USE OF A TARGETED BACTERIOPHAGE COCKTAIL FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE

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Introduction

Dysbiosis is associated with inflammatory bowel disease (IBD) and, together with genetic and environmental elements, is implicated in perpetuating chronic inflammation. Recent studies1 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

Prevalence of KP2H7 in IBD

- *US:* analyzed on pooled data (15 Gb) of longitudinal stool samples of IBD patients participating in the Human Microbiome Project, by reads mapping to unique KP2H7 regions.
- *France:* analyzed on metagenomic data (50 Gb) from stool samples, by reads mapping to unique KP2H7 regions.
- *Israel:* analyzed by KP2H7 real time (RT) qPCR, with 2 amplicons for KP2H7 unique regions

KP level determination

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).

Isolation of clinical KP2H7 strains

KP strains were isolated from fecal and mucosal samples of IBD patients using a series of biochemical tests and selective media. Strains with sequence homology of >99.9% to KP2H7 were defined as KP2H7 clinical isolates.

Induction of TH1 by clinical KP2H7 isolates

Analysis of TH1 (CD4+ IFNγ+ T cells) in the colonic lamina propria of monoassociated GF (germ free) wild type mice or IL10-/- mice at 3 or 2 weeks post-colonization, respectively.

Evaluation of potential cocktail compositions by liquid infection dynamics

Host bacteria were cultured to OD_{600nm} 0.2 in BHIS and 10^6 PFU/mL phage were introduced into the culture as indicated. The changes in bacterial cell density were then monitored by plate reader at OD_{600nm} for 20hr.

Results

1. Prevalence and abundance 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

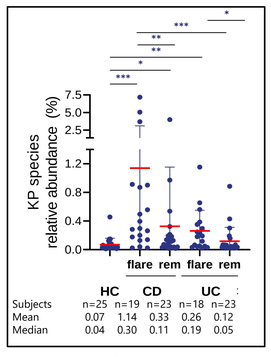
Cohort	UC			CD			IBD		
	Cohort Size	KP2H7 Pos	KP2H7 Prev	Cohort Size	KP2H7 Pos	KP2H7 Prev	Cohort Size	KP2H7 Pos	KP2H7 Prev
US	n=30	n=9	30%	n=50	n=23	46%	n=80	n=31	39%
France	n=46	n=11	24%	n=43	n=15	35%	n=89	n=26	29%
Israel	n=52	n=19	37%	n=57	n=17	30%	n=109	n=36	33%

n – number of participants, Pos – positive, Prev – prevalence
CD – Crohn's disease, UC – ulcerative colitis

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



HC – healthy controls, CD – Crohn's disease, UC – ulcerative colitis
Mean (red line) ± SD
*- p<0.05 , ** - p<0.01 , *** - p<0.001

KP2H7 is significantly enriched in the stool of patients in flare compared to remission and is present in higher relative abundance in samples from CD patients compared to UC patients.

2. Induction of TH1 by clinical KP2H7 isolates

Tens of strains with >99.9% sequence homology to KP2H7 were identified and examined for immune stimulation (TH1).

Representative KP2H7 strains were introduced into wild type and IL-$10^{-/-}$ gnotobiotic (germ free) animals to evaluate colonic TH1 response following bacterial colonization

Study design:

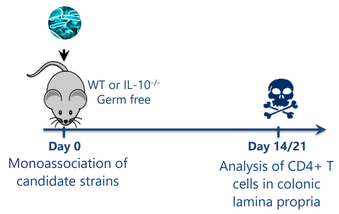
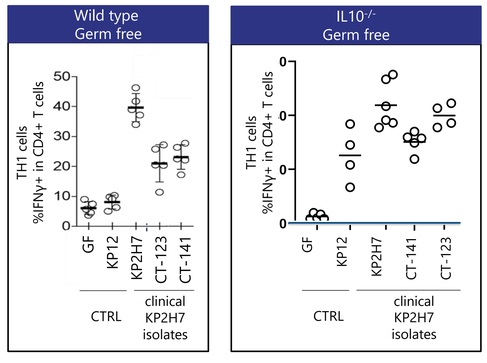


Figure 2: Induction of TH1 T cells following colonization with clinical KP2H7 isolates



Median (black line), CTRL - controls

Monocolonization with clinical KP2H7 isolates induced a higher percentage of TH1 cells (out of total CD4+ T cells) in the colonic lamina propria compared to colonization with a control *Klebsiella pneumoniae* strain KP12 (KCTC 2242) or no colonization.

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:

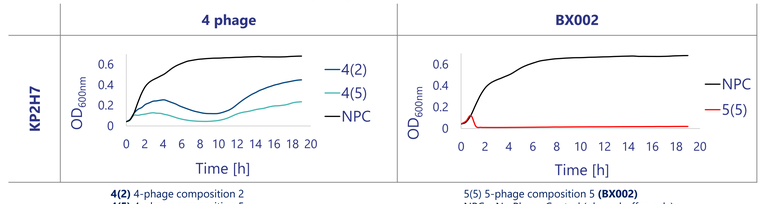
1. Creation of phage library → 2. "Wet" laboratory characterization → 3. In-silico characterization → 4. Phage scoring → 5. Cocktail combinations → 6. Cocktail characterization & optimization

Table 2: BX002 cocktail generations

Cocktail generation	Size
1st	3 phages
2nd	1st generation + 2 phages with different MoA

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



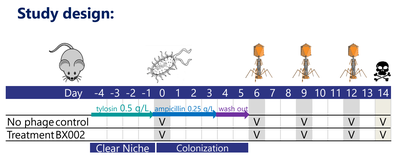
4(2) 4-phage composition 2
4(5) 4-phage composition 5
NPC – No Phage Control (phage buffer only)

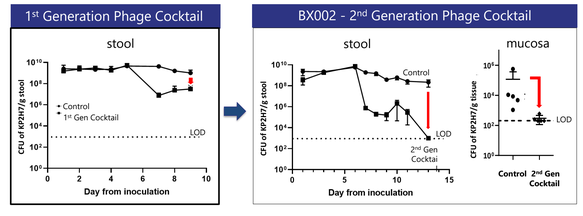
5(5) 5-phage composition 5 **(BX002)**
NPC – No Phage Control (phage buffer only)

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

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

Study design:

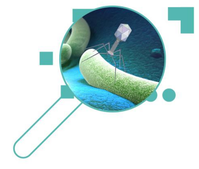




BX002 administration (3x) reduces target bacterial load by 4-5 logs in the stool and by at least 2 logs in the mucosa of colonized animals (down to the LOD of the assays).

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 mucosa of mono-colonized animals
- Clinical studies evaluating BX002 for the treatment of patients with IBD should be pursued



References: 1. Atarashi et al., 2017

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