Methylation in cell-free DNA from blood: universal media for clinical biomarkers.

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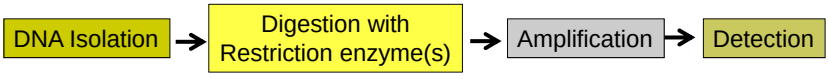
Technology

- ***Proprietary***; protected as Trade Secret
- Freedom to Operate confirmed
- Does ***NOT*** depend on ***bisulfite*** modification

Key elements of technology



Three steps to product:

- Discovery — *Genome-wide association study*
  - Goal: select informative fragments (***biomarker***)
- Biomarker transfer to clinical platform — *qPCR or NGS*
- Technical and clinical validation

Results of Feasibility Studies
(proof of principle)

Oncology

Breast:	differentiates benign, non-invasive, and invasive
Breast:	identifies response to treatment
Breast:	predicts outcomes of treatment
Colon:	differentiates pre-invasive and invasive cancer
Lung:	differentiates different forms of lung cancer
Pancreas:	differentiates pancreatitis and pancreatic cancer
Ovaries:	differentiates benign disease and ovarian cancer
Ovaries:	predicts response to treatment

Neurology (Relapsing-remitting multiple sclerosis, RRMS)

RRMS:	diagnosis, detection of relapse
RRMS:	monitoring of response

Psychiatry

possible but as yet untested

Performance of Feasibility Platform

Disease	Sensitivity vs specificity	
	Technology	Approved ***screening*** test
Breast	80% vs 88%[a]	87% vs 88%[1]
Colon	84% vs 68%[b]	82% vs 82%[2]
Lung	73% vs 87%[b]	94% vs 73%[3]
Ovaries	87% vs 79%[b]	None[4]
Pancreas	91% vs 91%[c]	None[4]
RRMS *(diagnosis)*	*79% vs 93%*[d]	75% vs 88%[5]

[a] pre-invasive (DCIS)
[b] Adenocarcinoma
[c] Pancreatitis vs Cancer
[d] RRMS in remission
[1] www.komen.org
[2] PMID: 27898666 (for stage IV)
[3] PMID: 23697514 (stage IIB – IV)
[4] www.cancer.org
[5] www.nationalmssociety.org

- ***Screening for ovarian and pancreatic cancer is not recommended due to a large number of false-positives.***
- ***Lung and colon cancers are usually detected too late to make a difference.***
- ***Breast cancer screening produces up to 35% of false-positive and misses interval cancers***

Feasibility data are published and available in PubMed

Performance of Discovery Platform

Results of the full-scale Discovery* performed for Colorectal cancer was evaluated by an independent biostatistician.

Excerpt from the report:

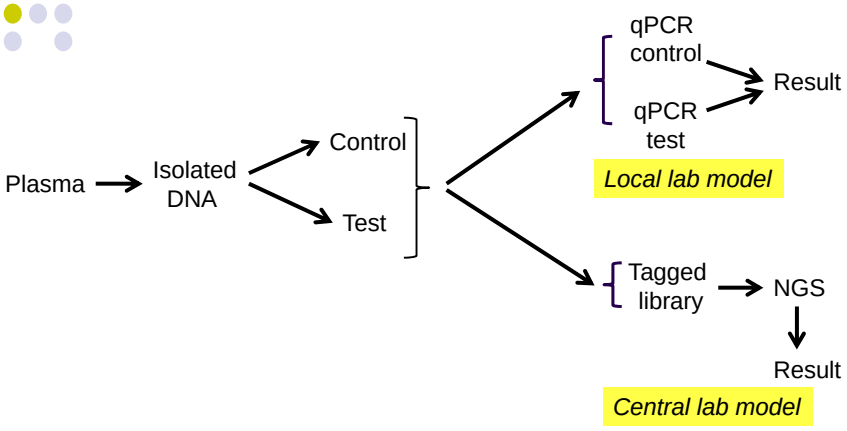
…using "20 individual samples and top 4 genes to do modeling, we get ***100% accuracy*** when training and validating"

Primary data can be made available.

Patents

Title	Number	Assignee
Methylation profile of breast cancer	US 7,666,589	Northwestern U
DNA Methylation based test for monitoring of efficacy of treatment	US 8,497,066	Rush University
Analysis of DNA methylation in ultra-small clinical samples	61/876,050	US Biomarkers, Inc
Biomarkers for detection of colorectal cancer	WO2015047910	US Biomarkers, Inc
Methylation Profile of Neuroinflammatory Demyelinating Diseases	60/880,130	Northwestern U

Clinical workflow



Typical Q & A

Q. Why use cell-free DNA? Why not tissues?

A. Tissues can be used when disease is detected and its location can be accessed. This cannot be done for EARLY detection when exact location is unknown

Q. Why use DNA methylation? Why not mutations?

A. Mutations usually involve a group of genes related to cell growth that are not specific for a particular cancer. DNA methylation reflects gene expression that is much more disease-specific.

Q. Why genome-wide association study is needed? Why not test known genes?

A. Limiting the scope of Discovery reduces its efficacy: less fragments are tested and important ones may be missed.

Q. Why use a new technology for methylation GWAS? Why not bisulfite process?

A. Bisulfite conversion eliminates >50% of input DNA and cannot be used when only a small amount of DNA is available. Sample loss with our technology is less than 20%.

Q. How many samples do you need to process for Discovery?

A. Discovery requires ~40-50 samples per group.

Q. How many fragments from Discovery are selected for biomarker?

A. The Colorectal biomarker has 12 fragments. If higher accuracy is needed, additional fragments are added.

Q. What about IP protection? Comment on *Mayo vs. Prometheus* and *AMP vs. Myriad*.

A. Both decisions state that "natural phenomenon" is not patentable. Out patent includes a *formula for calculating probability of correct test.* By US Supreme Court definition it is a product of human invention and thus patentable. Patents include the ID of fragments and the formula.

Unique position

Discovery phase

- Genome-wide discovery in small samples (at least **500x less than the closest competitor**) or **0.5 ml of blood *versus* 125 ml of blood**.
- Technology is designed for ***high-throughput analysis.***

Clinical analysis phase

- Technology requires **0.25 ml of blood** (*finger-stick*)
- Can be performed in **very high-throughput fashion**
- Complete test takes **less than 36 hr**.
- Test is done with **510k-approved instruments**.