Alterations in Microbial Diversity are Associated with Treatment Success with RBX2660, a Microbiota-Based Drug for the Prevention of Recurrent *Clostridium difficile* Infection: Results from PUNCH CD 2: a Randomized Double-Blind Placebo-Controlled Trial

Sahil Khanna, Gail Hecht, Erik Dubberke,
Robert Orenstein, Christine Lee and Dale Gerding.

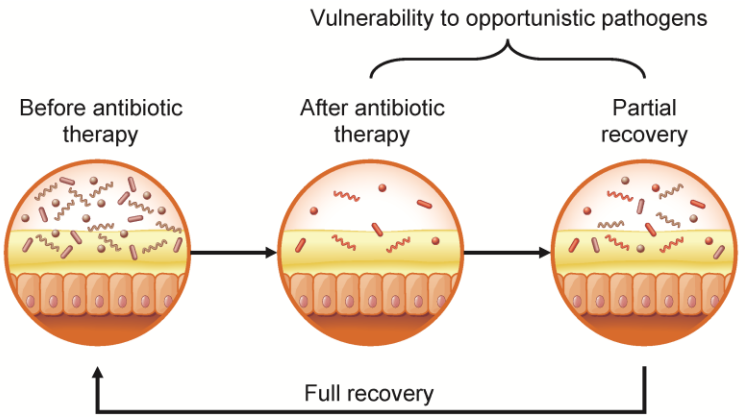
Digestive Diseases Week 2017
May 6th, 2017

Disclosures

- Research support
  - Rebiotix, Inc
  - Merck
- Consulting
  - Assembly biosciences
  - Rebiotix, Inc (paid to institution)

Recurrent *C. difficile* is a Difficult Problem

- ~83,000 cases of recurrent *C. difficile* infection (CDI) in the US every year
  - Difficult to treat with antibiotics for CDI
  - ~60% risk after 3 or more infections
- Disruption of the intestinal microbiome by antibiotics (both CDI and non-CDI)
  - Low colonization resistance
  - Increased spore survival



Khanna, Pardi. Expert Rev Gastroenterol Hepatol. 2016: Mar 16.
Lessa…McDonald. N Engl J Med 372:825-834

Microbiome based Therapies are Emerging

- Conventional fecal microbiota transplant
- Highly effective in preventing recurrent CDI
- Restore a disrupted gut microbiome
- Standard therapies such as RBX2660 are in clinical trials
- Lack of substantial data on microbiome changes with microbiota based therapies from placebo-controlled trials

Specific Aims

- Efficacy of a standard microbiome based therapy (RBX2660) for recurrent CDI
- Correlate efficacy of RBX2660 or placebo with longitudinal patient microbiome data
  - Do microbiome changes after therapy predict treatment response?

Investigational Product: RBX2660

- 50 g stool in 150 ml liquid suspension
  - ≥10^7 live organisms/mL
  - Single-dose ready-to-use enema bag
  - Stored and shipped frozen at ≤−80°C
- Thawed at 4°C for 24 hrs & delivered within 48 hrs
- Blinding: Investigational product or placebo shrouded in an opaque brown sleeve
  - Enema administrator (not involved in study follow-up) shrouds tubing and delivers enema

PUNCH CD 2 Clinical Trial Design

- Multi-center trial in the US & Canada between Dec 2014 & Nov 2015
- Participants randomized: 1:1:1 to the following
  - Group A: 2 doses of RBX2660
  - Group B: 2 doses of placebo
  - Group C: 1 dose each of RBX2660 & placebo
- Enemas administered on-site 7 days apart, no bowel preparation
- Failures in the blinded phase could receive up to 2 doses of open-label treatment

Patient Population

- Adults with ≥ 2 CDI recurrences or at least 2 CDI episodes resulting in hospitalization
- Positive *C. difficile* stool test with active symptoms within 60 days
- CDI symptoms controlled with antibiotics
- 24-48 hour antibiotic free period prior to dosing
- Common exclusions
  - Immunocompromized, IBS, IBD, Chronic diarrhea, celiac disease, chemotherapy, pregnancy, breast feeding

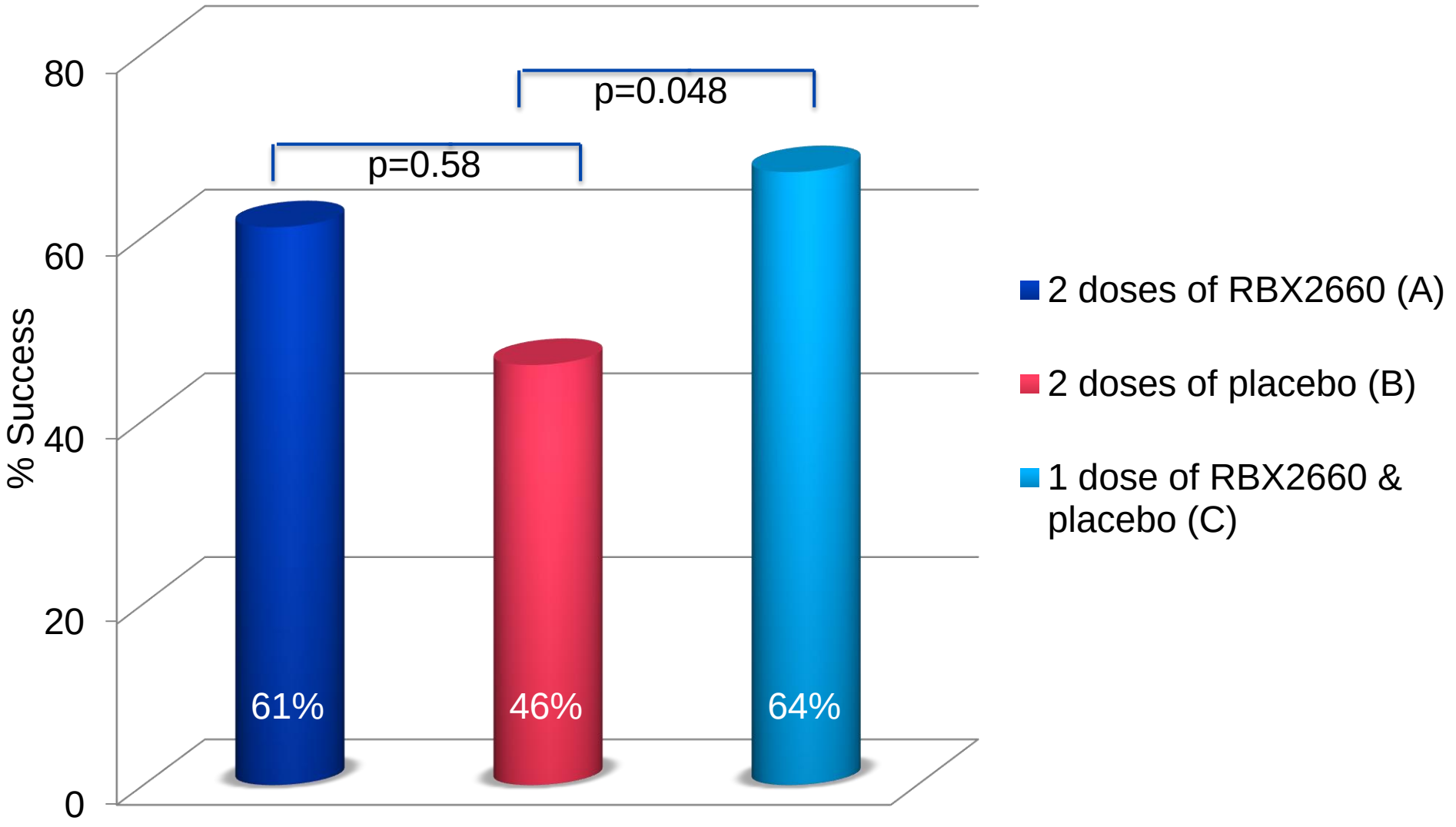
Endpoint Definitions: 8 Weeks

- Success:
  - Absence of diarrhea
  - No retreatment for CDI
- Failure (all 4 criteria required):
  - Presence of diarrhea, with or without other CDI symptoms
  - A positive stool test
  - Need for CDI retreatment
  - No other cause for diarrhea identified

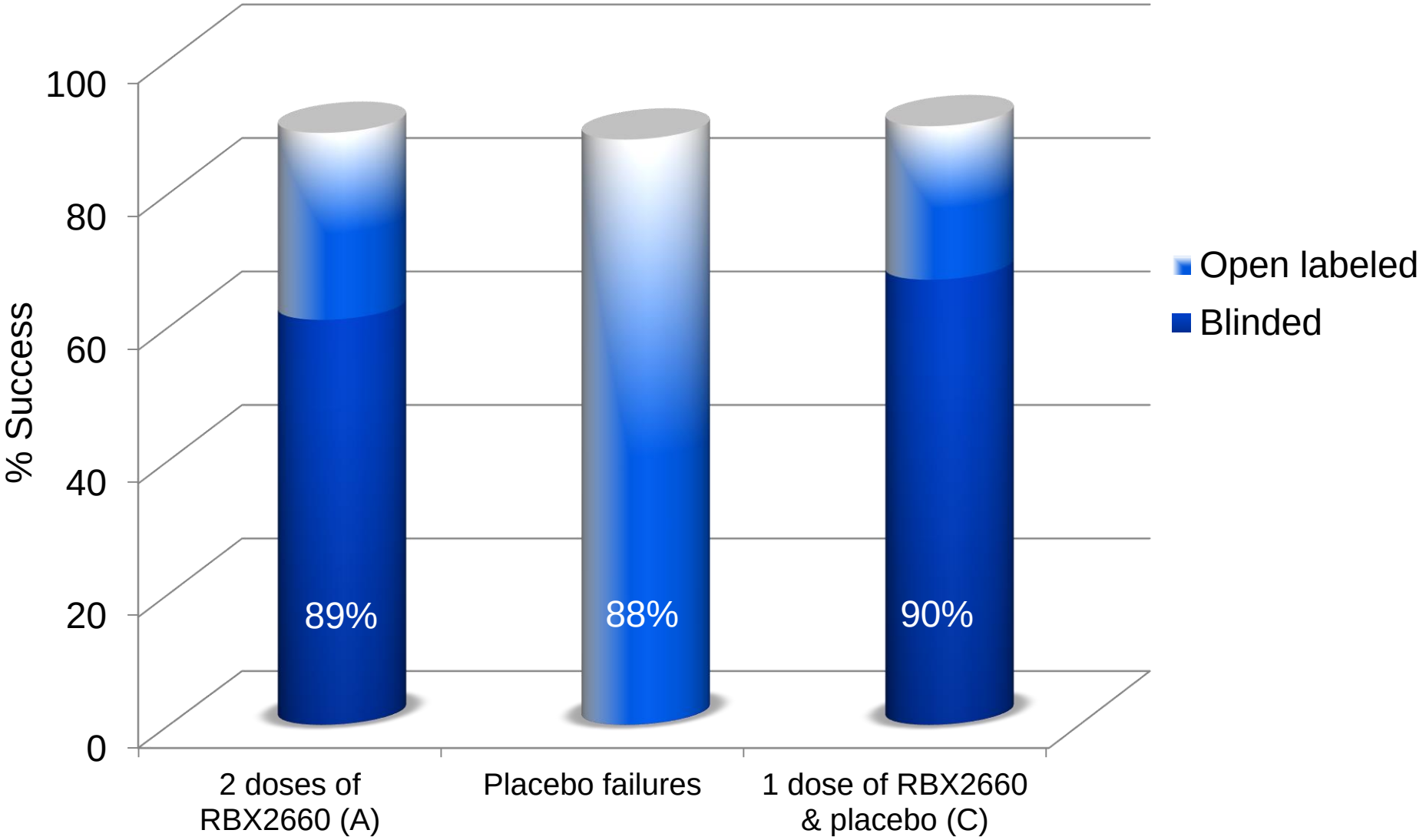
Baseline Characteristics

Characteristic	2 Doses of RBX2660 (N=41)	2 Doses of Placebo (N=44)	1 Dose of RBX2660 and 1 Dose of Placebo (N=42)
Mean age – years	62.8 ±19	58.8 ± 19	61.5 ± 20
Female sex- no. (%)	25 (61)	30 (68)	24 (57)
Race-White – no. (%)	39 (95)	42 (96)	40 (95)
Antibiotic screening – no. (%) Vancomycin Fidaxomicin Other	38 (93) 1 (2) 2 (5)	39 (89) 2 (5) 3 (6)	36 (86) 2 (5) 4 (10)
Median number of CDI episodes – no., (IQR)	4.0 (2)	3.0 (1)	4.0 (1)
Mean duration of CDI episode, days	18.8 ± 14	19.5 ± 17	16.4 ± 11
Healthcare-acquired CDI infection – no. (%)	10 (5.7)	10 (6.0)	13 (7.8)

RBX2660 is More Effective than Placebo



Overall Success for At least 1 dose of RBX2660 (blinded and open-label): 88.6%



Sample Collection – Microbiome Analysis

- Stool samples were collected at baseline, 7 days, and 30 days after the 2nd blinded dose
  - Group A (n=23)
  - Group B (n=28)
  - Group C (n=22)
- Active group includes subjects who received at least one dose of RBX2660 (Group A + Group C)

	Active (RBX2660)	Placebo	Total
Fail	18	15	33
Success	27	13	40
Total	45	28	73

Microbiome Analysis

- Longitudinal 16s rRNA analysis using the Illumina MiSeq platform
- The variable region V4 was targeted to identify the operational taxonomic units (OTUs) in each sample
- OTUs at each time point were compared to baseline and stratified by patient outcome

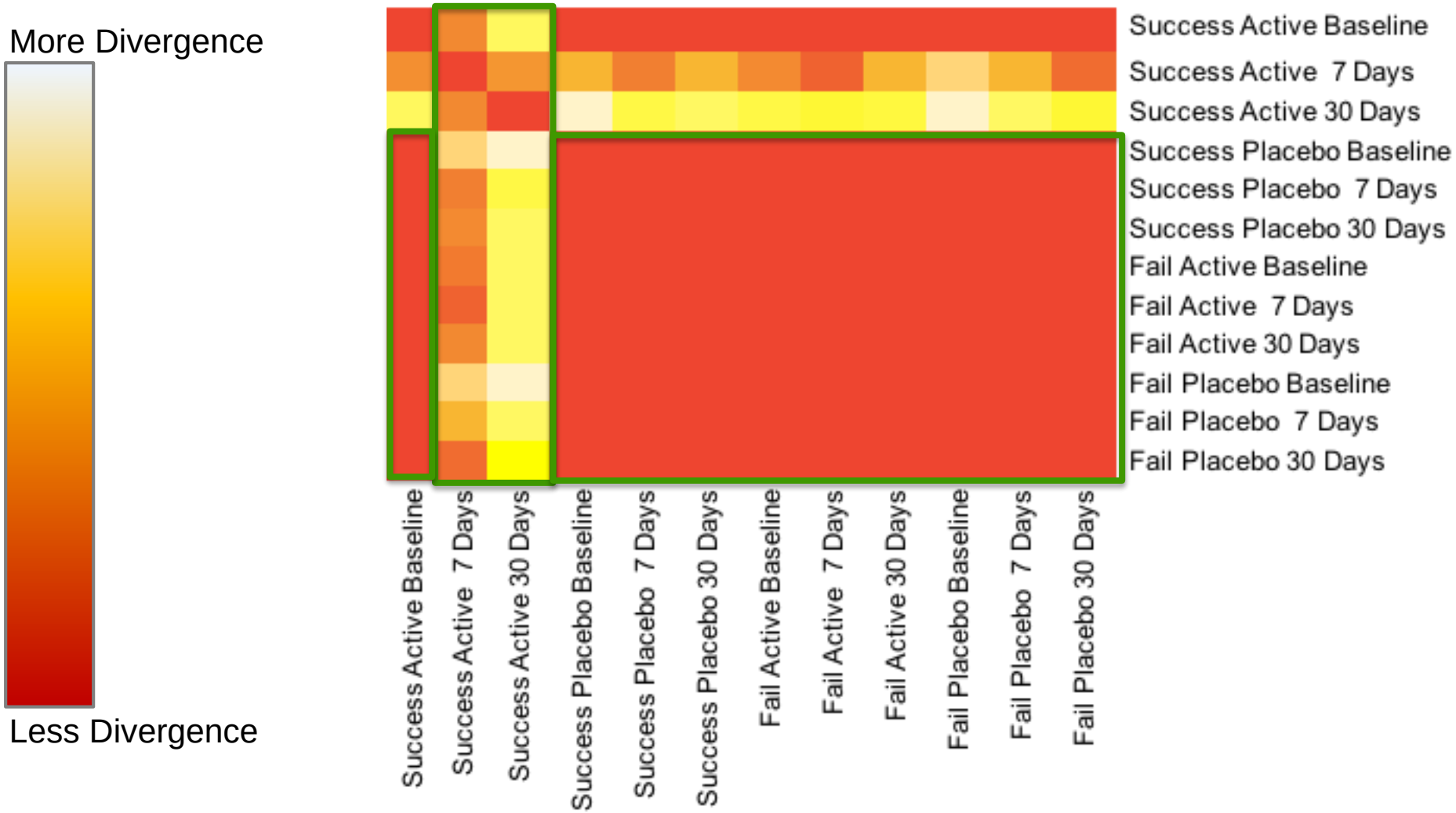
Microbiome Analysis

- Differentiation between sample community composition were visualized using a Kullback-Leibler (KL) divergence analysis model (BioRankings, St. Louis, MO)

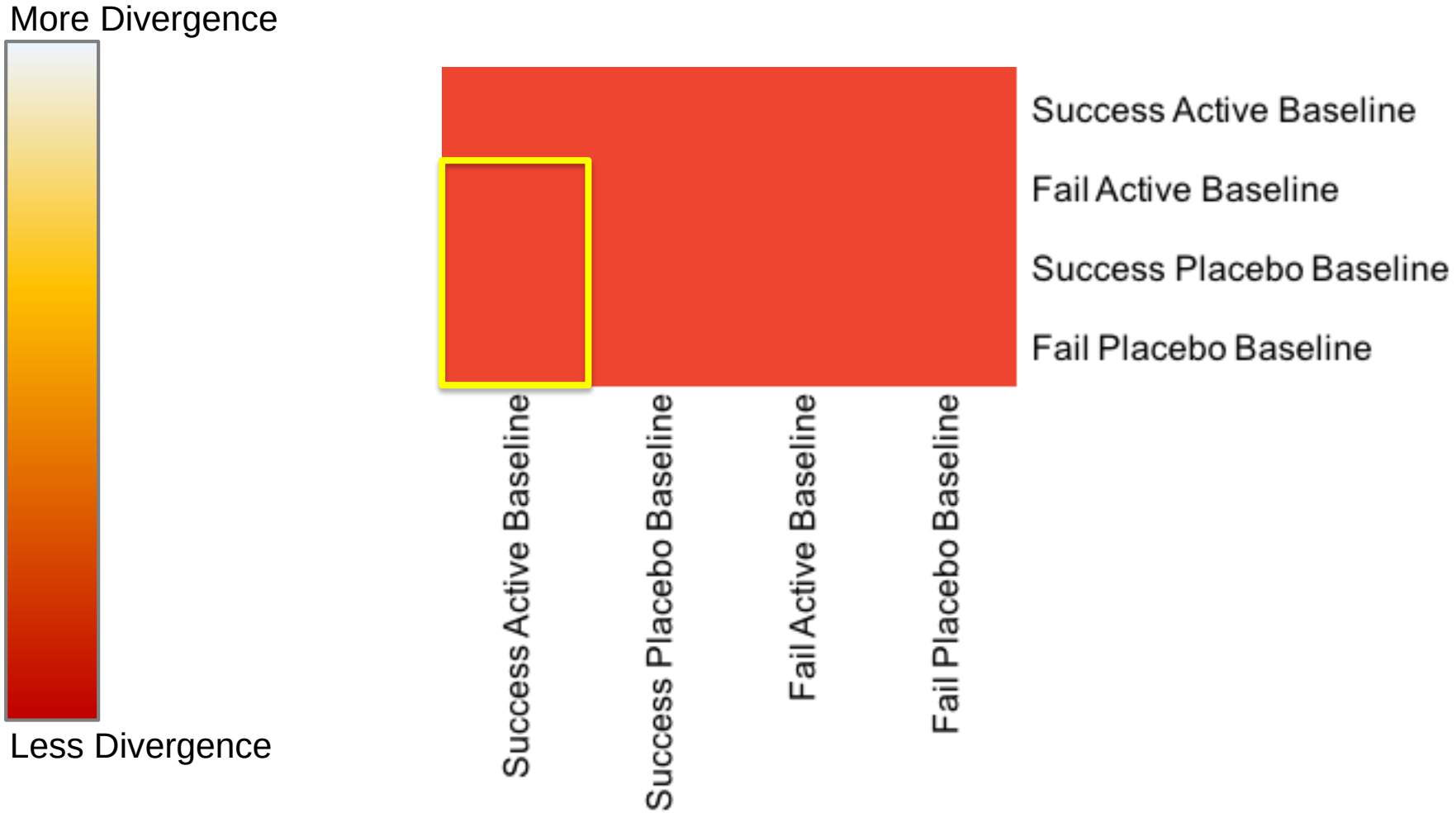
Less Divergence
Microbiomes are more similar

More Divergence
Microbiomes are less similar

Kullback-Leibler Divergence Analysis



No Differences in Baseline Microbiota Composition in Placebo and RBX2660 Treated Patients

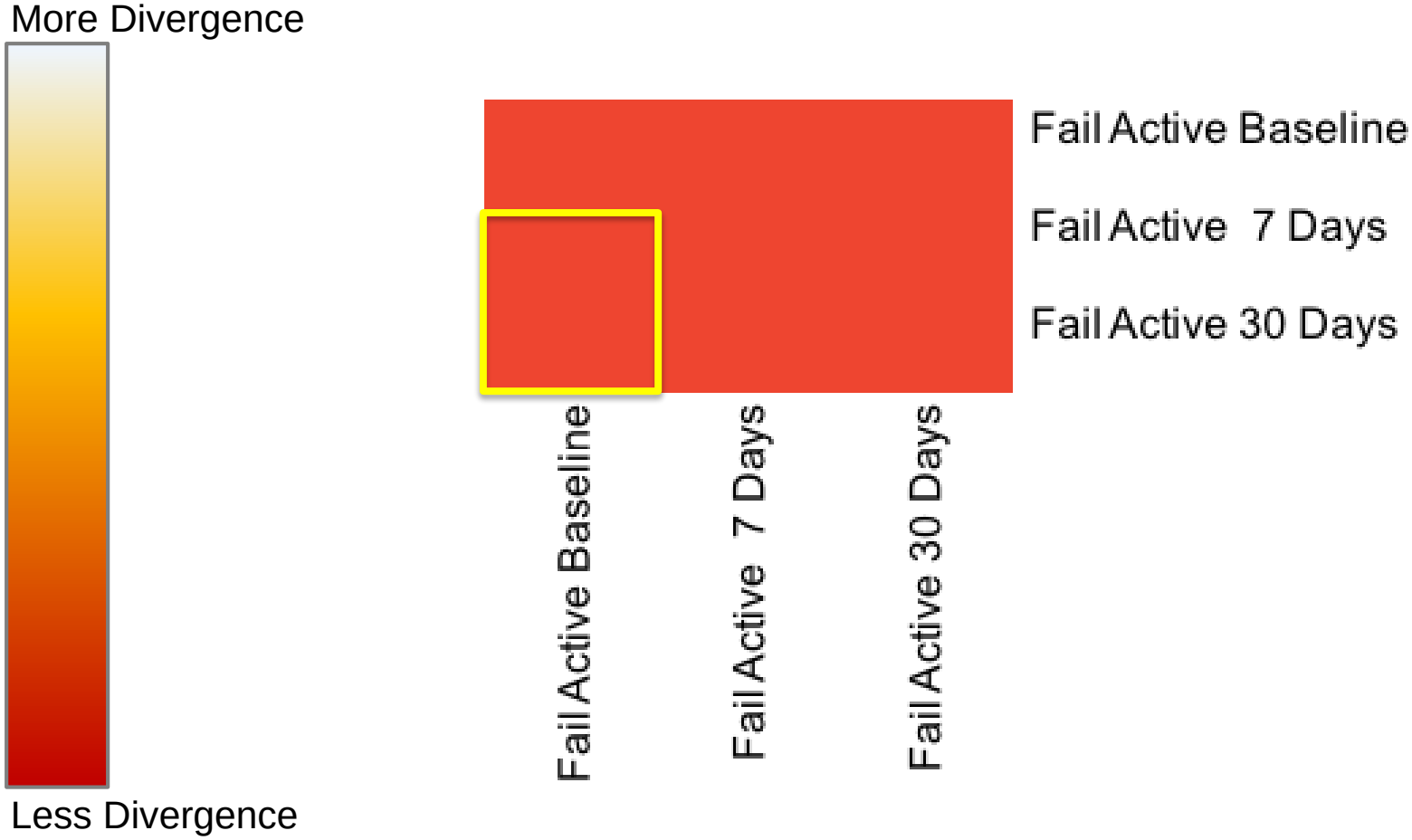


Kullback-Leibler Divergence Analysis

Placebo Responders have Similar Microbiota Composition at 7 and 30 days to Baseline

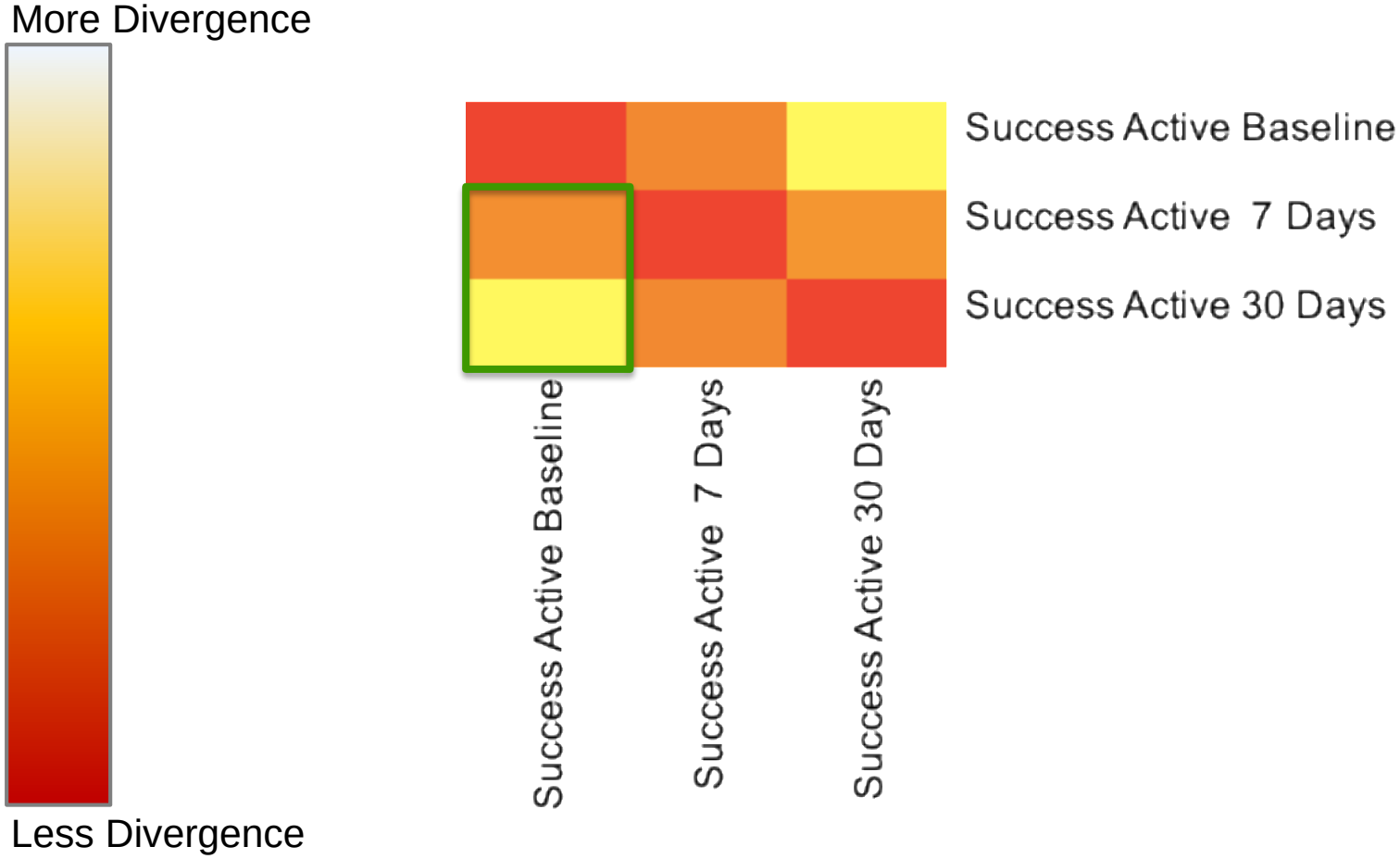
Kullback-Leibler Divergence Analysis

RBX2660 Non-responders have Similar Microbiota Composition at 7 and 30 days to Baseline



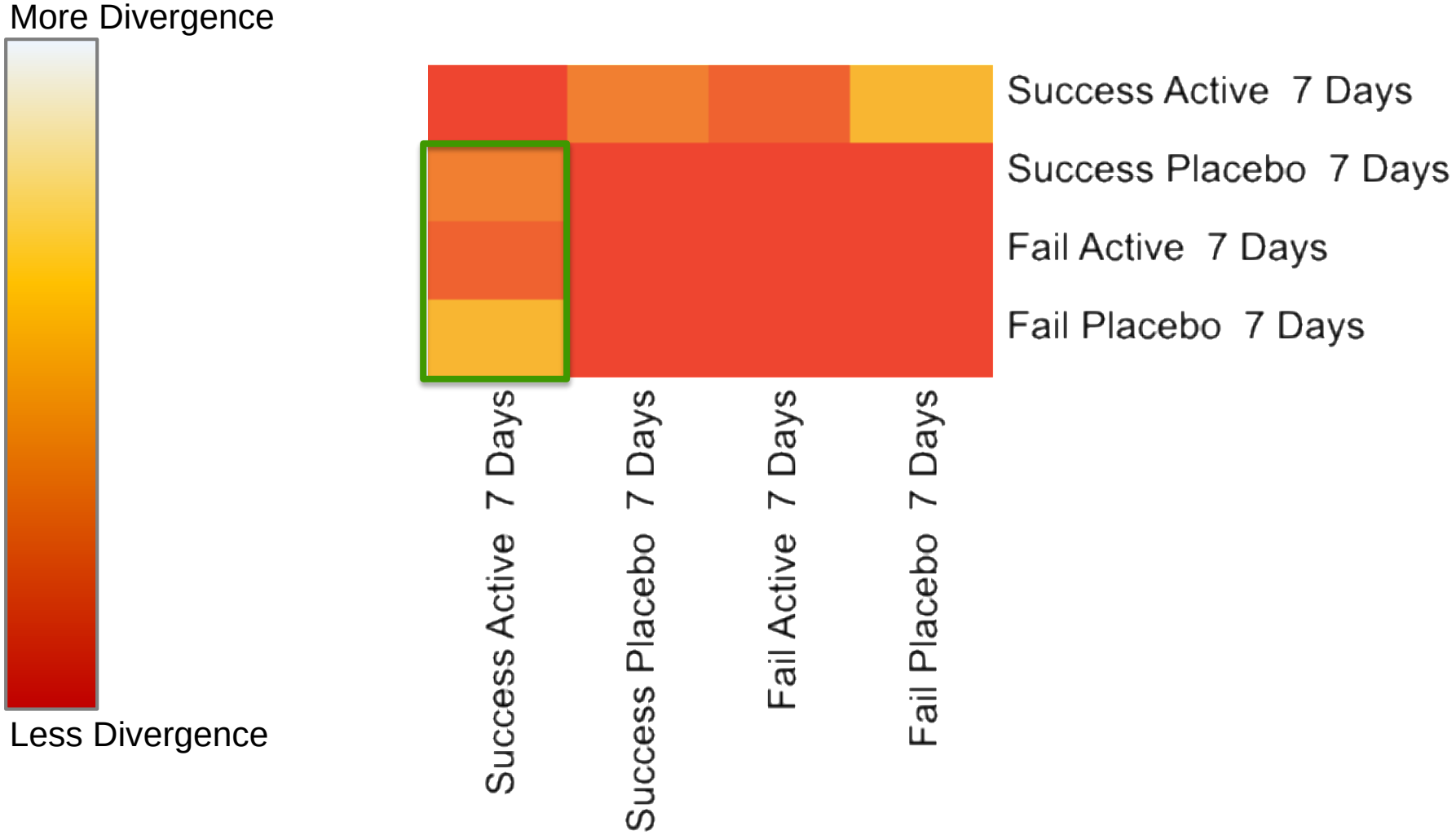
Kullback-Leibler Divergence Analysis

RBX2660 Responders have Different Microbiota Composition at 7 and 30 days from Baseline



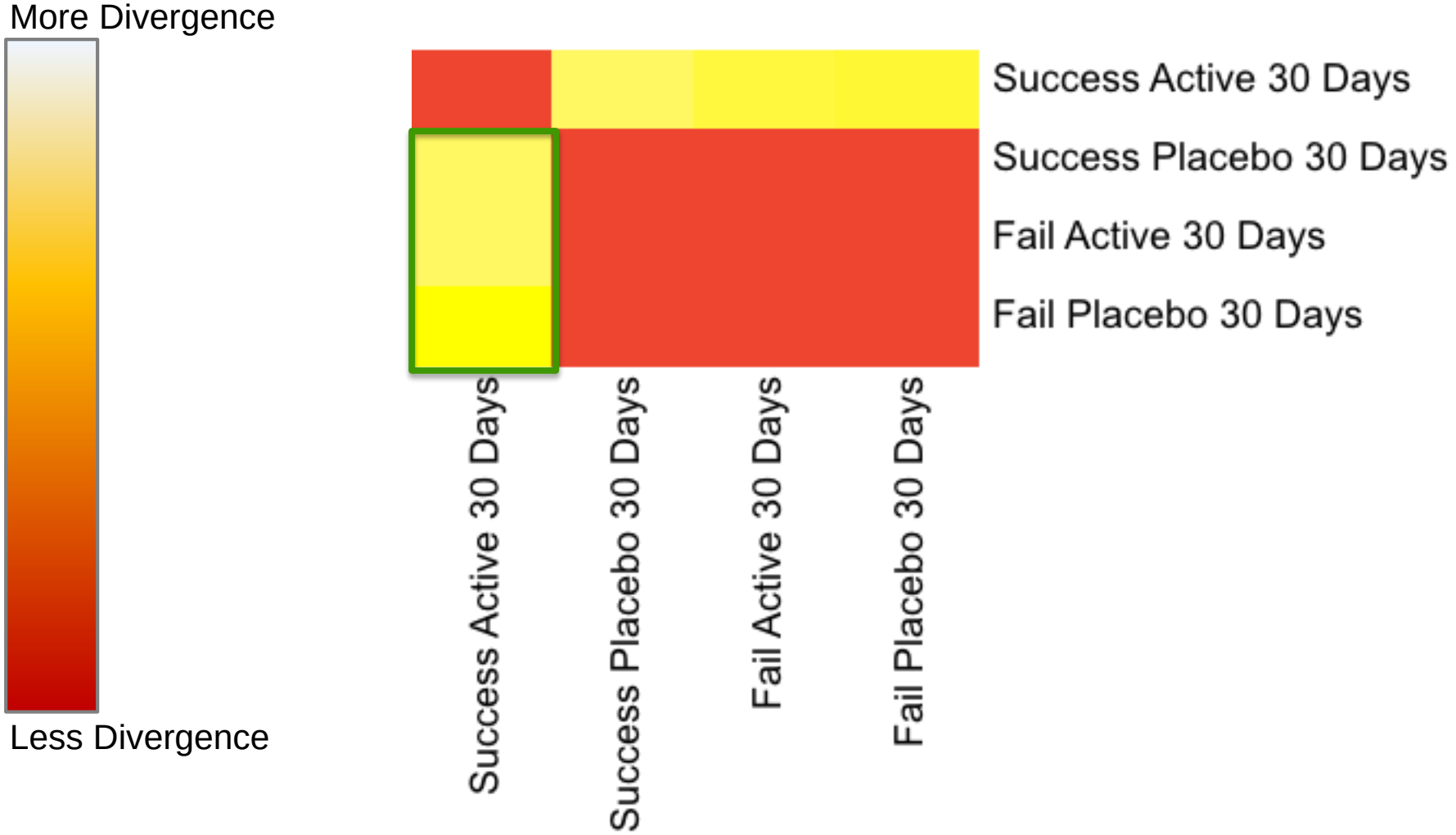
Kullback-Leibler Divergence Analysis

RBX2660 Responders Trend towards Different Microbiota Composition at 7 days from Failures



Kullback-Leibler Divergence Analysis

RBX2660 Responders have Different Microbiota Composition at 30 days from Failures and Placebo



Kullback-Leibler Divergence Analysis

Summary

- One dose of RBX2660 administered via enema is more effective than placebo for recurrent CDI
- Responders to RBX2660
  - Distinct microbiome changes from baseline in contrast to those who fail active treatment
- Responders to RBX2660
  - Different microbiota composition than responders to placebo
- Placebo responders demonstrate no changes in microbiota composition after treatment

Future Directions

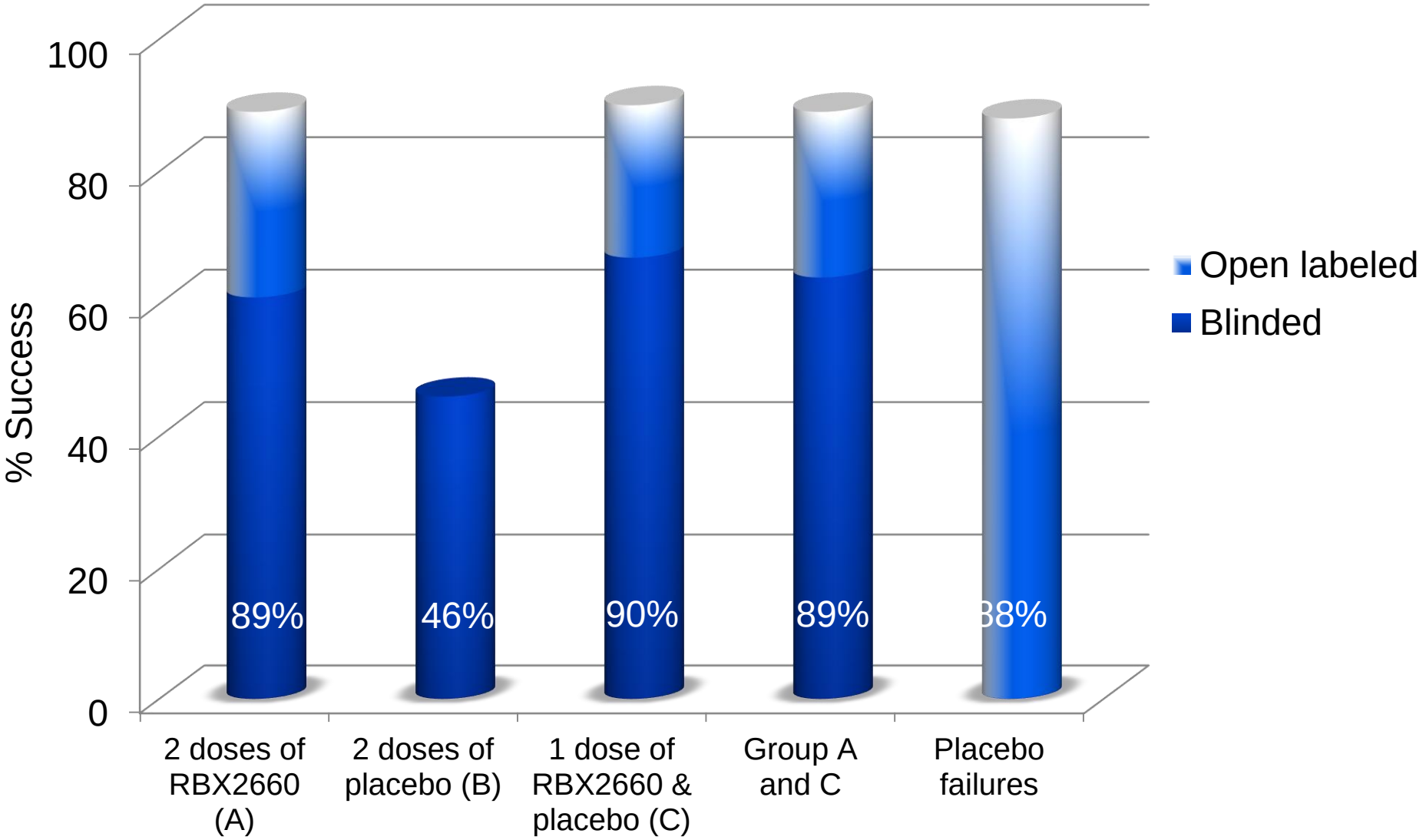
- Larger phase III trials are needed to demonstrate safety and efficacy of standardized microbiome based therapies
- In depth microbiome analyses to determine specific taxa at baseline and follow-up that may predict outcomes

Conclusions

- RBX2660 administered via enema is an effective treatment for recurrent CDI
- Responders to active therapy have distinct microbiome profiles compared to failures
  - Importance of bacterial engraftment
- Response to active therapy but not to placebo correlates with changes from baseline microbiome
- Changes in microbial profiles after microbiota-based therapies may predict treatment response

Questions?

Overall Success for At least 1 dose of RBX2660 (blinded and open-label): 88.6%



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