Clinical Development of a Live Biotherapeutic Product

Scott Plevy, MD
Chief Scientific Officer
Synlogic Inc.
Microbiome in Health and Disease

• The possibility of restructuring an unhealthy microbiome to modulate immunity and restore health represents a new direction in drug development and holds great promise for many patients
  • The gut microbiome is integral in regulating intestinal and systemic immune homeostasis
  • Dysbiosis has been identified as a major contributor in many diseases
Examples of Therapeutic Approaches

❖ **Bugs as Drugs:**
  o Minimally processed stool (e.g. FMT)
  o Purified Bacterial Strains
  o Cultured Bacterial Strains

❖ **Modified Bugs:**
  o Genetically modified bacterial strains

❖ **Drugs from Bugs:**
  o **Antibiotics**
    o Bacteria produce antibiotics
  o **Anti-inflammatory molecules**
    o E.g. Short chain fatty acids, secondary bile acids, tryptophan metabolites

❖ **Kill the Bugs:**
  o **Antibiotics**
  o **Bacteriophages**
    o Virus that infects and replicates with a bacterium
    o Estimated there are more bacteriophages on the planet than every other organism on Earth

❖ **Drug the Bugs:**
  o Bacterial Toxin Inhibitors
  o Inhibiting bacterial enzymatic pathways
A live biotherapeutic product (LBP)

An LBP as defined by the FDA is a biological product that:

• 1) contains live organisms, such as bacteria;
• 2) is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and
• 3) is not a vaccine

LBPs can also include genetically modified microorganisms, termed recombinant LBPs, that contain microbes with purposeful addition, deletion, or modification of genetic material.

Many challenges must be addressed to deliver LBPs as drugs

- Target ID & validation
- Screening & optimization
- Pre-clinical
- Clinical
- Production
- Commercial

What assay systems will we need for candidate screening?

How will we incorporate multi-omics?

What does a PK/PD study look like for bacteria?

How do we scale up production?

How do we sell bacteria?

How can we study microbes in living systems?

How will we culture different bacterial strains?

What will be required for toxicology studies?

How do we position this to the health authorities?

How can we ensure that strains engraft for sustained period of time?

How will we curate large data sets needed for in-human development?

How will we deliver the bacteria to patients?

How can we manufacture with consistent identity, purity, potency?

How do we position this commercially?

What advanced disease models will be needed?

What will the clinical development program look like?

How do we determine dose and regimen?

What patients will be candidates?
Fecal Microbial Transplant (FMT) has demonstrated proof of concept in treating Ulcerative Colitis with biologic-like activity.

There are theoretical and practical drawbacks to using FMT in patients

- Undefined product & donor variability
- Extensive stool pathogen and serological testing
- Potential safety risks, i.e. transferring infectious agents
- Risks from uncharacterized microbes
- Difficult routes of administration
Important Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Risk of Serious Adverse Reactions Due to Transmission of Multi-Drug Resistant Organisms

The Food and Drug Administration (FDA) is informing health care providers and patients of the potential risk of serious or life-threatening infections with the use of fecal microbiota for transplantation (FMT). The agency is now aware of bacterial infections caused by multi-drug resistant organisms (MDROs) that have occurred due to transmission of a MDRO from use of investigational FMT.

FDA Web site; June 13, 2019
An alternative approach to FMT: LBPs

**An oral drug product that**

- Circumvents the safety issues associated with FMT
- Contains **defined** microbes that are well **characterized**
- Produced and administered in a standardized manner
- Rely on a simplified preclinical package (i.e. no toxicology or DDI studies)
VE202: a rationally selected immune-modulating LBP to treat IBD

Clinical development of an LBP consortia: Current thinking from regulatory agencies

**Pre-clinical**

• “Traditional” toxicology package not applicable
  - ✓ Individual strains need to be sensitive to multiple classes of antibiotics
  - ✓ Intrinsic and transferable antibiotic resistance must be evaluated
  - ✓ High quality genomic sequencing of strains to assess:
    • Strain identity
    • Presence of antibiotic resistance genes
    • Virulence genes
    • Mobile genetic elements (transfer Abx resistance to GI bacteria)
• For consortia, inclusion of each strain needs to be justified
The Janssen experience: The path of drug development for VE202

2015
- Acquisition of 17 bacterial strains from Vedanta Biosciences
- Full genomic and antibiotic sensitivity characterization of all strains
- Established master cell banks
- Pre-clinical experiments validating and extending published work with novel findings
- Optimized manufacturing for clinical scale production & release testing
- Pre-IND regulatory meetings with the FDA and in Europe
- Microbiome bioinformatics analysis pipeline established
- Determined lead consortia for clinical development
- Learnings from the field applied to development of clinical study design (e.g., requirement for vancomycin pre-treatment)
- FIH clinical study initiated 4Q2018

2018
Current Thinking from Regulatory Agencies

**Clinical**

- Use of healthy volunteers in early phase studies and selection
  - Can and should factors be standardized (i.e. diet, geographic location, etc)?
  - Is microbiome stratification possible or required?

- Use of pre-treatment antibiotics must be justified

- Dose rationale and regimen
  - Use of existing data (i.e., FMT, probiotics) to estimate dose (CFU)
  - How is regimen determined? From dose/persistence/efficacy?

- General criteria for evaluation of safety
  - Blood culture evaluation in febrile subjects (suspected translocation)

- Microbiome specific endpoints
  - What is PK/PD for a microbial product?
  - Colonization, compositional changes, metabolites and durability of effects
  - Assay development must be able to detect these endpoints
First-in-human general design

• VE202(11) and VE202(16) will be tested in the first in human study
• Informed by previous work in the field (eg, vancomycin pretreatment and dose)
• Key Goals:
  • Assessment of Safety
  • Assessment of pharmacokinetics of colonization/engraftment/microbial community shifts under various conditions
    • Single dose (1-day) and Multiple dose (14-days)
    • $1 \times 10^{10}$ CFU/day vs $1 \times 10^{11}$ CFU/day
    • Vancomycin pre-treatment vs. No pre-treatment
  • Assessment of targeted biomarkers
    • Microbial composition
    • Short chain fatty acids
    • Secondary bile acids
• Study initiated Q4 2018
Questions in the clinic beyond phase 1

• Phase 2a
  • Will VE202 be safe, viable, detectable, colonize, and engraft in people?
  • Will VE202 have biological activity in UC patients?
  • Will any LBP recapitulate clinical signals seen with FMT?
    • If so, what is the best way to define an optimal consortium?
    • If not, what went wrong?

• Phase 2b
  • How will we define dose and dose regimen?

• Phase 2-3
  • What is the right patient population?
  • What are PD/response biomarkers?

• Development will be an iterative process driven by clinical signals
Current Thinking from Regulatory Agencies

**Manufacturing**

• Current FDA guidance is mostly focused on manufacturing requirements of LBPs
• Produce master and working cell banks to generate clinical drug substance material
  ▪ Aerobic vs anaerobic and manufacturing facilities
• Drug product (e.g. standard testing for capsules/tablets)
• Development of a potency assay that reflects the overall mechanism of action for late stage development
  ▪ For early development, the agency has been accepting CFU
  ▪ Challenge in defining mechanism of action for a consortium of strains
Manufacturing Challenges

Master Cell Bank

- Pre-culture
- Main culture
- Concentration
- Diafiltration
- Freeze-Drying
- Drug Substance
Synthetic Biotic™ Medicines
Designing A Novel Class of Engineered Living Medicines

**SYNTHETIC**
- Designed genetic circuits to execute biological functions
- Degradation of disease-causing metabolites
- Production of therapeutic molecules

**BIOTIC**
- Bacterial chassis
- Non-pathogenic
- Amenable to genetic manipulation

**PATHWAYS, COMBINATIONS, BIOMARKERS**

**PROGRAMMABLE POTENCY AND CONTROL**

**LOCAL ACTIVITY, REDUCED SYSTEMIC TOXICITY**
Synlogic’s EcN Platform has Numerous Favorable Drug Developmental Characteristics for Use as a Human Therapeutic

- **Fit-for-Purpose in Target Indications**
  - Oral Regulator of Metabolic Disease
    - Well tolerated and metabolically active in GI track
  - Oral Immunomodulatory Agent
    - Intrinsic anti-inflammatory properties from GI track
    - No detectable impact on microbiota composition in healthy human volunteers
  - Immunotherapeutic for Oncology
    - Intrinsic tumor tropism and colonization
    - Innate immune activation when localized within tumors

- **Manufacturing**
  - Bacterial production scalable to large scale fermenters at high cell densities
  - Tolerant to downstream processing (high viability after freezing and lyophilization)

- **Advantageous Regulatory Characteristics**
  - Extensive history of safety and tolerability in humans as probiotic
  - SYNb strains tested in healthy human volunteers at doses up to $5 \times 10^{11}$ CFU for up to 14 days
  - Safe and well tolerated in preclinical toxicology studies in mice and non-human primates
  - Favorable FDA interactions to date
Current Thinking from Regulatory Agencies

- Concerns mainly around genetically modified organisms
  - Genetic stability of transgenes
  - Potential for transfer to other bacteria
  - Biocontainment
- Otherwise, streamlined safety requirements as chassis and biomarkers are GRAS
Synthetic Biotic Portfolio: Breadth and Potential
Initial Applications Designed to Target Different Sites of Action in Metabolic and Immunomodulatory Diseases

- **METABOLIC DISEASES**
  - Rare Metabolic Disease
  - Broad Metabolic Disease

- **IMMUNOMODULATION**
  - Immuno-Oncology
  - "Cold" Solid Tumors
  - Inflammatory and Autoimmune

Small or Large Intestine
SYNB1618 Mechanism of Action

Amino acids from dietary proteins (absorption and recirculation)

Healthy

Phenylalanine (Phe)

PKU

Impaired PAH

Accumulation of Phe to toxic levels

SYNB1618

Manage Phe levels

Phenylalanine (Phe)

Phenylalanine Hydroxylase (PAH) converts Phe into Tyrosine

Tyrosine

Engineered Probiotic Bacteria: E. coli Nissle
Components of Synthetic Genetic Circuit

Phenylalanine

Phenylalanine

FNR

FNR

pheP

PAL3

trans-Cinnamic Acid (TCA)

Hippuric Acid (HA)

trans-Cinnamic Acid (TCA)

FNR

FNR

Metabolic Conversions

LAAD

AraC

AraC

Phenylalanine (Phe)

Phenylpyruvate (PP)

When Phe is not efficiently metabolized (PKU)

SYNB1618 provides an alternative mechanism

• PAL3: produces TCA which is converted to HA in the liver and is excreted in urine
• LAAD: produces phenylpyruvate (PP)
**SYNB1618 Phase 1/2a Study Design**

### Part 1: Healthy Volunteers
- **Single Ascending Dose (SAD)**
  - 6 cohorts, N = 24
  - Single Dose (SD)
    - N = 4
- **Multiple Ascending Dose (MAD)**
  - TID x 7 days
  - 4 cohorts, N = 32
  - Multiple Dose (MD)
    - TID x 7 days
    - N = 10

### Part 2: PKU Patients
- **Single Dose (SD)**
  - N = 4
- **Multiple Dose (MD)**
  - TID x 7 days
  - N = 10

Presented in September 2018

**PKU Clinical Trial Design**
- Randomized, double-blind placebo-controlled study at multiple sites in the US
- Primary outcome: establish safety/tolerability following single and multiple doses in HV and PKU patients
- Secondary outcome: SYNB1618 kinetics in feces
- Exploratory: change from baseline in plasma and urinary biomarkers of Phe metabolism

**New Data**
SYNB1618 in the Clinic: Activity
Statistically Significant and Equivalent Activity of SYNB1618 in Healthy Volunteers and Patients

Protein shake / meal
D5-Phe
SYNB1618 or placebo

Measure over 6hrs:
Plasma:
• Phe/D5-Phe
• TCA/D5-TCA
Urine: HA/D5-HA

Key: HA: Hippurate, D5-HA: labeled HA, CFB: change from baseline, CFP: change from placebo
• Rapid development of microbiome science in understanding fundamental processes of human health and disease are leading to novel therapeutic approaches

• Understanding and expectations of physicians and patients regarding opportunities is also growing

• Many different approaches in the clinic to alter the microbiome and utilize microbiome components as therapeutic tools.

• Different thinking about PK/PD will have to be applied

• Opportunities to define and shape global regulatory policy:
  • Clinical trial design
  • Manufacturing standards

• Learning in the clinic through iterative human experiments will be the norm