Antimicrobial peptides for tackling cystic fibrosis related bacterial infections: A review

Francesca Bugli [a,b], Cecilia Martini [a,b], Maura Di Vito [a,b], Margherita Cacaci [a,b], Daniele Catalucci [c], Alessandro Gori [d], Michele Iafisco [e,*], Maurizio Sanguinetti [a,b,**], Alberto Vitali [f]

[a] *Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy*
[b] *Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy*
[c] *IRCCS Humanitas Research Hospital, Rozzano (MI), Italy and Institute of Genetic and Biomedical Research (IRGB) - UoS Milan, National Research Council (CNR), Milan, Italy*
[d] *Institute of Chemical Sciences and Technologies "G. Natta" (SCITEC) - UoS Milan, National Research Council (CNR), Milan, Italy*
[e] *Institute of Science and Technology for Ceramics (ISTEC), National Research Council (CNR), Faenza (RA), Italy*
[f] *Institute of Chemical Sciences and Technologies "G. Natta" (SCITEC) - UoS Rome, National Research Council (CNR), Rome, Italy*

A B S T R A C T

Antibiotic resistance is a serious health and social problem that will have a substantial impact in the coming years on the world health and economy. Thus, the increasing demand for innovative antibiotics, has prompted many researchers in the medical, microbiological, and biochemical fields to exploit the properties of antimicrobial peptides (AMPs). When properly used, designed, and conveyed, AMPs can really represent a valid alternative to conventional drugs especially in situations that are particularly difficult to treat such as chronic infections found in Cystic Fibrosis (CF) patients. In this review we focused on the applications of AMPs in the specific field of CF, illustrating different types of peptides from natural, naturally modified, synthetic as well as the different strategies used to overcome the barriers, and the physiological conditions in which AMPs must operate.

1. Introduction

Cystic Fibrosis (CF) is the most frequent hereditary genetic disease among populations of Caucasian origin (Europe and North America), with an incidence of about one sick infant every 3000–4000 healthy births. The genetic basis for CF is well-characterized and arises from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Ratjen et al., 2015). In people affected by CF, secretions such as bronchial mucus or pancreatic juice are denser than normal, with harmful effects especially on the respiratory and digestive systems, but also other important physiological functions may be compromised (Elborn, 2016). These patients experience recurrent bronchitis or bronchopneumonia, caused by different bacteria species that give rise to infections and chronic inflammations, with progressive deterioration of respiratory function and lung tissues. Chronic bacterial infections and accompanying airway inflammations eventually lead to respiratory failure in 80–95 % of CF patients. Bacterial colonization arises in CF patients shortly after born. Deeper endobronchial colonization and

chronic inflammation develop later, within a year or two of life (Armstrong et al., 1995), with several organisms including Staphylococcus aureus, Haemophilus influenzae, and Gram-negative enteric organisms (Klebsiella pneumoniae and Escherichia coli). Pseudomonas aeruginosa infection is common to all CF patients, which leads to epithelial surface damage and airway plugging with a general deterioration of lung tissues finally resulting in a pulmonary function decline. Burkholderia cepacia and Stenotrophomonas maltophilia are also emerging multi-drug resistant organisms found in CF patients and their diffusion is increasing (Spencer et al., 2020). Life expectancy depends on the evolution of pulmonary complications, determined by a combination of genetic and non-genetic factors. The median survival age is 40 years, but progress in medical and surgical treatment options have improved the prognosis over the last few decades.

Antimicrobial treatment could improve the survival in CF patients. However, the particularly viscous composition of the sputum, rich in biomolecules (extracellular DNA, lipids, mucins, polysaccharides etc.), the presence of salts and the acid pH, make the administration of antibiotics more complicated, thus reducing their effectiveness. Novel antimicrobial agents and new more effective ways of administration that could replace or complement current therapies are consequently needed especially for the treatment of chronic infections. In this framework Antimicrobial Peptides (AMPs) are promising candidates due to special characteristics such as their lower toxicity, limited propensity to develop antibiotic resistance and their established anti-inflammatory and immunomodulatory effects (Waters and Smyth, 2015). AMPs are broadly expressed in all kinds of organisms (bacteria, archaea, protists, fungi, plants, and animals) as an essential component of their innate immune system which is the first line of defense against pathogens. AMPs are produced by epithelial cells and by circulating immune cells including neutrophils and macrophages. AMPs have demonstrated good antimicrobial activity against a wide range of microbes, and very interesting also against multidrug-resistant bacteria (Sala et al., 2021). Curiously, AMPs were discovered at the same time of antibiotics (in the early 1920 s) but were dominated by the success of this latter. To date more than 3000 peptides are registered in the AMPs database APD3 (https://aps.unmc.edu/AP). The emergence of antibiotics resistance, which is a major treat of human health, has resurrected the interest in AMPs. AMPs exhibit also synergistic effects upon co-administration with conventional antibiotics to treat both susceptible and multidrug-resistant bacteria at lower concentrations (Geitani et al., 2020). Even if the developed AMPs are very different in terms of compositions and lengths, they share common structural characteristics such as the most studied AMPs are short polypeptides of less than 50 amino acids having a positive net charge and a hydrophobic content. In the last years, a lot of companies tried to develop AMPs and, consequently, the research of peptide-drug has led to numerous FDA approved peptide therapeutics in the market, such as cyclic peptides gramicidins and polymyxins. Other peptides such as cathelicidin LL-37, omiganan, pexiganan, DPK-060 and PXL01 are nowadays objects of late-stage clinical studies. Although AMPs have very attractive qualities, drawbacks exist to produce an efficient peptide-drug for medical application. First, the in vivo antimicrobial activity of AMPs is not always the same as observed in vitro; peptides may undergo to proteolytic processes in serum, or to a reduced or suppressed in vivo efficacy due to the pH and the high salt concentrations present in physiological conditions (Rotem et al., 2009; Jahnsen et al., 2013). This aspect is particularly true if we consider the pulmonary environment related to CF, in which the pH level, the high concentration of salts, the viscosity conditions, and the presence of proteolytic enzymes could actively hamper the antibacterial properties of AMPs. These limitations are highlighted by several studies in which it is reported that the high-salt environment created on the apical side of CF epithelial cells impair AMPs effectiveness (Zhang et al., 2005) as observed for human cathelicidin LL37, histidine-richpeptide P-113, indolicidins, gramicidins, bactenecins, and magainins, all known to be salt sensitive (Mohanram et al., 2016). Second, an important element to

consider is the possibility that these peptides would trigger an immunogenic response, which could dramatically limit their efficiency. Some AMPs in fact, have a double role in modulating autoimmunity upon infection acting either as pro- or anti-inflammatory agents (Ganguly et al., 2009). Third, although AMPs are less exposed to antibiotic resistance phenomena, microorganisms are however able to express resistance towards peptides (Guo et al., 1998; Lyu et al., 2016). Fourth, another limit for the large-scale development and commercialization of AMPs as antibiotics may reside in their production cost, mainly depending on the peptide structure, which could be several times more expensive than the production of conventional antibiotics. Anyway, many of these obstacles are nowadays avoided or less important thanks to the advancements in many research fields related to peptide synthesis and delivery. Recently, researchers have focused on developing a series of AMP analogs often as derivatives of natural molecules, with better antibacterial, cytotoxic, and hemolytic properties. Other synthetic peptides (D-peptides, dimeric peptides, cyclized forms or composed of unnatural amino acids) have been designed, to mimic the structure, function, and mode of action of AMPs showing higher resistance to proteolytic degradation, resulting in prolonged half-lives and cost-effective molecules (Wang et al., 2019; Molchanova et al., 2017b). With the same aim, as discussed later in this review, diverse nano-systems have been designed to efficiently deliver AMPs. Furthermore, the progress in designing non-immunogenic peptides is improving quickly, and this should also increase the clinical success (Henninot et al., 2018; Holfeld et al., 2015). Finally, peptides are nowadays routinely produced on an industrial scale, and the production costs are taking benefit from new synthetic routes aimed at improving synthetic efficiency, such as microwave synthesis, and from the perspective of reduced environmental costs of large-scale synthesis (Ferrazzano et al., 2022; Sharma et al., 2022).

In this review we would like to give an update of the applications of AMPs with a special attention to their use as potential therapeutic agents in CF treatment of bacterial infections. Focus is given to the strategies used to overcome some of the barriers typical of the lungs and airways physiology found in CF infections. We have sub-divided the arguments into the following paragraphs taking in account that this division may not be accurate as some peptides could be equally described in a section or in another: i) "**AMPs related to Innate immunity applied to CF related infections**" in which are reported examples of natural peptides deriving from innate immune system and used to tackle bacterial infections. In particular, we pose our attention to defensins and cathelicidins; ii) **"Natural-derived AMPs applied to CF related infections",** where we have reported examples of natural peptides opportunely modified to counteract the harsh environment of CF patient lungs. Examples of cryptides deriving from natural proteins are reported; iii) **"Synthetic and peptidomimetics AMPs applied to CF related infections"** where some studies related to the employment of designed peptides of synthetic or semi-synthetic origin are described; iv) **"Delivery systems of AMPs applied to CF related infections"** in this paragraph we have pointed the attention on the delivery mediated by nano-systems to facilitate the application of AMPs in CF patients. We have tried to highlight the research papers illustrating efficient and innovative strategies to overcome the peculiar physiology and environmental conditions encountered by AMPs in CF airways physiology.; v) finally, in the paragraph "**Antibiofilm peptides (ABPs) applied to CF related infections**" we have reported some studies about the use of antimicrobial peptides specifically directed against bacterial biofilm, another challenging field of use of AMPs.

1.1. AMPs related to Innate immunity applied to CF related infections

Airways and lungs have different typologies of defense barriers to protect themselves from microbial injuries. The physical barriers are represented by mucociliary clearance, by the cell populations devoted to defense (i.e. neutrophils, NKCs, macrophages) (Rubin, 2007), and the
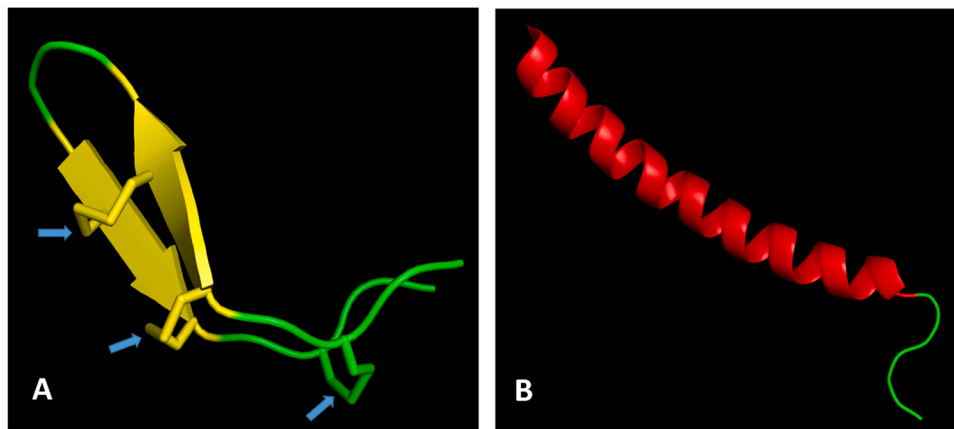
Fig. 1. The different colors evidence the secondary structures: yellow, a β-hairpin-like; green, coil; red, alpha helical. A) The θ-defensin RTD-1 (PDB: 1HVZ). The arrows indicate the disulfide bridges linking 7–12, 5–14 and 3–16 Cys residues B). The human cathelicidin LL-37 peptide (PDB: 2K6O). Images were rendered with PyMOL(TM) 2.3.2 (Incentive Product (C) Schrodinger, LLC).

presence of a thin layer, the Airway Surface fluid (ASL), in which a number of molecules with host-defense functions are dispersed. Along with antimicrobial proteins (lactoferrin, lysozyme), some peptides are present in the ASL and defensins and cathelicidin LL-37 are the most important representatives (Bals et al., 1999; Seiler et al., 2014). These components are constitutively and inducibility secreted by lung epithelial cells and are part of the innate immunity system, which is the fast acting non-specific first molecular barrier against microbial attacks and infections (Jirillo et al., 2018).

Defensins are a group of cysteine-rich cationic multifunctional peptides with molecular weights ranging from 3 to 6 kDa. Human defensins are divided into α-defensins, β-defensins, and θ-defensins in function of the different typologies of intramolecular disulfide bonds between the six cysteine residues typically found in their primary sequence (Bals, 2000; Ganz, 1999). The disulfide bonds drive the structure of defensins in a triple stranded β-sheet configuration.

As other AMPs, they are encoded as pro-peptides and they are fully active upon proteolytic processing. Defensins are expressed constitutively or following external stimuli, including bacterial and viral infection, cytokines, TNF, and LPS (Hazlett and Wu, 2011). In particular, β-defensins are expressed by different kind of epithelia (skin, eye, salivary glands and oral cavity, urogenital and respiratory tract) (Zasloff, 2007) and osteoblasts aiding in the modulation of bone tissue modeling (Luft, 2017). θ-defensins were firstly identified in rhesus macaque (Macaca mulatta) leukocytes (Tang et al., 1999), while in human genome are represented as pseudogenes (Lehrer et al., 2012). The structural peculiarity of these peptides resides in their cyclic arrangement achieved by a head to-tail link (Conibear et al., 2014). Their cyclic structure confers a high stability, resistance to proteases, and strong antibacterial and antiviral properties (Fig. 1). As antimicrobials, defensins show a broad spectrum of activity against gram-positive and gram-negative bacteria, fungi, and viruses. The mechanism of action is mainly based on the classical membrane perturbation effects driven by the electrostatic attraction between the anionic bacterial phospholipids and the cationic character of the peptide (hBD-2, −5) and the creation of a sort of molecular web promoted by reduction of disulfide bonds and subsequent bacterial entrapment (hBD-1) (Amerikova et al., 2019). The interaction with intracellular targets or other processes such as inhibition of DNA synthesis, are another mode of action observed for hNP-1 and − 5. Defensins behave as pleiotropic molecules being involved in other physiological functions. They are directly involved in the innate immunity and may serve to link the innate and adaptive immune response binding different receptors. hBD-2 was shown to be a ligand for TLR4 (Semple et al., 2011) and to be able to bind to the Chemokine Receptor 6 (CCR 6) (Yang et al., 1999) aiding in the inflammatory events

Table 1

Sequences of AMPs related to innate immunity system described in this work.

Peptide name	Sequence	Ref
θ-defensin RTD-1	GFCRCLCRRGVCRCICTR	(Lehrer et al., 2012)
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	(Saiman et al., 2001)
SMAP-29	RGLRRLGRKIAHGVKKYGPTVLRIIRIAG	"
CAP-18	GLRKRLRKFRNKIKEKLKKIGQKIQGLLPKLAPRTDY	"
CAP-18–18	KRLRKFRNKIKEKLKKIG	"
CAP18–22	RKRLRKFRNKIKEKLKKIGQKI	"
mCRAMP	GLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPEQ	"
rCRAMP	GLVRKGGEKFGEKLRKIGQKIKEFFQKLALEIEQ	"
OV-1	KNLRRIIRKIIHIIKKYG	"
OV-2	LRRIIRKIIHIIKK-NH2	"
OV-3	IRRIIRKIIHIIKK-NH2	"
BMAP-27	GRFKRFRKKFKKLFKKLSPVIPLLHLG	(Benincasa et al., 2003)
BMAP-28	GGLRSLGRKILRAWKKYGPIIVPIIRIG	(Mardirossian et al., 2016)

Note: Cysteine residues in θ-defensin RTD-1 are colored to evidence their involvement in the formation of the three disulfide bonds.

dampening the expression of pro-inflammatory genes. hBD3 was proven to have a role in the innate immune mediated response to microbial DNA, increasing inflammatory signaling and promoting activation of the adaptive immune system via antigen presenting cells (McGlasson et al., 2017). Other functions, as the wound healing ability, point to these peptides as potent tools in a wide variety of illness conditions ranging from bacterial and fungal infections, tissue repair and immunomodulatory effects. The θ-defensin RTD-1, a β-hairpin-like peptide stabilized by three disulfides in a ladder configuration structure (Fig. 1A, Table 1), was tested in vitro against multidrug resistant (MDR) mucoid and non-mucoid P. aeruginosa strains and in a in vivo model (Lehrer et al., 2012; Beringer et al., 2016) with mice bearing the CFTR F508del mutation. This peptide showed a rapid (within 30 min) and strong antibacterial activity (MIC90 8 μg/μl mucoid and 4 μg/μl non mucoids). Interestingly, it was also active against colistin resistant strains bearing pmrAB and/or phoPQ mutations, which confer resistance even to other cationic AMPs (McPhee et al., 2003). The in vivo test also showed promising data when RTD-1 was inhaled in mice, and no toxic effect was observed after animal treatment.

It is worth of mention the anti-inflammatory activity of RTD-1 with reductions in IL-1, TNF, CXCL8 and IL-6 concentrations and the inhibitory activity ($IC_{50} = 551$ nM) versus MMP-9 enzyme in a dose-dependent manner. As mentioned above, the θ-defensins seem to be very promising

Table 2

Sequence of the natural-derived AMPs described in this work. (Lowercase letters indicate D- amino acids).

Peptide	Sequence	Ref
Esculentin-1a (1–21)	GIFSKLAGKKIKNLLISGLKG-NH$_2$	(Cappiello et al., 2016)
Esc(1–21)– 1c	GIFSKLAGKKIKNlLIdGLKG-NH$_2$	"
Dermaseptin-PC	ALWKSILKNVGKAAGKAVLNAVTDMVNQ-NH$_2$	(Ying et al., 2019)
DMPC-10	ALWKSILKNVGKAAGKAVL-NH$_2$	"
DMPC-10A	ALWKKLLKKA-NH$_2$	"
DMPC-19	ALWKKLLKKA-Cha-NH$_2$	"
D-Bac8c^{2,5Leu}	rlwvlwrr	(Forde et al., 2014)
D-HB43	fakllaklakkll	"
D-P188	kwklfkklpkflhlakkf	"
AAG-D-WMR^{3,6}-leu (AAG-WMR)	AAGwglrrllkygkrs	(Reeves et al., 2012)
pro-D-WMR^{3,6}-leu (pro-WMR)	Ac-EEEEAAAGwglrrllkygkrs	"
VLL-28	VLLVTLTRLHQRGVIYRKWRHFSGRKYR	(Pane et al., 2017)

tools against CF bacterial infections (Tongaonkar et al., 2011). In fact, their cyclized structure confers a high resistance to proteases in comparison with natural β-defensins, and other features make this peptide a promising lead compound for designing new peptide-based antibiotics (Taggart et al., 2003). In a further study different aspects were investigated for a safe and potential use of RTD-1 in a therapeutic formulation, beside the antibacterial efficacy, the inflammatory response, the protease resistance, the structural stability in sputum and other parameters regarding the administration by aerosolizing were also taken in account (Bensman et al., 2017). Cathelicidins are an important group of antimicrobial peptides known to be key immunomodulatory mediators during infection events in vertebrates. LL-37 is the only cathelicidin antimicrobial peptide produced in humans (Fig. 1B, Table 1). In fact, hCAP18 is mainly produced by neutrophils, but also by airway epithelial cells and other cell types where it is processed upon proteolytic cleavage by neutrophil proteinase and epithelial kallikreins resulting in the release of the active form of LL-37 (Fabisiak et al., 2016). It is a typical pleiotropic peptide showing diverse biological activities beside the antimicrobial one, playing as an immunomodulatory agent by activating both pro- and anti-inflammatory pathways (Tjabringa et al., 2003). It also induces cell motility and wound healing, proliferation and differentiation, and regulates apoptosis of epithelial cells and neutrophils. It has also been shown to possess potent antiviral activity against a wide range of pathogens. In the ambit of CF related infections, cathelicidins and different typologies of their derivatives have been evaluated in various studies as potential therapeutic tools in the peculiar physiological conditions of CF. Cathelicidin peptides of different origin, including designed shorter sequences, were evaluated for their properties as new bactericidal agents against CF pathogens (Saiman et al., 2001). The in vitro activity of five cathelicidin peptides LL37, CAP18 (rabbit), mCRAMP (mouse), rCRAMP (rat), SMAP29 (sheep) (Table 1) and designed peptides derived from SMAP29 termed ovispirins (OV-1, OV-2, and OV-3, Table 1), and two derivatives of CAP18 were tested against clinical isolates from CF patients of P. aeruginosa, S. maltophilia, B.

cepacia and A. xylosoxidans using broth microdilution assays. SMAP29 peptide (MIC50, 1 μg/μl) and CAP18 (MIC50, 4 μg/ml) showed to be the most effective molecules against the different strains of P. aeruginosa. The same peptides showed the highest bactericidal activity also against S. maltophilia and A. xylosoxidans, but none of the peptides included in the study showed appreciable activity against the clinical isolate of B. cepacia. Antibacterial properties of SMAP-29 peptide were also tested in presence of a clinical sample of CF sputum where it showed a very low activity if compared to the broth microdilution assay. Only a 1000-fold concentration of SMAP-29 was able to restore the antibacterial activity against the two strains (one mucoid and one multiply antibiotic resistant) of P. aeruginosa identified in the patient mucus. SMAP-29 was also tested in combination with Tobramycin in checkerboard assays where a synergistic effect was evidenced against P. aeruginosa strain PAO1, but not towards the CF clinical isolate 50BK. In the study of Pompilio et al. (2012) two cathelicidins of bovine origin BMAP-27 and BMAP-28 (Skerlavaj et al., 1996; Benincasa et al., 2003) (Table 1) and the designed non-natural peptide P19(9/B) (Table 3) (Pacor et al., 2002) were assayed for their in vitro antibacterial and anti-biofilm (preformed and formed) activity potency, against selected CF clinical strains of S. aureus, P. aeruginosa, and S. maltophilia strains. All the three AMPs showed to be strongly effective against the tested strains, with BMAP-28 resulting more versatile versus the different strains. MBC/MIC ratio clearly indicated that all AMPs exert a bactericidal effect against the CF isolates, in agreement with the known capability of BMAP-27, BMAP-28 and P19(B/9) to kill target cells by rapid permeabilization of their membranes (Skerlavaj et al., 1996). An interesting evaluation was carried out by the authors between the MIC values obtained in a CF-like environment respect to the Clinical and Laboratory Standards Institute (CLSI) guidelines, for cathelicidins BMAP-27, – 28 (Benincasa et al., 2003) P19(9/B) and Tobramycin. This evaluation showed that mean $MIC_{CF-like}/MIC_{CLSI}$ and $MBC_{CF-like}/MBC_{CLSI}$ values obtained for Tobramycin (23.9 and 15.6, respectively) were significantly higher than those observed for BMAP-27 (1.5 and 1.2, respectively; $p < 0.001$), BMAP-28 (0.5 and 0.5, respectively; $p < 0.001$), and P19(9/B) (2.8 and 2.9, respectively; $p < 0.001$), irrespective of species tested, indicating a reduced antibiotic activity of Tobramycin in CF-like conditions. In any case, the three peptides showed different kind of activities exerting bacteriostatic or bactericidal effect depending on the microbial strain. An interesting synergic effect was observed against two S. aureus strains (Sa4 and Sa10) that became sensitive to Tobramycin when used in combination either with the cathelicidin BMAP-27 or the synthetic peptide P19(9/B) suggesting for these peptides a role as adjuvant in Tobramycin action at least in the case of these S. aureus strains. Further, the same research group, encouraged by the good results with BMAP-27 and – 28 and with the aim to design shorter peptides more feasible for a therapeutic use, produced various truncated versions of these cathelicidins that were tested both in vitro and in vivo assays (Mardirossian et al., 2016). The obtained peptides maintained in vitro a similar activity of parental peptides, and among them the 1–18 fragment of BMAP-27, resulted the less toxic in a C57BL/6NCrl mice model. Unfortunately, this peptide when intratracheally administered to P. aeruginosa infected mice, did not show any antibacterial activity, probably due to its degradation by pulmonary proteases, as suggested by observing its degradation within 10 min in a bronchoalveolar lavage (BAL) fluid sample. The authors suggested the use of D-peptides or peptidomimetics in order to enhance peptide resistance to pulmonary proteases in the

Table 3

Sequence of synthetic AMPs described in this work.

Peptide	Sequence	Ref
P19(9/B)	GZZOOZBOOBOOBZOOZGY-NH$_2$	(Pompilio et al., 2012; Pacor et al., 2002)
WLBU2	RRWVRRVRRVWRRVVRVCRRWVRR	(Chen et al., 2018)
lin-SB056–1	KWKIRVRLSA-NH$_2$	(Maisetta et al., 2017)

Note: Z: Norleucine; O: Ornithine, B: Aminoisobutiric acid.

design of therapeutics peptides for pulmonary applications.

1.2. Obstacles in using AMPs in CF airways and methods to circumvent them

The metabolic and mechanical defense mechanisms of lungs, which are set up to defend from foreign entities (microorganisms, molecules), can counteract the administration of peptides itself. Among these mechanisms, mucociliary clearance is definitely the primary one followed by proteolytic enzymes (cathepsin B, collagenases, prolyl-peptidases, Angiotensin Converting enzymes etc.) widely distributed in the lung's environment (Patton et al., 1998). AMPs activity may be also reduced in vivo by interactions with anionic proteins (e.g., mucin) or other polysaccharides (e.g., bacterial alginates or glycosaminoglycan) of lung extracellular environment (Wan et al., 2012).

In CF patients, the lung's environment is further complicated by the peculiar physiological ambient created by the CFTR dysfunction. Firstly, a highly sticky mucus is produced by bronchial epithelial (BE) cells, which can entrap external molecules neutralizing their efficacy. This mucus is also characterized by a high-salt concentration that, coupled to a reduced pH of the ASL (Pezzulo et al., 2012), may dramatically limit the potency of conventional antibiotics and AMPs (Souza et al., 2018). As an example, defensins and cathelicidin are, individually and synergistically affected by the acidic pH of ASL (Abou Alaiwa et al., 2014). High-salt conditions are well known to hamper the effectiveness of AMPs (Goldman et al., 1997), even if the real contribution of high-salt concentrations is still in debate (Hiemstra et al., 2007). Beside the physico-chemical ones, other obstacles of biochemical nature should be overcome. CF lung is characterized by high protease levels mainly due to neutrophil elastase (NE) (Rees et al., 1997), followed by other enzymes as cathepsins. These enzymes can degrade many proteins (matrix and plasma proteins, immunoglobulins, cytokines, protease inhibitors, hemoglobin) among which also those devoted to the defense from infective agents such as lactoferrin and AMPs defensins (Taggart et al., 2003) and human cathelicidin LL-37 (Bergsson et al., 2009). The cationic character of many AMPs is also the cause of a ionic binding to acidic mucins and other polysaccharides (alginates or glycosaminoglycans) (Bergsson et al., 2009; Benincasa et al., 2009), which are found in the lung extracellular compartment and produced by bacteria as constituents of biofilm (Batoni et al., 2011). This ionic-based effects thus leads to an inactivation of AMPs. A similar phenomenon is observed with DNA that is released in the mucus as cellular debris. Different approaches that will be discussed in the following paragraphs, may be used to overcome these drawbacks. They mainly comprehend the design and synthesis of natural-derived or non-natural (peptidomimetics) or the use of delivery systems in order to avoid systemic use and enhance bioavailability and peptide efficiency.

2. Natural-derived AMPs applied to CF related infections

The modification of natural AMPs by residue substitution or chirality modification, or by changing peptide lengths or by inserting appropriate modifications (amidation, lipidation, etc.) is often a successful strategy to maintain or enhance their antimicrobial potency. Amphibian AMPs are a rich source of effective antibacterial molecules (Mangoni et al., 2015) and some of their derivatives show interesting features to be exploited in treatment of CF infection. In this view two derivatives of Esculentin-1 have been studied in detail. A short form of esculentin-1 composed of 21 residue named Esc (1−21) was previously found to be effective against P. aeruginosa in planktonic and biofilm forms, well-functioning also in a high-salt environment (Luca et al., 2013). Furthermore a partial D-derivative named Esc(1−21)-c bearing the D-Leu 14 and the D-Ser 17 residues was found less susceptible for degradation by elastase action (Di Grazia et al., 2015) and it was also able to promote bronchial epithelium repair and wound healing (Cappiello et al., 2016). Chen et al. (2017) studied these peptides also in a in

vivo model of P. aeruginosa infection. Before the use in definitive animal models, they firstly tested the cytotoxic effects of Esc(1−21) and Esc (1−21)-c on primary bronchial cells by measuring the Trans Epithelial Electrical Resistance (TEER) and the effects on inflammation pathways by measuring IL-6, IL-10 or the tumor necrosis factor-α TNFα, and NF-kB gene expressions. As no deleterious effects were observed by using the peptides at 0.1 mg/kg at lung level, the in vivo efficacy was examined using a mouse model of acute lung infection induced by P. aeruginosa. An intratracheally administration of both peptides was performed 2 h after bacterial infection and after 6 h both peptides were effective in lower of 90 % the CFU both in BAL and lung, being the Esc(1−21)-c the most effective. In the following experiment the same Esc(1−21)-c effect was recorded after 24 h upon bacterial infection showing to be still capable to inhibit bacterial growth with a 2-log reduction compared with the 4 h effect. In the same conditions the control peptide LL-37 completely lost its effectiveness (Chen et al., 2017). These results coupled with a beneficial effect on inflammation pathways lead to consider this natural derivative peptide a molecule with a strong therapeutic potential to be further exploited in clinical trials. Dermaseptins derived from phyllomedusinae frogs, are another example from amphibian AMPs applied to bacterial CF isolated strains (Forde et al., 2014). The parent peptide identified from Phyllomedusa coelestis by molecular cloning and LC- MS/MS sequencing and named Dermaseptin PC (DM-PC), was found to be active against different bacterial strains derived from CF patients but showed a sensible hemolytic effect. This fact has prompted the researchers to selectively modify the length and the residues components, thus varying the charge and hydrophobicity of the original sequence producing three derivatives (DMPC-10, DMPC-10A and DMPC-19). The so obtained peptides were challenged towards MRSA and P. aeruginosa strains as a model to investigate the antimicrobial activity in the presence of divalent cations and to mimic the peculiar lung environment of CF patients. Interestingly, the peptides showed different activities in term of killing kinetics, MIC, and ability to prevent or bacterial biofilm eradication. On the basis of these evidence the authors indicate, in designing natural peptides derivatives, the importance to maintain a balance between charge, hydrophobicity, and peptides length. An interesting strategy to overcome susceptibility of natural AMPs to degradation by proteolytic enzymes and to resist to the hypertonic conditions found in pulmonary environment of CF patients, was proposed by Forde et al. (2014). The authors designed pro-peptides with tailored features to be employed in CF patients, showing a good resistance in high salt concentrations and to neutrophil elastases (NE) proteolytic activity. In particular, they designed peptides derived from diverse natural host defense peptides in their D-forms: D-Bac8c2 from Bactenecin[2,5 Leu] (Table 2) (Hilpert et al., 2005), D-HB43 from crustacean polyphemusin I (Zhang et al., 2005), and D-P188 Leu from cecropin A–magainin 2 hybrid (Shin et al., 2002). A further modification was represented by the addition of an N-terminal NE-sensible sequence, in free (EEEEAAG) or in acetylated forms (Ac-EEEEAAAG). The peculiar anionic pro-moiety reduces the net charge of the peptide limiting the potentially toxic effects of the AMP at level of the endobronchial cells where in turn, the NE activity is high. As a result, the authors found that some pro-peptides were activated by purified NE but not upon addition of NE containing BAL. In that case some antimicrobial activity could be restored only by adding 300 mM NaCl indicating that these peptides were salt-sensible (Forde et al., 2014). Thus, NE-labile peptides were produced, and the antimicrobial activity of parent and pro-drug peptides compared against P. aeruginosa, either in presence or absence of NE-rich CF human BAL fluid. Another example comes from the same group (Forde et al., 2016), who developed a pro-peptide, named pro-WMR, from innate immunity peptides previously found in the hagfish Myxine glutinosa to efficiently avoid cytotoxicity and reduce immunogenicity (Subramanian et al., 2009). Pro-WMR was produced in its D-form presenting the NE cutting sequence EEEEAAAG with an acetylated N terminus and an amidated C-terminus (Ac-EEEEAAAGwglrrll-kygkrs-NH$_2$). Pro-WMR and its activated fragment AAG-WMR showed
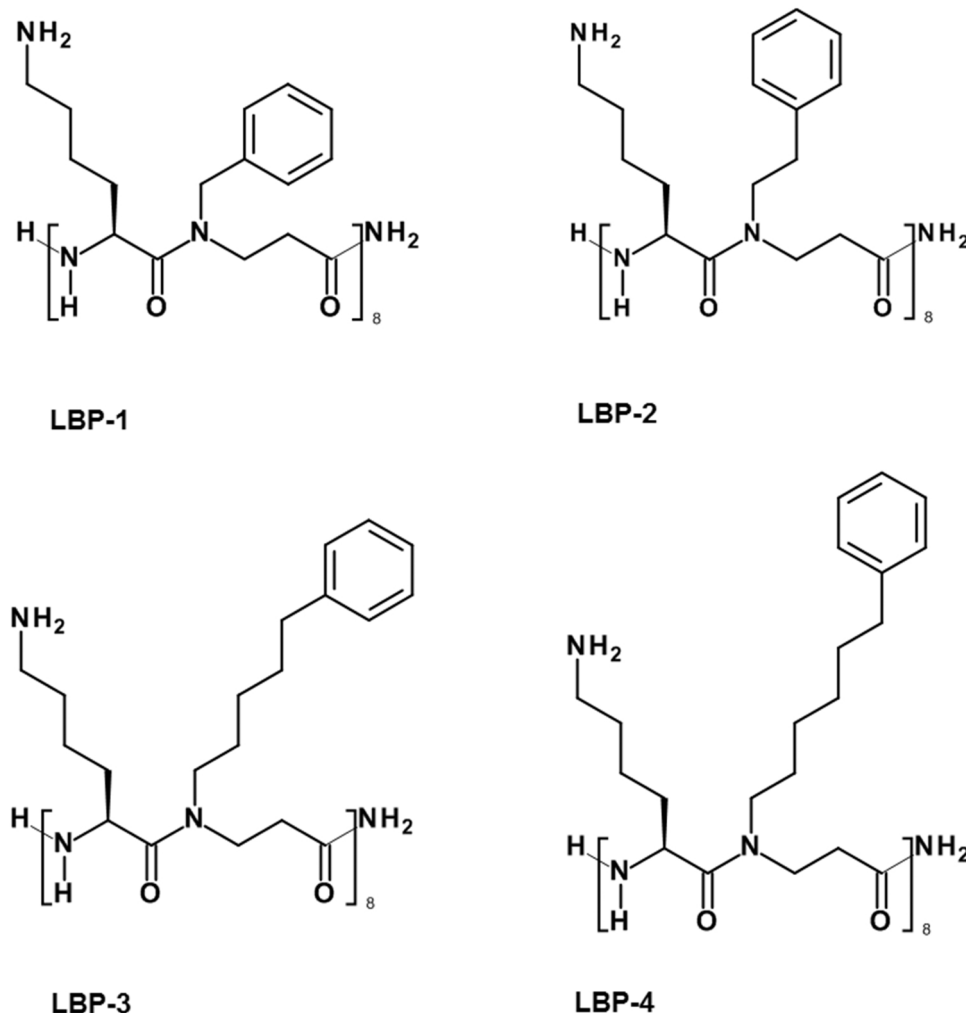
Fig. 2. Peptidomymetics LBP-1,− 2,− 3 and − 4 reported by Molchanova et al. (2019). Re-drawn from originals.

negligible effect on cytotoxicity both in vitro on Cystic Fibrosis Bronchial Epithelial (CFBE) cells and in a C57BL/6 mouse model, with low levels of pro-inflammatory cytokines IL-8 and IL-6. The authors observed an increase in TNF-release in mice treated with AAG-WMR but not with pro-WMR indicating for this pro-peptide a good safety. Along with a low cytotoxicity and immunogenicity, pro-WMR demonstrated a great salt tolerance. In fact, when incubated in high NaCl concentration condition (300 mM) and in presence of BAL with P. aeruginosa CF isolated strains, WMR maintained a good bactericidal activity. This is a favorable characteristic for an AMP because high salt tolerance would facilitate the delivery of these AMPs in a hypertonic saline solution, and their inhalation would improve lung function in patients with CF (Bergsson et al., 2009; Reeves et al., 2012). A promising approach to discover novel AMPs is to individuate them as embedded sequences within known proteins. It is a fact that different human proteins after undergoing proteolytic cleavage, may give rise to smaller biologically active fragments generally named as cryptides (Iavarone et al., 2018; Ciociola et al., 2016). As an example, hemorphins are opioid peptides deriving from hemoglobin (Lantz et al., 1991). Hemoglobin is also the origin of hemocidins natural AMPs found in different body comparts (Mak et al., 2008). Similarly, many antibody fragments originated by proteolytic degradation show potent antimicrobial activities (Magliani et al., 2009). In the last years, informatic tools have been developed allowing, through appropriate algorithms and Artificial Intelligence, to predict potential antimicrobial sequences within selected proteins (iAMPred (Meher et al., 2017), AmPEP (Bhadra et al., 2018), CAMP

(Waghu et al., 2016), iAMPl2 (Xiao et al., 2013). These approaches represent promising and increasingly accurate methods to discover new AMPs. The antimicrobial peptide VLL28 displaying chemical, physical and functional properties typical of AMPs, has been discovered and characterized following such predictive approach (pane et al., 2017). It was identified as a fragment hidden within the sequence of the Stf76 transcription factor from Sulfolobus islandicus. One of the most interesting features of this peptide is the maintenance of its antibacterial activity even in high-salt conditions, an attractive aspect if applied in CF environments (Pedone et al., 2016).

3. Synthetic and peptidomimetics AMPs applied to CF related infections

Although many natural AMPs are available to be used as promising new antibiotics in CF related infections, as previously described, they generally suffer from several shortcomings, e.g., poor bioavailability due to susceptibility to proteolytic degradation and their often unfavorable hemolytic and cytotoxic properties conferring a narrow therapeutic window (Rotem et al., 2009). For this reason, a great effort has been done in the last years to improve the design of de-novo synthesized biologically active peptides (Wiradharma et al., 2011) and peptidomimetics (Lohan et al., 2013; Bragonzi, 2010; Lachowicz et al., 2020). One of the followed strategies is to produce hybrid molecules provided with α and β-residues in order to improve the resistance to proteolytic enzymes. Molchanova et al. (2017a) following this approach, synthesized two
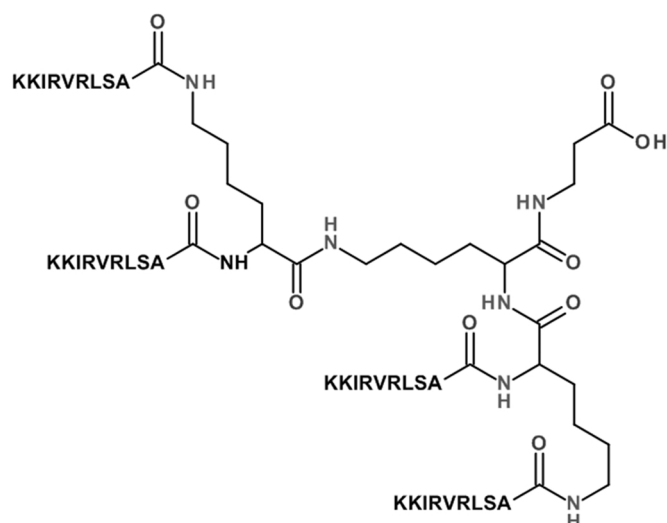
Fig. 3. The tetra-branched peptide M33 (Pini et al., 2012). Re-drawn from the original.

peptoids called LBP-1 (Molchanova et al. 2017a) and LBP-2 (Klodzinska et al., 2018) consisting of alternating lysine and β-peptoid phenylalanine-like hydrophobic residues, differing in length and hydrophobicity. These peptides showed a good antibacterial activity against gram-positive and gram-negative bacteria. To improve the activity of these peptides especially against gram-negative strains, the authors modified LBP-1 and LBP-2 peptides elongating the hydrophobic side chains to investigate the correlation between hydrophobicity and bactericidal activity, producing two new peptides, LBP-3 and LBP-4 (Molchanova et al., 2019). LBP-2, − 3 and − 4 were tested against P. aeruginosa CF clinical strains possessing phoQ and/or pmrB mutations, which render them resistant to colistin. The results showed that peptide LBP-2 was the most promising in terms of bactericidal activity against colistin-resistant strains (MIC 4 vs. >512 µg/ml of colistin) and possessed the most interesting cytotoxicity profile if compared to the more hydrophobic analogs LBP-3 and − 4 (Fig. 2).

Another example of synthetic AMP is represented by the engineered peptide WLBU2 composed of three amino acids in repeated sequences of Arg, Trp, and Val, (Table 3) developed through the principle of optimal amphipathicity while minimizing the diversity of amino acid composition using only hydrophobic (Val and Trp) and cationic (Arg) amino acids. The peptide was tested in vitro and in vivo in comparison with the natural cathelicidin LL-37, showing a broad-spectrum antibacterial activity, even in saline and divalent cation high concentrations, and demonstrating antiviral and antibiofilm activity. When challenged in animal murine models infected with PAO1, WLBU2 showed a prolonged effect on bacterial burden (through 24 h) while the effects of LL37 were only transitory (Chen et al., 2018). A semi-synthetic peptide named lin-SB056–1 (Table 3) and already described for its antimicrobial activity (Batoni et al., 2016) was tested in conditions resembling those of CF lung physiology (Maisetta et al., 2017). The bactericidal assays were carried out against P. aeruginosa ATCC 27853 (non-mucoid) and the mucoid strain PaM01 in Artificial Sputum Medium (ASM) to mimic the composition of CF sputum. The peptide was active against all tested strains at concentrations ranging between 1.56 and 3.12 µg/ml showing none or little differences in the activity between the mucoid and non-mucoid tested strains. The antibiofilm activity was also assessed with an interesting strategy. To counteract biofilm formation, the use of Lin-SB056–1 was coupled with the use of EDTA, which as cation chelator, is known to destabilize the biofilm structure. As a result, the combination of the two agents caused a reduction of more than 50 % of the biofilm biomass. Another approach to improve activity, stability and lowering cytotoxicity of AMPs, is the synthesis of dendrimeric
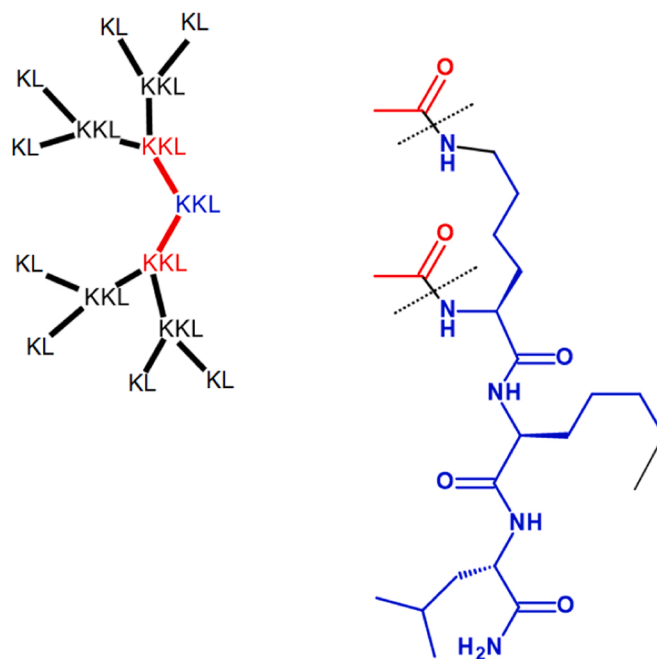

Fig. 4. G3KL dendrimeric peptide. On the left the branched peptide is schematized. On the right the first KKL moiety is reported evidencing the amine groups of the lysine involved in the branching.

compounds. Dendrimers are defined as hyperbranched polymeric molecules, and usually dendrimeric peptides show functionality completely different from linear ones (Tam et al., 2002). Dendrimeric peptides may offer a polyvalence to be exploited to enhance determined characteristics, as for example, the hydrophobic interaction with phospholipid bacterial membranes or the recognition of determined molecular patterns. In this context, a tetra-branched peptide called M33, and based on the KKIRVRLSA sequence (Fig. 3), has been developed and characterized as an efficient antimicrobial agent against CF isolated strains (Pini et al., 2012) and also further characterized for a pharmaceutical production (Castiglia et al., 2019). The peptide's toxicity against bronchial epithelial cells derived from CF patients (CFBE410) and bronchial epithelial cells from healthy individuals (16HBE14) was also evaluated.

More recently, two very interesting dendrimers have been characterized: G3KL, a 37 amino acids dendrimer, based on lysine and leucine dipeptides coupled via a branched lysine residue (Fig. 4), which proved to be active also against carbapenemase-producing P. aeruginosa and Acinetobacter baumannii clinical strains (Pires etal, 2015; Stach et al., 2014) and the 18 amino acids lipid-dendrimer TNS18 (Fig. 5), showing a wider antibacterial activity spectrum with respect to G3KL being active also against Gram-negative and Gram-positive MRSA pathogens (Siriwardena et al., 2018). Moreover, in a recent study these peptides showed the ability to inhibit P. aeruginosa biofilm formation (Han et al., 2019) by strongly affecting the bacterial swarming motility when used below their MIC value. On the contrary, preformed biofilms, could be eradicated only when the peptides were used above their MIC, highlighting how the concentration balancing is crucial to obtain a desired effect. These aspects further suggest these molecules for a therapeutic use in chronically CF related infections.

The mechanism of action of G3KL was further investigated (Gan et al., 2019) and demonstrated to rely on a classic lipid membrane perturbation mode of action as evidenced by the fast-killing kinetics and super-resolution stimulated emission depletion (STED) microscopy, time-lapse imaging, and transmission electron microscopy (TEM). With the aim to improve the low serum stability, the D-forms of these dendrimers were produced, dG3KL and dTNS18, demonstrating their in vitro activity comparable to Tobramycin against four P. aeruginosa
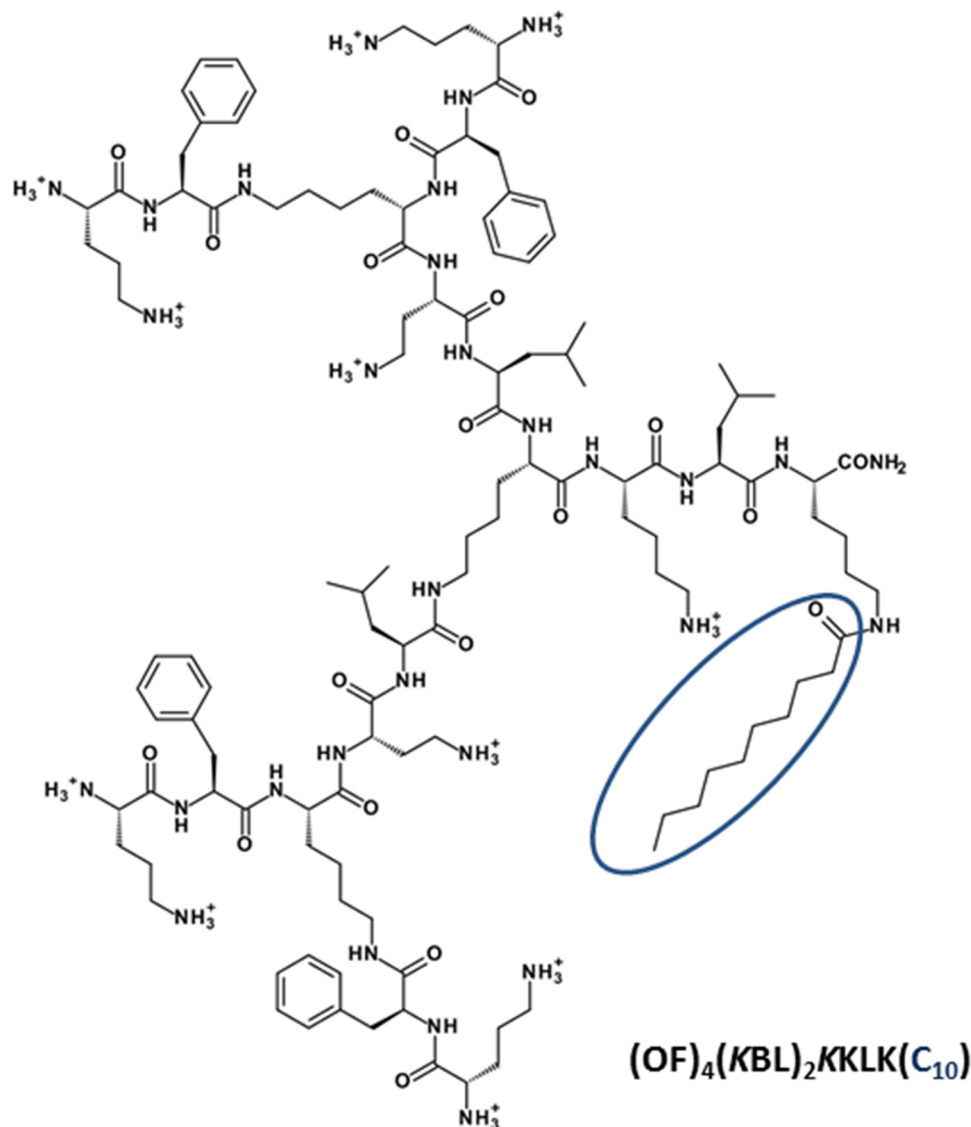
$(OF)_4(KBL)_2KKLK(C_{10})$

Fig. 5. TNS18 dendrimeric peptide. O is ornithine, B stays for diaminobutyric Acid, K represents the branching lysine residues. Blue circle evidences the lipidic moiety.

strains, representative of different stages of CF infection (Pompilio et al., 2018).

4. Delivery systems of AMPs applied to CF related infections

The natural development of CF antimicrobial treatments in alternative to systemic diffusion, is the direct inhalation of antibiotics in order to overcome the degradation of the molecules from proteolytic enzymes present in human serum (Charrier et al., 2014; d'Angelo et al., 2014).

Indeed, inhalation is the preferred route of administration for drugs targeting respiratory dysfunction in CF, given the possibility to locally target lung cells while concomitantly reducing side effects in off-target organs, compared to oral delivery (Sala et al., 2019).

The use of inhalation also minimizes the potential toxicity of the antimicrobial agent allowing the use of higher concentrations of drug to be delivered over a precise area of administration and thus enhancing its efficacy in the site of infection even for highly resistant pathogens. Moreover, for a better patient compliance, administration by inhalation is poorly or completely non-invasive, an important factor to be considered for long-term drug administration such as the one for CF patients. The respiratory tract has numerous physiological and structural features

that make it an excellent site for the administration of peptides: (1) a large area that can be exposed almost simultaneously to the drug, unlike for example the intestine where with a similar total surface area, it does not allow simultaneous exposure and absorption, (2) a very high blood flow that does not directly expose the absorbed drug to the liver elimination mechanisms, and (3) a relatively lower metabolism (Smith et al., 1997). In United States, the US Food and Drug Administration (FDA) has approved three inhalable antibiotics to treat P. aeruginosa infection in CF patients aged ≥ 6 years: aztreonam, tobramycin solution, and tobramycin powder. One additional product, colistin dry powder (Colistimethate) for inhalation, is approved by the European Medicines Agency (Daniels et al., 2017). Velino et al. (2019) have recently published a review describing the most interesting works about different nanocarrier-based inhalatory approaches for the pulmonary treatment of CF indicating that this way of delivery is highly practicable and powerful.

There are three main different modes of delivery drugs by inhalation: nebulization, metered dose inhalation and dry powders inhalation (Shoyele et al., 2006; Fröhlich et al., 2021) In the case of peptide inhalation, the delivery method should be compatible with the peptide characteristics. Nebulization of liquid formulations is the widest method

Table 4
Overview of the most representative works employed NPs as delivery systems for AMPs to counteract CF related infections.

Nanoparticle	Antimicrobial peptide	Key finding	Reference
Nano-structured lipid carriers	Sodium colistimethate	The obtained NPs displayed a 200–400 nm size, high drug entrapment (79–94 %) and a sustained drug release profile. Moreover, sodium colistimethate loaded nano-structured lipid carriers presented antimicrobial activity against clinically isolated P. aeruginosa.	(Pastor et al., 2014)
Solid-lipid NPs	Colistin	Colistin loaded NPs had the same in vitro antimicrobial activity as free drug against planktonic bacteria. However, nano-encapsulated colistin was much more efficient in the eradication of biofilms than free colistin.	(Sans-Serramitjana et al., 2016)
Poly(lactide-co-glycolide) NPs	Colistin	Col-loaded nano embedded microparticles (NEM) were found in vitro to kill P. aeruginosa biofilm and to display a prolonged efficacy in biofilm eradication compared to the free Col.	(d'Angelo et al., 2015)
Calcium phosphate NPs	Colistin	The antimicrobial and antibiofilm activity of colistin loaded NPs tested on P. aeruginosa RP73, a clinical strain isolated from a CF patient, was similar to that of free colistin demonstrating that the therapeutic effect of colistin adsorbed on NPs was retained.	(Iafisco et al., 2022)
Poly(lactic-co-glycolic acid) NPs	Plectasin	NPs displayed a high plectasin encapsulation efficiency (71–90 %) and mediated release of the peptide over 24 h. The antimicrobial efficacy of the peptide-loaded NPs was investigated using bronchial epithelial Calu-3 cell monolayers infected with S. aureus. The plectasin-loaded NPs displayed improved efficacy as compared to non-encapsulated plectasin, while the eukaryotic cell viability was	(Water et al., 2015)

Table 4 (*continued*)

Nanoparticle	Antimicrobial peptide	Key finding	Reference
		unaffected at the assayed concentrations.	
Poly(lactide-co-glycolide) (PLGA) NPs	Esc(1–21)	Esc(1–21)-loaded NPs (0.1 mg/kg) in a mouse model of acute P. aeruginosa lung infection significantly reduce the bacterial load by 17-fold compared to free esculentin-1a in solution	(Casciaro et al., 2019)
Dextran NPs	SET-M33	SET-M33 loaded on dextran NPs was effective against P. aeruginosa in time-kill kinetic experiments. Lung residence time of SET-M33, administered via aerosol in healthy rats, was markedly improved when loaded on dextran NPs. SET-M33 loaded NPs was also efficient in eradicating pulmonary infection in a BALB/c mouse model of pneumonia caused by P. aeruginosa.	(Falciani et al., 2020)
Anionic mesoporous silica NPs	LL-37	Anionic mesoporous NPs protect LL-37 from degradation by infection-related proteases.	(Braun et al., 2016)
Gold NPs	Esc(1–21)	Covalent conjugation of Esc(1–21) to AuNPs via a poly (ethylene glycol) linker increased by 15-fold the activity of the free peptide against the motile and sessile forms of P. aeruginosa without being toxic to human keratinocytes.	(Casciaro et al., 2017)
Iron oxide magnetic NPs	CSA-13	CSA-13 retains bactericidal activity against P. aeruginosa when immobilized on magnetic nanoparticles while biocompatibility increases when CSA-13 is covalently attached to the nanoparticle.	(Niemirowicz et al., 2015)
Albumin NPs	LL37	In a murine model of acute P. aeruginosa lung infection, LL37 encapsulated in albumin NPs significantly reduced TNF-α and IL-1β expression and alleviated lung damage. The accelerated clearance of P. aeruginosa indicates that LL37	(Yang et al., 2021)

Table 4 (*continued*)

Nanoparticle	Antimicrobial peptide	Key finding	Reference
Liposomes	Polymixin B	encapsulated in the nanoparticles could improve P. aeruginosa lung infection and the subsequent inflammation response more efficiently compared with free LL37. Polymixin B was encapsulated in liposomes comprising 1,2-dipal-mitoyl-sn-glycero-3-phosphocholine and cholesterol. The treatment of liposomal polymixin B showed a 2-fold lower bacterial burden in lung and longer survival time than free Polymixin B in a pneumonia model of infected mice with a clinical multidrug-resistant P. aeruginosa strain.	(He et al., 2013)
Silver NPs	Andersonin-Y1	In vitro antimicrobial effect of the peptide conjugate to NPs against the multidrug resistant strains of Klebsiella pneumoniae, P. aeruginosa, and Enterobacter species was higher than the sum of the activities of the peptide and the NPs taken separately	(Pal et al., 2019)
Lipid-core micelles / Dextran NPs	AA139	The bacterial killing activity of the AA139-nanomedicines in infected lungs was assessed in a rat model of pneumonia-septicemia caused by extended-spectrum β-lactamase-producing Klebsiella pneumoniae. Both AA139-nanomedicines showed equivalent in vitro antimicrobial activities (similar to free AA139). In uninfected rats, they exhibited longer residence times in the lungs than free AA139, as well as reduced toxicity, enabling a higher limiting dose. In rats with pneumonia-septicemia, both AA139-nanomedicines showed significantly improved therapeutic efficacy in terms of	(van der Weide et al. (2020)

Table 4 (*continued*)

Nanoparticle	Antimicrobial peptide	Key finding	Reference
β-cyclodextrin	ABP-CM4	an extended rat survival time In vitro antimicrobial activity results for the complex were similar to those for CM4. In vivo studies against P. aeruginosa performed in mice showed that the mice treated with the nano-complex had more viability (60 %) than those treated with CM4 (20 %) 12 h prior to P. aeruginosa infection, and after infection, both complex and CM4 alone protected from lung injury, with complex showing higher protection effi ciency by abdominal treatment.	(Li et al., 2017, 2020)

used for inhaling peptides because dry powder formulations require more complex manufacturing equipment, and use of excipients. However, commercial nebulizers including air-jet, ultrasonic and vibrating mesh nebulizers can exert different stresses on AMPs. Air-jet nebulization can apply high mechanical shear on compounds leading to denaturation of peptides (Agu et al., 2001) hence it can affect the antimicrobial activity of drugs as seen for example with polymixin B (Desai et al., 2003). Similarly, the heat produced by ultrasonic nebulizers may alter polypeptide structures affecting their bioactivity (Khatri et al., 2001). Another option is offered by vibrating mesh nebulizer which generates aerosols through a perforated plate with micrometric apertures that vibrates at a high frequency (e.g., 100–300 kHz) to extrude the liquid through the apertures as inhalable droplets. More recently, a novel surface acoustic wave nebulizer was developed that operates at a low input power (e.g., 1–5 W) to generate aerosols by passing acoustic wave along the liquid surface, which minimizes the risk of drug denaturation caused by hydrodynamic shear and cavitation (Wang et al., 2016). An example of this approach is given by Lange et al. (2001) where the α-helical cationic peptide CM3 was trapped in an optimal mixture of lipids (dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol, 3:1 molar ratio) in order to maximize its encapsulation and nebulization efficiencies. In this way using a valved jet nebulizer, the peptide was able to reach the most part of the tracheobronchial region. An efficient approach for overcoming biochemical and mechanical barriers of lung airways is represented by the use of nanocarriers that are able to transport drugs through mucus and biofilm (Günday et al., 2014). Moreover, due to higher drug amounts reaching the site of action and a sustained release provided by the nanoparticles (NPs), both the overall dose and the treatment time might be reduced. Drugs, including peptides, can be encapsulated within the NPs or can be attached onto its surface. Optimization strategies can be used to improve nanoparticles penetration, stability, and consequently the delivery and efficacy of inhaled peptides. NPs can be optimized in size and surface charge to improve the penetration in the mucus. NPs should be small enough (less than 300 nm) to diffuse through the network made by mucin fibers, DNA, and other macromolecules in CF mucus, since bigger particles would be trapped by physical exclusion.

Moreover, the interaction between NPs and the mucus can be

decreased by making the surface charges of NPs neutral or negative, given that mucins and other components of the thick layer, such as actin and free DNA, are negatively charged. Hence, while positively charged NPs would tend to interact electrostatically with these components of the mucus, neutral or negatively charged NPs would be repelled. Another possibility to reduce both electrostatic and hydrophobic interactions is to coat the surface of NPs with electrostatically neutral and muco-inert polymers. Among the most used is the low molecular weight polyethylene glycol (PEG).

The most widely employed approach for the administration of NPs in the lung relies on the nebulization of NP aqueous dispersions (Charrier et al., 2014). However, owing to the limitations of nebulized NPs like high tendency to agglomerate or aggregate which restricts their stability, processability and dispersibility, an alternative form of dry powder microparticles formulation was more recently proposed for the delivery of NPs to the lungs microparticles (Scherließ et al., 2022). This can be achieved by preparation of nano-embedded microparticles (NEMs) also known as Trojan particles, which facilitate the deposition of NPs into the lungs, following their release from microparticles (Scherließ et al., 2022). NEM powders are usually generated by spray drying a suspension of NPs with cryoprotectant excipients like mannitol or lactose, which are biodegradable and biocompatible polymers (Quarta et al., 2021). Examples of this approach applied to CF are reported in the review paper of d'Angelo et al. (2014).

Overall, different kinds of NPs functionalized with AMPs have been tested in the last years and the most representative studies are reported in Table 4 and described below.

Colistin is a cyclopeptide used in antibiotic therapies as a last resort due to its high toxicity and deleterious side effects (Gai et al., 2019). Thus, many studies have been carried out to produce efficient and safer colistin loaded into NPs (Wallace et al., 2012). An efficient delivery system for colistin was for example developed by Pastor et al. where lipid nanoparticles have been loaded with sodium colistimethate with a high efficiency (79–94 % of loading) and led to a sustained drug release profile. The two typologies of nanosystems used, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), both presented antimicrobial activity against CF clinically isolates of P. aeruginosa (Pastor et al., 2014). Furthermore, in vitro cell viability assays with A549 and H441 cell lines proved that lipid NPs are less cytotoxic than free sodium colistimethate. Finally, in vivo distribution experiments revealed that NPs were dispersed uniformly throughout the lung and that lipid NPs did not migrate to other organs. The obtained lipid NPs resulted also stable after nebulization.

Similarly, colistin loaded in SLN/NLC formulations was also used to eradicate bacterial biofilm produced by P. aeruginosa isolates from CF patients. In this study the free colistin and the nano-formulations showed a similar antimicrobial activity versus planktonic forms resulting the NLC formulation more efficient in terms of killing (Sans-Serramitjana et al., 2016).

Thus, this formulation was used to evaluate the minimal biofilm eradication concentration (MBEC) and biofilm prevention concentration (BPC) against different P. aeruginosa isolates comprising the colistin-susceptible 056SJD isolate and colistin-resistant P19 isolate. As a result, the NLC colistin complex was more active in eradicating preformed respect to free-colistin, while no difference was observed on the prevention of biofilm formation (Sans-Serramitjana et al., 2016).

Other kinds of nanomaterials have been loaded with colistin and tested in vitro and in vivo in mice; these formulations include gold and silver NPs (Pastor et al., 2014; Miller et al., 2016), human albumin NPs (Scutera et al., 2021), polydopamine nano-spheres (Ran et al., 2020) and poly(lactide-co-glycolide) nano-embedded microparticles (d'Angelo et al., 2015). More recently, biomimetic, and inhalable calcium phosphates (CaP) NPs were functionalized with colistin (Iafisco et al., 2022). The maximum colistin payload was of about 50 mg/g of CaP NPs. After functionalization, CaP NPs maintained a dimension and surface charge considered suitable for crossing mucus barrier. CaP NPs do not interact with mucin and are able to permeate a layer of artificial mucus. In vitro tests on pulmonary cells demonstrated that the NPs are not cytotoxic up to a concentration of 125 µg/ml. The antimicrobial and antibiofilm activity of colistin-loaded NPs tested on P. aeruginosa RP73, a clinical strain isolated from a CF patient, was similar to that of free colistin demonstrating that the therapeutic effect of colistin adsorbed on NPs was retained.

Poly(lactide-co-glycolide) (PLGA) NPs have been employed for the loading of the antimicrobial peptide plectasin previously isolated from Pseudoplectania nigrella. In this study a Calu-3 epithelial cells system infected with Staphylococcus aureus was used. As a result, the plectasin-loaded NPs displayed improved efficacy (EC_{50} 0.80 ± 0.12 µM) as compared to non-encapsulated peptide (EC_{50} 1.24 ± 0.15 µM) and free plectasin and non-loaded PLGA NPs did not affected Calu-3 cells viability at the highest assayed concentration (4 µM). The study further showed a different modality of internalization between Calu-3 cells (bronchial cells) and A549 cells (alveolar type epithelial cells), the formers were more efficient in the uptake probably due to non-specific interactions between NPs loaded plectasin and the Calu-3 membrane or its components (Water et al., 2015).

In the study of Casciaro et al. (2019) derivatives of esculentin, an extensively studied amphibian skin membrane-active peptide (Luca et al., 2013), have been used to functionalize PLGA NPs, stabilized with poly(vinyl alcohol) (PVA) to avoid aggregation and to promote the passage through biological barriers, in particular the lung mucus. NPs were loaded with Esc(1−21) or its diastereomer Esc(1−21)− 1c. The obtained formulation was tested in vitro, and in vivo in a murine lung infection model. The in vitro tests demonstrated that, although having lesser antibacterial activity than free soluble Esc peptides within 24 h, Esc peptide-loaded NPs were able to maintain bacterial.

growth inhibition (60 % inhibition) for up to 72 h. The in vivo data showed a remarkable antimicrobial effect without negative side effects on inflammation pathways, demonstrating that this formulation was highly effective also displaying very promising aerosolization properties and finally resulting in a reliable delivery system for AMPs to lungs.

Dextran based NPs have been recently employed in a very detailed study, to deliver the artificial peptide SET-M33 (Pini et al., 2012) M33-nano system (M33-NS) was shown to be effective against P. aeruginosa in a time-kill kinetic experiment and to be slightly cytotoxic both in vitro and in vivo. Pharmacokinetics studies revealed that M33-NS were more persistent in the lungs respect to the free peptide representing a strong advantage when developing therapeutics to be inhaled by aerosol (Falciani et al., 2020). Nano-delivery mediated by inhalation was also employed with synthetic AMPS from the group of Forde et al. (2016). The peptides AAG-D-WMR[3,6-leu] (AAGwglrrllkygkrs) and its acetylated pro-form pro-D-WMR[3,6-leu] (Ac-EEEEAAAGwglrrllkygkrs), designed to be specifically cut by NE were studied in a model of a mechanically ventilated patient employing a vibrating mesh nebulization (VMNs) a method which may allow to reduce the volume of the material inhaled. It was observed that this method of administration did not alter peptides properties before and after nebulization being no differences in MICs on P. aeruginosa strain PAO1 and of three CF clinical isolates (Forde et al., 2019).

Research on extracellular vesicles is increasingly broadening the horizons on their functions at the cellular level. It is now well established that these structures are critically important for cellular communication and many other cellular regulatory events. In addition to this, given the structural and functional characteristics of EVs, researchers have seen new uses for EVs as natural drug transporters. Recent studies have shown that AMPs are also stored and transported within extracellular vesicles and that they could efficiently be employed against bacterial infection and to counteract biofilm formation (Leiva-Sabadini et al., 2021). These studies thus point to properly characterized and manipulated EVs as potential nano-vehicles of precision medicine applied to infection diseases (Keshavarz Alikhani, 2021; Kim et al., 2021).

Table 5
ABPs described in this work and their sequences.

Peptide	Sequence	Ref
LL7–37	RKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	(Nagant et al., 2012)
LL-31	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNL	"
LL7–31	RKSKEKIGKEFKRIVQRIKDFLRNL	"
Gaduscidin-1 (Gad-1)	FIHHIIGWISHGVRAIHRAIH	(McDonald et al., 2015)
IDR-1018	VRLIVAVRIWRR-NH$_2$	(de la Fuente-Núñez et al., 2014)
H10	VRLIVRIWRR	"
HE12	RFKRVARVIW	"
α4	ILKPGGGTSGGLLGGLLGKVTSVIPGLNNI	(Yu et al., 2018)
α4M1	ILKKWWGTSGGLLGGLLGKVTSVIKGLNNI	"

5. Antibiofilm peptides (ABPs) applied to CF related infections

Bacterial biofilm is a complex polymeric matrix composed of different macromolecules known as extracellular polymeric substances (EPS) comprehending polysaccharides (alginate), proteins (enzymes, fibrin), lipids, and DNA, all contributing to create a biophysical barrier which renders bacteria more resistant to antibiotics treatment. Biofilms may develop on different inorganic and organic surfaces and so can colonize different human body compartments and tissues. Bacteria population living in a biofilm structure may be made of different species which communicate each other with chemical signals (quorum sensing) (Preda et al., 2019) and thus rendering the biofilm a multi organism environment. Biofilm preserves bacteria from human immune system surveillance thus allowing bacteria to grow slowly and adapt to environmental anoxia and nutrient limitation. In this setting, bacteria modify their gene and protein patterns and consequently their metabolism, leading to a lower metabolic rate and a reduced rate of cell division (Welp et al., 2020). Among the bacterial species, P. aeruginosa represents a major cause of morbidity and mortality in CF patients and this aspect is linked to the high propensity of the pathogen to form biofilm (Emerson et al., 2002). In CF, beside the sticky mucus produced by bronchial cells, biofilm represents a further physical barrier which can hamper the efficiency of conventional antibiotics (Luo et al., 2021) thus rendering more difficult the CF treatment. Among AMPs, several anti-biofilm peptides (ABPs) e.g. peptides specifically or mainly active against biofilms, are described in literature. Various webtools for the prediction of antibiofilm efficacy of peptides are also available (Sharma et al., 2016; Fallah Atanaki et al., 2020; Di Luca et al., 2015). Biofilm formation is a complex process that generally involves three stages: (i) primary adhesion to surfaces, (ii) accumulation of multilayered clusters of cells, and (iii) detachment. Therefore, some AMPs may act as inhibitor or disruptor or both of biofilm structure; consequently, many AMPs which are effective against planktonic forms are not equivalently efficient against the biofilm environment. In general, ABPs are structurally equivalent to AMPs sharing a cationic and an amphipathic character, but their specificity makes them more effective against biofilms at lower concentrations with respect to MICs of AMPs against planktonic bacterial forms (de la Fuente-Núñez et al., 2012). Different studies concerning the use of cathelicidin LL-37 have showed that fragments of this peptide, beside the antibacterial activity performed on planktonic bacterial forms, also possess strong antibiofilm activity. The reasons at the basis of exploring the activity of shorter forms of LL-37 to contrast proteases activity (Moncla et al., 2011) is a certain toxicity towards mammal cells (Johansson et al., 1998) and finally in reducing the synthesis costs. In the study of Nagant et al., 2012), 19-mer fragments of LL-37 were produced and their antibiofilm activity compared to native peptide. The strongest antibiofilm activity was observed with the peptides LL7–37, LL-31, and LL7–31 (Table 5), which decreased the percentage of biomass formation at a very low micromolar concentration. The peptide LL7–37 decreased the height of the biofilm and partly cause its disruption after

formation. LL7–37 and LL7–31 also resulted much less toxic than parent LL-37 and LL-31 confirming that the absence of the first 6 residues contributes to render these LL-37 deriving peptides more appealing for a therapeutic use. The metallo-histidine rich-AMP Gaduscidin-1 (Table 5) is a broad-spectrum AMP that is produced by Atlantic cod fish and binds two Cu^{2+} ions with high affinity at neutral and acidic pH conditions (McDonald et al., 2015). Other interesting features of this peptide are the ability to maintain its activity even at high salt concentrations as well as to act as a nuclease thanks to the capacity to produce ROS upon Cu^{2+} binding (Portelinha et al., 2021). These features are of particular interest in a potential application as AMP in CF patients. The amide peptide 1018 also called IDR-1018, presents a specific antibiofilm activity and it is one of the best characterized AMP (Rivas-Santiago et al., 2013). IDR-1018 is not only able to prevent biofilm formation, but also to eradicate mature biofilms produced by different P. aeruginosa and Burkholderia cenocepacia clinical isolates. Although its mode of action is not yet fully understood (Andresen et al., 2016), it has been the object of several studies to enhance its antimicrobial (anti-planktonic and anti-biofilm) activity. In particular, in the study by De La Fuente Nunez (2014), peptide 1018 was specifically tested against different CF clinical isolates of P. aeruginosa and B. cenocepacia, where it showed only just a minor effect on planktonic forms of P. aeruginosa and any effect on B. cenocepacia complex strains (de la Fuente-Núñez et al., 2014). Conversely, pre-formed biofilm from both isolated strains of P. aeruginosa and B. cenocepacia resulted to be susceptible to sub-lethal doses of 1018 (10 μg/ml) evaluated using either a flow cell model or hydroxyapatite surfaces upon treatment. In the same study different derivatives of 1018 were prepared mutating different positions of peptide residues. Some of these derivatives showed similar or reduced anti-biofilm activity in respect to the original peptide 1018. For example, derivative H10 lacking the hydrophobic pattern present in the parent peptide (Table 5), showed a reduced anti-biofilm activity against P. aeruginosa but interestingly, presented an enhanced activity vs. the Gram-positive methicillin resistant S. aureus (MRSA). 1018 is also known to modulate immune response through cytokines pathway production (Rivas-Santiago et al., 2013), but no modifications in these properties were observed in the mutated peptides, as they equally enhanced the production of chemokine MCP-1. The authors point to the importance of the residue sequence instead of the composition as main factor affecting peptide activity, as a proof of this hypothesis, the derivative peptide HE12 (Table 5), with same length and same content of charged and hydrophobic amino acids, but with a scrambled sequence, lacked anti-biofilm activity against Gram-negative and Gram-positive bacteria. Gram-positive bacteria biofilm was the object of another study in which MRSA strains from CF clinical isolates, were treated with derivatives of the Human SPLUNC1 (Short Palate Lung and Nasal epithelial Clone 1) protein. This is a 256 amino acid residues long multifunctional protein, and an important component of innate immunity endowed with a certain antimicrobial activity. In particular, the α4 domain displays a helical structure, and a cationic and an amphipathic feature, all typical features characterizing a "classic" AMP. The deletion of this motif from the WT structure (SPLUNC1 or the Δα4) confirmed the antimicrobial character of this portion. In fact, a strong reduction in antibiofilm activity was in fact observed when the α4 region deleted protein was used in comparison with the WT protein (Yu et al., 2018). The α4 portion was then synthesized as a peptide (Table 5), showing a lower activity in contrasting biofilm formation with respect to entire protein. To overcome this aspect, the authors designed a derivative called α4M1 (Table 5), with the aim to enhance the amphipathicity of the original peptide by replacing two Gly with two Trp, and the positive charge substituting two Pro residues on the hydrophilic side with two Lys. As a result, the derived peptide α4M1 retained antibiofilm activity of the parent protein even with diverse clinical strains of MRSA. In P. aeruginosa the lectins LecB and LecA are involved in the formation of antibiotic resistant biofilms, thus they represent a target for an efficient strategy to counteract biofilm formation. In the work of Reymond et al.
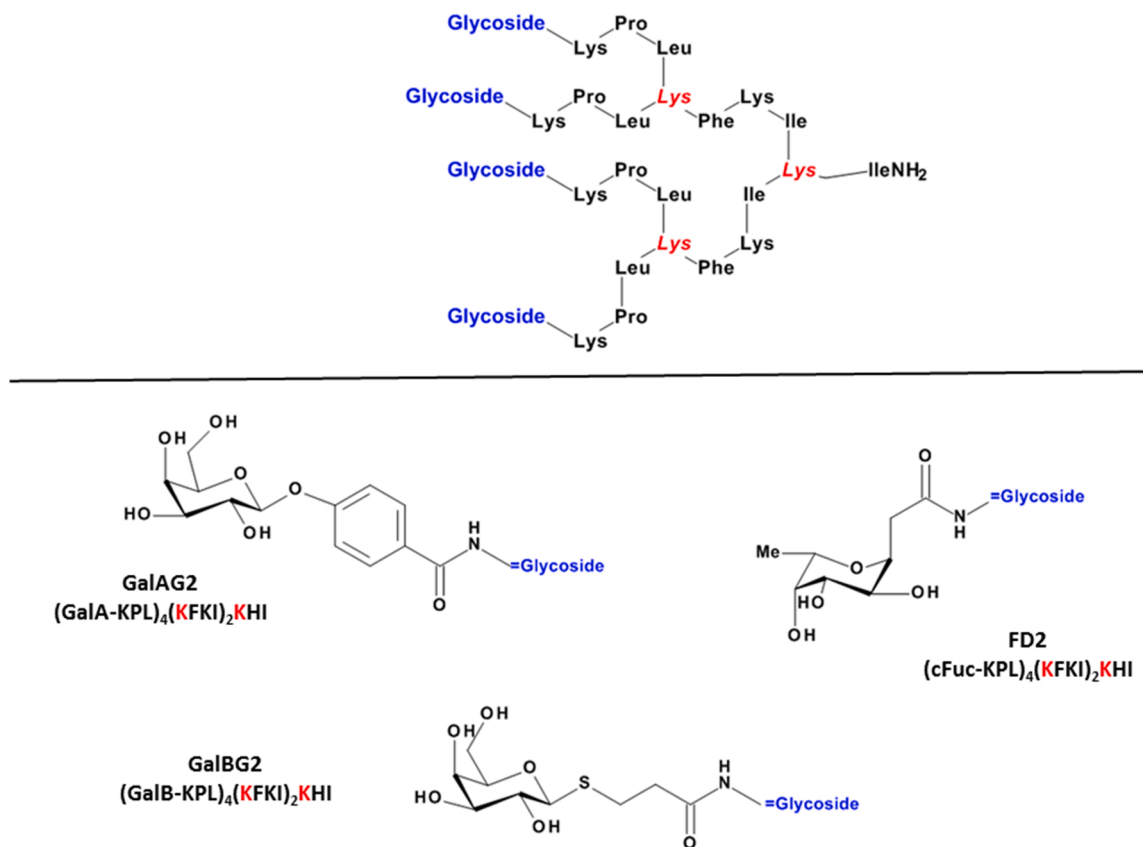
Fig. 6. Antibiofilm dendrimeric peptides GalAG2, GalBG2 and FD2.

synthetic glycopeptide dendrimers were selected through a synthetic combinatorial approach functionalizing diverse N-termini with sugars, as multivalent ligands to the P. aeruginosa lectins LecA and LecB. A tetravalent fucosylated peptide dendrimer FD2 binding to the fucose-specific lectin LecB, and galactosylated dendrimers GalAG2 and GalBG2 (Fig. 6), binding the galactose specific lectin LecA were hence produced. When tested in vitro against P. aeruginosa biofilm, they showed a strong activity in blocking biofilm formation and inducing biofilm dispersal (Reymond et al., 2013).

6. Conclusions

AMPs are gaining an important place in the landscape of future antibiotics, and this is due to several features that make them promising tools to fight and counteract antibiotic resistance. Many drawbacks that made peptides a forgotten resource for years have been solved, thus offering new possibilities for these molecules. CF, a complex pathology that compromises primarily the respiratory system, but also other compartments of the body, determines at the level of the lungs the creation of a harsh environment characterized by high salt conditions, low pH, high concentration of proteolytic enzymes, presence of mucus, all conditions particularly favorable for the growth of pathogenic microorganisms especially in the form of biofilm structures. This is the battleground in which AMPs must challenge microorganisms. In order to preserve or even enhance the efficiency of AMPs in CF related infections to overcome these difficulties, the successful road to be followed is probably the design, also on the basis of natural sequences, of synthetic and semi-synthetic peptidic derivatives and the development of dedicated systems for their delivery, as exemplified by the many examples reported in this article. In this view, is noteworthy the possibility in the next future to also exploit the potential of extracellular vesicles to transport and safely deliver AMPs.

Disclosure statement

The authors have disclosed no conflicts of interest.

Data Availability

No data was used for the research described in the article.

References

Abou Alaiwa, M.H., Reznikov, L.R., Gansemer, N.D., Sheets, K.A., Horswill, A.R., Stoltz, D.A., Zabner, J., Welsh, M.J., 2014. pH modulates the activity and synergism of the airway surface liquid antimicrobials β -defensin-3 and LL-37. Proc. Natl. Acad. Sci. USA 111, 18703–18708.

Agu, R.U., Ugwoke, M.I., Armand, M., Kinget, R., Verbeke, N., 2001. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir. Res. 2, 198–209.

Amerikova, M., Pencheva El-Tibi, I., Maslarska, V., Bozhanov, S., Tachkov, K., 2019. Antimicrobial activity, mechanism of action, and methods for stabilisation of defensins as new therapeutic agents. Biotechnol. Biotec Equip. 33, 671–682.

Andresen, L., Tenson, T., Hauryliuk, V., 2016. Cationic bactericidal peptide 1018 does not specifically target the stringent response alarmone (p)ppGpp. Sci. Rep. 6, 36549.

Armstrong, D.S., Grimwood, K., Carzino, R., Carlin, J.B., Olinsky, A., Phelan, P.D., 1995. Lower respiratory infection and inflammation in infants with newly diagnosed cystic fibrosis. B. M. J. 310, 1571–1577.

Bals, R., 2000. Epithelial antimicrobial peptides in host defense against infection. Respir. Res. 1, 141–150.

Bals, R., Weiner, D.J., Wilson, J.M., 1999. The innate immune system in cystic fibrosis lung disease. J. Clin. Invest 103, 303–307.

Batoni, G., Maisetta, G., Brancatisano, F.L., Esin, S., Campa, M., 2011. Use of antimicrobial peptides against microbial biofilms: advantages and limits. Curr. Med. Chem. 18 (2), 256–279.

Batoni, G., Casu, M., Giuliani, A., Luca, V., Maisetta, G., Mangoni, M.L., Manzo, G., Pintus, M., Pirri, G., Rinaldi, A.C., Scorciapino, M.A., Serra, I., Ulrich, A.S., Wadhwani, P., 2016. Rational modification of a dendrimeric peptide with antimicrobial activity: consequences on membrane-binding and biological properties. Amino Acids 48 (3), 887–900.

Benincasa, M., Skerlavaj, B., Gennaro, R., Pellegrini, A., Zanetti, M., 2003. In vitro and in vivo antimicrobial activity of two alpha-helical cathelicidin peptides and of their synthetic analogs. Peptides 24, 1723–1731.

Benincasa, M., Mattiuzzo, M., Herasimenka, Y., Cescutti, P., Rizzo, R., Gennaro, R., 2009. Activity of antimicrobial peptides in the presence of polysaccharides produced by pulmonary pathogens. J. Pept. Sci. 15 (9), 595–600.

Bensman, T.J., Jayne, J.G., Sun, M., Kimura, E., Meinert, J., Wang, J.C., Schaal, J.B., Tran, D., Rao, A.P., Akbari, O., Selsted, M.E., Beringer, P.M., 2017. Efficacy of Rhesus Theta-defensin-1 In Experimental Models Of Pseudomonas Aeruginosa Lung Infection And Inflammation. Antimicrob. Agents Chemother. 61, e00154–17.

Bergsson, G., Reeves, E.P., McNally, P., Chotirmall, S.H., Greene, C.M., Greally, P., Murphy, P., O'Neill, S.J., McElvaney, N.G., 2009. LL-37 complexation with glycosaminoglycans in cystic fibrosis lungs inhibits antimicrobial activity, which can be restored by hypertonic saline. J. Immunol. 183, 543–551.

Beringer, P.M., Bensman, T.J., Ho, H., Agnello, M., Denovel, N., Nguyen, A., Wong-Beringer, A., She, R., Tran, D.Q., Moskowitz, S.M., Selsted, M.E., 2016. Rhesus θ-defensin-1 (RTD-1) exhibits in vitro and in vivo activity against cystic fibrosis strains of Pseudomonas aeruginosa. J. Antimicrob. Chemother. 71, 181–188.

Bhadra, P., Yan, J., Li, J., Fong, S., Siu, S.W.I., 2018. AmPEP: Sequence-based prediction of antimicrobial peptides using distribution patterns of amino acid properties and random forest. Sci. Rep. 8, 1697.

Bragonzi, A., 2010. Fighting back: peptidomimetics as a new weapon in the battle against antibiotic resistance. Sci. Transl. Med. 2 (21), 21ps9.

Braun, K., Pochert, A., Lindén, M., Davoudi, M., Schmidtchen, A., Nordström, R., Malmsten, M., 2016. Membrane interactions of mesoporous silica nanoparticles as carriers of antimicrobial peptides. J. Colloid Interface Sci. 475, 161–170.

Cappiello, F., Grazia, Di, Segev-Zarko, A., Scali, L.A., Ferrera, S., Galietta, L., Pini, L., Shai, A., Di, Y., Mangoni, M.L. Y.P., 2016. Esculentin-1a-derived peptides promote clearance of pseudomonas aeruginosa internalized in bronchial cells of cystic fibrosis patients and lung cell migration: biochemical properties and a plausible mode of action. Antimicrob. Agents Chemother. 60, 7252–7262.

Casciaro, B., Moros, M., Rivera-Fernández, S., Bellelli, A., de la Fuente, J.M., Mangoni, M.L., 2017. Gold-nanoparticles coated with the antimicrobial peptide esculentin-1a(1-21)NH$_2$ as a reliable strategy for antipseudomonal drugs. Acta Biomater. 47, 170–181.

Casciaro, B., d'Angelo, I., Zhang, X., Loffredo, M.R., Conte, G., Cappiello, F., Quaglia, F., Di, Y.P., Ungaro, F., Mangoni, M.L., 2019. Poly(lactide- co-glycolide) nanoparticles for prolonged therapeutic efficacy of esculentin-1a-derived antimicrobial peptides against pseudomonas aeruginosa lung infection: in vitro and in vivo studies. Biomacromolecules 20 (5), 1876–1888.

Castiglia, F., Zevolini, F., Riolo, G., Brunetti, J., De Lazzari, A., Moretto, A., Manetto, G., Fragai, M., Algotsson, J., Evenäs, J., Bracci, L., Pini, A., Falciani, C., 2019. NMR study of the secondary structure and biopharmaceutical formulation of an active branched antimicrobial peptide. Molecules 24, 4290.

Charrier, C., Rodger, C., Robertson, J., Kowalczuk, A., Shand, N., Fraser-Pitt, D., Mercer, D., O'Neil, D., 2014. Cysteamine (Lynovex®), a novel mucoactive antimicrobial & antibiofilm agent for the treatment of cystic fibrosis. Orphanet J. Rare Dis. 9, 189.

Chen, C., Mangoni, M.L., Di, Y.P., 2017. In vivo therapeutic efficacy of frog skin-derived peptides against Pseudomonas aeruginosa-induced pulmonary infection. Sci. Rep. 7 (1), 8548.

Chen, C., Deslouches, B., Montelaro, R.C., Di, Y.P., 2018. Enhanced efficacy of the engineered antimicrobial peptide WLBU2 via direct airway delivery in a murine model of Pseudomonas aeruginosa pneumonia. Clin. Microbiol. Infect. 24, 547 e1-547.e8.

Ciociola, T., Giovati, L., Conti, S., Magliani, W., Santinoli, C., Polonelli, L., 2016. Natural and synthetic peptides with antifungal activity. Future Med. Chem. 8 (12), 1413–1433.

Conibear, A.C., Wang, C.K., Bi, T., Rosengren, K.J., Camarero, J.A., Craik, D.J., 2014. Insights into the molecular flexibility of q-defensins by NMR relaxation analysis. J. Phys. Chem. B 118, 14257–14266.

d'Angelo, I., Conte, C., La Rotonda, M.I., Miro, A., Quaglia, F., Ungaro, F., 2014. Improving the efficacy of inhaled drugs in cystic fibrosis: challenges and emerging drug delivery strategies. Adv. Drug Deliv. Rev. 75, 92–111.

d'Angelo, I., Casciaro, B., Miro, A., Quaglia, F., Mangoni, M.L., Ungaro, F., 2015. Overcoming barriers in Pseudomonas aeruginosa lung infections: Engineered nanoparticles for local delivery of a cationic antimicrobial peptide. Colloids Surf. B Biointerfaces 135, 717–725.

Daniels, L.M., Juliano, J., Marx, A., Weber, D.J., 2017. Inhaled antibiotics for hospital-acquired and ventilator-associated pneumonia. Clin. Infect. Dis. 64, 386–387.

Desai, T., Tyrrell, G., Ng, T., Finlay, W., 2003. In vitro evaluation of nebulization properties, antimicrobial activity, and regional airway surface liquid concentration of liposomal polymyxin B sulfate. Pharm. Res 20, 442–447.

Di Grazia, A., Cappiello, F., Cohen, H., Casciaro, B., Luca, V., Pini, A., Di, Y.P., Shai, Y., Mangoni, M.L., 2015. D-Amino acids incorporation in the frog skin-derived peptide esculentin-1a(1-21)NH$_2$ is beneficial for its multiple functions. Amino Acids 47, 2505–2519.

Di Luca, M.-, Maccari, G., Maisetta, G., Batoni, G., 2015. BaAMPs: the database of biofilm-active antimicrobial peptides. Biofouling 31, 193–199.

Elborn, J.S., 2016. Cystic fibrosis. Lancet 388, 2519–2531.

Emerson, J., Rosenfeld, M., McNamara, S., Ramsey, B., Gibson, R.L., 2002. Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr. Pulmonol. 34 (2), 91–100.

Fabisiak, A., Murawska, N., Fichna, J., 2016. LL-37: Cathelicidin-related antimicrobial peptide with pleiotropic activity. Pharmacol. Rep. 68 (4), 802–808.

Falciani, C., Zevolini, F., Brunetti, J., Riolo, G., Gracia, R., Marradi, M., Loinaz, I., Ziemann, C., Cossío, U., Llop, J., Bracci, L., Pini, A., 2020. Antimicrobial peptide-loaded nanoparticles as inhalation therapy for pseudomonas aeruginosa infections. Int. J. Nanomed. 15, 1117–1128.

Fallah Atanaki, F., Behrouzi, S., Ariaeenejad, S., Boroomand, A., Kavousi, K., 2020. BIPEP: sequence-based prediction of biofilm inhibitory peptides using a combination of NMR and physicochemical descriptors. ACS Omega 5, 7290–7297.

Ferrazzano, L., Catani, M., Cavazzini, A., Martelli, G., Corbisiero, D., Cantelmi, P., Fantoni, T., Mattellone, A., De Luca, C., Felletti, S., Cabri, W., Tolomelli, A., 2022. Sustainability in peptide chemistry: current synthesis and purification technologies and future challenges. Green. Chem. 24, 975–1020.

Forde, É., Humphreys, H., Greene, C.M., Fitzgerald-Hughes, D., Devocelle, M., 2014. Potential of host defense peptide prodrugs as neutrophil elastase-dependent anti-infective agents for cystic fibrosis. Antimicrob. Agents Chemother. 58, 978–985.

Forde, É., Schütte, A., Reeves, E., Greene, C., Humphreys, H., Mall, M., Fitzgerald-Hughes, D., Devocelle, M., 2016. Differential In vitro and in vivo toxicities of antimicrobial peptide prodrugs for potential use in cystic fibrosis. Antimicrob. Agents Chemother. 60 (5), 2813–2821.

Forde, É., Kelly, G., Sweeney, L., Fitzgerald-Hughes, D., MacLoughlin, R., Devocelle, M., 2019. Vibrating Mesh Nebulisation Of Pro-antimicrobial Peptides For Use In Cystic Fibrosis. Pharmaceutics 11 (5), 239.

Fröhlich, E., Salar-Behzadi, S., 2021. Oral inhalation for delivery of proteins and peptides to the lungs. Eur. J. Pharm. Biopharm. 163, 198–211.

de la Fuente-Núñez, C., Korolik, V., Bains, M., Nguyen, U., Breidenstein, E.B., Horsman, S., Lewenza, S., Burrows, L., Hancock, R.E., 2012. Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. Antimicrob. Agents Chemother. 56, 2696–2704.

de la Fuente-Núñez, C., Mansour, S.C., Wang, Z., Jiang, L., Breidenstein, E.B., Elliott, M., Reffuveille, F., Speert, D.P., Reckseidler-Zenteno, S.L., Shen, Y., Haapasalo, M., Hancock, R.E., 2014. Anti-biofilm and immunomodulatory activities of peptides that inhibit biofilms formed by pathogens isolated from cystic fibrosis patients. Antibiotics 3 (4), 509–526.

Gai, Z., Samodelov, S.L., Kullak-Ublick, G.A., Visentin, M., 2019. Molecular mechanisms of colistin-induced nephrotoxicity. Molecules 24 (3), 653.

Gan, B.H., Siriwardena, T.N., Javor, S., Darbre, T., Reymond, J.L., 2019. Fluorescence imaging of bacterial killing by antimicrobial peptide dendrimer G3KL. ACS Infect. Dis. 5, 2164–2173.

Ganguly, D., Chamilos, G., Lande, R., Gregorio, J., Meller, S., Facchinetti, V., Homey, B., Barrat, F.J., Zal, T., Gilliet, M., 2009. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. J. Exp. Med 206, 1983–1994.

Ganz, T., 1999. Defensins and host defense. Science 286, 420–421.

Geitani, R., Moubareck, C.A., Xu, Z., Karam Sarkis, D., Touqui, L., 2020. Expression and roles of antimicrobial peptides in innate defense of airway mucosa: potential implication in cystic fibrosis. Front. Immunol. 11, 1198.

Goldman, M.J., Anderson, G.M., Stolzenberg, E.D., Kari, U.P., Zasloff, M., Wilson, J.M., 1997. Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell 88 (4), 553–560.

Günday, N.T., Akif, E.T., Schneider, M., 2014. Inhalable antibiotic nanoformulations for the treatment of pseudomonas aeruginosa infection in cystic fibrosis a review. Drug Deliv. Lett. 4, 193–207.

Guo, L., Lim, K.B., Poduje, C.M., Daniel, M., Gunn, J.S., Hackett, M., Miller, S.I., 1998. Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides. Cell 16, 189–198.

Han, X., Liu, Y., Ma, Y., Zhang, M., He, Z., Siriwardena, T.N., Xu, H., Bai, Y., Zhang, X., Reymond, J.L., Qiao, M., 2019. Peptide dendrimers G3KL and TNS18 inhibit Pseudomonas aeruginosa biofilms. Appl. Microbiol. Biotechnol. 103 (14), 5821–5830.

Hazlett, L., Wu, M., 2011. Defensins in innate immunity. Cell Tissue Res. 343 (1), 175–188.

He, J., Abdelraouf, K., Ledesma, K.R., Chow, D.S., Tam, V.H., 2013. Pharmacokinetics and efficacy of liposomal polymyxin B in a murine pneumonia model. Int. J. Antimicrob. Agents 42 (6), 559–564.

Henninot, A., Collins, J.C., Nuss, J.M., 2018. The Current State of Peptide Drug Discovery: Back to the Future? J. Med. Chem. 61, 1382–1414.

Hiemstra, P., 2007. Antimicrobial peptides in the real world: implications for cystic fibrosis. Eur. Respir. J. 29, 617–618.

Hilpert, K., Volkmer-Engert, R., Walter, T., Hancock, R.E., 2005. High-throughput generation of small antibacterial peptides with improved activity. Nat. Biotechnol. 23 (8), 1008–1012.

Holfeld, L., Herth, N., Singer, D., Hoffmann, R., Knappe, D., 2015. Immunogenicity and pharmacokinetics of short, proline-rich antimicrobial peptides. Future Med. Chem. 7 (12), 1581–1596.

Iafisco, M., Carella, F., Degli Esposti, L., Adamiano, A., Catalucci, D., Modica, J., Bragonzi, A., Vitali, A., Torelli, R., Sanguinetti, M., Bugli, F., 2022. Biocompatible antimicrobial colistin loaded calcium phosphate nanoparticles for the counteraction

of biofilm formation in cystic fibrosis related infections. J. Inorg. Biochem 230, 111751.

Iavarone, F., Desiderio, C., Vitali, A., Messana, I., Martelli, C., Castagnola, M., Cabras, T., 2018. Cryptides: latent peptides everywhere. Crit. Rev. Biochem. Mol. Biol. 53 (3), 246–263.

Jahnsen, R.D., Haney, E.F., Franzyk, H., Hancock, R.E., 2013. Characterization of a proteolytically stable multifunctional host defense peptidomimetic. Chem. Biol. 20, 1286–1295.

Jirillo, E., Magrone, T., 2018. Editorial: antimicrobial peptides as mediators of innate immunity. Curr. Pharm. Des. 24, 1041–1042.

Johansson, J., Gudmundsson, G.H., Rottenberg, M.E., Berndt, K.D., Agerberth, B., 1998. Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. J. Biol. Chem. 273, 3718–3724.

Keshavarz Alikhani, H., Shokoohian, B., Rezasoltani, S., Hossein-Khannazer, N., Yadegar, A., Hassan, M., Vosough, M., 2021. Application of stem cell-derived extracellular vesicles as an innovative theranostics in microbial diseases. Front. Microbiol. 12, 785856.

Khatri, L., Taylor, K.M., Craig, D.Q., Palin, K., 2001. An assessment of jet and ultrasonic nebulizers for the delivery of lactate dehydrogenase solutions. Int. J. Pharm. 227 (1–2), 121–131.

Kim, G., Lee, Y., Ha, J., Han, S., Lee, M., 2021. Engineering exosomes for pulmonary delivery of peptides and drugs to inflammatory lung cells by inhalation. J. Control Release 330, 684–695.

Klodzinska, S.N., Molchanova, N., Franzyk, H., Hansen, P.R., Damborg, P., Nielsen, H.M., 2018. Biopolymer nanogels improve antibacterial activity and safety profile of a novel lysine-based a-Peptide/b-Peptoid peptidomimetic. Eur. J. Pharm. Biopharm. 128, 1–9.

Lachowicz, J.I., 2020. The best peptidomimetic strategies to undercover antibacterial peptides. Int. J. Mol. Sci. 19, 7349.

Lange, C.F., Hancock, R.E., Samuel, J., Finlay, W.H., 2001. In vitro aerosol delivery and regional airway surface liquid concentration of a liposomal cationic peptide. J. Pharm. Sci. 90, 1647–1657.

Lantz, I., Glämsta, E.L., Talbäck, L., Nyberg, F., 1991. Hemorphins derived from hemoglobin have an inhibitory action on angiotensin converting enzyme activity. FEBS Lett. 287, 39–41.

Lehrer, R.I., Cole, A.M., Selsted, M.E., 2012. θ-Defensins: cyclic peptides with endless potential. J. Biol. Chem. 287, 27014–27019.

Leiva-Sabadini, C., Alvarez, S., Barrera, N.P., Schuh, C.M.A.P., Aguayo, S., 2021. Antibacterial effect of honey-derived exosomes containing antimicrobial peptides against oral streptococci. Int. J. Nanomed. 16, 4891–4900.

Li, J.F., Zhang, J.X., Wang, Z.G., Yao, Y.J., Han, X, Zhao, Y.L., Liu, J.P., Zhang, S.Q., 2017. Identification of a cyclodextrin inclusion complex of antimicrobial peptide CM4 and its antimicrobial activity. Food Chem. 221, 296–301.

Li, J.F., Zhang, J.X., Li, G., Xu, Y.Y., Lu, K., Wang, Z.G., Liu, J.P., 2020. Antimicrobial activity and mechanism of peptide CM4 against Pseudomonas aeruginosa. Food Funct. 11, 7245–7254.

Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., et al., 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect. Dis. 16, 161–168.

Lohan, S., Bisht, G.S., 2013. Recent approaches in design of peptidomimetics for antimicrobial drug discovery research. Mini Rev. Med. Chem. 13 (7), 1073–1088.

Luca, V., Stringaro, A., Colone, M., Pini, A., Mangoni, M.L., 2013. Esculentin (1-21), an amphibian skin membrane-active peptide with potent activity on both planktonic and biofilm cells of the bacterial pathogen Pseudomonas aeruginosa. Cell Mol. Life Sci. 70 (15), 2773–2786.

Luft, F.C., 2017. Defensins defend against bone loss. J. Mol. Med. 95, 1255–1256.

Luo, Y., Yang, Q., Zhang, D., Yan, W., 2021. Mechanisms and control strategies of antibiotic resistance in pathological biofilms. J. Microbiol. Biotechnol. 31, 1–7.

Magliani, W., Conti, S., Cunha, R.L., Travassos, L.R., Polonelli, L., 2009. Antibodies as crypts of antiinfective and antitumor peptides. Curr. Med. Chem. 16, 2305–2323.

Maisetta, G., Grassi, L., Esin, S., Serra, I., Scorciapino, M.A., Rinaldi, A.C., Batoni, G., 2017. The Semi-synthetic Peptide Lin-SB056-1 in combination with EDTA exerts strong antimicrobial and antibiofilm activity against pseudomonas aeruginosa in conditions mimicking cystic fibrosis sputum. Int. J. Mol. Sci. 18 (9), 1994.

Mak, P., 2008. Hemocidins in a functional and structural context of human antimicrobial peptides. Front. Biosci. 13, 6859–6871.

Mangoni, M.L., Luca, V., McDermott, A.M., 2015. Fighting microbial infections: a lesson from amphibian skin-derived esculentin-1 peptides. Peptides 71, 286–295.

Mardirossian, M., Pompilio, A., Crocetta, V., De Nicola, S., Guida, F., Degasperi, M., Gennaro, R., Di Bonaventura, G., Scocchi, M., 2016. In vitro and in vivo evaluation of BMAP-derived peptides for the treatment of cystic fibrosis-related pulmonary infections. Amino Acids 48, 2253–2260.

McDonald, M., Mannion, M., Pike, D., Lewis, K., Flynn, A., Brannan, A.M., Browne, M.J., Jackman, D., Madera, L., Power Coombs, M.,R., Hoskin, D.W., Rise, M.L., Booth, V., 2015. Structure-function relationships in histidine-rich antimicrobial peptides from Atlantic cod. Biochim. Biophys. Acta 1848, 1451–1461.

McGlasson, S.L., Semple, F., MacPherson, H., Gray, M., Davidson, D.J., Dorin, J.R., 2017. Human β-defensin 3 increases the TLR9-dependent response to bacterial DNA. Eur. J. Immunol. 47, 658–664.

McPhee, J.B., Lewenza, S., Hancock, R.E., 2003. Cationic antimicrobial peptides activate a two-component regulatory system, PmrA-PmrB, that regulates resistance to polymyxin B and cationic antimicrobial peptides in Pseudomonas aeruginosa. Mol. Microbiol 50 (1), 205–217.

Meher, P.K., Sahu, T.K., Saini, V., Rao, A.R., 2017. Predicting antimicrobial peptides with improved accuracy by incorporating the compositional, physico-chemical and structural features into Chou's general PseAAC. Sci. Rep. 7, 42362.

Miller, S.E., Bell, C.S., Mejias, R., McClain, M.S., Cover, T.L., Giorgio, T.D., 2016. Colistin-functionalized nanoparticles for the rapid capture of acinetobacter baumannii. J. Biomed. Nanotechnol. 12 (9), 1806–1819.

Mohanram, H., Bhattacharjya, S., 2016. Salt-resistant short antimicrobial peptides. Biopolymers 106, 345–356.

Molchanova, N., Hansen, P.R., Damborg, P., Nielsen, H.M., Franzyk, H., 2017a. Lysine-based a-peptide/b-peptoid peptidomimetics: influence of hydrophobicity, fluorination, and distribution of cationic charge on antimicrobial activity and cytotoxicity. ChemMedChem 12 (4), 312–318.

Molchanova, N., Hansen, P.R., Franzyk, H., 2017b. Advances in Development Of Antimicrobial Peptidomimetics As Potential Drugs. Molecules 22, 1430.

Molchanova, N., Wang, H., Hansen, P.R., Høiby, N., Nielsen, H.M., Franzyk, H., 2019. Antimicrobial activity of a-peptide/b-peptoid lysine-based peptidomimetics against colistin-resistant pseudomonas aeruginosa isolated from cystic fibrosis patients. Front. Microbiol. 10, 275.

Moncla, B.J., Pryke, K., Rohan, L.C., Graebing, P.W., 2011. Degradation of naturally occurring and engineered antimicrobial peptides by proteases. Adv. Biosci. Biotechnol. 2, 404–408.

Nagant, C., Pitts, B., Nazmi, K., Vandenbranden, M., Bolscher, J.G., Stewart, P.S., Dehaye, J.P., 2012. Identification of peptides derived from the human antimicrobial peptide LL-37 active against biofilms formed by Pseudomonas aeruginosa using a library of truncated fragments. Antimicrob. Agents Chemother. 56, 5698–5708.

Niemirowicz, K., Surel, U., Wilczewska, A.Z., Mystkowska, J., Piktel, E., Gu, X., Namiot, Z., Kulakowska, A., Savage, P.B., Bucki, R., 2015. Bactericidal activity and biocompatibility of ceragenin-coated magnetic nanoparticles. J. Nanobiotech. 13, 32.

Pacor, S., Giangaspero, A., Bacac, M., Sava, G., Tossi, A., 2002. Analysis of the cytotoxicity of synthetic antimicrobial peptides on mouse leucocytes: implications for systemic use. J. Antimicrob. Chemother. 50, 339–348.

Pal, I., Bhattacharyya, D., Kar, R.K., Zarena, D., Bhunia, A., Atreya, H.S., 2019. A Peptide-nanoparticle System With Improved Efficacy Against Multidrug Resistant Bacteria. Sci. Rep. 9 (1), 4485.

Pane, K., Durante, L., Crescenzi, O., Cafaro, V., Pizzo, E., Varcamonti, M., Zanfardino, A., Izzo, V., Di Donato, A., Notomista, E., 2017. Antimicrobial potency of cationic antimicrobial peptides can be predicted from their amino acid composition: Application to the detection of "cryptic" antimicrobial peptides. J. Theor. Biol. 419, 254–265.

Pastor, M., Moreno-Sastre, M., Esquisabel, A., Sans, E., Viñas, M., Bachiller, D., Asensio, V.J., Pozo, A.D., Gainza, E., Pedraz, J.L., 2014. Sodium colistimethate loaded lipid nanocarriers for the treatment of Pseudomonas aeruginosa infections associated with cystic fibrosis. Int. J. Pharm. 477, 485–494.

Patton, J.S., Nagarajan, S., Clark, A. 1998. Pulmonary absorption and metabolism of peptides and protein. In Respiratory Drug Delivery VI, 17–24. Buffaloo Grove, IL: R. N. Dalby, P.R. Byron, S.J. Farr Interpharm Press (Eds).

Pedone, E., Notomista, E., Galdiero, S., Varcamonti, M., Conturs, P., 2016. A trans-kingdom antimicrobial peptide targeting cystic fibrosis. Pathog. J. Genet. Syndr. Gene Ther. 1000308.

Pezzulo, A.A., Tang, X.X., Hoegger, M.J., Abou Alaiwa, M.H., Ramachandran, S., Moninger, T.O., Karp, P.,H., Wohlford-Lenane, C.L., Haagsman, H.P., van Eijk, M., Bánfi, B., Horswill, A.R., Stoltz, D.A., McCray, P.B.Jr, Welsh, M.J., Zabner, J., 2012. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. Nature 487, 109–113.

Pini, A., Lozzi, L., Bernini, A., Brunetti, J., Falciani, C., Scali, S., Bindi, S., Di Maggio, T., Rossolini, G.M., Niccola,i N., Bracci, L., 2012. Efficacy and toxicity of the antimicrobial peptide M33 produced with different counter-ions. Amino Acids 43, 467–473.

Pires, J., Siriwardena, T.N., Stach, M., Tinguely, R., Kasraian, S., Luzzaro, F., Leib, S.L., Darbre, T., Reymond, J.L., Endimiani, A., 2015. In vitro activity of the novel antimicrobial peptide dendrimer G3kl against multidrug-resistant acinetobacter baumannii and Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 59, 7915–7918.

Pompilio, A., Crocetta, V., Scocchi, M., Pomponio, S., Di Vincenzo, V., Mardirossian, M., Gherardi, G., Fiscarelli, E., Dicuonzo, G., Gennaro, R., Di Bonaventura, G., 2012. Potential novel therapeutic strategies in cystic fibrosis: antimicrobial and anti-biofilm activity of natural and designed a-helical peptides against Staphylococcus aureus, Pseudomonas aeruginosa, and Stenotrophomonas maltophilia. BMC Microbiol 12, 145.

Pompilio, A., Geminiani, C., Mantini, P., Siriwardena, T.N., Di Bonaventura, I., Reymond, J.L., Di Bonaventura, G., 2018. Peptide dendrimers as "lead compounds" for the treatment of chronic lung infections by Pseudomonas aeruginosa in cystic fibrosis patients: in vitro and in vivo studies. Infect. Drug Resist. 11, 1767–1782.

Portelinha, J., Angeles-Boza, A.M., 2021. The antimicrobial peptide Gad-1 clears pseudomonas aeruginosa biofilms under cystic fibrosis conditions. Chembiochem 22, 1646–1655.

Preda, V.G., Săndulescu, O., 2019. Communication is the key: biofilms, quorum sensing, formation and prevention. Discov. (Craiova) 7 (3), e100.

Quarta, E., Sonvico, F., Bettini, R., De Luca, C., Dotti, A., Catalucci, D., Iafisco, M., Degli Esposti, L., Colombo, G., Trevisi, G., Rekkas, D.M., Rossi, A., Wong, T.W., Buttini, F., Colombo, P., 2021. Inhalable microparticles embedding calcium phosphate nanoparticles for heart targeting: the formulation experimental design. Pharmaceutics 13 (11), 1825.

Ran, H.H., Cheng, X., Gao, G., Sun, W., Jiang, Y.W., Zhang, X., Jia, H.R., Qiao, Y., Wu, F. G., 2020. Colistin-loaded polydopamine nanospheres uniformly decorated with

silver nanodots: a nanohybrid platform with improved antibacterial and antibiofilm performance. ACS Appl. Bio. Mater. 3, 2438–2448.

Ratjen, F., Bell, S.C., Rowe, S.M., Goss, C.H., Quittner, A.L., Bush, A., 2015. Cystic fibrosis. Nat. Rev. Dis. Prim. 1, 15010.

Rees, D.D., Brain, J.D., Wohl, M.E., Humes, J.L., Mumford, R.A., 1997. Inhibition of neutrophil elastase in CF sputum by L-658,758. J. Pharmacol. Exp. Ther. 283 (3), 1201–1206.

Reeves, E.P., Molloy, K., Pohl, K., McElvaney, N.G., 2012. Hypertonic saline in treatment of pulmonary disease in cystic fibrosis. Sci. World J. 2012, 465230.

Reymond, J.L., Bergmann, M., Darbre, T., 2013. Glycopeptide dendrimers as Pseudomonas aeruginosa biofilm inhibitors. Chem. Soc. Rev. 42 (11), 4814–4822.

Rivas-Santiago, B., Castañeda-Delgado, J.E., Rivas Santiago, C.E., Waldbrook, M., González-Curiel, I., León-Contreras, J.C., Enciso-Moreno, J.A., del Villar, V., Mendez-Ramos, J., Hancock, R.E., Hernandez-Pando, R., 2013. Ability of innate defence regulator peptides IDR-1002, IDR-HH2 and IDR-1018 to protect against Mycobacterium tuberculosis infections in animal models. PLoS One 8, e59119.

Rotem, S., Mor, A., 2009. Antimicrobial peptide mimics for improved therapeutic properties. Biochim. Biophys. Acta 1788 (8), 1582–1592.

Rubin, B.K., 2007. Mucus structure and properties in cystic fibrosis. Paediatr. Respir. Rev. 8, 4–7.

Saiman, L., Tabibi, S., Starner, T.D., San Gabriel, P., Winokur, P.L., Jia, H.P., McCray, P. B.Jr, Tack, B.F., 2001. Cathelicidin peptides inhibit multiply antibiotic-resistant pathogens from patients with cystic fibrosis. Antimicrob. Agents Chemother. 45, 2838–2844.

Sala, V., Murabito, A., Ghigo, A., 2019. Inhaled biologicals for the treatment of cystic fibrosis. Recent Pat. Inflamm. Allergy Drug Discov. 13, 19–26.

Sala, V., Cnudde, S.J., Murabito, A., Massarotti, A., Hirsch, E., Ghigo, A., 2021. Therapeutic peptides for the treatment of cystic fibrosis: Challenges and perspectives. Eur. J. Med. Chem. 213, 113191.

Sans-Serramitjana, E., Fusté, E., Martínez-Garriga, B., Merlos, A., Pastor, M., Pedraz, J.L., Esquisabel, A., Bachiller, D., Vinuesa, T., Viñas, M., 2016. Killing effect of nanoencapsulated colistin sulfate on Pseudomonas aeruginosa from cystic fibrosis patients. J. Cyst. Fibros. 15, 611–618.

Scherließ, R., Bock, S., Bungert, N., Neustock, A., Valentin, L., 2022. Particle engineering in dry powders for inhalation. Eur. J. Pharm. Sci. 172, 106158.

Scutera, S., Argenziano, M., Sparti, R., Bessone, F., Bianco, G., Bastiancich, C., Castagnoli, C., Stella, M., Musso, T., Cavalli, R., 2021. Enhanced antimicrobial and antibiofilm effect of new colistin-loaded human albumin nanoparticles. Antibiotics 10 (1), 57.

Seiler, F., Lepper, P.M., Bals, R., Beisswenger, C., 2014. Regulation and function of antimicrobial peptides in immunity and diseases of the lung. Protein Pept. Lett. 21, 341–351.

Semple, F., MacPherson, H., Webb, S., Cox, S.L., Mallin, L.J., Tyrrell, C., Grimes, G.R., Semple, C.A., Nix, M.A., Millhauser, G.L., Dorin, J.R., 2011. Human beta-defensin 3 affects the activity of pro-inflammatory pathways associated with MyD88 and TRIF. Eur. J. Immunol. 41, 3291–3300.

Sharma, A., Gupta, P., Kumar, R., Bhardwaj, A., 2016. dPABBs: a novel in silico approach for predicting and designing anti-biofilm peptides. Sci. Rep. 6, 21839.

Sharma, A., Kumar, A., de la Torre, B.G., Albericio, F., 2022. Liquid-phase peptide synthesis (LPPS): a third wave for the preparation of peptides. Chem. Rev. https://doi.org/10.1021/acs.chemrev.2c00132.

Shin, S.Y., Yang, S.T., Park, E.J., Eom, S.H., Song, W.K., Kim, Y., Hahm, K.S., Kim, J.I., 2002. Salt resistance and synergistic effect with vancomycin of alpha-helical antimicrobial peptide P18. Biochem. Biophys. Res. Commun. 290, 558–562.

Shoyele, S.A., Slowey, A., 2006. Prospects of formulating proteins/peptides as aerosols for pulmonary drug delivery. Int. J. Pharm. 314, 1–8.

Siriwardena, T.N., Stach, M., He, R., Gan, B.H., Javor, S., Heitz, M., Ma, L., Cai, X., Chen, P., Wei, D., Li, H., Ma, J., Köhler, T., van Delden, C., Darbre, T., Reymond, J.L., 2018. Lipidated peptide dendrimers killing multidrug-resistant bacteria. J. Am. Chem. Soc. 140, 423–432.

Skerlavaj, B., Gennaro, R., Bagella, L., Merluzzi, L., Risso, A., Zanetti, M., 1996. Biological characterization of two novel cathelicidin-derived peptides and identification of structural requirements for their antimicrobial and cell lytic activities. J. Biol. Chem. 271, 28375–28381.

Smith, P.L., 1997. Peptide delivery via the pulmonary route: a valid approach for local and systemic delivery. J. Control Release 46, 99–106.

Souza, L.M.P., Nascimento, J.B., Romeu, A.L., Estrada-López, E.D., Pimentel, A.S., 2018. Penetration of antimicrobial peptides in a lung surfactant model. Colloids Surf. B Biointerfaces 167, 345–353.

Spencer, H.K., Spitznogle, S.L., Borjan, J., Aitken, S.L., 2020. An overview of the treatment of less common non-lactose-fermenting gram-negative bacteria. Pharmacother 40, 936–951.

Stach, M., Siriwardena, T.N., Köhler, T., van Delden, C., Darbre, T., Reymond, J.L., 2014. Combining topology and sequence design for the discovery of potent antimicrobial peptide dendrimers against multidrug-resistant Pseudomonas aeruginosa. Angew. Chem. Int. Ed. Engl. 53, 12827–12831.

Subramanian, S., Ross, N.W., MacKinnon, S.L., 2009. Myxinidin, a novel antimicrobial peptide from the epidermal mucus of hagfish, Myxine glutinosa L. Mar. Biotechnol. 11, 748–757.

Taggart, C.C., Greene, C.M., Smith, S.G., Levine, R.L., McCray Jr., P.B., O'Neill, S., McElvaney, N.G., 2003. Inactivation of human beta-defensins 2 and 3 by elastolytic cathepsins. J. Immunol. 171, 931–937.

Tam, J.P., Lu, Y.A., Yang, J.L., 2002. Antimicrobial dendrimeric peptides. Eur. J. Biochem 269 (3), 923–932.

Tang, Y.Q., Yuan, J., Osapay, G., Osapay, K., Tran, D., Miller, C.J., Ouellette, A.J., Selsted, M.E., 1999. A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha-defensins. Science 286, 498–502.

Tjabringa, G.S., Aarbiou, J., Ninaber, D.K., Drijfhout, J.W., Sørensen, O.E., Borregaard, N., Rabe, K.F., Hiemstra, P.S., 2003. The antimicrobial peptide LL-37 activates innate immunity at the airway epithelial surface by transactivation of the epidermal growth factor receptor. J. Immunol. 171, 6690–6696.

Tongaonkar, P., Tran, P., Roberts, K., Schaal, J., Osapay, G., Tran, D., Ouellette, A.J., Selsted, M.E., 2011. Rhesus macaque θ-defensin isoforms: expression, antimicrobial activities, and demonstration of a prominent role in neutrophil granule microbicidal activities. J. Leukoc. Biol. 89, 283–290.

van der Weide, H., Cossío, U., Gracia, R., Te Welscher, Y.M., Ten Kate, M.T., van der Meijden, A., Marradi, M., Ritsema, J.A.S., Vermeulen-de Jongh, D.M.C., Storm, G., Goessens, W.H.F., Loinaz, I., van Nostrum, C.F., Llop, J., Hays, J.P., Bakker-Woudenberg, I.A.J.M., 2020. Therapeutic efficacy of novel antimicrobial peptide AA139-nanomedicines in a multidrug-resistant klebsiella pneumoniae pneumonia-septicemia model in rats. Antimicrob. Agents Chemother. 64 (9), e00517–e00520.

Velino, C., Carella, F., Adamiano, A., Sanguinetti, M., Vitali, A., Catalucci, D., Bugli, F., Iafisco, M., 2019. Nanomedicine approaches for the pulmonary treatment of cystic fibrosis. Front. Bioeng. Biotechnol. 7, 406.

Waghu, F.H., Barai, R.S., Gurung, P., Idicula-Thomas, S., 2016. CAMPR3: a database on sequences, structures and signatures of antimicrobial peptides. Nucleic Acids Res. 44 (D1), D1094–D1097.

Wallace, S.J., Li, J., Nation, R.L., Prankerd, R.J., Boyd, B.J., 2012. Interaction of colistin and colistin methanesulfonate with liposomes: colloidal aspects and implications for formulation. J. Pharm. Sci. 101 (9), 3347–3359.

Wan, F., Møller, Horn, Yang, E., Jørgensen, L., M., 2012. Formulation technologies to overcome poor drug-like properties. Drug Discov. Today.: Technol. 9, 141–146.

Wang, J., Dou, X., Song, J., Lyu, Y., Zhu, X., Xu, L., Li, W., Shan, A., 2019. Antimicrobial peptides: promising alternatives in the post feeding antibiotic era. Med. Res. Rev. 39, 831–859.

Wang, Y., Rezk, A.R., Khara, J.S., Yeo, L.Y., Ee, P.L., 2016. Stability and efficacy of synthetic cationic antimicrobial peptides nebulized using high frequency acoustic waves. Biomicrofluidics 10 (3), 034115.

Water, J.J., Smart, S., Franzyk, H., Foged, C., Nielsen, H.M., 2015. Nanoparticle-mediated delivery of the antimicrobial peptide plectasin against Staphylococcus aureus in infected epithelial cells. Eur. J. Pharm. Biopharm. 92, 65–73.

Waters, V., Smyth, A., 2015. Cystic fibrosis microbiology: advances in antimicrobial therapy. J. Cyst. Fibros. 14, 551–560.

Welp, A.L., Bomberger, J.M., 2020. Bacterial community interactions during chronic respiratory disease. Front Cell Infect. Microbiol. 10, 213.

Wiradharma, N., Khoe, U., Hauser, C.A., Seow, S.V., Zhang, S., Yang, Y.Y., 2011. Synthetic cationic amphiphilic a-helical peptides as antimicrobial agents. Biomaterials 32, 2204–2212.

Xiao, X., Wang, P., Lin, W.Z., Jia, J.H., Chou, K.C., 2013. iAMP-2L: a two-level multi-label classifier for identifying antimicrobial peptides and their functional types. Anal. Biochem. 436 (2), 168–177.

Yang, D., Chertov, O., Bykovskaia, S.N., Chen, Q., Buffo, M.J., Shogan, J., Anderson, M., Schröder, J.M., Wang, J.M., Howard, O.M., Oppenheim, J.J., 1999. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286, 525–528.

Yang, L., Liu, Y., Wang, N., Wang, H., Wang, K., Luo, X.L., Dai, R.X., Tao, R.J., Wang, H. J., Yang, J.W., Tao, G.Q., Qu, J.M., Ge, B.X., Li, Y.Y., Xu, J.F., 2021. Albumin-based LL37 peptide nanoparticles as a sustained release system against pseudomonas aeruginosa lung infection. ACS Biomater. Sci. Eng. 7, 1817–1826.

Ying, Y., Wang, H., Xi, X., Ma, C., Liu, Y., Zhou, M., Du, Q., Burrows, J.F., Wei, M., Chen, T., Wang, L., 2019. Design of N-Terminal Derivatives from a novel dermaseptin exhibiting broad-spectrum antimicrobial activity against isolates from cystic fibrosis patients. Biomolecules 9 (11), 646.

Yu, Z., Deslouches, B., Walton, W.G., Redinbo, M.R., D,i, Y.P., 2018. Enhanced biofilm prevention activity of a SPLUNC1-derived antimicrobial peptide against Staphylococcus aureus. PLoS One 13 (9), e0203621.

Zasloff, M., 2007. Antimicrobial peptides, innate immunity, and the normally sterile urinary tract. J. Am. Soc. Nephrol. 18 (11), 2810–2816.

Zhang, L., Parente, J., Harris, S.M., Woods, D.E., Hancock, R.E., Falla, T.J., 2005. Antimicrobial peptide therapeutics for cystic fibrosis. Antimicrob. Agents Chemother. 49, 2921–2927.