Molecular Diagnostics: Local vs. Central Lab

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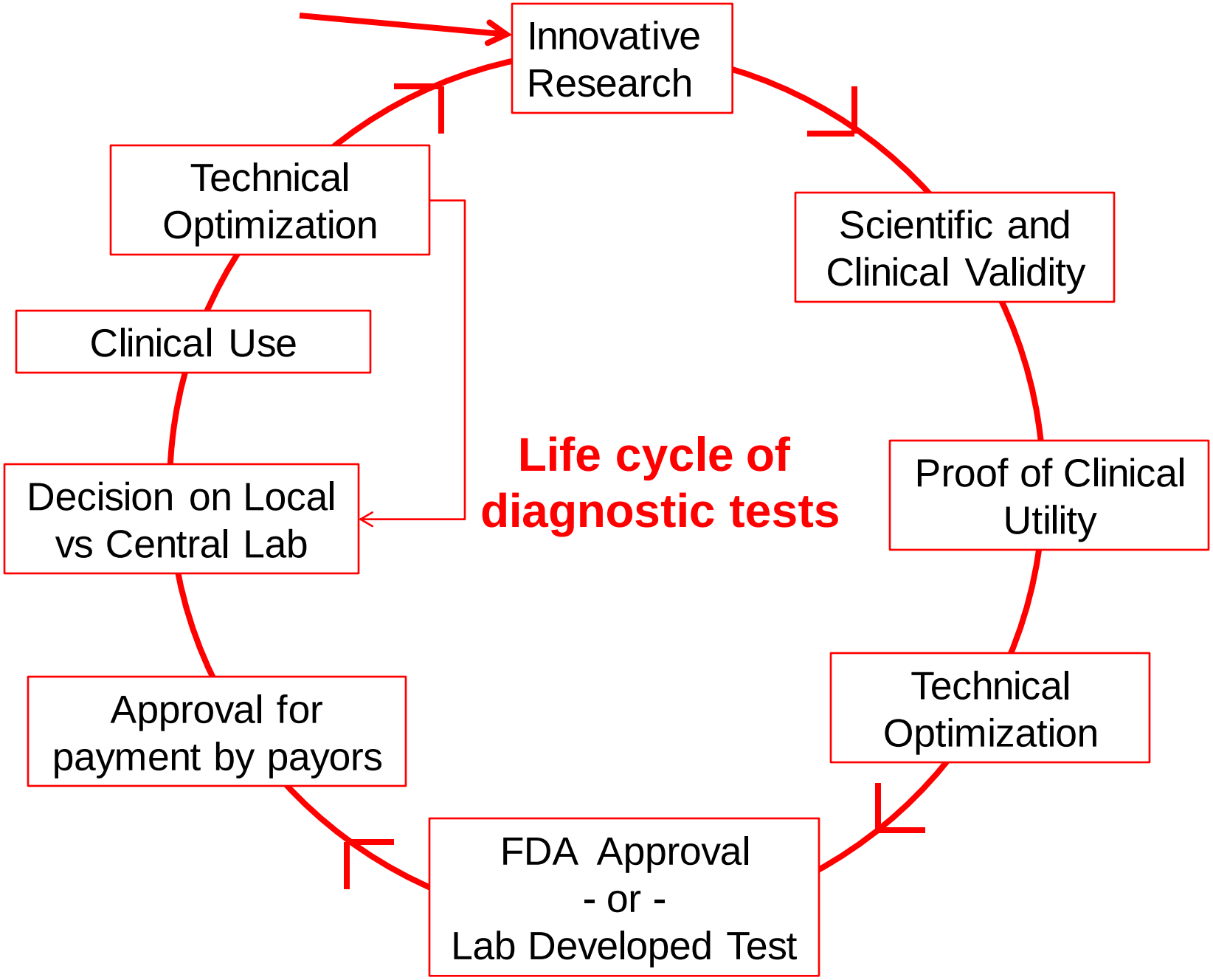

Innovative Research
Scientific and Clinical Validity
Proof of Clinical Utility
Technical Optimization
FDA Approval - or - Lab Developed Test
Approval for payment by payors
Decision on Local vs Central Lab
Clinical Use
Technical Optimization
Life cycle of diagnostic tests

How does MGH decide where to do lab tests?

1. Is the test turnaround time dependant? Regardless of volume or unit cost some tests must be available. For example carboxyhemoglobin for acute CO poisoning and other STAT tests that need to be done rapidly to permit clinical operations to function.

2. Is the specimen unstable and must be performed promptly regardless of clinical urgency? Example: Ionized calcium.

3. Is it a proprietary test or does it require patented/copyrighted interpretive software? We can't perform these tests: Example: Fibrosure for liver fibrosis.

4. Do we have the medical and technical expertise to perform and support the test: For example, nobody on our faculty knows enough about kidney stone analysis to interpret the test results so we send them out.

5. Then it is a make or buy decision. Moot if labs do 3rd party billing. Currently we send out 2,187 specific assays (n=161,000/yr; $7,000,000/yr)

From Kent Lewandrowski, MD, MGH Associate Chief, Laboratory & Molecular Medicine

Potential Advantages of Central Lab

Standardized methodology

?Peer reviewed methods

High volume

Build large multicenter comparison database for classifier development

?Cheaper (volume/fixed cost of equip)

?Turnaround time (runs every day)

High level of expertise

Examples of central better than local

Exotic lab send outs for low volume tests,

- Rare genetic diseases
- Rare infectious agents

Analysis of clinical trial samples

Example of a Central Lab
BRCA1,2 Breast Cancer risk(Myriad)

myriad® WHEN DECISIONS MATTER 25 YEARS

BRAC*Analysis*®

The BRAC*Analysis*® test assesses a person's risk of developing hereditary breast or ovarian cancer based on the detection of mutations in the *BRCA1* and *BRCA2* genes.

BRACAnalysis CDx™

BRACAnalysis CDx is an FDA-approved companion diagnostic test for germline BRCA1 and BRCA2 mutations intended to be used as an aid in treatment decision making for LynparzaTM (olaparib), a PARP inhibitor.[1]

Successful, developed database correlating outcome with individual mutations
Patented *BRCA1* and *BRCA2*– others couldn't perform the test

Association for Molecular Pathology v. Myriad Genetics

Decision of the Supreme Court

Justice Clarence Thomas, on June 13, 2013, delivered the opinion of the Court,[37][38][39] in which all other members of the Supreme Court joined, except Justice Antonin Scalia, who concurred in part and concurred in the judgment. The majority opinion delivered by Thomas held, "A naturally occurring DNA segment is a product of nature and not patent eligible merely because it has been isolated, but cDNA is patent eligible because it is not naturally occurring."[40]

He noted

- There are no method claims
- Does not involve patents on new *applications* of knowledge about BRCA1 and BRCA2
- Does not consider the patentability of DNA in which the order of the naturally occurring nucleotides has been altered

Potential Advantages of Local Lab

- Custom methodology (LDT)
- FDA Approval not necessary (may change!)
- Build comparison database for classifier development from local population samples
- ?Cheaper (Non-profit, no logistics)
- Turnaround time (no transportation)
- Data automatically entered into LIS
- Pathologist integrates results with pathology and clinical data
- Training of residents/faculty
- Familiarity breeds innovation

Examples of local molecular tests better than central

Common genetic diseases (Factor V Leiden)

Mutational analysis of tumors (high volume hosp)

Common infections

Pathology = Molecular Diagnostics

Research

Training

Practice

MGH Fellowships:

- Molecular Pathology
- Informatics

MGH Local Molecular Tests (Micro)

7 platforms
15 tests

Roche TaqMan

HIV

HCV

HBV

CMV

Seimens

HCV genotype

Cepheid GeneXpert

Influenza A/B and RSV

Cdif toxin (stool)

Enterovirus (on CSF)

MRSA/ MSSA (nasal swabs to detect colonization)

MTb (and Rifampin resistance) from sputum/BAL

Focus 3M

HSV 1 and HSV 2 (CSF)

Hologic/ Panther system

Chlamydia/Gonorrhea (Urine and cervical swabs)

Biofire

Ebola (emergency use) nucleic acid test

BD Max

Multiplex stool parasite panel

Multiplex Stool bacterial pathogen panel

Courtesy of Eric Rosenberg, MD PhD

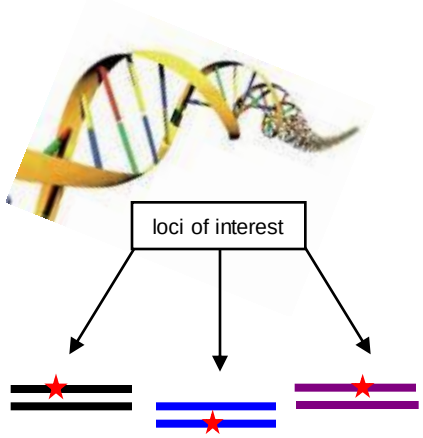
MGH Local Testing for Drugable Mutations in Cancer

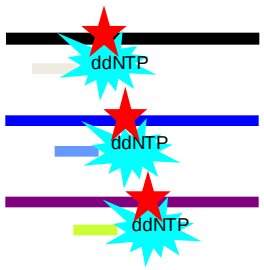
- SnapShot
- Next Generation Sequencing
  - ArcherDX
  - Illumina

John Iafrate, MD, PhD

SNAPSHOT Overview

Multiplex PCR → Single Base Extension Reaction → Capillary Electrophoresis

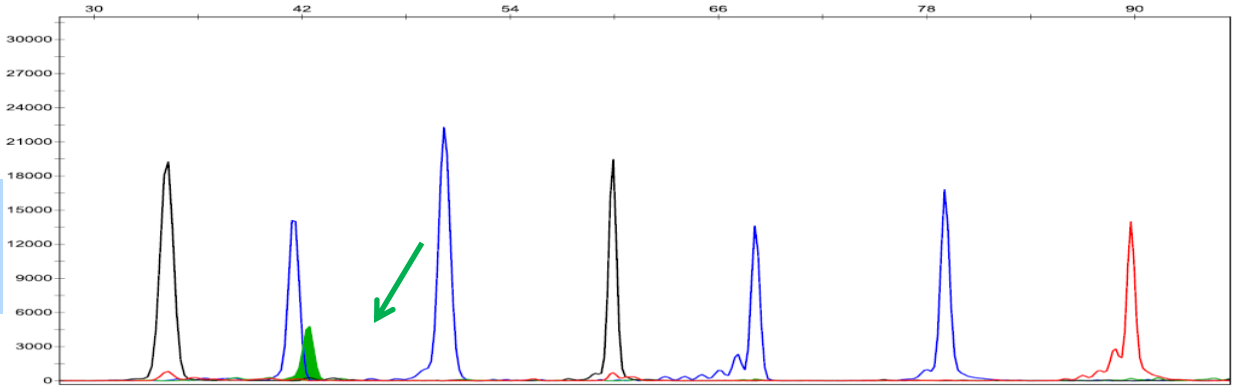




Electrophoretic Output

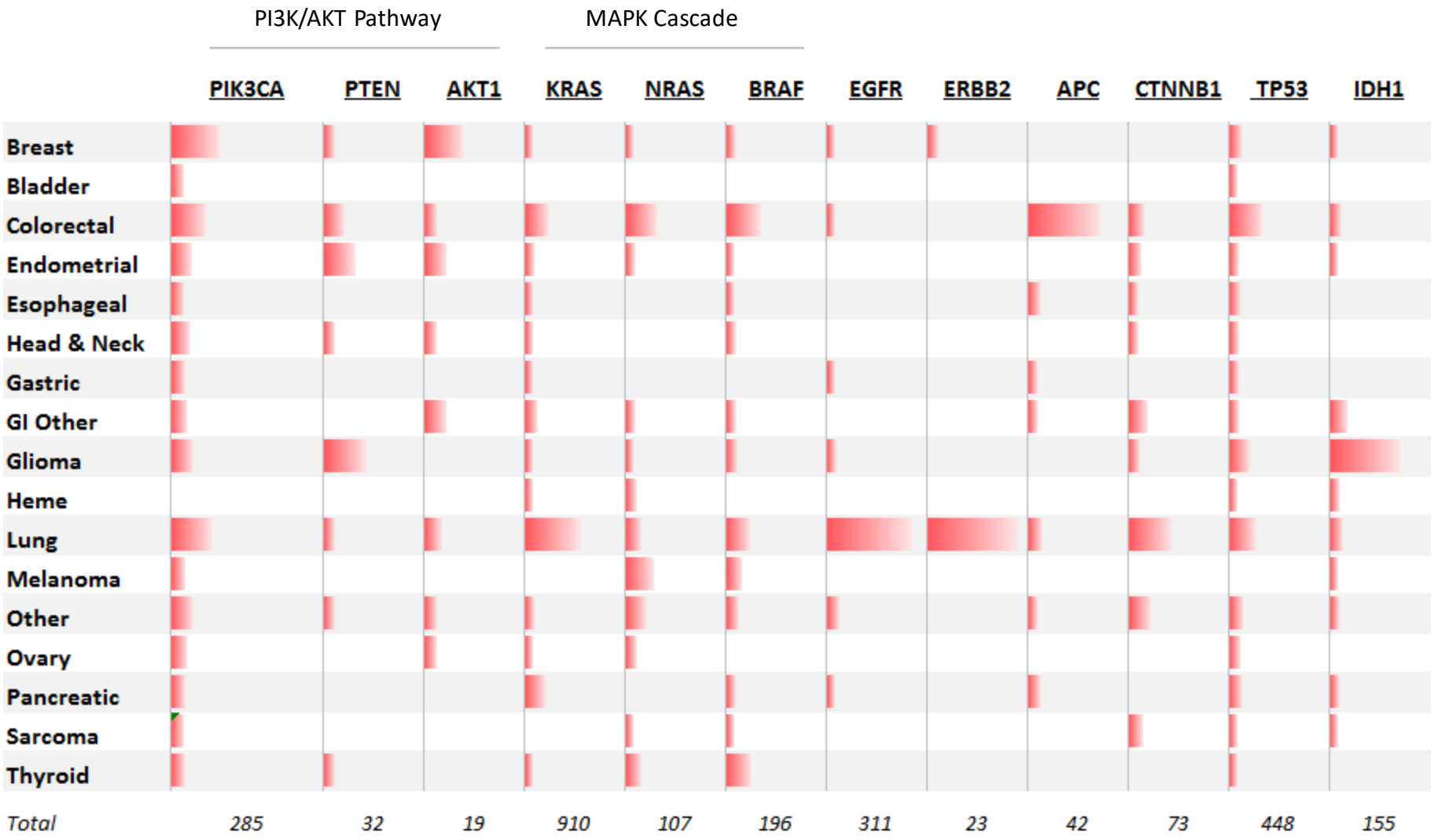
Lung cancer

EGFR mutation
Glu746_Ala750del
(c.2235_2249del)



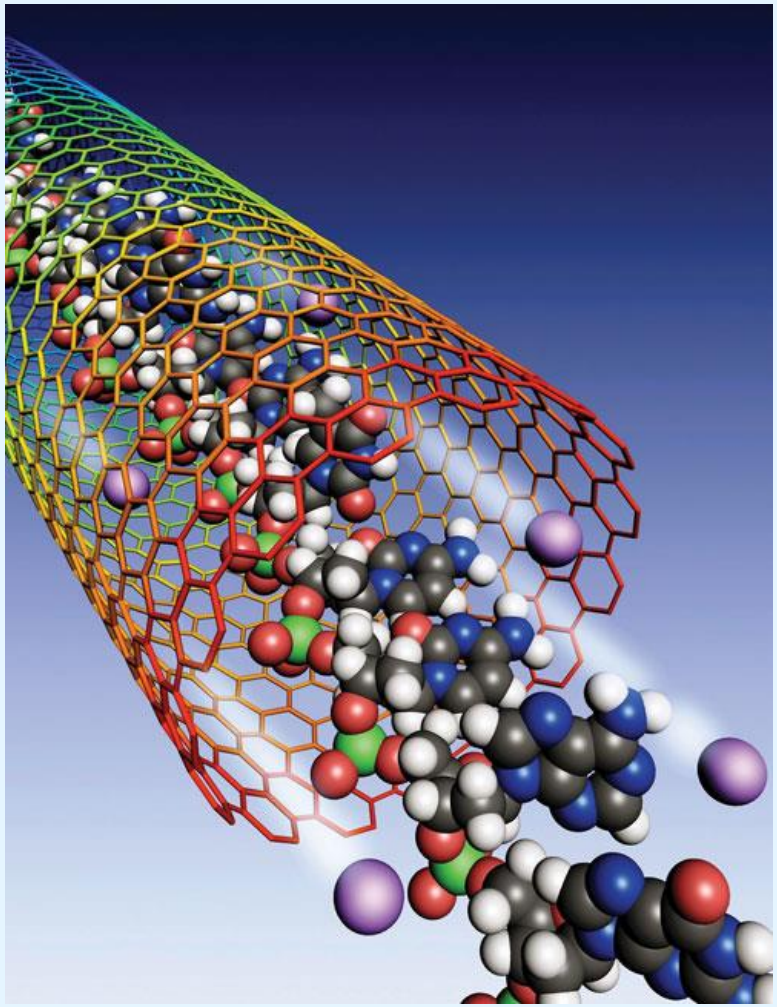
Dias-Santagata et al EMBO Mol 2010

Proportion of Mutations By Gene Across Disease Groups



Courtesy Darrell Borger

Next Generation Sequencing (NGS)
Clinical Cancer Genotyping



Clinical targeted sequencing of FFPE DNA

- 1000+ genes (~2.6 Mb)
- >100X coverage 10 bp into intron
- 5-10 Gb data per tumor-normal pair

- 5% analytical sensitivity
- 3-4 week turnaround time
- $700 raw reagent cost

- SNV, indel, copy number

J Iafrate

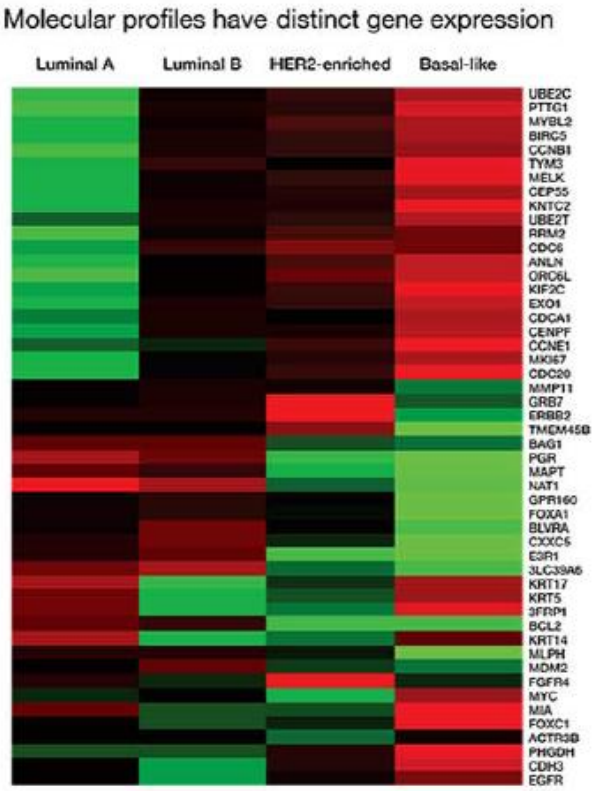
Example of a Local Lab Prosigna (Nanostring)

50 gene classifier

Every Prosigna Score is generated by a proprietary algorithm[1]

- The Prosigna Score is a numerical value on a 0-to-100 scale that correlates with the probability of distant recurrence within 10 years
- The gene expression profile of a patient's tumor is compared with each of the 4 PAM50 prototypical molecular profiles to determine the degree of similarity. The results in combination with a proliferation score and tumor size produce an individualized Prosigna Score

Intended use/indications for use: The Prosigna Breast Cancer Prognostic Gene Signature Assay is an in vitro diagnostic assay which is performed on the NanoString nCounter® Dx Analysis System using FFPE breast tumor tissue previously diagnosed as invasive breast carcinoma. This qualitative assay utilizes gene expression data, weighted together with clinical variables to generate a risk category and numerical score, to assess a patient's risk of distant recurrence of disease. The Prosigna Breast Cancer Prognostic Gene Signature Assay is indicated in female breast cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either as:



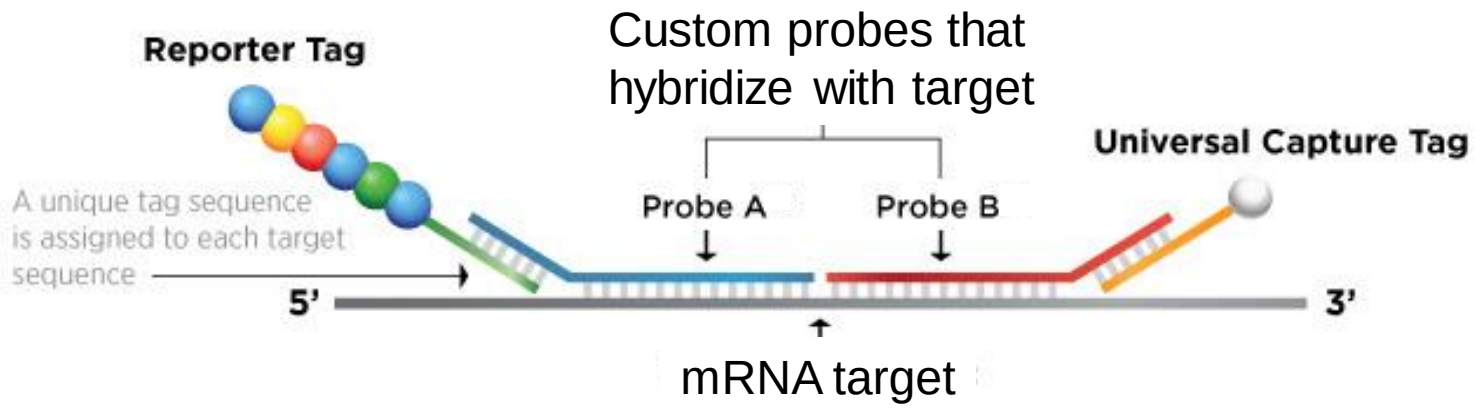
Nanostring Platform

FDA Approved Test for

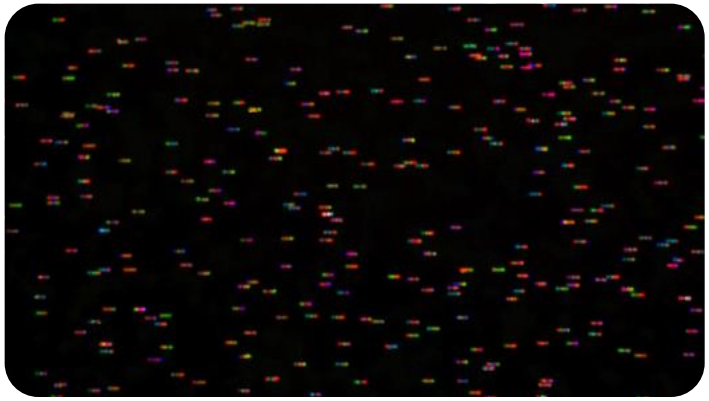
Breast Cancer Prognosis (Prosigna)

NanoString® Technique

- High sensitivity
  - > microarrays
  - = RT-PCR, without amplification
- Quantitative
  - Counts individual mRNA molecules



Fluorescent "Bar tags" detected and counted



Geiss et al
Nature Biotech 2008

Chronic Antibody-Mediated Rejection in Nonhuman Primate Renal Allografts: Validation of Human Histological and Molecular Phenotypes

B.A. Adam[1], R.N. Smith[2], I.A. Rosales[2], M. Matsunami[3], B. Afzali[1], T. Oura[3], A.B. Cosimi[3], T. Kawai[3], R.B. Colvin[2], M. Mengel[1]

[1]University of Alberta, Edmonton, Canada

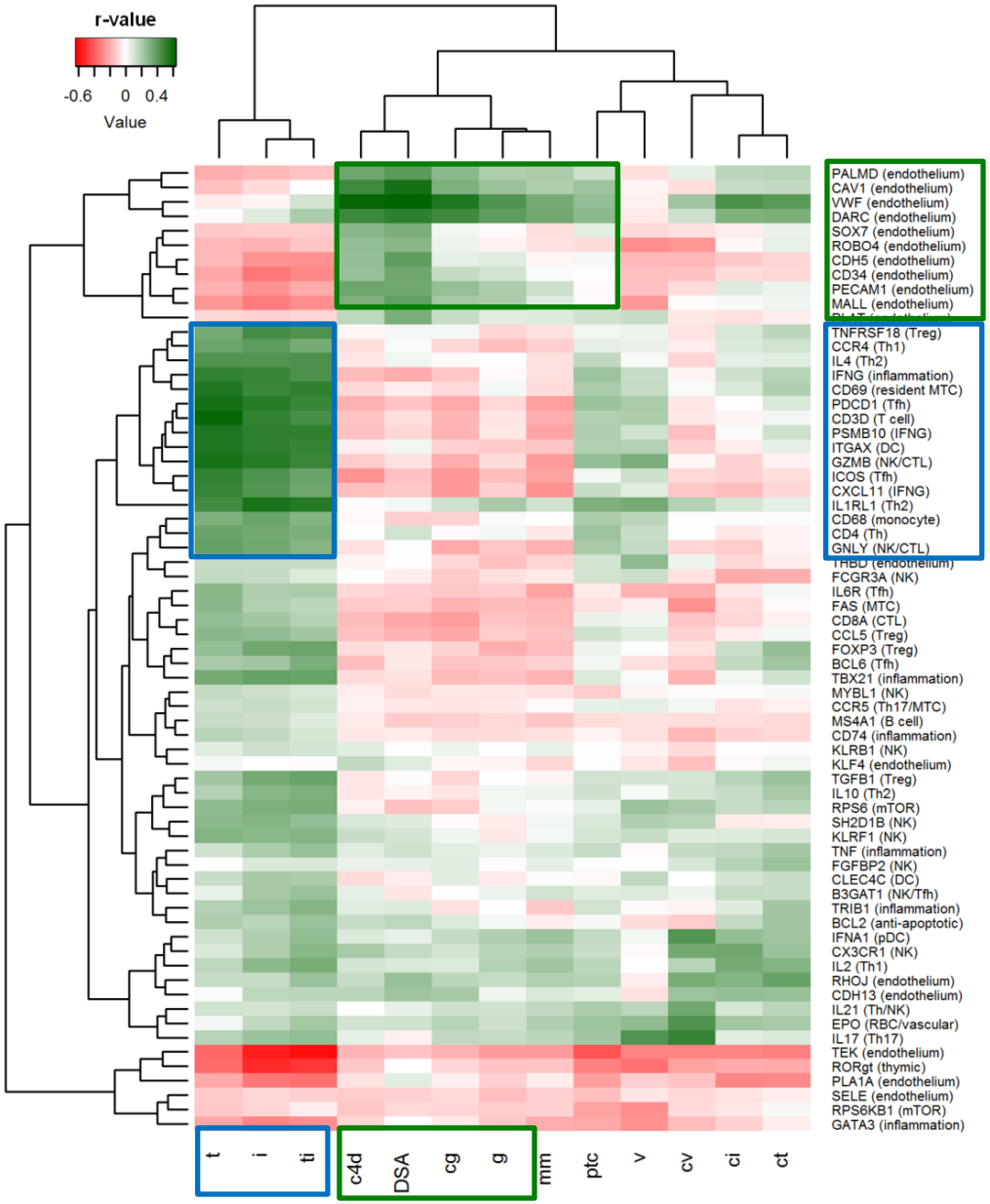
[2]Harvard Medical School and Massachusetts General Hospital, Boston, USA

Presented at the ATC 2016/Banff 2017

Under review AJT

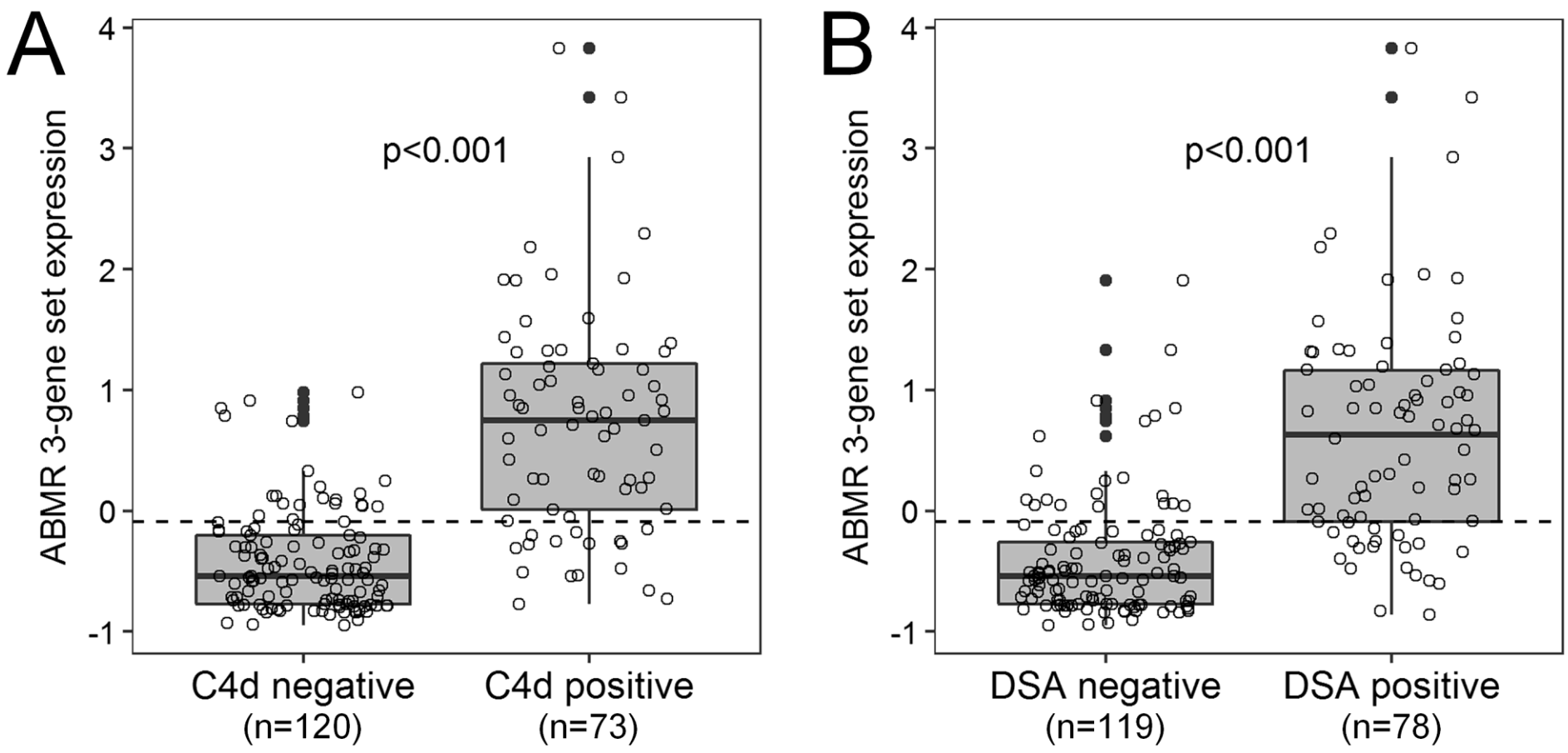
Endothelial genes correlate with C4d, DSA, cg, g, ptc

Inflammation-related genes correlate with t, i, ti



Ben Adam et al submitted

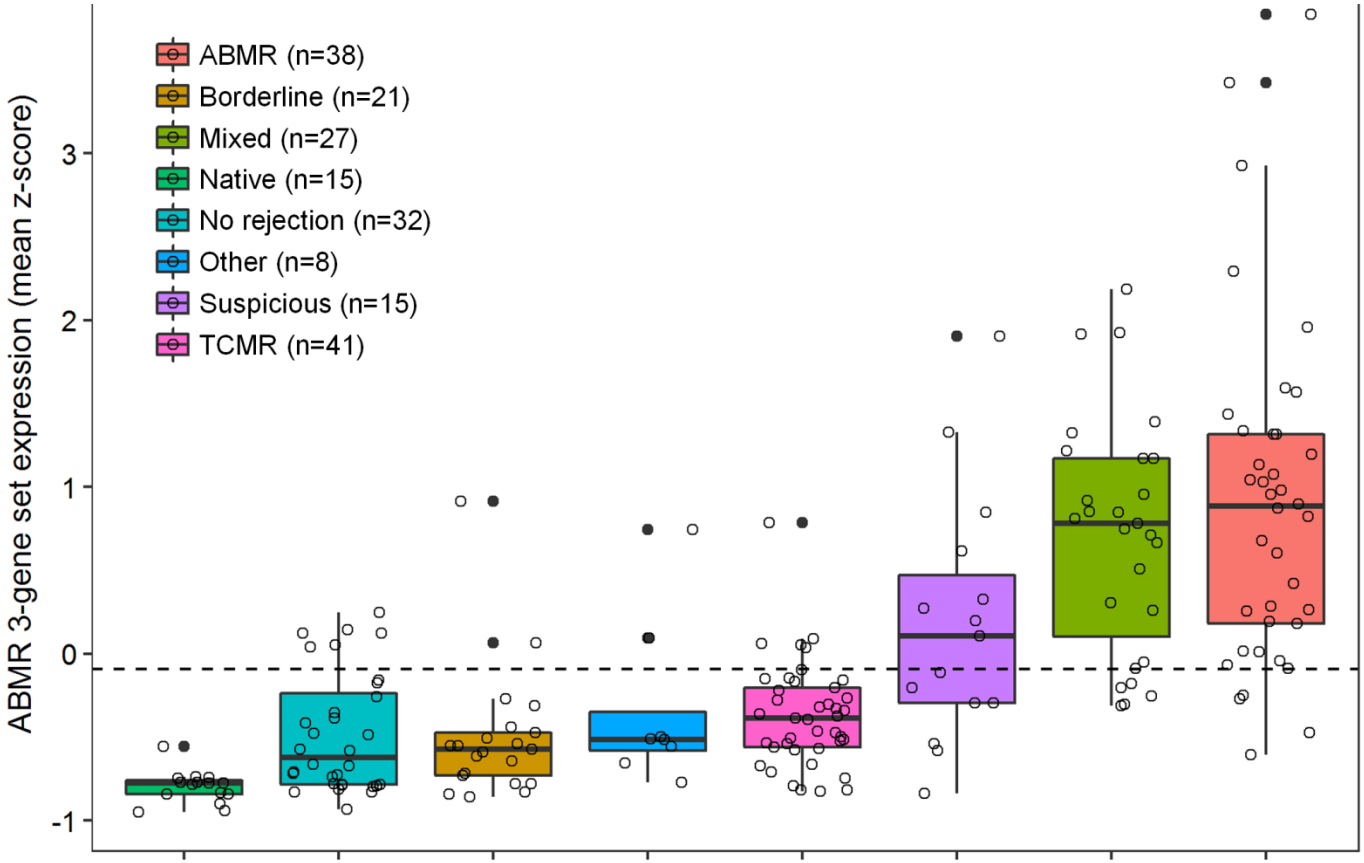
3 Gene AMR Set Correlates with C4d and DSA



Best performers in repeated ROC analysis: VWF, DARC, CAV1

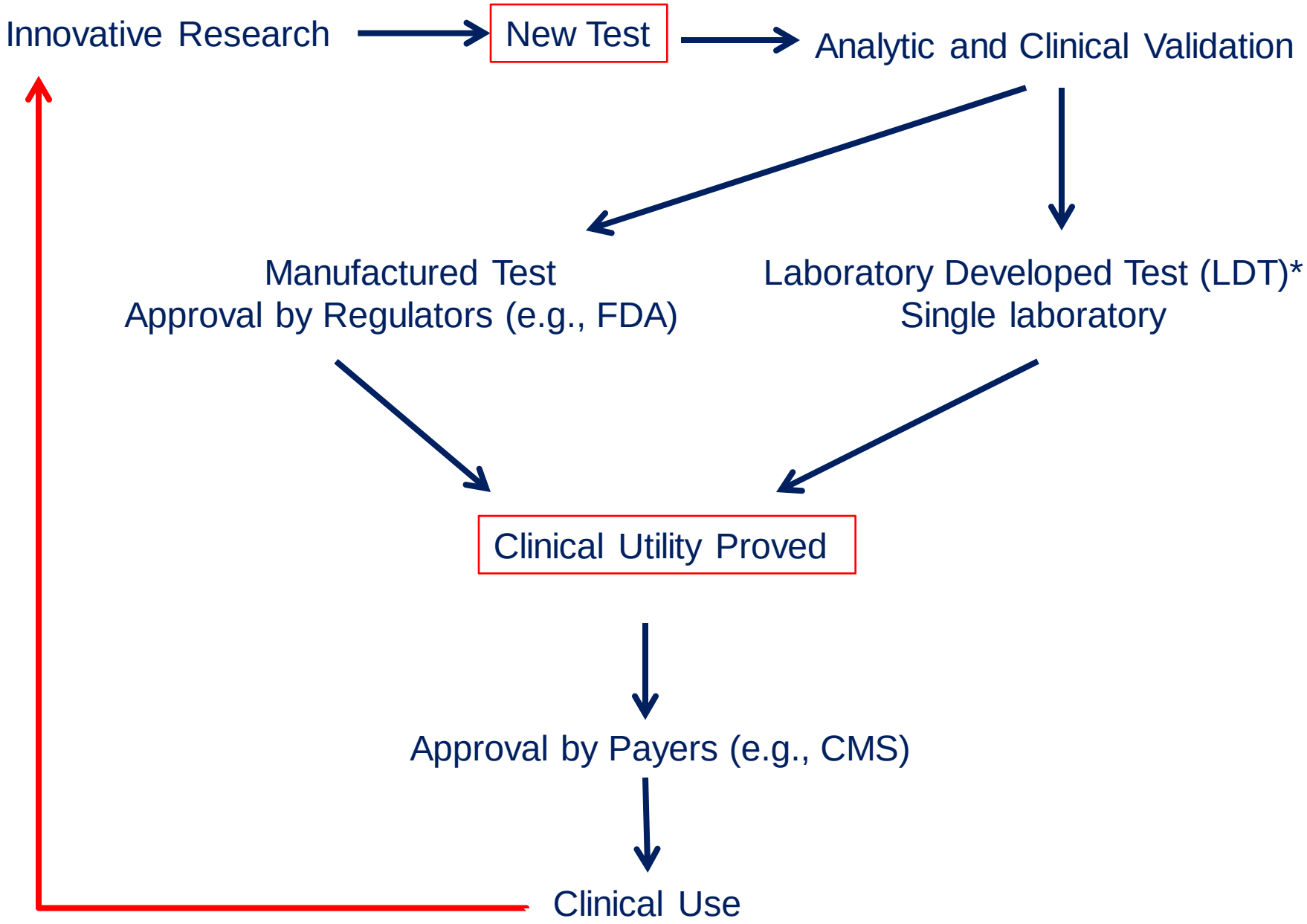
Ben Adam et al submitted

3 gene set distinguishes AMR



	Native	No rejection	Borderline	Other	TCMR	Suspicious	Mixed	ABMR
Native		p=0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
No rejection	p=0.001		ns	ns	ns	p<0.001	p<0.001	p<0.001
Borderline	p<0.001	ns		ns	p=0.016	p<0.001	p<0.001	p<0.001
Other	p<0.001	ns	ns		ns	ns	p<0.001	p<0.001
TCMR	p<0.001	ns	p=0.016	ns		p=0.004	p<0.001	p<0.001
Suspicious	p<0.001	p<0.001	p<0.001	ns	p=0.004		p=0.024	p=0.011
Mixed	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p=0.024		ns
ABMR	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p=0.011	ns	

Ben Adam et al submitted



*Federal regulations pending in US

mRNA Test Complexity

	Single Gene	Gene Set	Classifier
Examples	Granzyme B EBER	ENDAT (Halloran) ABMR/TCMR score (Halloran) Eculizumab Response (Lefaucheur)	Prosigna (Nanostring) Molecular Microscope (Transcriptome Sciences)
Technique	PCR In situ hybridization	PCR Affymetrix Nanostring	Affymetrix Nanostring
Interpretation	Value vs . disease control (mean $\pm$ std dev) Presence/Absence	Value vs . disease control (mean $\pm$ std dev) Geometric mean vs comparison group	Pattern vs large data set of classified samples Archetypes, PCA, random forest…

Steps for Molecular Dx in Transplantation

- Prove clinical utility
- Link results to specific therapy

- Optimize and simplify techniques

  Platform (Affymetrix, Nanostring, PCR...)
- Develop LDT or FDA approved tests

- Show cost effectiveness
- Get Payors to pay

- Then decide the optimal way to provide the test
  - local vs central