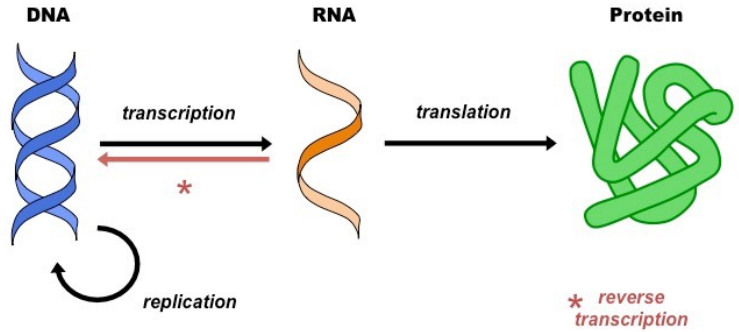
Non-invasive differential diagnostics of cancer using liquid biopsy

Paradigm shift in cancer standard of care

Victor Levenson MD, PhD

Central Dogma and Its Effects



Disease: abnormal expression of genes that can be detected as a change in DNA, RNA, or Protein.

Biomarkers for disease detection can be

- Genomic
- Transcriptomic
- Proteomic

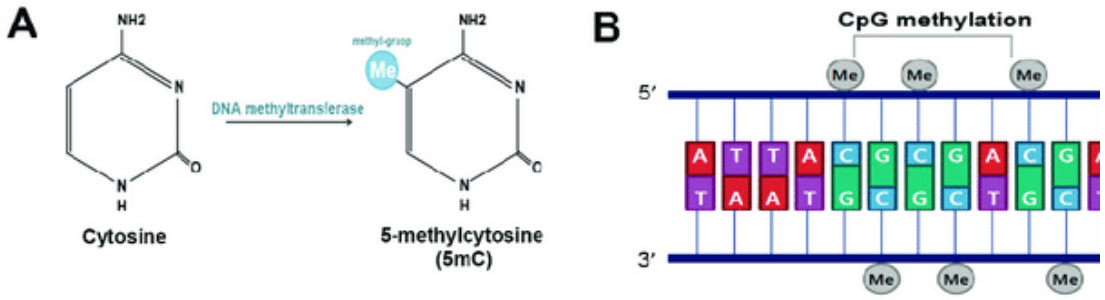
} Amplifiable

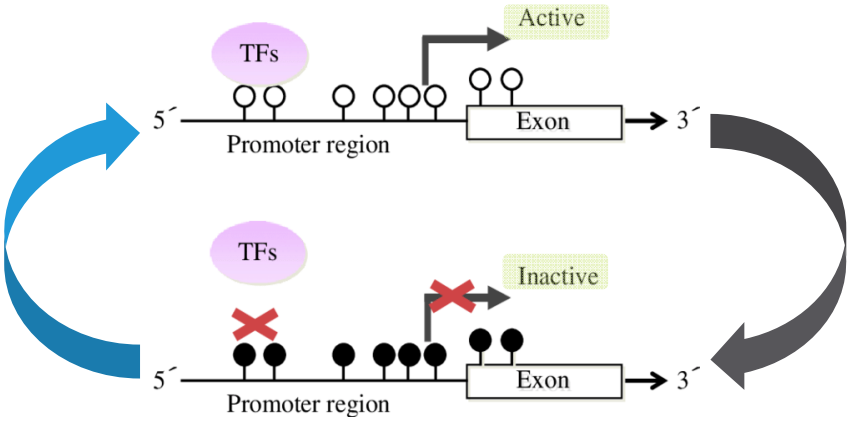
BUT

- **Genomic**: stable, unknown expression
- **Transcriptomic**: unstable, undefined expression
- **Proteomic**: stable, uneven representation

Epigenomic: stable, directly linked to expression, amplifiable

Central Dogma of Epigenomics and Its Effects





Epigenetic biomarkers

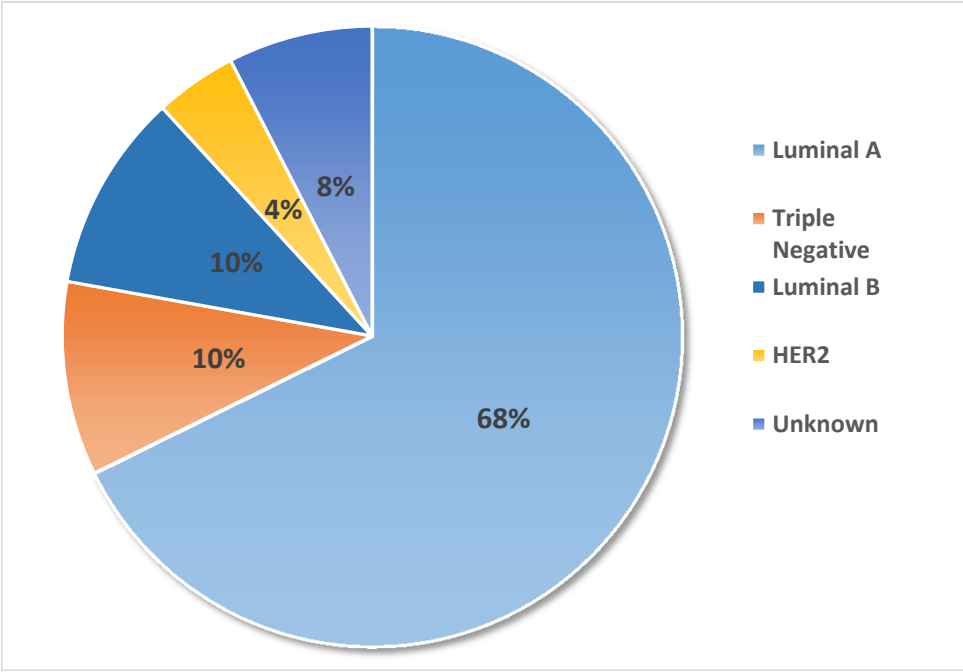
- Reflect gene expression
- Can be amplified

M-Test

- Discovery of epi-biomarkers

BREAST CANCER

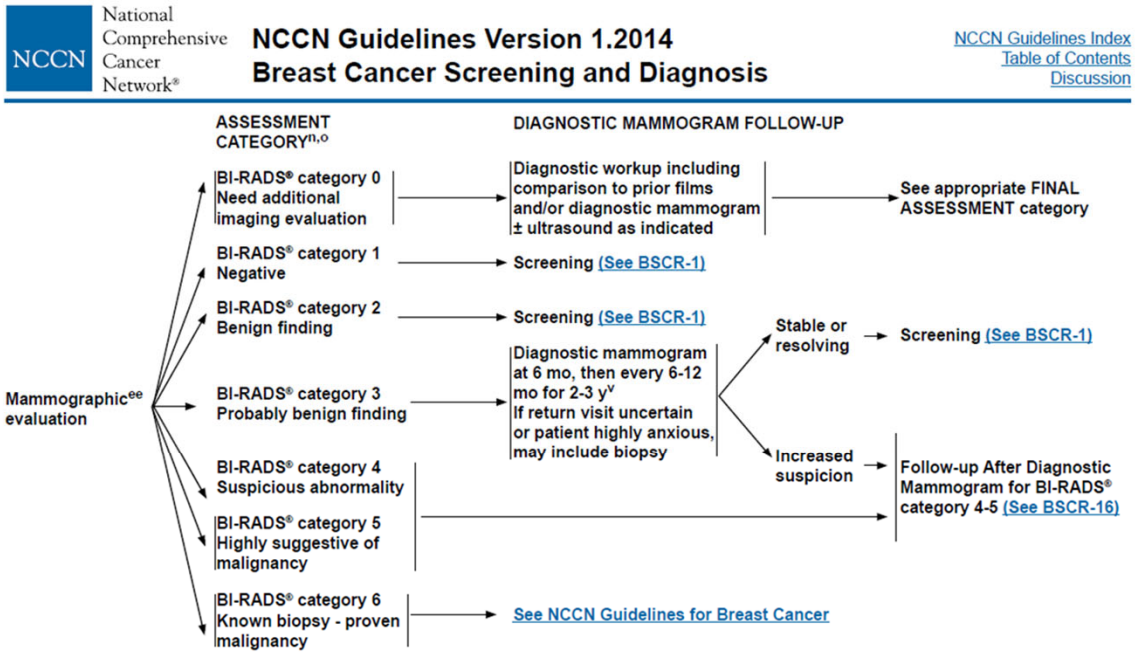
Breast Cancer is one the five deadliest cancers worldwide



- Estimated ~ 300,000 new cases of female breast cancer in the US and >2 million worldwide (2020)
- Accurate diagnosis is required to select the best treatment for each type
- Regular monitoring with diagnostic imaging is required if the lesions cannot be biopsied
- Tissue biopsy is the current standard to confirm breast cancer diagnosis ($535.8 M market in 2019)

https://seer.cancer.gov/statfacts/html/breast-subtypes.html

NCCN GUIDELINES FOR BREAST CANCER



[n] See Assessment Category Definitions (BSCR-C).
[o] Mammography results are mandated to be reported using Final Assessment categories (Quality Mammography Standards:Final Rule. Federal Register.1997;62:55988).
[v] There may be variability on the follow-up interval based on the level of suspicion.
[ee] Mammogram considerations: Specify if mammogram is screening or diagnostic and comparison should be made with prior noncopied films (original films), obtainable.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

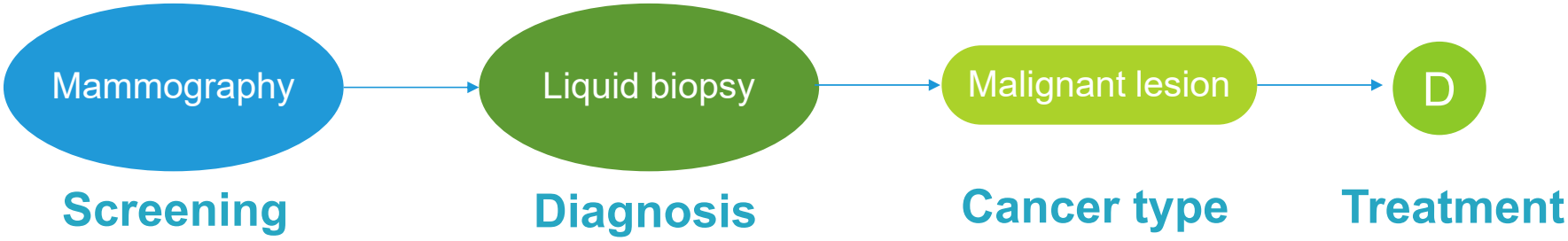
Regular screening

Repeat imaging
(Lesions can be malignant, but too small to biopsy)

Core biopsy or FNA (invasive)
(Lesions can be biopsied)

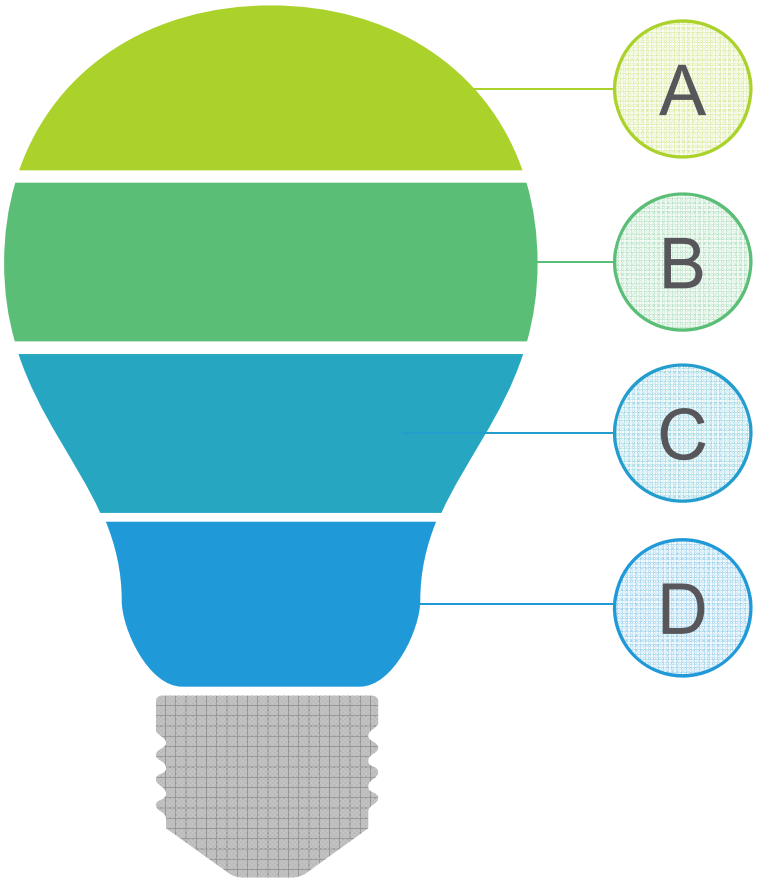
Our approach can
1. Replace repeat imaging and biopsy;
2. Reduce errors;
3. Improve screening results (interval cancers)

OUR SOLUTION FOR BREAST CANCER



- ✓ **Provide physicians and patients with actionable answers at critical decision points**
- ✓ **Replace invasive surgical sampling with non-invasive liquid biopsy**
- ✓ **Reduce patients' anxiety due to unknown or inconclusive diagnosis**
- ✓ **Eliminate false-negative and uncertain results**
- ✓ **Reduce the time to diagnose and select the best treatment option**
- ✓ **Fast-to-market opportunity (4-6 months to clinical grade test)**

KEY ADVANTAGES



A **Accessibility**
Sample is easy to collect

B **Convenience**
Only a small amount of blood is required

C **Affordable**
Much lower cost compared to imaging exams and biopsy

D **Actionable insights**
Provides actionable information for follow-up procedures

CLINICAL DATA
(proof-of-principle M-Test-56 platform)

- **4 peer-reviewed publications and 1 issued patent**

DISEASE*	CLINICAL DIFFERENTIATION**	SENSITIVITY, %	SPECIFICITY, %
Breast cancer	No cancer vs **Benign**	70	80
	No cancer vs **non-invasive cancer**	80	88
	No cancer vs **invasive cancer**	87	85
	Benign vs non-invasive cancer	79	82
	Benign vs invasive cancer	79	82
	Invasive cancer vs non-invasive cancer	87	76

** The feasibility is also shown for Lung, Colon, Ovarian and Pancreatic cancers and some chronic diseases*

***At least 30 samples per group,*

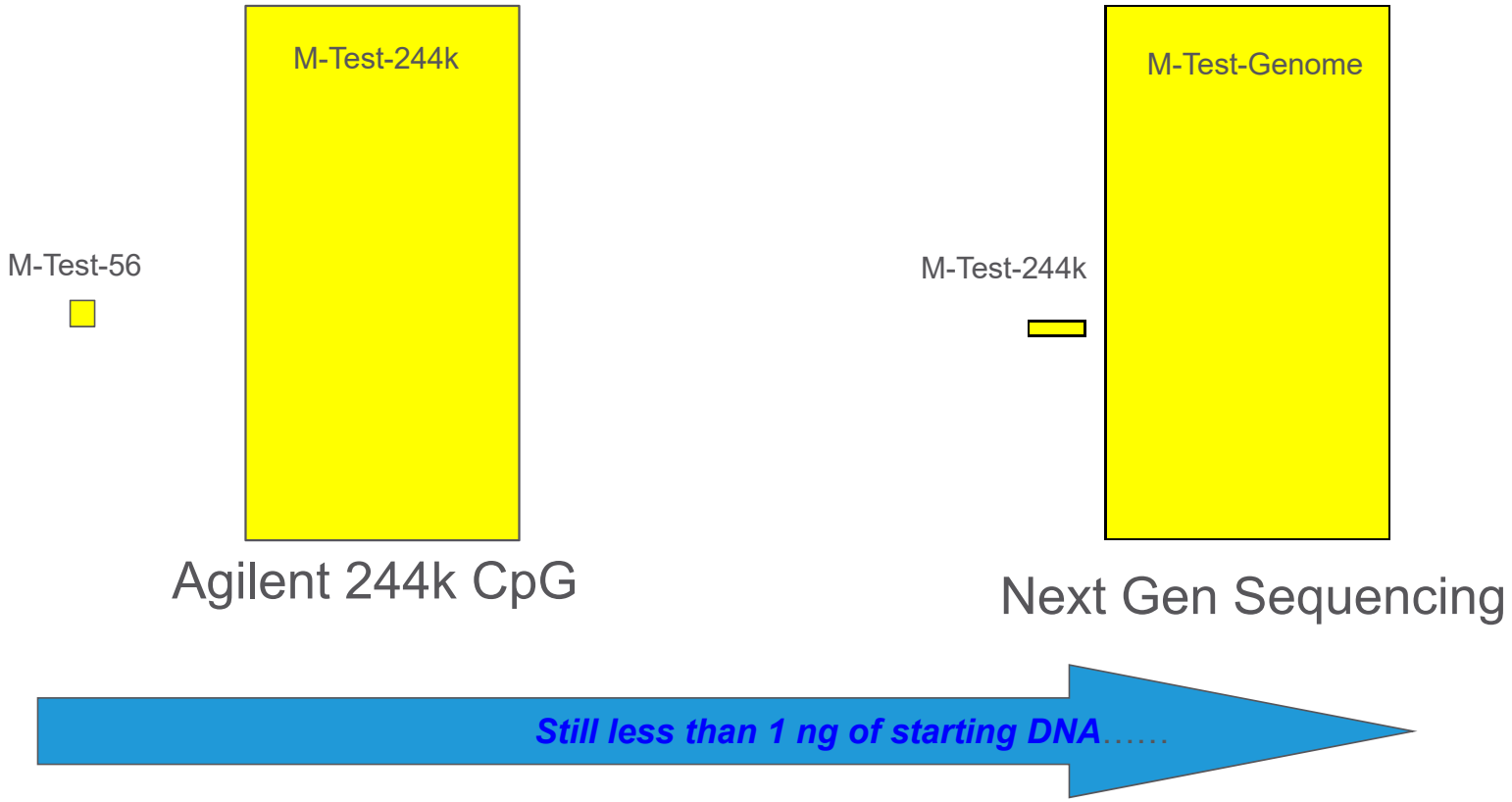
IMPROVING CLINICAL PERFORMANCE

Improved performance by increasing selection space (Colon Cancer)

Platform	# of fragments	Sensitivity	Specificity
M-Test-56	6	84%	68%
M-Test-244k	6	100%	100%

There are 4800 additional fragments with significantly different ($p < 0.05$) methylation and more than 2 fold difference between patients with colon cancer and healthy controls.

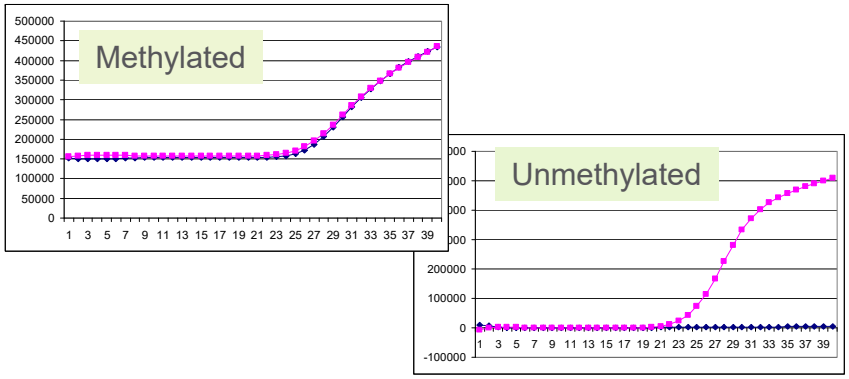
PROOF of PRINCIPLE vs PRODUCTION



CLINICAL TEST

Principle: cfDNA → Treatment → Amplification of selected fragments

Hospital lab (low volume):
qPCR for individual fragments



Centralized lab (high volume):
NGS for selected fragments

MULTI-CANCER APPLICATIONS

Clinical feasibility has been shown for five different cancers

01

Breast:

- differentiates benign, non-invasive, and invasive
- identifies response to treatment

02

Colon:

- differentiates pre-invasive and invasive cancer

03

Lung:

- differentiates different forms of lung cancer

04

Pancreas:

- differentiates pancreatitis and pancreatic cancer

05

Ovaries:

- differentiates benign disease and ovarian cancer
- predicts response to treatment

None of the existing methods can differentiate chronic disease and cancer!

Epigene Diagnostics
Accuracy in our blood

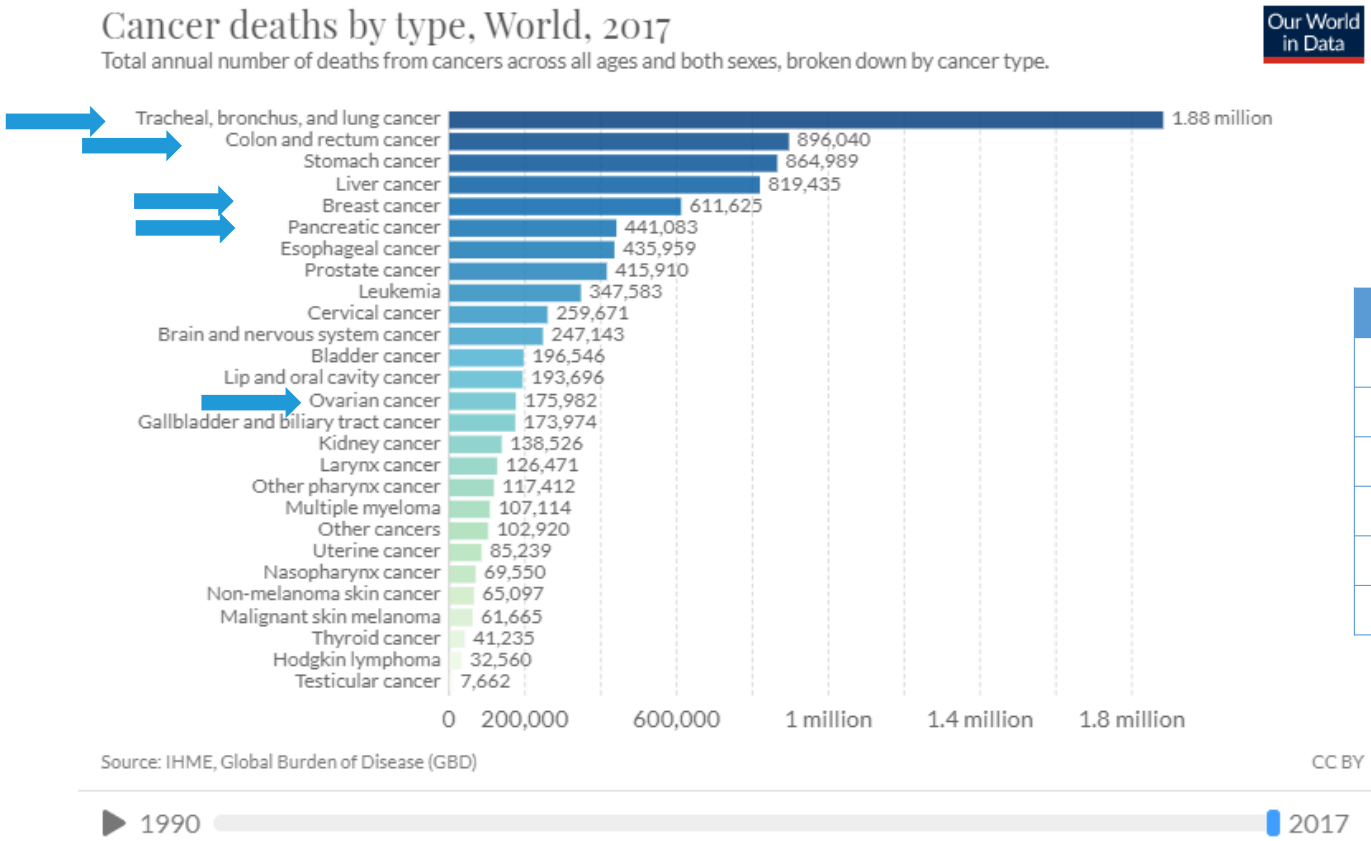
SUMMARY OF CLINICAL DATA
(proof-of-principle M-Test-56 platform)

ORGAN	CLINICAL DIFFERENTIATION	SENSITIVITY, %	SPECIFICITY, %
Ovaries	No cancer vs **Benign**	79-90	74-77
	No cancer vs **Cancer**	79-90	87-87
	Benign vs Cancer	73-82	72-80
Pancreas	No cancer vs **Pancreatitis**	78	82
	No cancer vs **Cancer**	76	59
	Pancreatitis vs Cancer	91	91
Lung	No cancer vs **Adenocarcinoma**	87	73
	No cancer vs **Squamous**	80	87
	Adenocarcinoma vs Squamous	87	90
Colon	No cancer vs **Cancer**	84	68
	No cancer vs **Advanced Adenoma**	55	65

** At least 30 samples per group, published in >* ***15 peer-reviews papers and 6 patents***

TARGETED MARKET SIZE

Our test covers 45% of all cancers worldwide



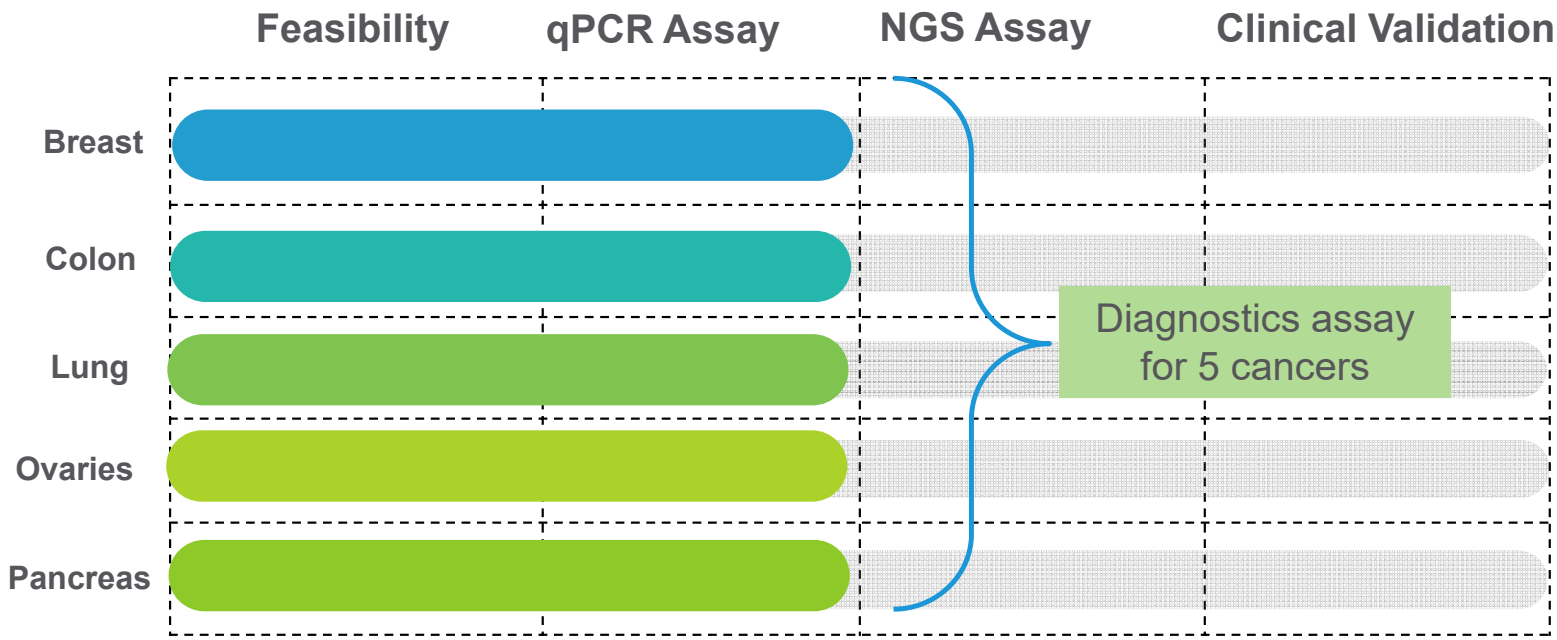
8.9M total of cases in 2017

Cancer type	# cases
Tracheal, bronchus and lung	1,880,000
Colon and rectum	896,040
Breast	611,625
Pancreatic	441,083
Ovarian	175,982
total	4,004,730

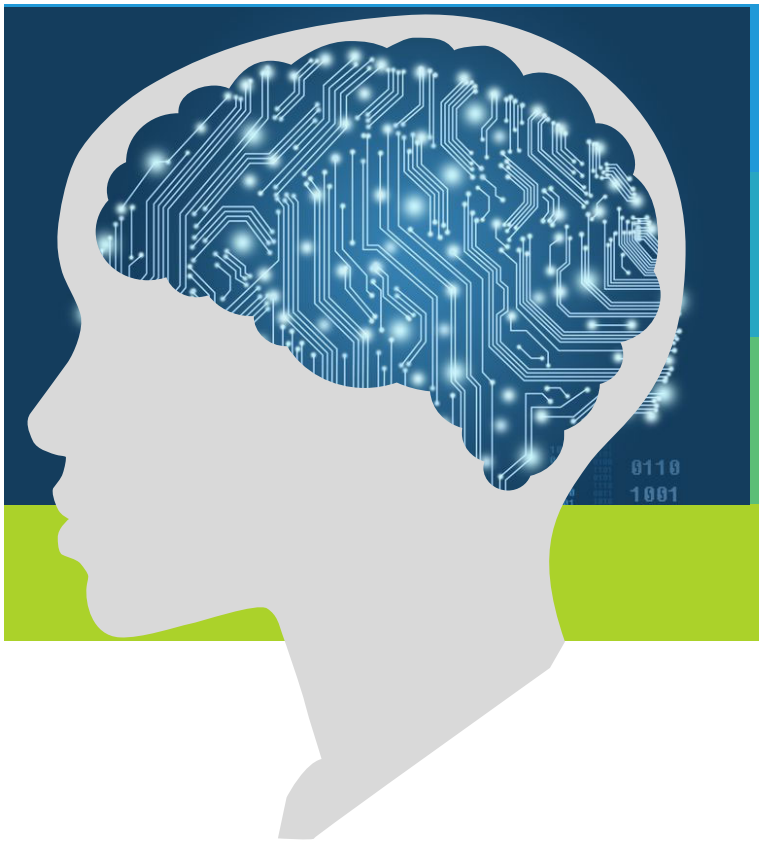
https://ourworldindata.org/cancer

EXTENDED PRODUCT PIPELINE

- The overall goal is to develop clinical **differential diagnostics assay** for 5 major cancer types
- The roadmap considers developing and validating NGS assays for individual conditions to address more focused indications first starting **with Breast Cancer**



AI DRIVEN MODELS TO EXPLAIN CANCER



Creating new AI models
To predict signatures of body response to different cancers and molecular mechanisms involved

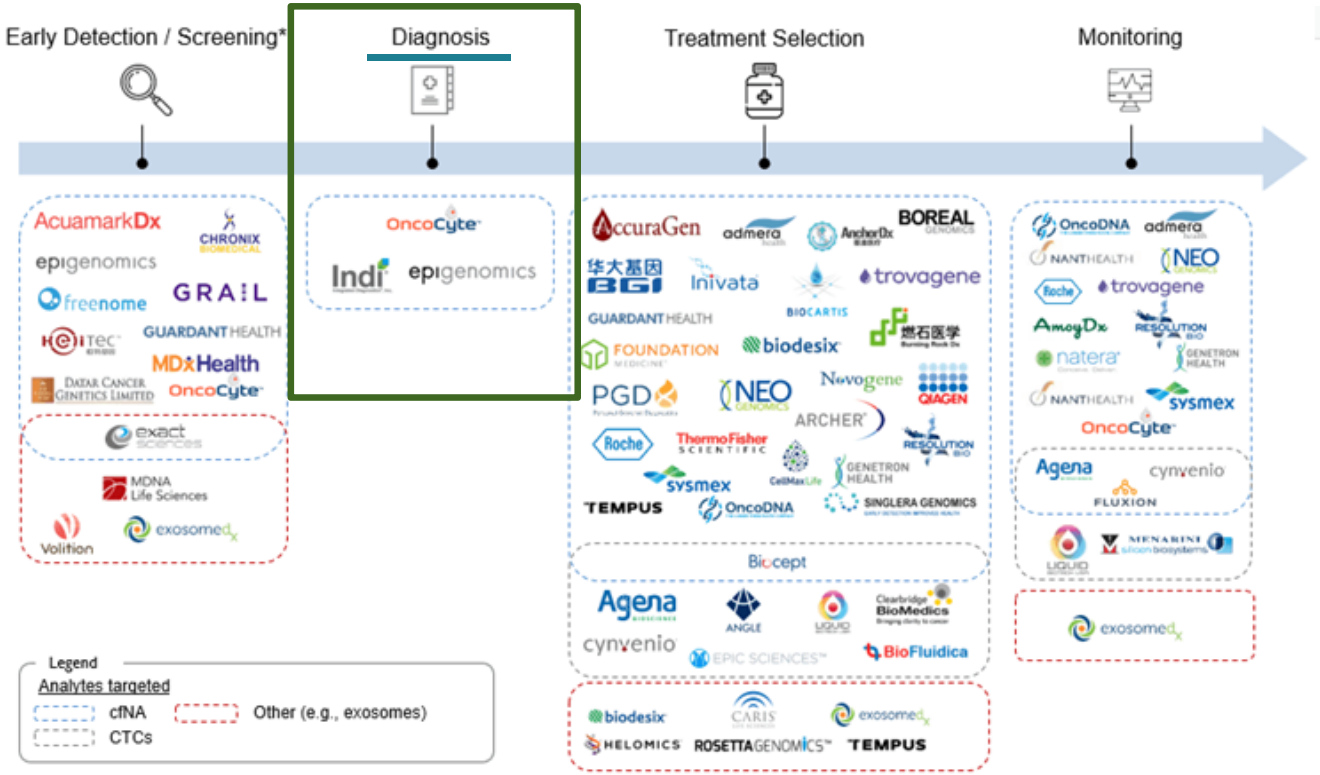
Composite Biomarker
For early screening and differential diagnosis of multiple cancers

Novel Marker Selection
To identify sets on biomarkers specific for each cancer type

Genome-wide analysis of methylation
To build methylation database

LIQUID BIOPSY MARKET 2017

- Liquid biopsy has become a very crowded market with > 100 active companies
- Our market segment is **Diagnosis** and <u>has significantly smaller competition</u>



Plan

- **Develop of the Breast Cancer test.**
- **Milestones:**
  1. Expand the assay to include additional fragments of cell-free DNA
  2. Convert prototype qPCR assays into NGS-assay for **Breast Cancer**.
  3. Perform CLIA-validation with the goal to secure FDA, CE-IVD and NMPA approvals in the future.
  4. Conduct clinical validation on a large number of samples for each **Breast Cancer** type.
- **Develop multi-cancer diagnostic test**

Technical Approach
(Grail *vs* M-Test)

COMPLETELY DIFFERENT APPROACHES

Grail:

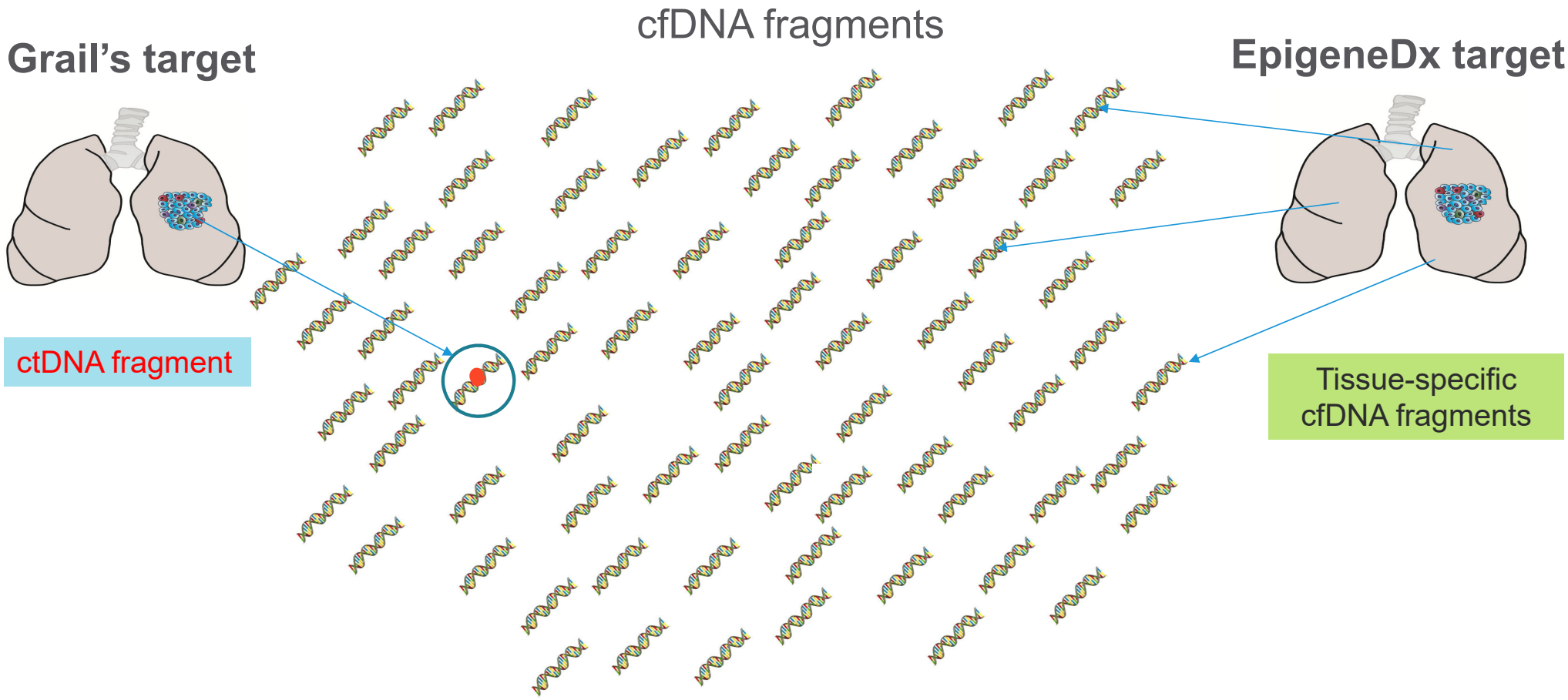
- Focusing on detecting ctDNA fragments originated from tumor tissue
- Methylated fragments discovered by sequencing of **tumor** may NOT appear in **blood** early on
- Biomarkers are not informative for pre-cancerous lesions
- Bisulfite conversion eliminates >50% of cell-free DNA
- High demand for input cell-free DNA (>1,000 ng ideally)

Repeating the errors of Epigenomics!

M-Test:

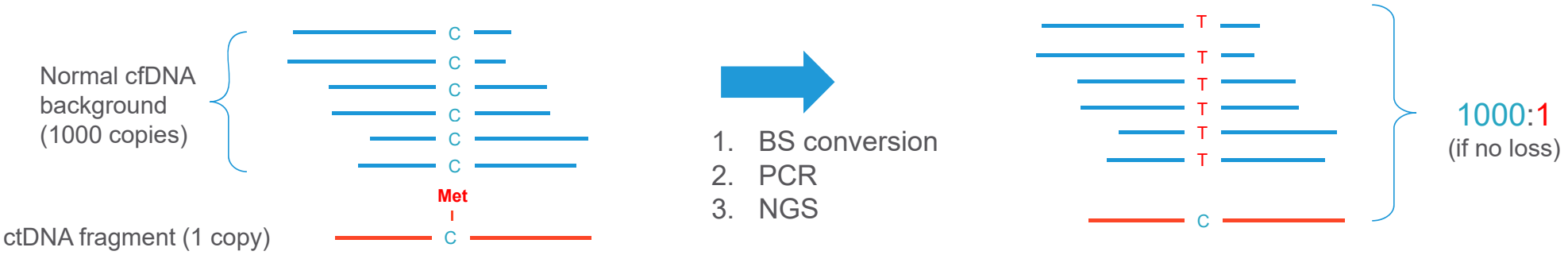
- Focusing on a **systemic whole body response to cancerous or pre-cancerous processes**
- The assay targets methylation patterns in total cell-free DNA rather than in tumor-specific fragments
- Enzymatic digestion instead of bisulfite conversion preserves > 80% of starting cell-free DNA
- Only 0.7 ng of cell-free DNA (0.5 ml of plasma) is required
- Designed to work with cell-free DNA fragments

ASSAY TARGETS

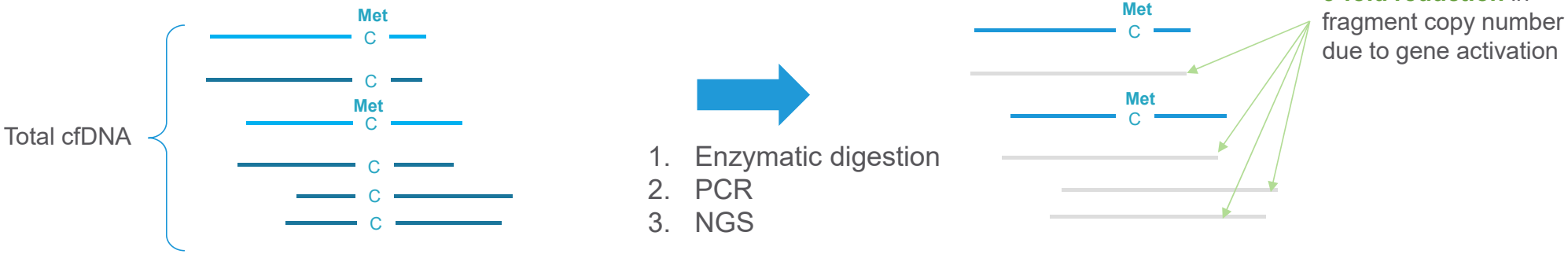


COMPARISON OF TWO METHODS

Grail's approach, 0.1% ctDNA fraction (early stages)

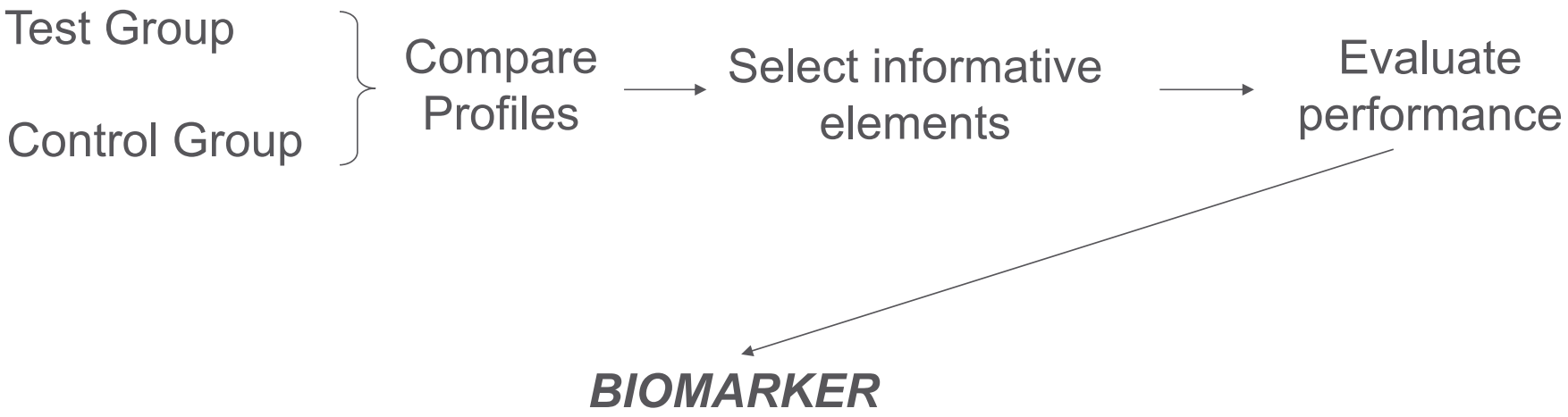


M-Test



Principles of the M-Test Method

Differentiate methylated and unmethylated fragments using ENZYMATIC DIGESTION



Appendix

INTRODUCTION TO THE APPROACH

- The core approach is based on **detecting methylation profiles in blood** and is **fundamentally superior and different** from what Grail, Cirina, BioChain and others are using currently.
- The only molecular method which allows to **differentiate between chronic organ disease (such as pancreatitis) and early stage cancer.**
- Relatively low price of the test (**estimated cost is under $100** compared to >$5000 Guardant360) makes it very affordable for large, currently underserved populations.
- **Non-invasive sample collection** (finger stick or venous draw) makes it suitable for routine annual check ups with minimal discomfort to patients.
- Discovery and clinical feasibility phases **for 5 cancer types including: lung, colon, ovarian, breast and pancreatic** were completed using $4.5M funding from NIH and private investors.
- Several patents on biomarkers and their use have been filed and issued.
- Key elements of the technology such as proprietary reagents and AI algorithm for data interpretation are kept as trade secrets.
- The current goal is to convert existing assays for individual cancers into a single pan-cancer test (NGS or microarray) and conduct clinical validation on larger number of samples.

PEER-REVIEWED PUBLICATIONS

1. A seven-gene CpG-island **methylation** panel predicts breast cancer progression.
   Li Y, Melnikov AA, **Levenson V**, Guerra E, Simeone P, Alberti S, Deng Y.
   BMC Cancer. 2015 May 19;15:417. doi: 10.1186/s12885-015-1412-9.
   PMID: 25986046 **Free PMC Article**

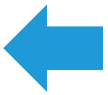
2. Commonality and differences of **methylation** signatures in the plasma of patients with pancreatic cancer and colorectal cancer.
   Melson J, Li Y, Cassinotti E, Melnikov A, Boni L, Ai J, Greenspan M, Mobarhan S, **Levenson V**, Deng Y.
   Int J Cancer. 2014 Jun 1;134(11):2656-62. doi: 10.1002/ijc.28593. Epub 2013 Nov 29.
   PMID: 24288256 **Free Article**

3. Molecular biomarkers in 2013.
   Levenson VV, Melnikov AA.
   Expert Rev Mol Diagn. 2013 Nov;13(8):773-6. doi: 10.1586/14737159.2013.850419. No abstract available.
   PMID: 24151845

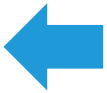
4. **Methylation** of death-associated protein kinase is associated with cetuximab and erlotinib resistance.
   Ogawa T, Liggett TE, Melnikov AA, Monitto CL, Kusuke D, Shiga K, Kobayashi T, Horii A, Chatterjee A, Levenson VV, Koch WM, Sidransky D, Chang X.
   Cell Cycle. 2012 Apr 15;11(8):1656-63. doi: 10.4161/cc.20120. Epub 2012 Apr 15.
   PMID: 22487682 **Free PMC Article**

5. DNA **methylation** as clinically useful biomarkers-light at the end of the tunnel.
   Levenson VV, Melnikov AA.
   Pharmaceuticals (Basel). 2012 Jan 18;5(1):94-113. doi: 10.3390/ph5010094.
   PMID: 24288045 **Free PMC Article**

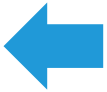
6. The MethDet: a technology for biomarker development.
   Levenson VV, Melnikov AA.
   Expert Rev Mol Diagn. 2011 Nov;11(8):807-12. doi: 10.1586/erm.11.74.
   PMID: 22022943 **Free PMC Article**



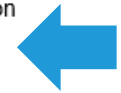
Breast cancer



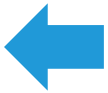
Pancreatic and CRC



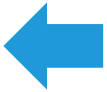
Invited Expert Review



CDx application



CDx review



Core technology

PEER-REVIEWED PUBLICATIONS

7. DNA **methylation** patterns in blood of patients with colorectal cancer and adenomatous colorectal polyps.
Cassinotti E, Melson J, Liggett T, Melnikov A, Yi Q, Replogle C, Mobarhan S, Boni L, Segato S, **Levenson V**.
Int J Cancer. 2012 Sep 1;131(5):1153-7. doi: 10.1002/ijc.26484. Epub 2011 Nov 19.
PMID: 22020530 **Free PMC Article**

CRC and pre-cancer

8. Distinctive DNA **methylation** patterns of cell-free plasma DNA in women with malignant ovarian tumors.
Liggett TE, Melnikov A, Yi Q, Replogle C, Hu W, Rotmensch J, Kamat A, Sood AK, **Levenson V**.
Gynecol Oncol. 2011 Jan;120(1):113-20. doi: 10.1016/j.ygyno.2010.09.019. Epub 2010 Nov 6.
PMID: 21056906 **Free PMC Article**

Ovarian Cancer

9. **Methylation** patterns in cell-free plasma DNA reflect removal of the primary tumor and drug treatment of breast cancer patients.
Liggett TE, Melnikov AA, Marks JR, Levenson VV.
Int J Cancer. 2011 Jan 15;128(2):492-9. doi: 10.1002/ijc.25363. Epub 2010 Apr 5.
PMID: 20473856 **Free PMC Article**

Breast cancer therapy

10. DNA **methylation** as a universal biomarker.
Levenson VV.
Expert Rev Mol Diagn. 2010 May;10(4):481-8. doi: 10.1586/erm.10.17. Review.
PMID: 20465502 **Free PMC Article**

Invited Expert review

11. Differential **methylation** of cell-free circulating DNA among patients with pancreatic cancer versus chronic pancreatitis.
Liggett T, Melnikov A, Yi QL, Replogle C, Brand R, Kaul K, Talamonti M, Abrams RA, **Levenson V**.
Cancer. 2010 Apr 1;116(7):1674-80. doi: 10.1002/cncr.24893.
PMID: 20143430 **Free Article**

Pancreatic cancer

PEER-REVIEWED PUBLICATIONS

12. **Methylation** patterns of cell-free plasma DNA in relapsing-remitting multiple sclerosis.
Liggett T, Melnikov A, Tilwalli S, Yi Q, Chen H, Replogle C, Feng X, Reder A, Stefoski D, Balabanov R, **Levenson V**.
J Neurol Sci. 2010 Mar 15;290(1-2):16-21. doi: 10.1016/j.jns.2009.12.018. Epub 2010 Jan 12.
PMID: 20064646 **Free PMC Article**

Multiple sclerosis

13. CpG **methylation** analysis--current status of clinical assays and potential applications in molecular diagnostics: a report of the Association for Molecular Pathology.
Sepulveda AR, Jones D, Ogino S, Samowitz W, Gulley ML, Edwards R, **Levenson V**, Pratt VM, Yang B, Nafa K, Yan L, Vitazka P.
J Mol Diagn. 2009 Jul;11(4):266-78. doi: 10.2353/jmoldx.2009.080125. Epub 2009 Jun 18.
PMID: 19541921 **Free PMC Article**

Expert article

14. Differential **methylation** profile of ovarian cancer in tissues and plasma.
Melnikov A, Scholtens D, Godwin A, **Levenson V**.
J Mol Diagn. 2009 Jan;11(1):60-65. doi: 10.2353/jmoldx.2009.080072. Epub 2008 Dec 12.
PMID: 19074590 **Free PMC Article**

Ovarian cancer

15. **Methylation** profile of circulating plasma DNA in patients with pancreatic cancer.
Melnikov AA, Scholtens D, Talamonti MS, Bentrem DJ, Levenson VV.
J Surg Oncol. 2009 Feb 1;99(2):119-22. doi: 10.1002/jso.21208.
PMID: 19065635

Pancreatic cancer

16. Array-based multiplex analysis of DNA **methylation** in breast cancer tissues.
Melnikov AA, Scholtens DM, Wiley EL, Khan SA, Levenson VV.
J Mol Diagn. 2008 Jan;10(1):93-101. doi: 10.2353/jmoldx.2008.070077. Epub 2007 Dec 28.
PMID: 18165279 **Free PMC Article**

Breast cancer

PEER-REVIEWED PUBLICATIONS

Biomarkers for early detection of breast cancer: what, when, and where?

17. Levenson VV.
Biochim Biophys Acta. 2007 Jun;1770(6):847-56. Epub 2007 Feb 12. Review.
PMID: 17368950

Breast cancer

DNA **methylation** biomarkers of cancer: moving toward clinical application.

19. Levenson VV.
Pharmacogenomics. 2004 Sep;5(6):699-707. Review.
PMID: 15335290

Original discovery

Total > 50 peer-review articles were published by 2 co-founders