Early and accurate diagnosis of cancer, moving towards a solution.

Early cancer diagnosis using a new bio-marker (HAAH) reduces time-to-treatment, patient anxiety and health care costs

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HAAH is a Cancer Biomarker

- Panacea discovered that HAAH is elevated in the sera of individuals with cancer
- Panacea has developed a specific and sensitive immunoassay to detect HAAH in serum
- Panacea has also developed a sensitive qRT-PCR methods for detecting HAAH gene expression
- These assays may be applied as companion diagnostics in various scenarios where there is currently unmet medical need
Human Aspartyl (Asparaginyl) $\beta$-Hydroxylase

- HAAH catalyzes the following reaction:

$$\text{Asp} + \text{O}_2 + \alpha\text{-Ketoglutarate} \xrightarrow{\text{Fe}^{++}} \beta\text{Hya} + \text{CO}_2 + \text{Succinate}$$

- Potential substrates for HAAH include proteins with an EGF-like Domains; including receptors (e.g. Notch), ECM and ECM interacting proteins (e.g. Tenascin) and clotting factors.
- Expression of HAAH in normal cells results in their transformation, while its down-regulation in cancer cells results in the normalization of their phenotype.
- Normal cells express very low levels of HAAH only intracellularly; Cancer cells overexpress HAAH on their plasma membrane.

Panacea
Pharmaceuticals
HAAH Over-Expression in Cancer by IHC

• HAAH has been detected in all cancer types tested (n>20) including: liver, bile duct, brain, breast, prostate, colon, lung, ovary, pancreas and in nearly 100% of all cancer specimens (n>1,000)

• HAAH was *not* detected in over 500 non-cancerous tissues including proliferative disorders

• Staining for HAAH is found throughout the cancerous tissue but is generally more prominent at the infiltrating margins of the tumor
HAAH Expression in Leukemia

- Splenic sections from patients with AML or CML were isolated and immunohistochemically stained.

- Diseased-leukocytes stain (brown) with anti-HAAH antibodies.

  - Acute Myeloid Leukemia stained with Anti-HAAH Ab.
  - Chronic Myelomonocytic Leukemia stained with Anti-HAAH Ab.
  - Chronic Myelomonocytic Leukemia stained with non-relevant Ab.
HAAH as a Prognostic Marker

HAAH can be used to determine disease prognosis

• High levels of HAAH expression correlated with:
  • Tumor size (p<0.05)
  • Infiltrative growth pattern (p<0.01)
  • Poor histological grade (p<0.01)
  • Vascular invasion (p<0.05)
  • Poor prognosis (p<0.05)

• High HAAH expression strongly correlates with poor prognosis
HAAH is a Cancer Biomarker
The Companion Diagnostic Strategy

• We have applied HAAH testing:

1- For early detection where currently no other test exists (*Lung Cancer*)

2- To select individuals at risk for cancer and suggest: invasive & expensive testing (*Colon Cancer*)

3- To detect cancer where current biomarkers fail (*Prostate Cancer*)

4- To monitor for disease recurrence (*Breast Cancer & AML*)

5- To determine drug sensitivity prior to treatment (*CML*)
Example I: Early Detection of Lung Cancer

• Lung Cancer - Highest Cancer deaths worldwide, (men and women)

• 226,160 new cases of lung cancer –

• No approved serum biomarkers for lung cancer. Current screening:
  X-ray and/or - CT scanning and/or - MRI and /or Bronchoscopy methods, all low sensitivity and high cost.

• The 5-year survival rate for lung cancer is only 15%, however, Early Detection= survival rate of 50%.
Serum HAAH Concentration in Lung Cancer

[Graph showing scatter plot with data points for Lung Cancer (x = 34.5, n = 160) and Non-Cancer* (x = 0, n = 93).]

Specificity = 91%, Sensitivity 99%

*Includes 50 smokers.
HAAH is elevated in all Stages - Lung Cancer

Specificity* = 90%, Sensitivity 98%

*Among smokers
Example II: Who should have a Colonoscopy?

• Colorectal Cancer - 3rd Cancer - in the USA
• 143,460 new cases and 51,690 deaths in 2011

• FOBT is an imperfect screening tool; ~9 out of every 10 positive tests are false positives.

• Thus, a more specific initial screening test for Colorectal Cancer is highly desirable. To date, no serum biomarkers have been approved for Colorectal Cancer screening primarily due to relatively low specificities and sensitivities.
Serum HAAH in Colon Cancer

![Graph showing the distribution of HAAH levels across different stages of colon cancer]

- Stage I: n = 37, x̄ = 33.0
- Stage II: n = 55, x̄ = 28.8
- Stage III: n = 40, x̄ = 24.0
- Stage IV: n = 13, x̄ = 34.3

**n = 145, x̄ = 29.0, Sensitivity = 99%**
Example III: When Other Biomarkers Fail

- PSA >4 ng/ml is suggestive of PC and these patients undergo biopsy. However, only 25% of patients with a PSA of 4-10 ng/ml who undergo biopsy have PC.

- A more specific biomarker for PC, especially in men with PSA values <4 ng/ml, would be of significant value.
Serum HAAH Concentration in Prostate Cancer

- Non-Cancer: n = 43, \( \bar{x} = 0 \)
- PSA <2: n = 100, \( \bar{x} = 23.0 \)
- PSA 2-4: n = 49, \( \bar{x} = 21.1 \)
- PSA >4: n = 84, \( \bar{x} = 31.60 \)

Prostate Cancer: n = 233, \( \bar{x} = 25.7 \)

Specificity = 93%  Sensitivity = 95%

*Men > 50 years of age, cancer-free
Example IV: Monitoring for Disease Recurrence

- Breast cancer = accounting for 26% of female cancers.

- > 200,000 new cases/ in USA, 2012
  >2 million women are currently survivors.

- 5-year survival rates are 98% for localized disease, 26% for distantly spread cancer. However, 25% of all metastases occur > 5 years after the initial diagnosis.

  - Current screening modalities rely on mammography, and breast self exam – highly in-sensitive, in recurrence.
    MRI is far more sensitive but more Expensive

  - Thus the development of a sensitive biomarker to monitor for disease recurrence would greatly enhance survival rates.
Serum HAAH Concentration in Breast Cancer

Specificity* = 91%  Sensitivity = 94%

* “False” positive within expected range for this population.
Companion Diagnostics - Monitoring

• If a treatment reduces tumor load and/or neutralizes cancer cells, serum HAAH should be reduced

• Monitoring could be individualized to the treatment agent and the patient

• Preliminary results and observations support the use of, serum HAAH levels, to monitor treatment
Companion Diagnostics - Recurrence

• HAAH is produced only by active cancer cells

• As the cancer cells become active and grow, serum HAAH levels should increase significantly

• Preliminary studies suggest that serum HAAH levels can be used for early detection of recurrence of cancer

• HAAH gene expression can determine very early minimal residual disease at extremely low levels
HAAH Gene Expression in Leukemia

- Leukocytes isolated from
  - Normal individuals
  - CML patients
  - AML patients

- CML patients have a ~4.2-fold increase in HAAH expression over normals (p<0.0001)

- AML patients have a ~8.6-fold increase in HAAH expression over normals (p<0.0002)

![Graph showing HAAH expression in different groups](image)
Mechanism of Action/
Relationship to
Cancer Phenotypes
HAAH Transfection Promotes Cell Motility and Invasiveness

A. Western blot analysis showing a band at ~85 kDa.

B. Micrographs showing variations in cell morphology after transfection with pcDNA3 and pcDNA3-AAH.

C. Graph comparing the area cleared by cells for pcDNA3 and pcDNA3-AAH, with a significant difference indicated by an asterisk.

D. Graph comparing the number of cells invaded per 200x field for pcDNA3 and pcDNA3-AAH, with a significant difference indicated by an asterisk.
Inhibition of HAAH Expression Inhibits Motility
Inhibition of Cellular Proliferation

Anti-HAAH antibodies inhibit tumor cell proliferation, thus they are cytostatic agents.
Inhibition of Cellular Motility and Invasiveness

- Anti-HAAH antibodies inhibit tumor cell motility, as well as invasion, thus they are anti-metastatic agents.
HAAH and Cancer Detection in 857 samples

HAAH test scatter chart

The scatter chart shows the result of HAAH test as a cancer biomarker on a group of 857 patients composed of 211 individuals known not to have cancer and 646 individuals who are diagnosed with cancer.

The cancer group is composed of mix of individuals with different types of cancer (Breast, Prostate, Lung, Colon) in various stages from 1-4.

Combining the 12 false positive and 34 false negative results, the test has less than 4.6% error even in such a large group of patients.
New Screening Techniques Show Potential for Early Detection of Lung Cancer

Tracy Hampton, PhD

ATLANTA—Because about 90% of all lung cancer cases are caused by smoking but only 10% to 1% of heavy smokers will develop the disease, researchers are developing new and sensitive detection techniques to better identify those individuals at risk due to other factors, such as heredity.

Researchers presenting findings on some of these efforts at the American Association for Cancer Research annual meeting and International Conference on Molecular Targets and Cancer Therapeutics in Philadelphia stressed that new tests are needed because current techniques such as high-resolution computed tomography often detect lung cancer at later stages. Approximately 213,000 people in the United States are expected to be diagnosed with the disease this year, and more than 160,000 deaths are anticipated.

ONCOGENE DETECTION

Assessing the expression of certain cancer-causing genetic pathways may be effective for identifying smokers at the highest risk for developing lung cancer. One such pathway under investigation involves phosphatidylinositol 3-kinase, an important regulatory protein. A recent study of 129 airway brushings collected during clinical bronchoscopy revealed that PIK3 signaling pathway levels in patients who responded to treatment with imatinib (Gleevec) were lower than those who did not, said Gustafson. He noted that the results need further validation because only 9 patients were assessed, 3 of whom did not experience a treatment response.

QUIET GENES

Tumors also may arise when certain genes that protect against lung cancer become inactive in lung cells. Researchers at the University of Toronto, in Ohio, have found that this may occur in at least 12 genes that encode antioxidant and DNA repair proteins in lung array cells. In their study, they measured the abundance of messenger RNA (mRNA) transcribed from these genes in lung cells taken from 23 individuals with lung cancer and 24 without the disease.

Gustafson and colleagues also found that this information may help identify targets for lung cancer chemoprevention. In their experiments, they profiled gene expression in histologically normal bronchial epithelium that was collected from high-risk smokers with dysplastic airway lesions before and after chemopreventive therapy with the drug oncostatin M. There was a greater reduction from baseline in mRNA expression in the IGFBP3 signaling pathway in the patients who were followed up.

The researchers noted that, although the study was small, the results suggest that monitoring the expression of these genes in patients who are exposed to high-risk environments may help identify individuals at risk of developing lung cancer from smoking.

BLOOD PROTEIN

Other researchers have detected a protein in blood that is linked to all stages of lung cancer but is rarely found in the blood of individuals without the disease.

“We can detect a cancer signature in blood as early as stage 1,” said lead author Mark Semenov, of Dana-Farber Cancer Institute, in Cambridge, Mass. Semenov and colleagues tested the specificity and sensitivity of a blood test for an enzyme, human apoptosis 33 (APG33), which is produced in H460 lines. This protein, which is important for early cell differentiation, is normally expressed in mice, but in malignant cells it is often expressed on the cell surface and can be found in the blood of individuals with cancer.

Researchers found that 99% of serum samples from 100 patients with early stages of lung cancer showed elevated levels of H460 protein, compared with 9% of samples from 93 non-smokers without lung cancer. Also, in a group of 20 smokers not known to have cancer, 4 patients had high levels of H460, but because the researchers did not have access to future patient records, they could not determine whether these individuals developed lung cancer. Semenov said that testing for H460 cannot confirm whether a person has lung cancer, but “it is a strong indicator that an individual needs further examination, so it may be a good tool for screening asymptomatic individuals.”
#8. Early-Stage Test for Lung Cancer

Lung cancer is the leading cancer killer in America and is responsible for more deaths than the next three most common cancers combined (colon, breast, and prostate). One reason lung cancer is so deadly is that by the time tumors are diagnosed, usually as a result of physical symptoms like coughing up blood, the cancer is often advanced and tough to treat. But a new blood test may improve the odds of catching the disease earlier, at a more curable stage. Called 

"TrakTumor," the test measures blood levels of a protein present in all stages of lung cancer, but barely seen in healthy people. While the test alone can't confirm a diagnosis of lung cancer, it can be used in conjunction with chest X rays, CT scans, and other imaging technology to hone in on early-stage tumors.
Thank You