Developing A Companion Diagnostic for Detection Of Hyaluronan, A Key Component Of The Tumor Microenvironment

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PEGPH20 is a new investigational drug product whose safety and efficacy profile have not been established. PEGPH20 is not available for commercial distribution.
About Halozyme Therapeutics

- Biopharmaceutical company headquartered in San Diego
- Partnerships exist with leading pharmaceutical companies for the ENHANZE™ subcutaneous drug delivery platform
  
![Partnership Logos](https://example.com/partnership-logos)

- Recent focus on developing and commercializing novel oncology therapies that target the tumor microenvironment
- A partnership exists with a leading pharmaceutical company for the investigational PEGylated human hyaluronidase PEGPH20

**About PEGPH20**

PEGPH20 is a new investigational drug product whose safety and efficacy profile have not been established. PEGPH20 is not available for commercial distribution.
Introduction To Companion Diagnostics

- Biomarkers may be used to assess risk, diagnosis, prognosis, predictiveness (drug response), or monitoring of response or adverse events

- To be useful as a test, a biomarker may be developed as a companion diagnostic:

> An *IVD companion diagnostic device* is an in vitro diagnostic device that provides information that is essential for the safe and effective use of a corresponding therapeutic product. The use of an IVD companion diagnostic device with a therapeutic product is stipulated in the instructions for use in the labeling of both the diagnostic device and the corresponding therapeutic product, including labeling of any generic equivalents of the therapeutic product. (Guidance for Industry, No. 1737; 6 Aug 2014)

- Objective of this presentation
  - Describe some of the challenges overcome during development of a CDx for a drug that alters a primarily noncellular target that is a key component of the tumor microenvironment/ extracellular matrix

22 US FDA Cleared or Approved Companion Diagnostics for Oncology as of 15 July 2015

- [www.fda.gov](http://www.fda.gov)
The Challenge: Targeting Hyaluronan – A Physical Barrier To Cancer Therapy Access

- Hyaluronan (HA)
  - Nonsulfated glycosaminoglycan polymer of repeating disaccharides, consisting of D-glucuronic acid and D-N-acetylglucosamine
  - Key component of the tumor microenvironment
  - Stabilizes TME via physicochemical properties and binding to cell surface receptors and extracellular components
  - Coordinates water molecules so interstitial pressure may increase
  - Present in normal tissues

- Implications for cancer therapy
  - Barrier to entry of cancer therapeutics into the tumor
  - HA may impede host immune cell access (e.g., hinders ADCC)
  - Structurally identical between species, so difficult to raise antibodies directed against it for a diagnostic

Kultti. *JBC* 2006;281:15821.
The Tumor Microenvironment Frequently Accumulates High Levels of Hyaluronan

This phenotype results in:
• Increased tumor interstitial pressure (IP)\textsuperscript{1,2}
• Compression-narrowed blood vessels \textsuperscript{3,4}
• Reduced anti-cancer therapy delivery to tumor

Tumor Hyaluronan Accumulation Is Associated With Shorter Overall Survival In Pancreatic Cancer Patients


- Retrospective analysis of survival categorized according to staining intensity for HA in tumor biopsies
- Inverse correlation observed between level of deposition of TME HA and patient survival
PEGPH20 Improves Delivery Of Co-Administered Cancer Therapeutics

- PEGPH20 is an investigational PEGylated form of Halozyme's recombinant human hyaluronidase rHuPH20
  - Targets hyaluronan, a key component of the TME
  - Increases access of anticancer drugs into tumors that accumulate high levels of HA
  - Improves survival in animal models with high HA in combination with chemotherapy

- Encouraging early efficacy data in Phase 1b and 2 trials of Stage IV pancreatic ductal adenocarcinoma in combination with
  - Gemcitabine
  - Gemcitabine and nab-paclitaxel (Abraxane®)

Hingorani. ASCO GI 2015, #359.
Hingorani. ASCO 2015, #4006.
PEGPH20 Actions Include Removing HA From The TME

TME post PEGPH20 plus chemotherapy

- Malignant cell
- HA
- Collagen
- Cancer-associated fibroblasts
- Endothelial Cell
- Lymphocyte
- Pericyte
- Myeloid Cell
- Neutrophil
- Capillary

Expected TME Post Chemotherapy plus PEGPH20:
- Tumor interstitial pressure reduced
- Vascular perfusion improved
- Access of anti-cancer therapeutics increased
- Therapy-induced cell death

PEGPH20 Has Preferential Antitumor Activity in Tumors With High Levels of HA

Tumors high in HA may be dependent on HA to maintain their phenotype

**NSCLC PDx**
- **HA\textsubscript{low}**
  - 0% TGI

**HA\textsuperscript{high}**
- 78% TGI

**CRPC Xenografts**
- **HA\textsubscript{low}**
  - 0% TGI

**HA\textsuperscript{high}**
- 70% TGI

PDx = patient-derived xenograft
P = PEGPH20 monotherapy
V = vehicle
TGI = tumor growth inhibition

Cowell. AACR 2015, #2547.

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Clinical Focus Was Informed By Hyaluronan Level, Disease Incidence and Patient Prognosis

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High Correlation Between The Predicate Research Probe And A Novel Halozyme-Patented Recombinant Binding Protein

- Predicate research probe: bHABP = biotin-Hyaluronan Binding Protein
- Novel recombinant HA binding protein: biotin-TSG-6-Δhep-Fc (HTI-601), where TSG-6 = tumor necrosis factor-stimulated gene 6

Halozyme-Patented Recombinant Binding Protein Enables Improved Measurement of Hyaluronan

 Predicate Research Probe

 Animal sources
 HABP Protease fragments of Aggrecan + LINK
 HA binding region

 Cartilage-Derived Protein
 Contains impurities
 Lower specificity
 Batch-to-batch variability

 Recombinant Protein
 Pure
 Higher specificity
 Consistent staining

 HTI-601
 HTI-601 = biotin-TSG-6-ΔHep-Fc

Acquiring / Staining Specimens For Hyaluronan

Diagnostic Biopsy from referring institution

Prepare formalin-fixed paraffin-embedded slides

Perform ligand binding assay (affinity histochemistry) using HTI-601 on an automated immunostainer

- Similar to immunohistochemistry except that probe is technically an immunoadhesin, not an antibody
Positivity was defined by a ratio of pixels above a defined intensity threshold relative to the total using a positive pixel count algorithm.
Hyaluronan Accumulation Is Similar In Primary Tumor and Metastases

- Example shows primary breast tumor and metastasis samples staining similarly for hyaluronan using bHABP
- This suggests that biopsies of metastatic lesions may be used to assess patient eligibility

Rationale For Developing An HA Companion Diagnostic

- HA Accumulation in tumors is a negative prognostic indicator
- HA level in tumor predicts response to PEGPH20 in preclinical studies
- Novel probe is sensitive, specific and can be reliably manufactured
- HA levels can be measured using an IHC-based assay with HTI-601
- Estimated incidence of high HA phenotype in pancreatic cancer (40-60%) may enable patient selection

Measuring HA level in tumors with an IHC-based assay using HTI-601 yields a distribution of staining intensities

Low HA | High HA

Jiang. AACR-EORTC 2011, #B35.
Analytical Validation Studies Were Performed Following Regulatory Guidelines

• Halozyrne's research assay was transferred and analytically validated at Clarient Diagnostics

• Within-Laboratory Precision
  – Repeatability and reproducibility across multiple batches of probe, instruments, operators and pathologists
    ▪ On nonconsecutive days in randomized order with "wild card" cases and wash-out period between readings

• Sensitivity
  – Dilution linearity of probe
  – "Immunohistochemistry" Sensitivity
    ▪ Percentage of samples from cancer indication that stained strongly

• Specificity
  – Analytical specificity
    ▪ Stain with hyaluronidase pre-digestion to remove substrate
  – "Immunohistochemistry" Specificity
    ▪ Percentage of samples of other histotypes that stained weakly

Analysis of Within-Laboratory Precision Demonstrates Assay Is Robust

Staining Variables

<table>
<thead>
<tr>
<th></th>
<th>%CV</th>
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<tbody>
<tr>
<td>Batches of Probe</td>
<td>5%</td>
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<tr>
<td>Operator</td>
<td>7%</td>
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<tr>
<td>Instrument</td>
<td>9%</td>
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Variance Component Estimate by HA Level

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<tr>
<th></th>
<th>%CV for Reader</th>
<th>%CV for Repeatability</th>
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<tbody>
<tr>
<td>Weak</td>
<td>4%</td>
<td>14%</td>
</tr>
<tr>
<td>Moderate</td>
<td>2%</td>
<td>15%</td>
</tr>
<tr>
<td>Strong</td>
<td>&lt;1%</td>
<td>14%</td>
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Repeatability and Reproducibility

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<tr>
<td>Repeatability</td>
<td>17%</td>
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<tr>
<td>Between-Day Reproducibility</td>
<td>5%</td>
</tr>
<tr>
<td>Between-Run Reproducibility</td>
<td>12%</td>
</tr>
</tbody>
</table>

Gelb. AACR 2015, #576.
High Agreement Between Scores Derived From Different Readers' Annotations For Image Analysis

Note near-zero bias (solid line) and that most samples fall within 95% limits of agreement (dotted lines)
The Prototype Assay Method Is Sensitive And Specific

• Sensitivity
  – Dilution linearity testing demonstrated detection down to 0.013 μg/mL
  – Frequency of tumors staining strongly was 63% (47 of 75) of archival pancreatic cancer specimens in the validation set

• Specificity
  – 5% (4 of 75) pancreatic cancers and 3 normal adjacent tissue samples had faint, focal staining after pre-digestion with recombinant PH20 hyaluronidase
  – But no cross-reactivity that would interfere with reading was identified in a panel of 32 different normal human tissues (x 3 each)

Gelb. AACR 2015, #576.
Summary And Next Steps

- A tissue-based assay for HA can be used for patient enrichment in clinical studies of PEGPH20-associated therapies in pancreatic cancer and other cancer indications.

- The within-laboratory precision, sensitivity and specificity of the prototype assay met all acceptability criteria.

- Work is ongoing with Halozyeme's partner Ventana Medical Systems (Roche Group) for the final stages of our CDx development and commercialization.
  - The proprietary diagnostic will be used to prospectively identify and select patients with high levels of HA, with the goal of identifying the patients we believe are most likely to benefit from PEGPH20 treatment. We are making good progress with Ventana, including transferring our process to Ventana’s platform; and refining and validating the staining and scoring methodology.
Recommendations For Developing A Novel CDx

- Begin co-development of the CDx early in the process of drug development
- Expect that commercially-available critical reagents may not be suitable for a novel companion diagnostic
- Carefully evaluate its performance characteristics to ensure the assay system is robust, sensitive and specific
- Involve CDRH promptly by holding Pre-Submission meeting(s) to get Agency buy-in on the validation plan and development path
- Initiate technology transfer of the prototype assay to a CDx development partner for feasibility studies and subsequent development as soon as appropriate
- Collaborate with the clinical team on the design of registration studies to ensure clinical samples and patient outcome data needed for clinical validation of the CDx
  - Careful planning may avoid the need for bridging studies
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Q&A

Thank You For Your Attention

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