Introduction

Dysbiosis is associated with inflammatory bowel disease (IBD) and, together with genetic and environmental elements, is implicated in perpetuating chronic inflammation. Recent studies demonstrated that a specific Klebsiella pneumoniae strain (KP2H7) isolated from IBD patients causes a strong TH1 pro-inflammatory response when used to colonize wild-type mice and moderate-severe colitis in IL-10/-/- mice. The present study aims to validate the KP2H7 bacteria as a disease-associated target in IBD patients and to initiate development of a bacteriophage-based therapy.

Methodology

1. Prevalence of KP2H7 in IBD patients

The presence of KP2H7 in stool samples of IBD patients from the US, France, and Israel was examined.

Table 1: Percentage of KP2H7 carriers among IBD patients across geographical regions

<table>
<thead>
<tr>
<th>Region</th>
<th>KP2H7 positive (%)</th>
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</thead>
<tbody>
<tr>
<td>US</td>
<td>50</td>
</tr>
<tr>
<td>France</td>
<td>40</td>
</tr>
<tr>
<td>Israel</td>
<td>30</td>
</tr>
</tbody>
</table>

KP2H7 is present in stool samples of approximately 30% of IBD patients from multiple geographies.

Next, we evaluated the levels of KP species across disease states in the French cohort using a proprietary algorithm for relative abundance assessment.

Figure 1: KP relative abundance in IBD patients at different disease stages and in healthy controls

2. Induction of TH1 by clinical KP2H7 isolates

Induction of TH1 by clinical KP2H7 isolates

KP species level were determined using a BiomX proprietary tool that employs both complete reads and species unique 21-mers and calculates the frequencies compared to those of reference assemblies (RefSeq & in-house isolates).

Figure 2: Induction of TH1 cells following colonization with clinical KP2H7 isolates

3. BX002 - a phage cocktail which targets KP2H7

Development of a phage cocktail targeting KP2H7 and KP2H7 clinical strains was carried out through the following steps:

Table 2: BX002 cocktail generations

<table>
<thead>
<tr>
<th>Generation</th>
<th>1st generation</th>
<th>2nd generation</th>
</tr>
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<tbody>
<tr>
<td>10 phages</td>
<td>3 phages</td>
<td>2 phages</td>
</tr>
<tr>
<td>1st generation + 2 phages with different MAs</td>
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To develop the 2nd generation KP2H7-targeting cocktail, 3-, 4- and 5-phage combinations were evaluated in vitro by a liquid infection dynamics assay and in vivo in KP2H7-colonized animals. The optimal cocktail was selected based on its ability to reduce KP2H7 levels and suppress growth of phage-resistant mutants both in vitro and in vivo.

Figure 3: Liquid infection dynamics of 2nd generation KP2H7-targeting combinations on KP2H7

Only the combination comprised of 5 phages (BX002) was capable of preventing the appearance of resistant mutant bacteria.

Figure 4: Comparison of activity of 1st and 2nd generation phage cocktails in KP2H7-colonized animals

Discussion and Conclusions

- Approximately 30% of IBD patients across the US, France, and Israel are colonized by KP2H7 strains.
- Klebsiella pneumoniae is present at a higher relative abundance in active disease (flare) in CD patients compared to UC patients.
- KP2H7 clinical isolates were demonstrated to be pro-inflammatory (TH1) in colonized wild-type and IL-10/-/- animals.
- A 5-phage cocktail (BX002) that is effective in eradicating KP2H7 and preventing the appearance of phage-resistant mutant bacteria has been developed.
- The BX002 phage cocktail significantly reduces KP2H7 load in stool and intestinal mucus of mono-colonized animals.
- Clinical studies evaluating BX002 for the treatment of patients with IBD should be pursued.

References:

1. Atarashi et al., 2017

For more information visit www.biomx.com

Conflict Of Interests: All authors are employees of BiomX Ltd.