111 Characterization of bacterial strains isolated from the CF patients in Georgia and evaluation of the efficacy of phage treatment

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We reviewed the patients attending the "Cystic Fibrosis National Center" in Tbilisi, Georgia from November, 2006 until October, 2008. All strains isolated from the patients' specimens were subjected to taxonomic analysis. For the genus- and species-level identification of the bacterial strains PCR analysis was used. Genetic relatedness of bacterial strains was determined by PFGE. Antibiotic susceptibility of the strains was determined by the disc diffusion method. 30 strains of *P. aeruginosa* and 76 strains of *S. aureus* have been identified. Phage sensitivity in vitro was determined using Pyophage. Four patients who were diagnosed to have cystic fibrosis were selected for Pyophage application (by nebulizer administration). According to the bacteriological testing, in two out of four patients *S. aureus* and *P. aeruginosa* were the main causative agents of the secondary infections. The other two were infected only by *S. aureus*. All patients were treated by the standard scheme of therapy, including antibiotics, anti-mucus medications and vitamins. We studied the bacterial cultures before, after and during the treatment, including checking antibiotic- and phage-resistance and PFGE. The existence of phage-neutralizing antibodies was checked in all patients' blood sera. The treatment continued for 6–10 days (several times); titter of bacterial cells isolated has been drastically decreased and the general state of the patients was clearly improved: expectoration was facilitated, and no crepitating was observed. Bacteriophage treatment provided long infection-free periods between colonization episodes

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112 A gift of Nature for the treatment of *Pseudomonas aeruginosa*, *Burkholderia cepacia* and MRSA in Cystic Fibrosis cases

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The CFTR gene is responsible for the production of a protein regulating outflow of water and salts (like Cl– or SCN–) from epithelial cells. Inactivation of CFTR is likely to cause multiple defects in the airway that together alter local innate immunity. As shown in recent publications, hypothiocyanite (OSCN–) antimicrobial molecule is deficient in CF condition.

OSCN– is well known by ALAXIA scientists who demonstrated with appointed experts during MEVEOL development its antimicrobial efficacy on various strains including MRSA, *B. cepacia*, and mucoid *P. aeruginosa*.

MEVEOL tackles a wide range of microorganisms including biofilm due to its composition associating naturally linked compounds. OSCN– and lactoferrin, main biomolecules, are normally present in human body and especially in human airways suggesting low risk of bacterial resistance.

Effects of MEVEOL have been evaluated in vitro and in vivo.

MEVEOL (inhalation use) will be of potential significant benefit for the treatment of lung microorganisms proliferation in CF condition thanks to its combined antimicrobial and local mechanism of action.

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In vitro efficacy vs *B. cepacia* (log CFU/mL)

	Contact time (h)					
	0	1	2	4	6	24
Control	4.41	4.48	4.54	4.56	4.58	4.53
MEVEOL	4.41	3.49	2.99	2	1.84	1.56
MEVEOL + peroxidase system	4.41	0.3	0	0	0	0

Same kind of results on MRSA, *Pseudomonas*.

In vivo demonstration of efficacy on mice, previously infected with mucoid *P. aeruginosa* (log CFU/mL)

	Control	Treated with Meveol
Mice lung 72 h after infection	3.1	1.5

Same kind of results on MRSA, *Pseudomonas*.

113 Orphan drug designation to Anti-pseudomonas IgY

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Pseudomonas aeruginosa (PA) lung infections are main causes of morbidity and mortality in CF. Repeated courses of antibiotics give fewer infections and improved prognosis, but the threat of serious side effects by the use of antibiotics is a reality – especially the rapidly increasing antibiotic resistance. Alternatives and/or complements to antibiotics are urgently needed.

Passive immunization is a known alternative to fight infections. Hens produce large amounts of antibodies, IgY, which can be retrieved from egg yolk. Hens are vaccinated with PA in order to obtain specific Anti-pseudomonas IgY (Anti-PA IgY), which are used for oral treatment to prevent PA lung infections. Humans do not produce anti-IgY antibodies to orally given IgY and the toxicity is very low. Moreover, the risk that bacteria should develop resistance to IgY is extremely small. Anti-PA IgY treatment to CF patients in a clinical trial reduced the number of PA positive cultures and tended to delay the onset of chronic PA infections. Thus, the need for antibiotics was reduced. Atypical mycobacteria and A. xylosoxidans only appeared sporadically and there have been no cultures positive for B. cepacia. BMI was normal or close to normal and pulmonary function was preserved within the group at the end of the study (Ped Pulm, 2008).

Due to these results Anti-PA IgY has been granted orphan drug designation by EMEA for the orphan indication: treatment of cystic fibrosis. EMEAs motivation was: "...justifications have been provided that avian polyclonal IgY antibody against *Pseudomonas aeruginosa* may be of significant benefit to those affected by the condition."

We are now searching financial support for a Phase III study.

114* Activity of liposomal formulations on sputum isolated from CF Patients

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Opportunistic pathogens like the Gram-negative *Pseudomonas aeruginosa* develop resistance by forming biofilm, controlled by Quorum Sensing (QS) molecules. We have developed a novel liposomal formulation by co-encapsulating the bismuth metal (Bi) with tobramycin (Tob). It is hypothesized that delivery of bifunctional liposomes containing both Bi and Tob would increase their antibacterial efficacy. The stability of the liposomal-bismuth-tobramycin formulation (LipoBT) was measured in bronchoalveolar lavage (BAL) and sputum, and their activities against clinical isolates of *P. aeruginosa* grown on biofilms were compared to the conventional forms by using the Calgary Biofilm Device. QS reduction on isolates of *P. aeruginosa* was determined by monitoring the N-acyl homoserine lactone (AHL) production after exposure to LipoBT formulation using an *Agrobacterium tumefaciens* reporter strain. Sputum penetration by LipoBT was determined by comparing its penetration with latex bead particles equaling liposome particle size. The LipoBT formulations tended to be stable in sputum and BAL. Encapsulated Tob in LipoBT formulation eradicated bacterial growth in biofilms at concentrations of 64 to 512 mg/L, whereas, other Tob formulations were only able to reduce the growth log compared to positive control. Our LipoBT formulation was able to prevent QS signaling production at concentrations as low as 0.012 mg/L of Tob in absence of the bactericidal activity. LipoBT formulation was also able to penetrate diluted sputum samples more efficiently than latex beads. In conclusion, co-encapsulating Bi with Tob establishes a new strategy for higher efficacy of these agents by promoting their antimicrobial activity and eradicating biofilms produced by mucoid bacterial strains.