Lenima Field Diagnostics

Molecular Diagnostic Point of Care Testing

Wan Shih
Founder
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Lenima Diagnostics Vision

- Improving healthcare by developing accurate, easy to use, rapid, and cost effective molecular diagnostic tests to the point of care.
MDX Fastest Growing Segment Within IVD Space

- $5.9 Billion (2011) and estimated to grow to $10.9 billion 2015 (Research and Markets, 2/12 Molecular Diagnostics: Market Segmentation and opportunities)
  - Chronic Diseases of Aging Population
  - Increased Availability of Tests
  - Further adoption of pharmacogenomics/personalized medicine
- Major competitors include BioPharma (Abbott, Roche), IVD/MDX pure play companies (Myriad Genetics, Cepheid, Gen-Probe), and research tool based companies (Illumina, Life Technologies)
- M&A deals valued at $4.7 Billion in 2010 with 45 deals
  - Growth will continue with drivers that include companion diagnostics and early detection attracting interest from large diagnostic companies and pharma
First Application

A rapid, low-cost, accurate and point-of-care *Clostridium difficile* infection (CDI) diagnostic tool
Team

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—Chair & Professor of Emergency Medicine, Drexel University College of Medicine

Joe Zack
—Business Advisor
**Clostridium difficile** Infection (CDI)

- *Clostridium difficile* (CD) is an anaerobic, spore forming, gram-positive rod-like bacterium that produces toxins A and B.
- CD spores persist on surfaces for months, can only be destroyed by bleach.
- CD spores are transferred to patients via hands of healthcare personnel who have touched a contaminated surface or item.
- CDI is a serious healthcare-associated infection (HAI) for all types of healthcare facilities, including hospitals, nursing homes, and outpatient clinics.
CDI Prevalence & Mortality are Increasing

- CDI prevalence have more than quadrupled in the past two decades and remain at historically high levels while most types of hospital-associated infections (HAIs) are declining.
- Deaths related to CDI increased 400% between 2000 and 2007, due in part to a stronger germ strain.

CDI Transmission / Financial Burden

- 3 million CDI cases annually in the US
- Accounts for 20-30% of hospital-associated diarrhea
- Causes 14,000 annual deaths in the US
- Cost > $3B to treat in the US annually
- ~50% CDI occur in people younger than 65, but >90% of deaths occur in people 65 and older
- CDI risk generally increases with age; children are at lower risk
- About 25% of CDI first show symptoms in hospital patients; 75% first show in nursing home patients or in people recently cared for in doctors' offices and clinics
Treatment / Patient management

Treatment

- First step is discontinuation of antibiotic therapy
- Mild diseases are treated with oral Metronidazole
- Severe diseases are treated with Vancomycin
- In rare cases, surgery may be needed
- Relapse or reinfections occurs in 12-24% of patients

Patient management

- CDI patients are isolated in a single room or cohorted with other CDI patients
- All healthcare workers and visitors must wear gloves and gowns when entering the room of CDI patients
Current CDI Diagnosis

(A) Combination of EIA and NAAT

- GDH EIA test
  - GDH N
  - GDH P
  - CDI N

- Toxins EIA test
  - Toxins EIA P
  - Toxins EIA N
  - CDI P

- NAAT test
  - NAAT N
  - NAAT P
  - CDI P

(B) NAAT stand alone test

- NAAT test
  - NAAT N
  - NAAT P
  - CDI N
  - CDI P

NAAT: Nuceic Acid Amplification Test (of toxin genes)

EIA: Enzyme enhanced ImmunAassay

GDH: surface antigen
Emerging Epidemic Hyper-virulent Strains

- Since 2005, hyper-virulent strains such as BI/NAP1/027 are emerging
- Hyper-virulent strains possess a third toxin, **binary toxin gene**

- CDI 30-day mortality rate
  - 17% without binary toxin gene
  - **28% with binary toxin gene**

- CDI recurrence rate
  - 17% without binary toxin gene
  - **28% with binary toxin gene**

- Early detection and correct treatment is critical to reduce severe outcomes
- Detection of the binary toxin gene in addition to the toxins genes is important to combat CDI
Unmet Need
Accurate, Affordable, multiplexed, Rapid and Point-of-Care test

Sensitivity

Low

High

Cost

Low

High

Unmet need

NAAT
Meridian, Cepheid,
Nanosphere, BD, Prodesse

$35-$58/gene
Sensitive (>90%) but expensive
Does not multiplex, adding binary toxin gene would further increase the cost

$15/target
Toxins EIA/toxins & G DH EIA
Meridian, Remel,
Quick Chek

Sensitivity of toxins EIA is only 60%
Piezoelectric Plate Sensor (PEPS) Array

- Rapid, sensitive, and yet low-cost detection using PEPS with
  - \textit{in situ} bacteria lysing,
  - \textit{in situ} DNA release,
  - \textit{in situ} DNA denaturing,
  - \textit{in situ} DNA detection All in 40 min

- With \textit{PCR-like sensitivity} but no DNA extraction, concentration, and amplification
- \textbf{Real-time genetic} detection using array piezoelectric plate sensors (PEPS) with a $500$ impedance analyzer
PMN-PT piezoelectric plate sensor (PEPS)

PMN-PT PEPS: (1) 1 mm x 0.5 mm made
(2) made of PMN-PT freestanding film 8 μm thick
(3) operated at length extension mode (LEM)
or width extension mode (WEM)

In air
In PBS
With MPS insulation
k_{31} = 0.34

(a)
PMN-PT Piezoelectric Plate Sensor (PEPS)

Rapid, Label-Free Sensing

Phase Angle ($\theta$)

Target antigen/analyte

PMN-PT PEPS

Impedance analyzer

frequency

$f_2$

$f_1$
WYS and WHS have worked on PEPS and its predecessor, PEMS

- For more than 15 years
- With more than $4M federal/state funding
- More than 10 PhD theses
- 10 patents/patent applications
- More than 40 published journal papers

- The piezoelectric-material and sensor development is ripe
1000 times Self Enhancement of Detection $\Delta f/f$

- Due to crystalline orientation switching in “thin” PMN-PT layer induced by binding stress---No such enhancement in other piezoelectric sensor (QCM, SAW…)
- The enhancement increases inversely with a decreasing thickness
- Enhancement is further amplified in DNA detection due to the highly negatively charged nature of DNA
Testing on 40 Blinded Patient Stools

- **PEPS exhibited**
  - 95% sensitivity-- positive 19/20 CDI positive stools
  - 95% specificity-- negative 19/20 CDI negative stools

- The same as 
  Cepheid Xpert
  (the best genetic test)

40 stool samples: 
20 CDI positive
20 CDI negative
According to stool culture/sub/toxins EIA
### Table 2.1 Competitive Comparison between PEPS and commercially-available CD diagnosis alternatives

<table>
<thead>
<tr>
<th></th>
<th>Equipment</th>
<th>Detection time</th>
<th>CDI diagnosis</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>severity test</th>
<th>Cost/test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GDH+toxins EIA</strong></td>
<td>$20 – 50k</td>
<td>Hours</td>
<td>No</td>
<td>50-60%</td>
<td>95%</td>
<td>No</td>
<td>$17.5</td>
</tr>
<tr>
<td><strong>Genetic test</strong></td>
<td>Free to $150 – 180K</td>
<td>1 hour</td>
<td>Yes</td>
<td>95%</td>
<td>95%</td>
<td>No</td>
<td>$30-$58</td>
</tr>
<tr>
<td><strong>GDH/toxin/Genetic test</strong></td>
<td>$150 – 180K</td>
<td>Hours</td>
<td>Yes</td>
<td>87%</td>
<td>&gt;90%</td>
<td>No</td>
<td>$40</td>
</tr>
<tr>
<td><strong>PEPS</strong></td>
<td>Free to $3K</td>
<td><strong>40 min</strong></td>
<td>Yes</td>
<td>95%</td>
<td>95%</td>
<td>Yes</td>
<td>$20</td>
</tr>
</tbody>
</table>

Reimbursement from the Centers for Medicare and Medicaid Services
- $17.5 for GDH test
- $50.27 for bacterial detection using amplification
$20/test makes it a +$100 million opportunity

Cepheid Xpert penetrates only 30% and 10% of mid-size and small hospitals, respectively due to its costs.

Even large hospitals like Temple University Hospital moved away from using Cepheid Xpert and is trying to develop their own PCR method.

Small and mid-size hospitals accounts for 53% and 27% (together 80%) of the market, or $92MM a year based on $20/test

<table>
<thead>
<tr>
<th># of hospitals</th>
<th>Bed size</th>
<th>Avg Estimated CDI tests*</th>
<th>Total Estimated CDI tests*</th>
<th>Revenue Potential</th>
<th>% of Revenue potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>XL-size</td>
<td>75</td>
<td>&gt;800</td>
<td>3,000</td>
<td>225,000</td>
<td>$4,500,000</td>
</tr>
<tr>
<td>L-size</td>
<td>430</td>
<td>400-799</td>
<td>2,250</td>
<td>967,500</td>
<td>$19,350,000</td>
</tr>
<tr>
<td>M-size</td>
<td>1500</td>
<td>150-399</td>
<td>1,032</td>
<td>1,547,932</td>
<td>$30,958,640</td>
</tr>
<tr>
<td>S-size</td>
<td>3400</td>
<td>&lt;149</td>
<td>891</td>
<td>3,029,323</td>
<td>$60,586,460</td>
</tr>
<tr>
<td>Total</td>
<td>5405</td>
<td></td>
<td>6,282</td>
<td>5,769,756</td>
<td>$115,395,120</td>
</tr>
</tbody>
</table>

*Estimate is based on Hahnemann, a 400-bed hospital that performed 1,500 tests last year.
IP*


*Optioned from Drexel University*
We are targeting fecal CDI detection as the first application

FDA Approval: 510K Pre-Market Notification

- Predicate Devices:
  - K091109: Cepheid Xpert C. difficile
  - K100818: Meridian Illumigene C. difficile
  - K123197: Nanosphere Verigene C. difficile

Reimbursement from the Centers for Medicare and Medicaid Services
$17.5 for GDH test
$50.27 for bacterial detection using amplification
Development Plan

• Development of the core technology-24 month process in total
  ➢ Manufacturing of PMN-PT films and PEPS (In House)
    − PMN-PT films and PEPS are the heart of the technology
    − PMN-PT films fabrication has been perfected on the lab scale
    − Will work on mass production of PMN-PT films and mass production of PEPS cutting
      Development cost / duration: $0.7M / 24 months
  ➢ Fabrication of PEPS Array (NextFab)
    − PEPS reproducibility issues was solved on the lab scale.
    − Presently working with Nextfab to get an estimate for assemble array PEPS by 3D printing
      Development cost / duration: $0.1M / 24 months
  ➢ Automated total system w/ flow & electronic circuitry (Imet, ION Design, NextFab w/ Lenima)
    − a sieve to strain the stool
    − a reservoir at 95°C
    − a cooling module
    − a detection chamber at around 50-60°C
    − AIM 4170 impedance analyzer ($500)
      Development cost / duration: $1.2M / 24 months
      Permanent unit price: $3K
      Disposal unit: $0.5-10

Total Development Cost: ~$2M
Business Strategy

- Build Awareness and Positive Disposition toward Technology Pre-Launch
  - Work with Key Opinion Leaders (KOL’s)
  - Publications
  - Build relationships with all the key infectious disease organizations, and get incorporated into appropriate testing guidelines.

- Develop payer strategy to optimize reimbursement

- Assess U.S. Commercialization Requirements
  - Hospital Market is Accessible

- Alternatively, partner with BioPharma or Molecular Diagnostic Companies (MDX) following validation or FDA approval
  - U.S.
  - Europe
  - Developing Countries

- Work closely with WHO and other key international organizations/non-profits to leverage their relationships and infrastructure
Other Applications

- **Infectious Disease**
  - MRSA
  - Antibiotic susceptibility
  - Blood infections
  - Meningitis

- **Oncology**
  - Blood test for cancer mutation markers
    - For example, T790M mutation test for **AQUIRED resistance to TKI treatment in EGFR-positive lung cancer** and other EGFR-positive cancer
  - Blood test for glycoprotein cancer markers
    - For example, Serum Tn antigen and Anti- Tn antigen malignancy test to accompany imaging tests
Capital Formation Plan-Early On, Non-Dilutive Funding

- Signed an option agreement with Drexel
- Actively pursuing NIH STTR/SBIR grants as part of the funding strategy
- A Phase-I STTR of $300K on CDI detection has started April 15, 2014.
- Phase II STTR grant up to $3M on product development of CDI detector is planned on August 5th.
- 2nd Phase-I STTR on blood malignancy test to prescreen lung cancer will be submitted on August 5th.
Time Line

Phase-I
STTR, $300K
April 2014

ION Proposal

Phase-II
STTR, $3M*
April 2015

First Prototype
April 2017**

FDA Approval
December 2018

*Based on the fact that current results had exceeded the milestone set for Phase-I grant
**With additional funding this date can move up substantially
Summary

- PEPS technology brings the precision of genetic testing to the field
- CDI is a serious health care infection. Early detection and correct treatment is critical to reduce mortality, morbidity, as well as financial burden
- Although CDI is the initial focus, this platform technology has wide applications
- Performance, speed, and relatively low cost appears to be attractive to hospitals
- Our data provides confidence that the sensor works, it’s simply a question of developing it into a working prototype, and validating on the instrument
- FDA pathway is straightforward
- A strong core team is in place to take it to the next level
Thank you
Appendix
**PEPS Target Product Profile Background**

- *Clostridium difficile* (CD), a bacterium causing diarrhea and other intestinal problems with high mortality of 18-30% that links to 14,000 annual deaths in the US. CDI is an antibiotic-associated infection as well as a health-care-associated infection.

- Current CDI diagnosis relies on CD toxins enzyme immunoassay (EIA) together with antigen (GDH) EIA. However, the sensitivity of stool toxin EIA is only 60%.

- Nucleic acid amplification test (NAAT) using quantitative real-time polymerase chain reaction (qPCR) or loop-mediated isothermal amplification (LAMP) to detect the toxin gene tcdB or tcdA is sensitive and specific but qPCR and LAMP requires expensive fluorescent probes ($30-$58 per kit).

- Neither qPCR nor LAMP are widely available as rapid qPCR requires expensive equipment (>150K) and LAMP requires users to isolate DNA prior to LAMP amplification.
PEPS Target Product Profile

**Profile**
- Platform technology features a piezoelectric plate sensor (PEPS), that permits genetic testing without amplification, that is low cost (less than $3K for instrument, less than $10 for test), rapid (40 minutes), and point of care. Been in development for 10-years
- PEPS detection is different from other non-amplified DNA detection. Those sensors still need the steps of DNA extraction and denaturation before detection and they can only detect purified, denatured DNA in high concentrations in PBS. In comparison, PEPS can detect the DNA of bacteria directly in stool at 60 copies/ml all within a continuous flow system in 40 min without the need to extract the DNA or amply the DNA.
- One-step, multiplexed test detects multiple bacterial genes (Toxin B (tcbB) gene and binary toxin gene cdtB-associated with severity and recurrence) from stool to diagnose CDI and assess the severity and risk of recurrence at the point of care.

**Specimen type**
- Serum, sputum, stool, urine. 1 ml.

**Time for results**
- 40 minutes

**Sensitivity**
- Sensitivity 95%; specificity 95%. As good or better than PCR

**Through-put**
- 36 samples per day/per module

**Portability**
- 8” by 6” by 6”

**Data Management**
- PC connection. Upload to existing system

**Value Proposition**
- This one-step, multiplexed test detects multiple bacterial genes (Toxin B (tcbB) gene and binary toxin gene cdtB-associated with severity and recurrence) from stool to diagnose CDI and assess the severity and risk of recurrence at the point of care. This would allow earlier and better treatment decisions, and minimize the mortality rate and recurrence risk, as well as prevent CDI from spreading.
- This rapid, accurate, quantitative, and low-cost CDI test does not require highly trained personnel and can be widely available at point of care such as small- and medium-size hospitals, outpatient clinics, and nursing homes for rapid and accurate CDI diagnoses.
**Clostridium difficile Infection (CDI)**

- CDI is a common cause of antibiotic-associated diarrhea (AAD).
  - It accounts for 25% of all AAD.
- CDI can lead to:
  - pseudomembranous colitis,
  - toxic megacolon,
  - perforations of the colon,
  - sepsis, and
  - death.
- Symptoms of CDI include:
  - watery diarrhea, fever, nausea, loss of appetite, abdominal pain.
- CDI risk factors include:
  - antibiotic exposure, proton pump inhibitors,
  - gastrointestinal surgery/manipulation,
  - long stay in healthcare settings,
  - a serious underlying illness,
  - immunocompromising conditions,
  - advanced age.