung disease develops as a consequence of mutations in the CF transmembrane conductance regulator (CFTR) gene [2], which encodes a membrane-bound cAMP-regulated chloride channel: diminished chloride and water secretion leads to viscous secretions in the affected airways [3,4]. This impairs mucociliary clearance [3], thereby facilitating chronic bacterial infections, which may start at a very early age [1,5,6].

Among the bacterial pathogens isolated from airways of CF patients the triad *Haemophilus influenzae*, *Staphylococcus*

aureus and *Pseudomonas aeruginosa* [7,8] has been isolated most frequently. Infections with some members of the *B. cepacia* complex are associated with a markedly shortened median survival [9]. Other microbial pathogens isolated from CF patients include *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Mycobacteria* ssp., *Aspergillus fumigatus* [10] and strict anaerobes [11,12].

P. aeruginosa, a Gram-negative bacterium found in many natural and man-made water sources, is present in approximately 27% of patients aged 2–5 years and approximately 80% of patients aged 25–34 years [1]. Thus this opportunistic bacterium pathogen is regarded as the most important pathogen in CF [13–16]. Respiratory infections with *P. aeruginosa* are difficult to treat due to growth of the pathogen in biofilm-like macrocolonies [10,17]. Nevertheless, various treatment strategies have been developed

during the past few decades that have a significant positive impact on prognosis [10]. The predicted median survival age of CF individuals in the USA increased from 14 years in 1969 to 36.5 years in 2005, and 43% of patients are 18 years of age or older [2]. European registries report similar increases in median survival ages [6,18]. Repeated courses of inhaled antibiotics using high doses for the treatment of lung disease in CF patients have been applied increasingly in the last two decades [10]. This strategy has circumvented the problem of the poor penetration of intravenously administered antibiotics into lung parenchymal tissue and bronchial secretions, and their potential systemic toxicity when given over prolonged periods of time.

One of the most striking characteristics of *P. aeruginosa* is its extraordinary capacity to develop resistance to virtually all anti-pseudomonal agents through the selection of genetic mutations. Repeated and prolonged treatment strategies may therefore increase the resistance of the pathogen to the applied antibiotics, as demonstrated in trials using tobramycin [19], leading to a strategy of intermittently administration of this drug [14]. Development of antibiotic resistance in *P. aeruginosa* is also facilitated by the occurrence of hypermutable (or mutator) strains, deficient in the DNA mismatch repair system [20,21]. To avoid the development of resistance and in an attempt to eradicate non-mucoid *P. aeruginosa*, many European CF centres started antibiotic treatment early after the first detection of the pathogen with great success [22–27]. In CF patients initially colonized with mucoid *P. aeruginosa* strains, or patients in whom initially nonmucoid strains have already switched to mucoid strains, it may not be possible to eradicate pathogens from their airways.

Chronic airway inflammation is uniformly observed in patients with CF [10,28,29]. Chronic lung inflammation with episodes of acute exacerbations initiates several physiological and metabolic changes with deleterious effects including weight loss, anorexia, and metabolic breakdown. Thus, as an adjunct to optimal antibiotic therapy, anti-inflammatory therapy is warranted to avoid a decline in lung function, tissue remodeling and tissue destruction. Compared to inhaled corticosteroids, the non-steroidal anti-inflammatory drug ibuprofen gave promising results in children and adolescents with CF [30,31], while a phase III trial in CF patients with the LTB₄-receptor antagonist BIIL 284 [32] was terminated due to adverse effects of the drug. Trials with protease inhibitors including aerosolized recombinant secretory leukocyte protease inhibitor (SLPI) or α_1 -proteinase inhibitor (α_1 -PI) have not been consistently successful [10,33], while antibiotics with anti-inflammatory effects, such as macrolides, have improved lung function in CF children and adults, infected with chronic *P. aeruginosa* [34–36].

One of the open questions in this context is which markers of inflammation and which diagnostic techniques or molecules should be employed to monitor the success of anti-inflammatory therapy in people with CF.

Since purulent CF sputum impairs the activity of aerosolized drugs, administration of aerosolized antibiotics is generally preceded by physiotherapy, and/or bronchodilators or mucolytic agents such as recombinant human deoxyribonuclease (rhDNase, Dornase alfa) [37,38]. Additionally, drugs improving mucociliary clearance such as hypertonic saline may be beneficial [39,40].

Since inhalation therapy was discussed as part of a ECFS Consensus Conference in 1999 [41] and 2003 [10], several new drug formulations and new inhalation devices have been developed. Here we review the current status of inhaled medication in CF, including the mechanisms of action of the various drugs, their optimal administration and important indications, their effects on lung function, exacerbation rates, survival and quality of life, as well as side effects. Specifically we address antibiotics, mucolytics/mucous mobilizers, anti-inflammatory drugs, bronchodilators and combinations of solutions. Additionally, we review the current knowledge on devices for inhalation therapy with regard to the characteristics of wet nebulisers, dry-powder inhalers (DPIs) and metered dose inhalers (pMDIs) and their interaction with the drug formulation and patients. Finally, we address the subject of testing new devices before they are introduced onto the market.

2. Inhaled medications

2.1. Antibiotics

2.1.1. Tobramycin

The aminoglycoside tobramycin is a bactericidal drug that inhibits protein synthesis by irreversibly binding to the 30S bacterial ribosome. It is active against most Gram-negative bacilli, but typically displays no significant activity against BCC strains or *S. maltophilia* while it is active against strains of *Enterococcus* and *Staphylococcus*. Tobramycin Solution for Inhalation (TSI) is registered as TOBI[®] (300 mg/5 ml) in combination with a PARI LC PLUS[™] reusable jet nebuliser and a suitable compressor resulting in a flow rate of 4–6 l/min. Additionally, tobramycin is present in Bramitob[®] (300 mg/4 ml) in combination with a PARI LC PLUS[™] reusable jet nebuliser and the PARI TURBO BOY[™] compressor.

Uptake across the bacterial cell wall is energy-dependent and is impaired in anaerobic environments [42]. Thus, the low oxygen partial pressure in CF sputum plugs [17] may limit the efficacy of this drug. Tobramycin is positively charged and thought to be bound in CF airways to the negatively charged DNA fibers and *P. aeruginosa* alginate. Despite these considerations, intermittent (28-day on/28-day off) treatment, using 300 mg of tobramycin twice daily, significantly improved lung function and reduced sputum *P. aeruginosa* density compared with placebo in CF patients [43(1a)].² Increases in lung function of about 10% at week 20 were most marked in adolescent patients (aged 13–17 years) and maintained for up to 96 weeks in an open-label extension study [14(1a)]. Fewer TSI than placebo recipients required parenteral anti-pseudomonal agents or hospitalisation [44(2b),45(2b),46]. Two open-label uncontrolled trials have shown that aerosolized tobramycin safely eradicated *P. aeruginosa* in the majority of CF patients for up to three months [24(2b),27(2b)]. *P. aeruginosa* eradication was associated with reduced neutrophilic airway inflammation.

TSI is generally well tolerated. Renal toxicity or hearing loss has not been reported in clinical trials, although transient mild or moderate tinnitus occurred more frequently in TSI than placebo

² Numbers and letters in parentheses refer to the grading of medical scientific publications. For details see Table 1.

Table 1
Grading of medical scientific publications.

Level	Description
1a	Systemic review of 2 or more unrelated randomised controlled trials of level 1b.
1b	Individual randomised controlled trial of good quality and sufficient patient numbers included
2a	Systemic review cohort studies of level 2b
2b	Individual cohort study (including low quality RCT; e.g., <80% follow-up)
3a	Systemic review of case–control studies of level 3b
3b	Individual case–control study
4	Case-series (and poor quality cohort and case–control studies)
5	Expert opinion without explicit critical appraisal, or based on physiology, bench research or “first principles”

Ref: Oxford Centre for Evidence-based Medicine Levels of Evidence (May 2001).

recipients [14(1a)]. Bronchoconstriction following inhalation of TSI has been reported in both preservative-free TSI and tobramycin solutions containing preservatives such as phenol [47(2b)]. The use of inhaled beta-agonists may prevent the post-inhalation decline in lung function [47(2b),48(2b)].

2.1.2. Colistimethate sodium

Colistimethate sodium (Colomycin[®], Promixin[®]) is a cyclic polypeptide antibiotic, derived from *Bacillus polymyxa varietas colistinus*, and belongs to the polymyxin group. Due to their cationic nature, polymyxin antibiotics can damage cell membranes and are bactericidal for Gram-negative bacteria. There are no specific requirements concerning inhalation devices for colistimethate sodium and thus the drug can be administered by ultrasonic or jet nebulisers or by vibrating mesh devices.

Although colistimethate sodium for inhalation has been prescribed for more than 20 years in people with CF for the treatment of *P. aeruginosa* infections, controlled trials are few. A trial in 40 CF patients showed that inhalation with colistimethate sodium reduces symptom scores and may have a protective effect on lung function [49(2b)]. When colistimethate sodium was compared with TSI, the latter drug was superior, concerning lung function improvement, while both treatment regimens decreased *P. aeruginosa* sputum density [50(2b),51(2b)]. The greater improvement in lung function seen with TSI might have resulted from the fact that all patients had previously used colistimethate sodium but were naïve to TSI. Also the dose of colistimethate sodium used in the trial (80 mg twice daily) was lower than most physicians would prescribe in adult CF patients. Finally, results were only reported for a half cycle of TSI as the study was not continued for the month off. In combination with oral ciprofloxacin inhaled colistimethate sodium effectively eradicated *P. aeruginosa* for a period of 24 months in more than 80% of treated CF patients [52(2b)]. A European wide randomized double-blinded phase III study of colistimethate sodium administered by a dry-powder inhaler (Colobreathe[®]) has been carried out but results have not been reported to date.

Colistimethate sodium is generally well tolerated in CF patients. However, bronchoconstriction following inhalation is quite common, especially in CF patients, suffering from asthma

or airway hyperresponsiveness [53(2b),54(2b)]. Colistimethate sodium must be inhaled promptly after reconstitution, since after prolonged times, the drug is hydrolyzed into the bases colistin A (polymyxin E1) and colistin B (polymyxin E2). Polymyxin E1 has been shown in animal studies to cause localized airway inflammation and eosinophilic infiltration (FDA, 2007). Colistin (sulfate) is not suitable for treating CF patients due to severe adverse effects [55].

2.1.3. Aztreonam lysine

Aztreonam is a synthetic monobactam (monocyclic beta-lactam) antibiotic, which is active against Gram-negative aerobic organisms and stable to most β -lactamases. Aztreonam inhibits synthesis of bacterial cell walls and has shown to produce clinically significant synergy with aminoglycosides against *P. aeruginosa*. Aztreonam lysine (AZLI) is a new, currently unlicensed, formulation for aerosolized treatment of *P. aeruginosa* infection in CF patients. The AZLI formulation makes this compound safe for inhalation, whereas inhalation of aztreonam arginine, used for intravenous treatment, can cause airway inflammation after chronic inhalation therapy in CF patients [56(1b)]. It is delivered by the eFlow[®] electronic nebuliser which produces an aerosol with a narrow size distribution allowing peripheral lung deposition after 2 min of inhalation [57].

A double-blind, placebo-controlled, dose-escalation Phase 1b trial of single daily doses of 75 mg, 150 mg and 225 mg AZLI or placebo, self-administered by clinically stable CF patients >12 years of age, showed retention of anti-pseudomonal activity after nebulisation and no inhibition by CF sputum [58(1b)]. AZLI was active against multiply resistant *P. aeruginosa*, and in moderate sputum concentrations showed activity when tested against BCC complex strains of genomovar I to V. AZLI was well tolerated in CF patients. The most common adverse events were increased cough particularly in patients with the highest dose. Further mild to moderate side effects were chest tightness and nasal congestion. AZLI sputum concentrations exceeded the MIC₅₀ for at least 4 h post dose. [58(1b)].

In another double-blind, randomised, placebo-controlled Phase 2 study [59(1b)], the safety, tolerability and efficacy of 75 mg and 225 mg AZLI, inhaled twice daily for 14 days were investigated in 105 CF patients with chronic *P. aeruginosa* infection. The drug significantly reduced *P. aeruginosa* CFU density after 7 and 14 days but did not lead to an increased isolation of *S. aureus*, *B. complex*, *S. maltophilia*, or *Alcaligenes xylosoxidans*. FEV₁ did not change. AZLI caused a possible dose-related trend in the incidence and severity of cough in the higher dose. Therefore, the 75 mg dose thrice daily was tested against placebo in a Phase 3 study [60(1b)]. Patients in the active arm showed a significant improvement in clinical symptoms, percent change in FEV₁, and in *P. aeruginosa* CFU density at 28 days. Adverse events did not differ between the groups.

In a further study, 75 mg of AZLI, inhaled twice or thrice daily, was tested against placebo [56(1b)] in 246 CF patients. At day 28, a significant improvement in clinical symptoms, percent change in FEV₁ and in *P. aeruginosa* CFU density was noted in both treatment groups and at the end of the 56 day follow-up

period, the treated groups showed a significantly lesser need for additional inhaled or intravenous antibiotic therapy. Adverse events did not differ between the groups. In an open-label follow-up study of 75 mg AZLI twice or thrice daily with an alternating 28 days on/off design, improvements in patient reported symptoms, pulmonary function, and *P. aeruginosa* CFU density were greater in the thrice daily group (1b). A six-month Phase 3 comparator study of 75 mg of AZLI thrice daily against 300 mg of TSI twice daily in a 28-day on/28-day off design is currently in progress.

2.1.4. Liposomal ciprofloxacin

Ciprofloxacin, a fluoroquinolone which affects gyrase function in bacteria, has been broadly used by the oral route in patients with CF and other diseases. Aerosolization of ciprofloxacin as small particle aerosol or encapsulated in liposomes into guinea pigs, infected with *Legionella pneumophila* [61], or in mice infected *Francisella tularensis* [62], prevented the death of the animals and suggested aerosol delivery to the lower respiratory tract of CF patients to be effective. When used with appropriate nebuliser devices liposomal disruption was minimal [245]. After a successful Phase 1 safety, tolerability and pharmacokinetic trial in healthy volunteers and a preclinical toxicology programme currently, a Phase 2 safety and efficacy study of inhaled liposomal ciprofloxacin in 24 CF patients is carried out using *P. aeruginosa* CFU change in sputum as the primary endpoint. Pharmacokinetic data suggested that once daily dosing may be possible.

2.1.5. Aerosol MP-376

A formulation of the fluoroquinolone levofloxacin for aerosol administration (MP-376) is currently undergoing clinical evaluation in patients with CF after results in healthy volunteers have demonstrated that it is well tolerated [63(1b)]. In the single within-subject ascending dose study of 78, 175 and 260 mg levofloxacin, there were no serious adverse events or significant changes in respiratory function between treatment groups and placebo. Systemic absorption appears to be the major route for drug elimination from the lungs.

2.1.6. Amphotericin B

Amphotericin B is a widely used antifungal drug with activity against *Cryptococcus neoformans*, *Candida albicans*, *A. fumigatus* and other species. The drug binds to sterols in the plasma membranes of fungi, thereby interfering with membrane permeability. Its potentially severe nephrotoxicity and neurotoxicity is a disadvantage of this drug. A liposomal amphotericin B preparation (AmBisome®) reduces drug toxicity whilst maintaining antifungal activity in murine models of pulmonary aspergillosis [64; Gilbert et al., 1996]. Although nebulised liposomal amphotericin B has been studied in different patient populations, data on clinical efficacy and tolerability are inconclusive, possibly because of the lack of uniformity in drug doses and administration methods [65(1b),66,67(2b),68(2a)].

No controlled trials with nebulised liposomal amphotericin B have been carried out in CF patients, suffering from *A. fumigatus* pulmonary infection. Nebulisation of 50 mg of liposomal am-

photericin B once a week, administered by an adaptive aerosol delivery nebuliser (HaloLite™) in five CF patients suffering from aggressive bronchopulmonary aspergillosis (ABPA) was well tolerated, although the 8 ml drug dose required an up to 150 min inhalation period [69(2b)]. In another study, two persistently infected CF patients became *A. fumigatus* culture negative for one to four months after 10 days of treatment with 25 mg aerosolized liposomal amphotericin B twice daily [70(4)].

2.2. Mucolytics/mucous mobilizers

2.2.1. Dornase alfa

Dornase alfa inhalation solution is a purified solution of recombinant human deoxyribonuclease (rhDNase), an enzyme cleaves sputum DNA, thereby reducing sputum viscoelasticity. Dornase alfa is used in jet nebulisers (and not in ultrasonic nebulisers) connected to a compressor. Multiple short and long-term studies have demonstrated significant improvements in FEV₁ after Dornase alfa treatment compared to placebo in CF patients [37(1b),71(1b)] and a good tolerance of the drug. Side effects include voice alteration and rash. In some studies a significant decrease in the exacerbation rate [37(1b),72(1b)] and air trapping [73(2b)] was observed. A Cochrane review concluded that dornase alfa improves lung function in short as well as long-term trials [74(1a)].

In clinical trials device combinations such as Durable Sidestream® with MOBILAIRE™, Durable Sidestream® with Porta-Neb®, Hudson T Up-draft II® with Pulmo-Aide®, Respigard II Nebulizer® with Pulmo-Aide®, PARI LC PLUS with PARI PRONEB®, PARI BABY™ with PARI PRONEB® have been used.

2.2.2. Hypertonic saline

Nebulised hypertonic saline in CF treatment is water for injection (sterile) with a concentration of 3% to 7% sodium chloride. Increasing salt concentrations on the luminal side of the respiratory epithelium is thought to hydrate the viscous mucus, thereby improving mucociliary clearance and hence lung function [75(2b)]. Several studies have assessed the efficacy of hypertonic saline in CF patients. A Cochrane review concluded that nebulised hypertonic saline improves mucociliary clearance in CF patients in short-term clinical trials and appears to increase lung function compared to control [76(1a)]. In a parallel placebo-controlled trial over 48 weeks, FEV₁ and FVC increased in 82 patients receiving 7% hypertonic saline to 3.2% and 2.8%, respectively, compared to controls [40(1b)]. Hypertonic saline also reduced the percentage of exacerbations (56%) compared to placebo in this study. In another study, an increase of FEV₁ of 15% was observed after 14 days of treatment with hypertonic saline [77(2b)]. When hypertonic saline was compared with dornase alfa once daily and on alternate days in 48 children in an open cross over study, FEV₁ increased in the daily dornase alfa group (16%), followed by alternate day dornase alfa (14%) and only a modest improvement (3%) in patients treated with hypertonic saline [78(2b)]. However, large individual differences in response to dornase alfa and hypertonic saline were found, suggesting that patients should be

tested on an individual basis before long-term prescription is started [79(2b)]. Side effects of nebulised hypertonic saline include bronchospasm and cough.

2.2.3. Denufosol tetrasodium

Denufosol tetrasodium (Denufosol) inhalation solution is a selective P2Y₂ receptor agonist which activates an alternative chloride channel [80]. This activation is thought to result in an increase in the hydration of the respiratory epithelium, thereby improving mucociliary clearance and lung function. Compared to UTP and diquafosol, Denufosol shows a prolonged stability [81–83]. In a phase II study in patients with CF, the aerosolized drug improved several lung function parameters [84(2b)]. Adverse effects, such as cough and immediate decline in lung function after inhalation, were similar in the placebo and the treatment group. Based on these findings, a placebo-controlled double-blind phase III trial was initiated using 60 mg of Denufosol over a period of 6 months.

2.2.4. Lancovutide (Moli1901)

Lancovutide (Moli1901) is thought to activate intracellular calcium in alternative chloride channels, thereby increasing chloride transport and fluid secretion onto the apical surface of the airway [85]. Indeed, in a phase II trial, a significant improvement of FEV₁ was observed by Moli1901 treatment in CF patients [86]. Aerosolized Lancovutide was well tolerated. The most frequent adverse events were non-clinically significant cough and throat irritation. An exploratory multi-center phase IIb study is currently ongoing in Europe to establish the optimal dose of Moli1901 in CF patients. Patients receive either placebo, 2.5 mg Lancovutide daily, every other day, or twice weekly for two months. The primary endpoint is the change in the percentage of predicted FEV₁.

2.3. Inhaled anti-inflammatory therapies

2.3.1. Inhaled corticosteroids

Inhaled corticosteroids are used to reduce endobronchial inflammation in CF [87,88] and to minimize systemic adverse effects, experienced with oral prednisolone [89]. In clinical trials involving CF patients, different doses of budesonide, beclomethasone or fluticasone propionate (400–1600 µg/day) were used for treatments of 3 to 52 weeks [90(2b),91(2b),92(2b),93(2b),94,95(1b),96(2b)]. Decreased bronchial hyperreactivity in non-asthmatic CF patients was observed in two studies [90(2b),95(1b)].

No study has shown a statistically significant increase in lung function, although patients receiving beclomethasone for 30 days showed a significant change in DLco (diffusing capacity for carbon monoxide) [91]. There was no beneficial change in sputum inflammatory markers [92,93,95] but airway markers of inflammation fell markedly in lavage fluid [96]. Van Haren et al. demonstrated small but significant improvements in daily symptom scores for cough and dyspnoea in a small group of 12 patients but no improvement in mean overall respiratory symptom, wellbeing or appetite scores was seen in a larger study [92].

Inhaled corticosteroid treatment was generally well tolerated and the treatment did not affect urine and blood cortisol, did not cause any decrease in adrenal reserve or any increase in airway infection [96]. However, a recent study showed a significant slowing in linear growth in pre-pubertal children receiving dry-powder fluticasone propionate over 12 months compared to placebo [94].

The largest study tested the safety of a withdrawal of corticosteroid after switching all study patients to fluticasone inhalation for a two month run in period. Patients were then randomised to continue fluticasone or start placebo for the next six months [97(1b)]. There was no difference in the primary outcome measure of time to first exacerbation between the two groups, nor in lung function changes, oral or intravenous antibiotic use, or rescue bronchodilator use. This study supports the conclusion from the Cochrane review that there is neither evidence nor benefit or harm from corticosteroid use in CF [98]. The authors suggested that the majority of patients taking inhaled corticosteroids probably do not need to do so.

2.3.2. Antiproteases

α₁-AT and secretory leukoprotease inhibitor (SLPI) are two endogenous serine protease inhibitors which inactivate neutrophil elastase a protease which has been shown to be present in high concentrations in CF sputum and BALFs [29]. Short-term aerosol delivery of α₁-AT to 12 CF patients suppressed neutrophil elastase in the epithelial lining fluid and restored anti-neutrophil elastase capacity [99]. However, a phase II trial to assess the clinical efficacy and safety of nebulised transgenic α₁-AT did not show any evidence of reduction of airway inflammation [100(1b)]. In another open short-term study, a decrease in neutrophil elastase activity, neutrophils, pro-inflammatory cytokines and *P. aeruginosa* numbers was observed, however, aerosolized α₁-AT treatment had no positive effect on lung function in CF patients [101(2b)]. It is generally agreed, that studies longer than four weeks in young children with moderate lung disease are necessary to show potential drug efficacy of aerosolized α₁-AT [102]. Aerosolized SLPI at a dose of 100 mg twice daily for one week reduced epithelial lining fluid neutrophil elastase in patients with CF, but 50 mg twice daily for two weeks were ineffective [103(2b),104(2b)]. The drug has not been further evaluated in clinical trials.

2.4. Bronchodilators

Inhaled bronchodilators are frequently prescribed for CF patients with atopy or those who develop airway hyperreactivity secondary to bronchial damage [105(2b)]. Bronchodilator therapy may increase mucociliary transport, decrease inflammatory damage to the airways, increase exercise tolerance and decrease dyspnoea [106]. Often the short acting salbutamol or the long acting salmeterol is used by inhalation.

Most patients show a positive response at some time if repeatedly treated [107(2b),108(2b)]. However, there are no long-term controlled trials of inhaled β₂-stimulants. A two month double-blind crossover trial of 90 µg salbutamol four times daily significantly improved peak expiratory flow rate

(PEFR) in patients with bronchial hyperresponsiveness [109(2b)]. While lung function was not changed in this study, treatment with inhaled salbutamol (pMDI, 180 µg b.i.d.) significantly improved respiratory function in a 12 month observational study [110(2b)]. However, in a subsequent placebo-controlled double-blinded trial, CF patients, receiving six months of 180 µg inhaled salbutamol twice daily, did not differ significantly compared to placebo in lung function tests [111]. In another study 18% of CF patients, who had salbutamol showed a significant increase in FEV₁ [112(2b)]. Inhaled short-acting β-agonists did not improve exercise performance or post exercise dyspnoea in CF patients despite significantly improving FEV₁ [113(1b),114(2b)].

Greater benefits have been reported with the long acting bronchodilator salmeterol. In an unblinded study [115(4)], dyspnoea improved even in patients not showing a positive FEV₁ response, when treated with 50 µg salmeterol twice daily for two weeks. In a 24 week treatment period, 100 µg salmeterol given twice daily was well tolerated and associated with better pulmonary function, fewer interventions, and fewer respiratory symptoms compared to treatment with salbutamol in CF patients with mild to moderate disease [116(2b)]. Stable CF patients who responded to day time salbutamol showed significant increases in nocturnal oxyhaemoglobin saturation, following salmeterol administration before sleep [117(1b)]. A Cochrane review concluded that both short and long acting β-sympathomimetics can be beneficial in CF patients with bronchodilator responsiveness or bronchial hyperresponsiveness [118(2a)].

Bronchial smooth muscle relaxation may increase airway compression and reduce cough efficiency by inducing large airway collapse [119(2b)] but negative responses are unusual and collapse is unlikely during normal breathing [120(2b),108(2b)]. No paradoxical responses were found with forced oscillation technique measurements in CF children [121(2b)].

During exacerbations, the efficacy of inhaled bronchodilator therapy may be reduced [122(2b),107]. However, this concern has not been confirmed in later studies with inhaled salbutamol [123] and high dose salmeterol [124(1b)].

Also short-term studies of anticholinergic agents have shown benefit in some CF studies [125(2b),126(2b),127(2b),128(2b)]. However, combinations of β-sympathomimetic and anticholinergic drugs did not result in synergistic or additive effects in CF patients [125(2b),126(2b),127(2b),128(2b)].

2.5. Drug combinations

Inhaled drug combinations have been used in CF patients since nebulisation via a jet nebuliser is generally time-consuming. Inhaling a mixed drug solution for inhalation saves time.

Also CF patients sometimes refill their nebuliser with another drug without cleaning in between treatments. Another objective for inhaled drug combinations is to overcome adverse effects of one drug by another. An example of the latter case is bronchoconstriction caused by some antibiotics which can be overcome by co-administration of salbutamol. The following drugs have been studied in various combinations: tobramycin, colistimethate sodium, salbutamol, budesonide, hypertonic saline, dornase alfa, cromolyn, ipratropium bromide and N-acetylcysteine.

By mixing different drugs, the following questions arise: Does mixing affect the physico-chemical stability of the drugs, their particle-size distribution or the therapeutic outcome? Which effects have preservatives?

2.5.1. Chemical stability and particle-size distribution

The best way to study chemical stability is a visible judgement followed by high-performance liquid chromatography (HPLC) analysis of the combined solution and a search in relevant handbooks, e.g., in the Handbook on Injectable Drugs [129], the Drugdex database [130,131] and The King Guide to Parenteral Admixtures [130,131]. Table 2 summarizes studies on chemical stability of mixtures of inhalation solutions. Stability has been proven for combinations of cromolyn with salbutamol, ipratropium, N-acetylcysteine and budesonide; furthermore, combinations of salbutamol with ipratropium, colistimethate sodium, tobramycin, N-acetylcysteine and

Table 2
Chemical stability of combinations of inhalation solutions.

Drugs	CROMO	SAL	IPRA	COLI	TOBRA	NAC	BUDE	FENO	HTS	DORNA	BENZA
CROMO	–	C [1] ^a	C [1,2]	n.d.	n.d.	C [1]	C [1,2]	n.d.	n.d.	IC	IC [1]
SAL		–	C [1]	C [1] ^b	C [1]	C [1]	C [1,2]	n.d.	n.d.	IC	n.d.
IPRA			–	n.d.	C [1]	C [3]	C [1]	C [4]	n.d.	IC	n.d.
COLI				–	n.d.	C [4]	n.d.	n.d.	n.d.	IC	IC [1]
TOBRA					–	n.d.	n.d.	n.d.	n.d.	IC	n.d.
NAC						–	n.d.	C [3]	n.d.	IC	n.d.
BUDE							–	C [1]	n.d.	IC	n.d.
FENO								–	n.d.	IC	n.d.
HTS									–	IC	n.d.
DORNA										–	IC[5]

Drugs: CROM: cromolyn; SAL: salbutamol; IPRA: ipratropiumbromide; COLI: colistimethate sodium; TOBRA: tobramycin; NAC: N-acetylcysteine; BUDE: budesonide; FENO: fenoterol; HTS: hypertonic saline; DORN: dornase alfa; BENZ: benzalkonium chloride; C : compatible; IC :incompatible; n.d. : no data.

^a [References] 1: [134]; 2: [242]; 3: [243]; 4: [246]; 5: [244].

^b Preservative-free salbutamol.

budesonide are stable as well as combinations of ipratropium with tobramycin, N-acetylcysteine, budesonide and fenoterol or N-acetylcysteine with fenoterol. N-acetylcysteine is inactivated by oxygen. This is prevented in the commercial product by including EDTA, which has no influence on pulmonary function but is capable of chelating metal ions. EDTA increases the activity of azithromycin [132] and colistimethate sodium [133] by chelating divalent cations such as calcium.

Dornase alfa should not be mixed with any other drug for inhalation due to stability problems of the protein. Sometimes the preservative and not the pharmacologically active drug causes an incompatibility. For instance benzalkonium chloride in combination with colistimethate sodium or cromolyn forms a hazy cloud [134]. Benzalkonium chloride is present in multi dose formulations of salbutamol and ipratropium. Benzalkonium chloride is a pulmonary irritant and thus preservative-free solutions are preferred.

Combination of different inhalation fluids may affect particle-size distribution due to changes in surface tension of the aerosol. Only one study addressed the aerosol characteristics after mixing different drugs for inhalation [135]. The authors studied the mean mass aerodynamic diameter (MMAD), respirable fraction (RF) and respirable mass (RM) of combinations of salbutamol (albuterol) and cromolyn, ipratropium bromide, tobramycin, N-acetylcysteine and flunisolide in continuous nebulisation and in breath-actuated nebulisation. Most combinations with salbutamol gave no difference in aerosol characteristics except cromolyn in continuous nebulisation (MMAD decreases), ipratropium bromide in breath-actuated nebulisation (RM increased), tobramycin in breath-actuated nebulisation (RF decreased), and flunisolide in breath-actuated nebulisation (RF and RM decreased). Results make clear that not only chemical stability must be studied but also aerosol characteristics, such as is shown in Table 3.

3. Devices for inhaled medication

Basic knowledge on inhalation of drugs will help the prescriber in choosing the right device for each patient. This relates

to the dose of the drug, inhaler specifications and patient characteristics. Table 4 presents a non exhaustive overview of inhaler specifications versus available inhaler devices.

3.1. Physical parameters

3.1.1. Particle mass, inhaled mass and respirable mass

The particle mass can be described as the fraction of the nominal dose that leaves the inhaler during inhalation. The availability of an aerosol is affected by the choice of nebuliser, volume of fill, residual volume, surface tension of the nebuliser solution, and the nebulising flow [136]. The inhaled mass is the fraction of a nebuliser charge that is actually inhaled by the patient. It is not a fully recognized quality criterion for nebulisers that affects therapeutic efficacy [137]. The inhaled mass may differ considerably between nebulisers [138]. It is therefore possible that the effectiveness of an inhaled drug is dependent on the delivery system. Part of the inhaled mass may be exhaled again, resulting in a smaller lung dose [139]. An inhaled mass of the nebuliser charge of approximately 20–40% has been found in a study with children and adults inhaling isotonic saline [140]. In CF children, the inhaled mass ranged from 9% to 14% using a conventional jet nebuliser and 17% to 19% using a Venturi jet nebuliser [141,142]. The inhaled mass can be measured by putting an inspiration filter on the nebuliser. This estimated deposition probably differs from real life deposition, as the latter will be influenced by the particle-size distribution of the drug, the age and tidal volume of the patient. The amount of drug on an inspiratory filter may be comparable, while the mass median aerodynamic diameter (MMAD) of the generated aerosol may differ significantly, possibly resulting in central or more peripheral pulmonary deposition [141,142].

The respirable mass, also called the fine particle fraction, is the portion of the inhaled mass that is in the particle-size range expected to bypass the upper airways and deposit in the lower airways. It is generally considered to consist of particles with an aerodynamic diameter between 1 and 5 μm and these dimensions are thought to result in optimal drug deposition in the

Table 3
Physico-chemical stability of combinations of inhalation solutions.

Drug	Cromolyn	Salbutamol	Ipratropium bromide	Colistimethate sodium	Tobramycin	N-acetyl cysteine	Budesonide	Fenoterol	Hypertonic Saline	Dornase alfa
Cromolyn	–	A	B	n.d.	n.d.	B	B	n.d.	n.d.	X
Salbutamol	–	–	A	B	A	A	B	n.d.	n.d.	X
Ipratropium bromide	–	–	–	n.d.	B	B	B	n.d.	n.d.	X
Colistimethate sodium	–	–	–	–	n.d.	B	n.d.	n.d.	n.d.	X
Tobramycin	–	–	–	–	–	n.d.	n.d.	n.d.	n.d.	X
N-acetylcysteine	–	–	–	–	–	–	n.d.	B	n.d.	X
Budesonide	–	–	–	–	–	–	–	B	n.d.	X
Fenoterol	–	–	–	–	–	–	–	–	n.d.	X
Hypertonic Saline	–	–	–	–	–	–	–	–	–	X
Dornase alfa	–	–	–	–	–	–	–	–	–	–

A: miscible, grade A evidence, B: miscible, grade B evidence, n.d.: no data, X: not miscible. The highest grade of evidence for safe combination (A) is for combinations where chemical stability has been proven and aerosol characteristics are not altered. The second highest grade of evidence for safe combination (B) is for combinations where only chemical stability has been proven without study of aerosol characteristics. When chemical or physical incompatibility has been proven, drugs should not be mixed (X).

Table 4
Inhaler specifications versus available inhaler devices.

Inhaler specifications	Jet	Ultrasonic	Soft mist, vibrating mesh	Dry-powder inhaler (DPI)	Pressured metered dose inhaler (pMDI)
Evidence-based/based on clinical efficacy studies in CF patients	Yes	Yes	No	No	Yes
For all ages	Yes	Yes	Yes	No (for age > 6 years)	Yes (holding chamber for infants)
General/generic use; useful for many drugs and/or disease states	Yes	Yes	Yes	No	No
Fast (nebulisation time)	No	No	Yes, intermediate	Yes	Yes
Small size, easy to carry/portability	No	No	Yes	Yes	Yes
Noise	Yes	Yes	No	No	No
External power source (electricity, battery) needed	Yes	Yes	Yes	No	No
Durability	Yes	Yes	No data	Not applicable	Not applicable
Price (initial expense)	Low-intermediate	Low-intermediate	High	Low	Low
Large fraction of the output of the inhaler has a particle size of 1–5 micron	Yes/no ^a	Yes/no ^a	Yes	Yes	Yes
Multiple dose capacity	No	No	No	Depends on design	Yes
Variable payload possible	Yes	Yes	Yes	No	No
Breathing coordination required	No	No	No	Yes	Yes
Useful in tidal breathing/low velocity of the aerosol	Yes	Yes	Yes	Yes and no ^a	No; yes (holding chamber)
Dead volume	Yes	Yes	Yes, but generally smaller than jet/ultrasonic devices	Not applicable	Not applicable
High risk on bacterial contamination	Yes	Yes	Yes	No data/no ^b	No data ^b
Preparation and cleaning is easy	No	No	No	Yes	Yes
Cleaning after each use (bacterial contamination and maintenance)	Yes	Yes	Yes	No ^c	No ^c
Periodical maintenance and/or replacement to keep up efficiency	Yes	Yes	Yes	No	No

Adapted from Wolff RK, Niven RW. Generation of aerosolized drugs. *J Aerosol Med* 1994;7:89. and Rau 2002.

^a Depends on device(s) used.

^b No data/no: unknown risk in case of a multiple use design/no risk on bacterial contamination in case of disposable design.

^c Manufacturers' instructions vary.

peripheral airways. Smaller particles will be exhaled while larger particles are predominantly lost because of inertial impaction in the oropharynx. Optimal peripheral deposition has been found to occur with a MMAD of 2–3 μm , combined with an inhalation flow rate of approximately 15–30 l/min and the largest inhalation volume convenient for the subject [143,144]. The range of the respirable mass is related to the desired target area. Relative humidity appears to influence the respirable mass, depending on the type of nebuliser and the drug solution [145].

3.1.2. Lung dose

The lung dose describes the amount (in mg or fraction of the nominal dose) of the drug that enters the airways, e.g., passes the vocal cords. A lung dose can be quoted as a percentage of the nominal dose, and also as a percentage of the particle mass or a percentage of the inhaled mass. For example, an intrathoracic deposition of ~85% of the emitted aerosol (particle mass) was measured of which ~77% was deposited in the peripheral lung [146]. A lung dose can be estimated by measuring the cumulative excretion of the drug during 24 h in the urine [147–150] or by radiodeposition studies [139,141, 142,151–159]. For newly developed inhalers, theoretical equivalent doses to current, standard inhalation treatment have been calculated, based on *in vitro* testing [160,161] or using a dose

escalating method [162]. In older studies lung doses ranged between ~3% to 8% using conventional jet nebulisers [139,140, 151,153]. These percentages improved when newer breath-enhanced and breath-actuated nebulisers were used: estimated mean lung doses between 9% and 15% were found using a PARI LC[®] Plus–PARI MASTER[®] combination [163,164], a Ventstream[®]–Porta-Neb[®] combination [165,166], a PARI LC[®] Plus–Pulmo-Aide[®] combination [167] and a PARI LC[®] Plus or PARI LL[®] connected to a PARI BOY[®] compressor [168].

Ultrasonic nebulisers produce a mean lung dose of approximately 14%–18% [147,163]. However, also higher mean lung doses were reported. A mean lung dose of 22% was estimated using the PARI LC[®] Star–AKITA[®] system compared to 16% for the PARI LC[®] Star–PARI MASTER[®] combination [169]. Comparison of a PARI LC[®] Plus–PARI BOY[®] with an adapted aerosol delivery (AAD) system (HaloLite[®]) showed a lung uptake of 20% versus 31% respectively [158]. The delivered dose to the lungs with an Aerodose[®] breath-actuated inhaler was ~35% versus ~9% with the PARI LC[®] Plus nebuliser [170]. A mean lung dose of 32% was measured using the eFlow[®] vibrating mesh device compared to 16% using the PARI LC[®] Plus–ProNeb[®] [171]. A lung dose of 63% to 73% was found with the I-neb[®] AAD[®] System [172], expressing a fraction of the emitted dose.

After inhalation of 25 mg of tobramycin dry-powder formulations using an Aerolizer[®] capsule inhaler, lung doses of ~53% and ~34% were observed, compared to ~8% using a PARI LC[®] Star–PARI TurboBOY[®] [159]. In a study on lung deposition of budesonide dry-powder Turbohaler in CF children, a deposition of 10% to 50% of the inhaled mass was measured [141,142]. Deposition data of dry-powder formulations relative to liquid nebulisation have been collected [160–162] but these data do not provide insight into the absolute lung doses due to the chosen study design. An advantage of dry-powder inhalation is a reduced loss of aerosolized drug due to exhalation [159] and leakage.

3.1.3. Output and output rate

3.1.3.1. Jet and ultrasonic nebulisers. Drug output from a jet or ultrasonic nebuliser is characterized by the generated particle-size distribution and the particle mass. Drug output also depends on a number of different variables including the nebuliser type and the flow rate or (ultrasonic) frequency. A higher flow results in a higher output rate and a larger drug output and (central) deposition [173]. Increasing the nebulising flow also results in a smaller particle-size distribution (MMAD) and a higher respirable fraction [136,174,175], which may also improve peripheral lung deposition. However, the total drug output levels off at a certain point: the optimal inspiratory flow rate has been found to be ~15 l/min to 30 l/min, depending on the jet nebuliser used [144,165,166,176–178]. Further variables are the humidity of the generating gas, temperature, concentration, viscosity, density, the physical state (solution versus suspension) and surface tension of the fluid during aerosolization [165,166,176,179–183,247]. Importantly the nebuliser configuration can affect the particle size and the amount of aerosol inhaled [184]. It has been suggested that the nebuliser configuration should be precisely specified in treatment protocols.

Each jet nebuliser has its own resistance, and it is therefore mandatory to test a nebuliser–compressor combination for output and flow rate prior to starting therapy. Data on output rates of compressors, provided by manufacturers, are often based on a configuration without a nebuliser connected to it. With an identical driving airflow, the resulting output rate will differ between various jet nebulisers, as each device has its own internal resistance. Similarly, ultrasonic devices had a comparable or greater output than jet devices when comparing nebuliser output with normal saline [183]. Different methods have been applied to assess drug output rate, which can be expressed in volume (ml/min) and amount of drug (mg/min). Methods include weighing a nebuliser [183] or measuring the change in osmolarity or concentration before and after nebulisation [178,185]. Additionally, direct measurement of the aerosol on filters can be used [248,249]. Results from estimating the aerosolized volume may be misleading when the increase in drug concentration within the nebuliser, caused by evaporation of the solvent, is not taken into account [185]. Therefore, drug output rate in mg/min is a better parameter to describe nebuliser output than ml/min [165,166].

3.1.3.2. Other inhalers. Inhaling from a *pressurized metered dose inhaler* (pMDI) without a spacer/holding chamber has a similar principle to jet nebulisation. A high external driving flow is generated which is responsible for drug output and output rate. The patient inhales the drug using its own inspiratory flow, preferably with good hand–mouth coordination. Output and output rate from vibrating mesh devices also depends to some extent on the inhalation technique of the patient. If using a passive DPI, the inspiratory flow of the patient is the only driving source for drug release and dispersion from the inhaler. A DPI has an internal resistance which has to be overcome by the inspiratory flow. However, this interplay may be used to guide effective regional drug deposition in the lung.

3.1.4. Residual volume

3.1.4.1. Jet and ultrasonic nebulisers. The residual volume, or dead volume, is defined as the volume of solution remaining in the nebuliser at the endpoint of nebulisation [183], which is typically in the range of 1 ml to 3 ml [186] or 38% to 61% of a drug dose [139,141,142,147,187], depending on the nebuliser used. Small doses are especially affected. The residual volume depends on the design of the nebuliser, particularly the extent of the internal surface, the surface tension, the viscosity of the drug solution and the wetness of the nebuliser [181,186,188]. A higher fill volume may reduce the relative extent of the residual volume [203]. The drug loss may also be decreased and the output improved by tapping the nebuliser [186]. Residual volumes of ultrasonic nebulisers are often larger than for jet nebulisers [181].

3.1.4.2. Other inhalers. Residual volumes in novel electronically operated vibrating mesh devices are generally lower than traditional nebulisers. The residual volume of the I-neb[®] AAD[®] system is approximately 0.1 ml [189]. The residual volumes in percentage of the nominal dose depend on the fill volume (0.25–1.4 ml) of the devices. No clinical observations on residual volume are available. The eFlow[®] Rapid has a residual volume of ~1 ml, while ~28% of the drug dose was found in a clinical study with the eFlow[®] [171,190]. In general, powder retention in dry-powder inhalers is low, minimising drug wastage.

3.1.5. Particle-size distribution

3.1.5.1. Jet and ultrasonic nebulisers. The particle-size distribution of an aerosol is to a great extent defined by the design and operating principle (f.e. jet or ultrasonic technique) of the nebuliser. Additionally, it depends on the applied driving air flow or ultrasonic frequency, the inspiratory flow generated by the patient, the temperature of the solution and the physical characteristics of the nebulised drug [165,166,184]. Manufacturer's data on particle-size distribution are frequently based on normal saline solution, and nebulising drug solutions may result in altered particle-size distribution [141,142,168,191–193] and variable administration times. Due to a lower surface tension, colistimethate sodium tends to foam during nebulisation,

resulting in smaller droplets with uncompromised biological activity [137,154,155,183]. Due to this foaming, administration of colistimethate sodium with an ultrasonic nebuliser is problematic [183].

3.1.5.2. Other inhalers. Some important parameters that affect drug dispersion from a DPI are the inhaled flow rate [159,194], the inhaled volume and the internal resistance of the device [195]. Large fine particle fractions with a peripheral deposition of approximately 10–20% have been found using a newly developed DPI [159]. No similar data have been published to date on the newer electronically operated devices, like the I-neb AAD[®] and eFlow[®].

3.1.6. Polydisperse and monodisperse aerosols

The majority of drug aerosols have a polydisperse, asymmetrical distribution, according to the log-normal law [137]. Monodisperse aerosols have uniformly sized particles with a narrow size distribution, which can be imitated with a polydisperse aerosol, as both distributions are comparable provided the width of the distribution is not too large ($\sigma_g < 2$) [144]. Hygroscopic changes in particle size appear to be negligible if the concentration of the monodisperse aerosol is high (Finlay et al., 1998). Generated at an optimal drug particle size for a target region in the airways, a monodisperse aerosol might result in the most effective treatment [196]. Clinical data on the use of monodisperse aerosols are scarce and these aerosols are currently not used in the treatment of CF lung disease. However, they are studied in aerosol research to gain knowledge on aerosol particle behaviour *in vivo* [144,197].

3.1.7. Administration time

3.1.7.1. Jet and ultrasonic nebulisers. To define the administration time of a nebulised drug, an endpoint has to be defined when aerosolization is finished. In clinical studies, definitions of endpoints vary including the absence of mist [163,181,187,198,199], sputtering from the nebuliser [183,200], and the absence of mist for 10 to 30 s [136,141,142,177,187,192,201–204].

Others defined three possible end points for nebulisation: sputtering time, total time and clinical time [187]. Sputtering is the point when aerosolization becomes erratic. This point in time corresponds with an 8-fold drop in the total number of particles, read by a laser diffraction analyzer [205]. Total time is when production of aerosol ceases and clinical time is somewhere between sputtering and total time and approximates the point when a patient or therapist typically stops a treatment. Delivery time by a jet nebuliser may vary when connected to a compressor compared to hospital dry compressed air [178]. Furthermore, tapping of the nebuliser may introduce greater subjectivity in the measurement [187]. Delivery time by an ultrasonic nebuliser may be negatively influenced by a higher drug concentration or higher viscosity [183]. Determination of aerosol output and residual volume depends on the definition of the end of the nebulisation and it is therefore important that this parameter is clearly defined.

3.1.7.2. Other inhalers. The newer electronically operated nebulisers generally switch off at a point when the dose in the reservoir is aerosolized or at a set time (i.e., after 10 min). However, it is not clear whether a dose is always completely nebulised within this time frame, which has been shown with a 6 month old eFlow[®] Rapid [206]. A mean nebulisation time of 5 min with the I-neb[®] AAD[®] System was found in a 3-month observation period [207]. DPIs require one or several inhalation manoeuvres which generally take about 1 min to 2 min, just as pMDIs.

3.1.8. Drug waste during aerosolization

3.1.8.1. Jet and ultrasonic nebulisers. Using a constant output jet nebuliser, a substantial part of a nominal drug dose may be lost because of aerosols, generated during the non-inspiratory part of the respiratory cycle [140,179]. Since the introduction of the Venturi-nebulisers/breath-enhanced and breath-actuated nebulisers, drug delivery has improved considerably [141,142,168,178,193,208,209]. Additionally, drug loss due to exhalation contributes to drug wastage.

3.1.8.2. Other inhalers. Data on drug wastage in CF patients using pMDIs, vibrating mesh devices and DPIs are sparse. Using the eFlow[®], ~34% of the nominal dose charge was found on the expiratory filter [171] and ~1% of the emitted drug dose (particle mass) was wasted using the I-neb AAD System [172].

3.1.9. General purpose nebuliser

Only drug–device combinations tested in clinical studies for efficacy and safety, particularly concerning drugs with a small therapeutic window, should be used by CF patients. This is especially relevant for jet nebulisers, which often are used with various compressors, each with its own specification. For new drugs, characterization of the drug–device combination in a clinical study is essential for *in vitro* bridging studies. Bridging studies may be useful in finding an alternative nebulising device when the preferred device is unavailable.

There are many general purpose nebuliser devices available worldwide but availability differs from country to country. Therefore, pharmaceutical companies, marketing drugs for inhalation, are responsible to provide evidence-based recommendations for their aerosol device in each country in which the drug is marketed. The use of inhaled drugs in children should receive specific attention. A pediatric investigation plan (PIP), already in use by the EMEA, on drugs for inhalation in CF should include testing appropriate inhaler devices in combination with a specific drug in children. Regulatory authorities should promote the use of specific drug–device combinations in CF patients and set guidelines for bridging studies [210].

3.2. Bacteriological safety and performance of nebulisers over time

Cleaning of inhaler devices that are used for aerosolization of liquids is important for bacteriological safety and to ensure that the performance is not compromised. As DPIs and pMDIs are

far less influenced by hygienic threats, this paragraph focuses on wet nebulisers.

3.2.1. Bacteriological safety

Wet nebulisers may become a source of bacterial infection of the respiratory tract and contamination of home equipment with bacterial pathogens after suboptimal cleaning procedures has been documented [211–218]. Reports that CF patients would have acquired bacterial infections from respiratory therapy equipment during home use however, are lacking [219] and bacterial organisms grown from patients' sputum specimens and the respective devices did not correlate [214,220] in contrast to another study [215]. Nevertheless, early *P. aeruginosa* acquisition in young children was associated with the use of aerosolized drugs and clinic exposures [221]. A low risk of microbial contamination of CF inpatients with CF pathogens from the interior of a disposable nebuliser over a 24 h period was reported [222]. Also, the bacterial flora from environmental sources, for example from tap water [219,223,224] may contaminate a nebuliser, as well as the colonising flora of the oropharynx [213]. Importantly, nebuliser devices should not be shared between CF patients as this has been associated with the acquisition of *B. cepacia* complex strains [225]. Cleaning and drying of nebulising equipment between uses decrease the risk of acquiring pathogens, including *B. cepacia* complex [213,214,220,226].

3.2.2. Performance over time

Without cleaning or proper maintenance between runs, some nebulisers may require a longer time to complete aerosolization, although particle-size distribution and output are not necessarily affected. Unwashed devices fail to produce an optimal aerosol after long-term use [201]. Patients may assess the functionality of their devices by visual inspection for mist production, cracks or leaks, and checking the nebulisation time [201]. In a study using vibrating mesh-nebulisers, no modification of the membrane function could be detected [227]. After daily use for six to twelve months of an eFlow[®] Rapid nebuliser and a PARI LC[®] Plus–PARI TurboBOY[®] combination, changes in droplet size distribution and a decrease in output rate were reported [206].

3.2.3. Cleaning of nebuliser equipment

Soaking and rinsing with tap water [216,217], warm soapy water [201,216,217,227] and boiling water [219] as well as using a dish washer [201,219] have been proposed to clean nebuliser devices. However, *P. aeruginosa* is only killed at temperatures of ~70 °C. Sodium hypochlorite, isopropylalcohol and ethanol (70% to 90%) are effective [36,219], in contrast with the suboptimal results obtained with acetic acid and quaternary ammonium salts [228–230]. Drying of the equipment after disinfection is important [213,214,220,226]. Patients should receive clear, written and oral instructions, about how to keep the bacterial contamination risk as low as possible [214,221,220,231] to ensure patient adherence to the cleaning of the nebulising equipment several times daily. Also, home visits by nurses have been advocated to improve compliance

[214,220]. As a result of improved compliance with cleaning protocols, bacterial contamination may be prevented or decreased [232,213].

Disposable nebulisers are frequently re-used to reduce expenses and for convenience [233]. But the consequences of long-term use of disposable nebulisers are poorly understood [215] and suggestions in this context differ widely. For instance, the nebuliser should be changed every 24 h to reduce the risk of infection [234]; old plastic tubing and atomizing chambers should be replaced at six-month intervals [213], or at longer intervals up to four years [231]. The interior tubes should be dried with the aid of a compressor, attached to the nebuliser [233] and the service of equipment once a year is advised [214].

3.3. Patient parameters

3.3.1. Deposition pattern and breathing pattern

Causal relationships between the sites of drug deposition and the patients' response have been established in diseases of the respiratory tract [235], but no data exist for cystic fibrosis. Effective targeting of a given region in the lung, for instance the peripheral airways, has been defined when >50% of the total drug deposition occurs in that region [197,144]. Studies with radiolabelled aerosols in CF have measured regional distribution of deposited aerosol throughout the lung, but often without a direct relationship to efficacy [139,156,173]. Ideally, the radiolabel should be tied to the drug in a 1:1 ratio, to visualize pulmonary drug dispersion and drug efficacy.

Many factors affect total and regional deposition. The underlying disease process is a major determinant of the final deposition pattern [236]. The deposition pattern is influenced by age and by the lung function of the individual patient [141,142,154,155]. Patients with high FEV₁ values tend to have a more homogeneous distribution in the lung including peripheral airways than those with a low FEV₁ [139]. However, the total amount of drug in the lung can be equivalent in both groups of patients. The wide variation of aerosol deposition in CF patients may, among other parameters, be caused by the different breathing patterns of patients [139,154,155,201,237]. Indeed, controlled breathing has resulted in a narrower range of deposition [139].

An optimal inspiratory flow rate (l/min) results in an optimal deposited dose. The deposition rate is related to the patient's respiratory rate and the type of nebuliser used. An increased inspiratory flow rate led to an increased deposition rate with a breath-actuated nebuliser (Halolite), while such a relation was not seen with a breath-enhanced (PARI LC[®] Plus) or a breath-enhanced/breath-actuated device (AeroEclipse) [178]. Slow and deep inhalation using adaptive aerosol delivery devices such as the Akita[®] or the I-neb[®] AAD[®] System may improve the lung deposition [169,189].

3.3.2. The optimal particle size

Drug particles are characterized by size, shape and density. Deposition properties of drug particles are described by the aerodynamic behaviour and geometric standard deviation of the

particles [144]. Sedimentation, inertial impaction and diffusion are the mechanisms by which inhaled particles from an inhaled aerosol deposit upon airway surfaces. The overall deposition is a result of the interaction of these mechanisms. During slow deep breathing sedimentation is efficient in the peripheral airways and during rapid breathing inertial impaction is efficient in the large tracheobronchial passages. Major determinants of the deposited fraction and distribution of the aerosol in the lung include the inspiratory flow rate, the particle size and the inhaled volume. By varying these factors, drug delivery to specific regions in the lung may be accomplished, while minimizing losses in the oropharynx [173]. By controlling the inhalation flow rate and the inhaled volume, a higher peripheral deposition and reduced dose variability can be obtained [238].

Because of these variables, the optimal particle size for peripheral deposition in CF airways is not known and probably does not exist, as the particle size alone does not determine the deposition result. Furthermore, penetration of particles in the CF lung is influenced by a decrease in airway caliber, caused by airway infection, airway inflammation, by an increased mucus layer, mucus plugging or a combination of these factors. As a possible consequence, the optimal particle size may differ between patients according to the disease state. The impact of infection and inflammation upon airway caliber has not been studied systematically in CF patients, [239]. Nevertheless, two clinical trials have given insight concerning the particle size of drugs in CF patients. Using dornase alfa, both studies showed a trend for more improvement in pulmonary function tests with smaller particles versus larger particles, suggesting that targeting peripheral airways with this drug may be advantageous [202], Geller et al., 1998).

3.3.3. Infants and small children

It is generally agreed that many therapy strategies for CF would have the greatest benefit in infants and young children, before the onset of irreversible lung disease. However, this patient group is the most challenging to treat with an aerosol (Geller et al., 1997, Tiddens et al., 2007), since infants and young children have smaller upper and lower airways, faster respiratory rates and lower inhaled volumes. Also, infants tend to prefer nasal breathing, which may filter the aerosol and reduce the lung dose of the drug. Some children become fussy or cry during aerosol administration, which dramatically decreases the lung dose of the drug (Geller et al., 1997). Even though the lung dose in infants is several-fold smaller than in older children and adults, their lungs are also much smaller. Therefore lung deposition is proportionate to size, [152] so adjusting the nominal dose for children is not always necessary. As an example, Rosenberg reported serum tobramycin levels in young CF children that were similar to those of older subjects, using the same nominal dose and delivery system [216]. Pulmonary scintigraphy is probably the most valuable method for studying deposition of drugs in lungs, also in young children. In scintigraphic lung deposition studies in CF infants a ~10-fold lower lung deposition has been observed compared to adults [240]. Deposition can be

reduced by bronchial obstruction and inhalation without a correct facemask. A mouthpiece must be used as early as possible when children get older [241].

4. Questions and answers

(Grade of recommendations according to the Oxford Centre For Evidence Based Medicine Levels of Evidence (Table 5))

Q1 What are the advantages and disadvantages of inhalation medication in CF patients compared to other drug administrations?

Advantages:

- Generation of high drug levels in CF airways
- Limited systemic toxicity due to low systemic drug absorption
- Fast onset of action
- No drug inactivation before reaching the target organ
- Direct drug action on target site
- Suitable for home therapy

Potential disadvantages:

- Uncertainty about drug dose at the target site
- Severely affected lung areas may not be reached
- Drug delivery depends on inhalation technique and device performance
- Local side effects (e.g., cough, airway narrowing, hoarseness)
- Variable systemic drug absorption
- Time consuming drug administration
- Need for education and training
- Limited information on drug interactions in the lung
- Specific drugs may need specific delivery devices
- Poor adherence
- Potential pollution of the environment
- Potential device contamination and patient infection
- Need for hygiene control and maintenance of the equipment
- Limits social functioning

Q2 What are the current indications for inhalation medication in CF?

Current indications, based on level A or B clinical evidence, include:

- Maintenance therapy for chronic *P. aeruginosa* infection
- Early eradication therapy for *P. aeruginosa*
- Improvement of airway hydration
- Improvement of mucus clearance
- Documented bronchial hyperreactivity

Q3 What are optimal endpoints in studies testing the efficacy of inhaled medication in CF?

The optimal endpoint is survival which is difficult to test in clinical trials in CF patients. Established surrogate endpoints are:

- FEV₁

- Pulmonary exacerbations
- Quality of life

Potential surrogate endpoints are:

- Lung function parameters other than FEV₁
- Imaging techniques such as HRCT
- Exercise tolerance
- Markers of lung inflammation
- Prevention of lung infection

Surrogate endpoints have serious limitations. They may depend on age and disease severity of the included patients as well as on the drug tested. Only few exacerbations occur in many CF patients, especially in those with mild lung disease. The terms pulmonary exacerbations and stable lung function lack a consistent definition. Limited information for quality of life can be obtained in young patients. In general, clinical endpoints in children below the age of 6 years are difficult to achieve. Independent of the endpoints chosen, novel drugs for inhalation should be tested in CF patients, treated according to the best standards of care.

Q4 Should the effect of inhaled medication be evaluated in individual patients?

Drugs for inhalation which have obtained market authorization should be repeatedly monitored in eligible CF patients with regard to potential side effects and the need for continuous administration. The treatment with a given drug should be prescribed for the patient in the way its efficacy has been determined.

Q5 How should the priority of different inhaled medications be established?

Inhaled medications are difficult to compare, since there are only few comparative trials. For drug prioritization, the level of clinical evidence and the potential benefit for the patients should be taken into consideration.

Q6 How should the sequence of different inhaled medications during a treatment session be determined?

Information is limited. Mucus clearance and bronchodilator therapies should precede antimicrobial treatment by inhalation. Drug interactions should always be considered.

Q7 Which inhaled antibiotics can be recommended?

Tobramycin [A] and colistimethate sodium [B] preparations for inhalation are recommended. Microbiological breakpoints for systemic infections (susceptible, intermediate, resistant) do not predict the clinical efficacy of inhaled antibiotics [B].

Q8 Should inhaled antibiotics be used on alternate months or continuously?

The decision between a continuous or alternate month antibiotic therapy strategy depends on the drug and the

clinical status of the patients. Alternate month therapy reduces the selective antibiotic pressure and may thus reduce the development of antibiotic resistance, observed during continuous therapy [B]. Comparative trials between both strategies are lacking.

Q9 Can microorganisms be eradicated using aerosolized antibiotics, and if so, is monotherapy as effective as combination therapy with other aerosolized antibiotics or antibiotics administered by other routes?

Eradication of early *P. aeruginosa* infection and prevention of chronic *P. aeruginosa* infection can be achieved in the majority of patients and should be attempted with aerosolized antibiotics or aerosolized antibiotics combined with oral antibiotics in patients with CF [A]. Different antibiotic regimens have been successful. Due to lack of comparator studies, it is unclear whether monotherapy or aerosol/systemic combination therapy is more effective. Re-occurrence of *P. aeruginosa* in CF airways after successful eradication should lead to new attempts to eradicate the pathogen by using the same or more intensive therapy strategies [B].

Q10 Should inhaled antibiotic therapy be continued upon eradication of *P. aeruginosa*, and if so, for which time period?

Clinical data supporting continuous use of antibiotics after eradication of *P. aeruginosa* in CF airways are lacking. Regardless of undetectable *P. aeruginosa* in throat swaps and negative serum antibody titers against *P. aeruginosa*, the administration of inhaled antibiotics may be continued for longer period of time, in case *P. aeruginosa* is suspected to be present in the sinuses or the small airways [D].

Q11 Is aerosol and intravenous administration of a given antibiotic drug at the same time superior to the administration of the drug by either route?

For the treatment of *P. aeruginosa* infections, antibiotics could be administered by aerosol and intravenously at the same time to reach high drug concentrations in the lung [D]. However, there is no scientific evidence to answer this question.

Q12 Are novel inhaled antibiotics for the treatment of *P. aeruginosa* and other CF-related bacterial pathogens needed?

For the treatment of *P. aeruginosa* and other CF-related bacterial pathogens new developments of inhaled antibiotics are urgently needed, since tobramycin and colistimethate sodium are not sufficiently effective and are not tolerated by all CF patients.

Q13 Should inhaled corticosteroidal drugs be used for the treatment of CF lung inflammation and how effective are they?

Inhaled corticosteroidal drugs should be considered in CF patients with clinical diagnosis of concomitant asthma,

not controlled by short-acting bronchodilators. A 2–3 month treatment is recommended [D]. Regular anti-inflammatory therapy, regardless of symptoms, is not recommended [A]. Inhaled corticosteroidal drugs can be safely withdrawn even in CF patients who have been treated for years, and who are not symptomatic. It is recommended to reduce the dose of inhaled corticosteroidal drugs or withdraw the drug whenever possible, particularly when clinical benefit has not been demonstrated [A]. There is no clinical evidence for the benefit of the use of inhaled corticosteroidal drugs in aggressive bronchopulmonary aspergillosis in CF patients.

Q14 Should the use of recombinant human DNase be recommended for CF patients regardless of age and if not, which criteria should be used for implementing this treatment strategy?

CF patients ≥ 6 years with mild, moderate and severe lung disease should be treated with recombinant human DNase [A]. Evidence for efficacy is lacking in patients < 6 years of age.

Q15 Should the use of hypertonic saline be recommended for CF patients regardless of age and if not, which criteria should be used for implementing this treatment strategy?

CF patients ≥ 6 years should be treated with hypertonic saline for short-term use to improve lung function [A] and for long-term treatment to improve lung function, reduce exacerbations [B] and improve the quality of life. Evidence for efficacy is lacking in patients < 6 years of age. Clinical trials comparing the established dose of 7% saline in 4 ml, twice daily, to lower concentrations or less frequent dosing have not been performed. The therapeutic effect of hypertonic saline differs from the mode of action of recombinant human DNase and therefore the two drugs cannot replace each other.

Q16 Which CF patients should be treated with bronchodilators?

For CF patients with persistent wheeze or exercise-induced bronchospasm, potentially suffering from CF asthma, who experience symptomatic relief from this treatment, short-acting bronchodilators should be used [D]. A significant response to treatment may support the use of bronchodilators, but responses may be quite variable. Evidence for benefit from regular use of bronchodilators prior to physiotherapy is lacking. Evidence for a sustained benefit on mucociliary clearance is lacking. Bronchodilators may be necessary before inhaled antibiotics and hypertonic saline are administered [B]. Long-acting bronchodilators should be used in CF patients with asthma who cannot be controlled with short-acting bronchodilators and inhaled corticosteroids alone [A]. There is insufficient evidence to support the use of short-acting anticholinergic agents.

Q17 Should systemic absorption of marketed inhaled antibiotics be routinely measured and if so, when and how often should drug levels be measured?

Aminoglycosides: Measurement of serum trough levels may be performed on a regular basis in CF patients with reduced renal function, and in CF patients with normal renal function but at risk for nephrotoxicity (e.g., potential nephrotoxic co-medication such as non-steroidal anti-inflammatory drugs and immunosuppressants). Colistimethate sodium cannot be measured in routine laboratories.

Q18 What tests are required before a new inhalation device is introduced for use in CF patients?

The introduction of a new inhalation device must be accompanied by clinical comparative studies, including the medications recommended by the manufacturer. The design of such studies is dependent on the drug.

Q19 Can drugs for inhalation be mixed in one device?

It is not recommended to mix medications for inhalation prior to their use. If mixing is needed, the mixture should have been tested for chemical and physical compatibility.

Q20 How should a new medication for inhalation be tested before its use in CF patients.

Safety studies in animals and phase I, II and III studies should be performed according to regulatory requirements. New medications for inhalation should be tested for pharmacokinetics, including systemic absorption, safety and efficacy, in young CF children, in adequate numbers, and in CF adults. Studies should always be performed using combination(s) with predefined device (s). As drug concentrations are highly variable in sputum specimens, they are likely to be the wrong parameter for pharmacokinetics, peripheral drug deposition and efficacy.

Table 5

Grades of recommendations.

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- A Good scientific evidence suggests that the benefits of the clinical service substantially outweigh the potential risks. Clinicians should discuss the service with eligible patients (consistent level 1 studies).
 - B At least fair scientific evidence suggests that the benefits of the clinical service outweigh the potential risks. Clinicians should discuss the service with eligible patients (consistent level 2 or 3 studies *or* extrapolations from level 1 studies).
 - C At least fair scientific evidence suggests that there are benefits provided by the clinical service, but the balance between benefits and risks are too close for making general recommendations. Clinicians need not offer it unless there are individual considerations (level 4 studies *or* extrapolations from level 2 or 3 studies).
 - D At least fair scientific evidence suggests that the risks of the clinical service outweigh potential benefits. Clinicians should not routinely offer the service to asymptomatic patients (level 5 evidence *or* troublingly inconsistent *or* inconclusive studies of any level).
 - E Scientific evidence is lacking, of poor quality, or conflicting, such that the risk versus benefit balance cannot be assessed. Clinicians should help patients understand the uncertainty surrounding the clinical service.
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Ref: Oxford Centre for Evidence-based Medicine Levels of Evidence (May 2001).

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