NUCLEOSOMICS® – HOW IT WORKS

The Nucleosomics® technology measures and identifies signatures of nucleosomes circulating in the blood.

Nucleosomes and DNA

- The DNA in every cell is wound around protein complexes in a “beads on a string” structure, forming chromosomes
- Each individual “bead” is called a nucleosome, and consists of DNA wrapped around a core of histone proteins
- Each core consists of four pairs of variants of H2A, H2B, H3 and H4 histones
- Histones and the DNA are subject to a variety of post translational modifications
- Various proteins interact with nucleosomes to modulate gene expression
- When a cell dies, the body breaks the DNA string up into individual nucleosomes which are released into the blood to be naturally “recycled”
- Competitors’ blood DNA tests for cancer mutations involve extraction of DNA fragments from these nucleosomes for gene sequence analysis. Less than 1 in a million of the DNA fragments will contain the target gene sequence. This means the tests use a lot of blood and are extremely difficult and expensive. None have reached the market
- VolitionRx’s Nucleosomics® tests involve epigenetic analysis of all of the intact nucleosomes with their associated DNA using simple low cost NuQ® ELISA assays on less than a drop of blood
EPIGENETICS MEETS ONCOLOGY

During cell death chromatin is fragmented into nucleosomes which are released into the bloodstream. Cancer is characterized by high levels of cell turnover which results in an increase in average level of nucleosomes in the blood of cancer patients. This characteristic is not specific to cancer, as elevated cell turnover can increase blood nucleosome levels in a number of other instances, including heart attack, immune and inflammatory disease.

VOLITIONRX'S SOLUTION

VolitionRx's tests have shown, in several trials, the ability to distinguish cancer patients from healthy patients and those with other blood nucleosome-elevating conditions, and to distinguish one type of cancer from another (prostate from colorectal cancer).

Nucleosomes of cancer origin are structurally different (i.e. have different patterns of histone modifications, DNA modifications, DNA methylation or certain protein adducts) from nucleosomes of other cells.

VolitionRx has developed five families of NuQ® double antibody ELISA assays, each of which captures intact nucleosomes and labels (identifies) a specific structural feature:

- NuQ®-T: Assay to quantify total nucleosomes
- NuQ®-X: Assays to detect nucleosomes containing modified nucleotides
- NuQ®-V: Assays to detect nucleosomes with specific histone variants
- NuQ®-M: Assays to detect nucleosomes containing specific modified histones
- NuQ®-A: Assays to detect nucleosome-protein adducts

4-5 assays from a multiple families are combined to form a “panel” test for specific diseases

PRODUCT DEVELOPMENT

NuQ® tests can be adapted onto one or more of four existing ELISA diagnostic formats:

I. ELISA microtiter plate (current platform)
II. Automated ELISA machine in a hospital or centralized pathology laboratory
III. Point of care test administered at an oncologist office, in-patient or out-patient clinic
IV. Disposable point of care test administered at MD office or, in the case of remission patients, potentially at home
RESULTS TO DATE

Colorectal Cancer

Hvidovre Hospital, Copenhagen, Denmark

- 4,800-subject CRC study design – symptomatic population
- Initial representative 938-subject sample analysis
- 938 subjects with colorectal cancer, precancerous polyps or adenomas, benign bowel diseases and other malignancies
- All subjects had undergone a colonoscopy
- NuQ® CRC panel diagnostic test demonstrated (CRC versus no findings on colonoscopy and no comorbidities):
  - 84% sensitivity (accurate detection) at 78% specificity
  - 60% detection of adenomas (polyps)
  - Detection of early (I or II) and late-stage (III or IV) disease with similar accuracy

Lung Cancer

Centre Hospitalier Universitaire (CHU) de Liege, Liege, Belgium

- Pilot study evaluating NuQ® performance detecting lung cancer in blood and sputum (obtained by coughing)
- 46 subjects with non-small cell lung cancer, chronic obstructive pulmonary disease (COPD) or with no disease (healthy)
- Blood:
  - Detected 16 of 21 lung cancer cases (76% sensitivity) with a single false positive result for a healthy subject (1 of 13) (92% specificity)
- Sputum:
  - Detected 18 of 21 lung cancer cases (85% sensitivity) with no false positive results for healthy subjects (0 of 13) (100% specificity)
  - Discriminated lung cancer from COPD

Pancreatic Cancer

Lund University, Lund, Sweden

- Pilot study evaluating NuQ® performance detecting pancreatic cancer
- 60 subjects with stage II operable pancreatic cancer, healthy subjects and subjects with other pancreatic diseases
- Detected 21 of the 25 pancreatic cancer cases from healthy subjects (84% sensitivity), with only two false positive results among the 25 healthy subjects (92% specificity)
- Detected 19 of the pancreatic cancer cases (76% sensitivity) from all other subjects including healthy subjects and those with other pancreatic diseases with only a single false positive for one healthy subject and two false positives for subjects with other pancreatic diseases, one of which was a subject with pre-cancerous IPMN condition (91% specificity).
VolitionRx has a number of clinical trials planned and ongoing for a variety of cancers including colorectal cancer and for the 27 most prevalent cancers (including lung, breast, prostate and pancreatic cancers).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Institution</th>
<th>Sample Collection</th>
<th>Start Date (approx.)</th>
<th>No. of Subjects (approx.)</th>
<th>Study Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer (symptomatic population)</td>
<td>Hvidovre Hospital (Denmark)</td>
<td>Retrospective: took place 2010-2012</td>
<td>Analysis began late 2013</td>
<td>4,800</td>
<td>Subjects with colorectal cancer, polyps or adenomas, benign bowel diseases, or other malignancies. All subjects underwent colonoscopy. Full access to medical history through electronic audit.</td>
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<tr>
<td>Colorectal cancer (screening study)</td>
<td>Hvidovre Hospital (Denmark)</td>
<td>Prospective: commenced April 2014</td>
<td>Analysis expected to begin 2015</td>
<td>14,000 (8,000 FIT +ve, 6,000 FIT -ve)</td>
<td>Population screening trial. All subjects will have a fecal immunochemical test (FIT). FIT-positive subjects will have a colonoscopy. Full access to medical history through electronic audit.</td>
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<tr>
<td>Colorectal polyps (pre-cancerous colorectal adenomas)</td>
<td>Hvidovre Hospital (Denmark)</td>
<td>Retrospective</td>
<td>Analysis expected to begin Q3 2015</td>
<td>800</td>
<td>Study to identify a NuQ® biomarker panel for the identification of patients with precancerous colorectal polyps.</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>CHU Dinant Godinne UCL</td>
<td>Namur (Belgium)</td>
<td>Prospective: 2012-2015</td>
<td>Analysis underway</td>
<td>250 Longitudinal study subjects with suspected colorectal cancer. Study to evaluate NuQ® for early detection and prognosis of CRC.</td>
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<tr>
<td>27 most prevalent cancers</td>
<td>Bonn University Hospital (Germany)</td>
<td>Prospective</td>
<td>Analysis expected to begin Q3 2015</td>
<td>4,200</td>
<td>Study to evaluate NuQ® for early detection of 27 most prevalent cancers; and to evaluate differences in nucleosome structures between cancers. Subjects with cancers including respiratory cancer, gastrointestinal cancer, gynecological cancers, urinary cancers, hematological cancer, melanoma, sarcoma and cancers of the thyroid and brain; as well as control patients with 24 other conditions and healthy individuals.</td>
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<tr>
<td>Lung cancer</td>
<td>Bonn University Hospital (Germany)</td>
<td>Retrospective</td>
<td>Analysis expected to begin 2015</td>
<td>600</td>
<td>Study to evaluate NuQ® for early detection of lung cancer. Subjects with lung cancer with different histological subtypes and diverse stages of disease; subjects with benign lung diseases that are relevant for differential diagnosis; as well as samples from healthy subjects.</td>
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<tr>
<td>Prostate cancer</td>
<td>MD Anderson Cancer Center (USA)</td>
<td>Retrospective</td>
<td>Analysis expected to begin 2015</td>
<td>TBC</td>
<td>Study to evaluate NuQ® for early detection of anaplastic cancer, a particularly aggressive form of prostate cancer, from typical castration resistant prostate cancer (CRPC), the less aggressive form.</td>
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<tr>
<td>Prostate cancer</td>
<td>ImmuneHealth (Belgium)</td>
<td>Prospective</td>
<td>Analysis expected to begin 2015</td>
<td>120</td>
<td>Multicenter study to evaluate ability of NuQ® to detect prostate cancer.</td>
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<tr>
<td>Ovarian cancer</td>
<td>Singapore General Hospital (Singapore)</td>
<td>Retrospective</td>
<td>Analysis expected to begin 2015</td>
<td>40</td>
<td>Pilot study to evaluate NuQ® for early detection of ovarian cancer.</td>
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<tr>
<td>Endometriosis</td>
<td>The University of Oxford (England)</td>
<td>Prospective</td>
<td>Analysis expected to begin 2015</td>
<td>500</td>
<td>Study to evaluate NuQ® for detection of endometriosis. Subjects comprise healthy and endometriosis-positive individuals confirmed by laparoscopy, with samples taken across the menstrual cycle.</td>
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