Targeting NETs in human disease

Demetrios Braddock
Team

Reinaldo M. Diaz, MBA: Lead investor and Board of Directors, Inozyme Pharma, Venture partner at Longitude Capitol Management, LLC; Managing director of DA Advisors LLC; co-founder of Diaz & Altschul Capital Management, LLC.

Gene Griffin, D.V.M., M.S: Senior Director at Alexion Pharma (Solaris, Strensiq, ENPP1-Fc in collaboration with Braddock Lab), VP Therapeutic Head at CureVac, 30 years of drug development experience in pharmaceutical and biotechnology companies including Biogen, Sandoz, Bristol-Myers Squibb, and Warner lambert-Parke-Davis

Demetrios Braddock MD/PhD: Assoc. Prof. Yale; Expertise in enzyme therapeutics and biologic development; Scientific Founder of Inozyme (Series A Jan 2017, IPO July 2020); Scientific Founder of Petragen (Series A Oct 2020); Founder and Medical Director of Precipio (2011-2017).

David Lewin PhD: Sr. Assoc. Dir. Bus. Dev. Yale, OCR; David and Demetrios have worked closely together for over 15 years; collaborations include the founding of Inzoyme, Precipio, and Petrogen.
Background and Rationale: Overview

A. Increased NET formation and reduced degradation drives the morbidity/mortality in
- Thrombotic conditions including
  - ANCA Vasculitis, Anti-phospholipid syndrome
  - Acute Kidney Injury, ST-elevation following MI
- Autoimmune disorders such as Lupus, Hypocomplementemic urticarial vasculitis syndrome (HUVS), others
- Severe COVID-19 infection
- Cancer Metastasis

B. Neutrophils ‘self destruct’, shooting ‘NETs’ of DNA in the presence of:
- Foreign viral and micro-organisms
- Specific High Grade (highly metastatic) Tumor Cells including
  - Breast Cancer
  - Esophageal Cancer
  - Lung Cancer
  - Esophageal Cancer

Neutrophils casting a net entrapping Helicobacteria

Dr. Volker Brinkman, Max Planck Institute for Infection Biology, Berlin Germany (ref 28)
A. Several human enzymes are reported to degrade NETs
   - DNAse1
   - Dnase1L3
   - Others

B. Efficacy of enzymes limited by poor bioavailability and pharmacologic properties
   - A 1999 study to determine efficacy of DNAse1 in Lupus (Genetech & NIH)
     - drug was well tolerated
     - no neutralizing antibodies
     - failed to achieve therapeutic bioactive concentrations and was therefore ineffective

C. Braddock Lab has expertise engineering stable and bioavailable enzyme therapeutics.
   - ENPP1 enzyme for GAC1:
     - Can be lyophilized to a powder and rehydrated, retaining full activity
     - Lyophilized enzyme can be heated to 100° F for 3 months, rehydrated, without aggregation or loss of activity
     - Half-life of 36 hours
     - Predicted human dose is 2 mg/Kg sub-Q, twice a week
     - First in human dosing to begin in first quarter, 2021
     - Recently increased potency of ENPP1 ERT to enable bimonthly dosing at 0.3-0.6 mg/Kg

D. Braddock lab has applied their experience in enzyme biologics to design and optimize stable and long acting DNA degrading Biologics (DDB’s)

CMC

Rigorous, scalable, amenable to GMP production
Pharmacodynamics

Bioactivity for over 10 days following single sub-Q dose

1 mg/Kg 1671

1 mg/Kg 1687

166 hrs. after dosing

257 hrs. after dosing
Murine models of NET driven Thrombosis and Tumor Metastasis

**Thrombosis:** DNAse1 and DNase1L3 knockout mice stimulated with GCSF: Mortality in 7 days

**Acute Kidney Injury:** Rodent model of renal injury on ventilator

**Tumor Metastasis:**
1. Tail vein injection of high grade metastatic tumors
   - Breast
   - Lung
   - Esophageal
   - Pancreas
2. Spontaneous Metastasis
   - Breast: implantation into mammary fat pad w/ and w/o resection
   - Lung/esophageal/pancreas: Implantation into flank
Timelines and Budget

- **Preclinical animal models of Thrombosis and Oncology:** Begin upon funding, Thrombosis models of Acute Kidney Injury, Oncology models to include Triple neg breast, Pancreatic, and Esophageal cancer.
- **Clinical cell bank and Master cell lines:** To begin within 3 months of successful preclinical animal models.
- **Series A raise:** To begin after Master Cell Bank established.
Summary

We have developed optimized DDB ready for *in vivo* efficacy studies in established animal models of NET driven thrombotic and oncologic diseases

- DBB’s are highly stable
  - Retain activity for 3 months at room temperature
  - Can frozen and stored in an aqueous buffer at -80°C without loss of activity
  - Amenable to multiple freeze-thaw cycles without loss of activity
- Rigorous purification amenable to high-throughput bio-production has been established.
- PK and PD have been determined
  - *in vivo* bioactivity of lead constructs is confirmed out to at least 257 hours.
- IP on composition of matter and indications filed by Yale OCR (OCR7857)
- *In vivo* efficacy studies are now underway