Development of a Bacteriophage Cocktail Targeting *Staphylococcus aureus* to Treat Atopic Dermatitis

Tamar Lin[1], Nufar Buchshtab[1], Yifat Elharar[1], Julian Nicenboim[1], Rotem Edgar[1], Iddo Weiner[1], Lior Zelcbuch[1], Ariel Cohen[1], Sharon Kredo-Russo[1], Inbar Gahali-Sass[1], Naomi Zak[1], Sailaja Puttagunta[2], Merav Bassan[1]

[1]BiomX Ltd., Ness Ziona, Israel

[2]BiomX Inc. Branford, CT, USA

Background

Multiple lines of evidence suggest that atopic dermatitis (AD) is associated with increased colonization of Staphylococcus *aureus. S. aureus* contributes to AD pathogenesis through the release of virulence factors such as superantigens, phenol soluble modulins (PSMs), toxins and lipoproteins that affect both keratinocytes and immune cells leading to disruption of the skin barrier and immune dysfunction including T helper cell 2 lymphocyte skewing. The link between atopic dermatitis exacerbation and *S. aureus,* suggests that reducing *S. aureus* abundance has the potential to improve the clinical outcomes of patients with atopic dermatitis. However, systemic and topical antibiotics, as well as bleach baths, often failed to show direct killing activity of surface bacteria or improvement in skin inflammation. This suggests that the classic approach of broad-spectrum antibiotic therapy lacks efficiency in the treatment of AD and that targeted modulation of the skin microbiome may be more beneficial. The aim of the current study is to develop a bacteriophage therapy that specifically targets *S. aureus* while maintaining the normal skin microbiome.

Methods

Natural phages were discovered by screening environmental samples on 118 *S. aureus* strains isolated from skin samples, followed by multiple enrichment steps. After isolation, the phages were subjected to Next generation Sequencing (NGS) and analyzed using proprietary bioinformatics tools for taxonomic classification, undesirable genes (toxins, antibiotic resistance genes, lysogeny potential), and purity. The host range was determined by an efficiency of plating (EOP) value above 0.1 and the ability of the cocktail to completely lyse liquid bacterial culture under different growth conditions (e.g., temperature, bacterial stage). Phages with divergent specificities were combined in a cocktail to address the diversity of S. aureus strains in AD and to address the emergence of resistance.

Results

50 lytic phages from different taxonomies, including Silviavirus, Kayvirus, Podoviridae, were discovered. Host range results of the individual phages by EOP ranged between 41% (48/118) to 79% (93/118). Host range studies in liquid culture revealed that a subset of the phages can infect a broad range of S. aureus strains in different metabolic states, including stationary state. Combining the single-phage EOP results of selected phages resulted in a broad host range cocktail which infected 92% (109/118) of the strains. Furthermore, clearance in an *in-vitro* liquid infection assay was achieved in 87% (103/118) of the strains, with no evidence of phage resistance after 24 hours (Figure 1).

A *S. aureus* host that can be used for production of all the phages in the cocktail at high titers suitable for large scale manufacturing has been identified. Advanced NGS methods combined with multiple production cycles confirmed the absence of contaminating prophages in this host. The phages are being used for the development of a topical formulation (BX005) that may be administered to patients with atopic dermatitis in an upcoming clinical trial.

Conclusions

A cocktail of natural phages targeting S. aureus was effective in reducing bacterial burden across multiple assays. This development holds the potential to offer a novel therapeutic approach for atopic dermatitis.

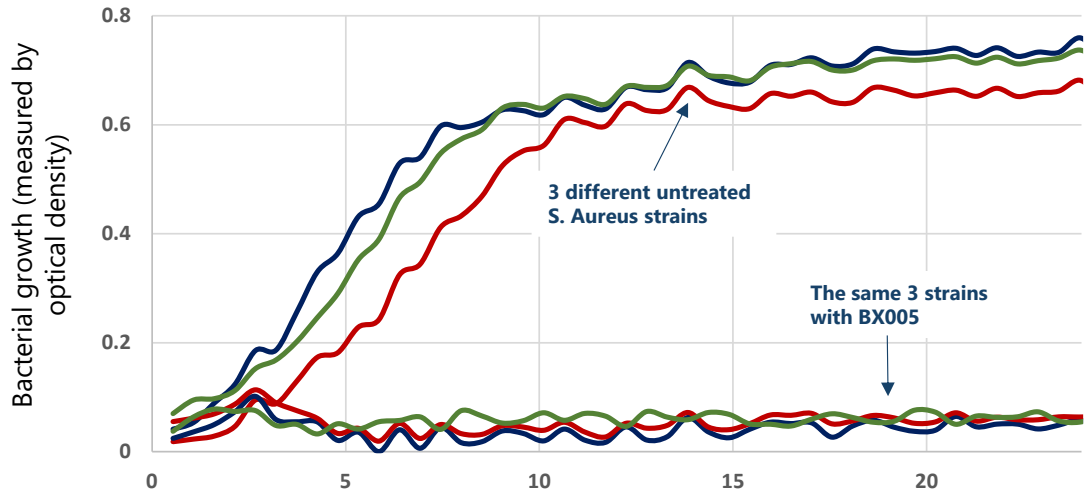

Figure 1: BX005 eradicates S. aureus strains in a in-vitro liquid infection assay. Untreated strains showed normal growth curve while the same strains with the BX005 phage cocktail were eradicated with no mutants arising after 24 hours. Similar results were achieved in 87% of the 118 tested S. aureus strains.