Developing RNA-based molecular diagnostics in the post-genomic era

National Cancer Policy Forum

Policy Issues in the Development and Adoption of Molecularly Targeted Therapies for Cancer

11/10/2014

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UNC LINEBERGER

UNC CANCER CARE

Introduction

- Computational biologist / translational researcher (The Cancer Genome Atlas 10,000 transcriptomes)
- Physician and clinical trialist
- Not pathologist
- Co-Founder of diagnostics company (GeneCentric)

Diagnostic Test Adoption

- Science
  - Evidence (hypothesis generation, validation)
  - Platform (tissue and technology)
- Regulatory (federal, state, accrediting bodies)
- Payment
- Practice (adoption in clinical practice)

Science

Evidence for RNA

Mutation Detection

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Integrated RNA and DNA sequencing improves mutation detection in low purity tumors

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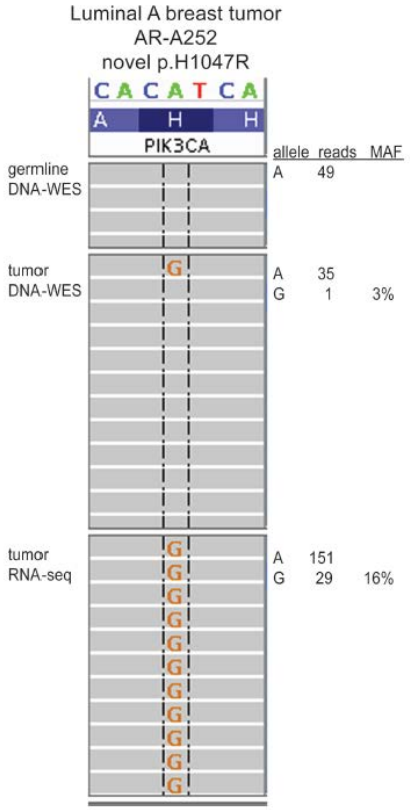
- Somatic (cancer causing) alterations
- Driver versus passenger
- “Mutant expression”

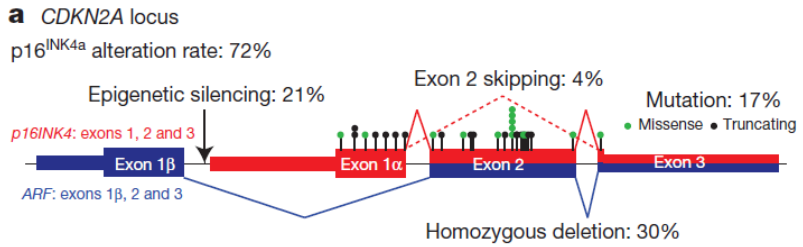


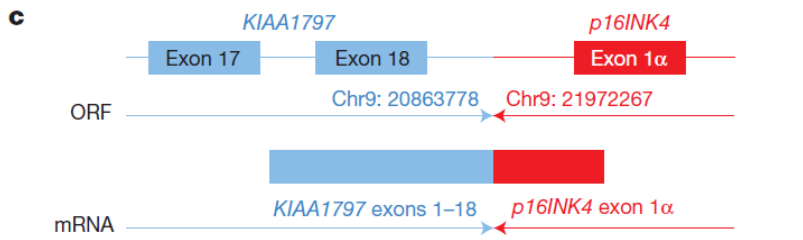
Structural Alteration

Detection of Structural Alterations

- Whole genome sequencing (expensive)
- Whole exome (limited)
- In situ hybridization (clinical assay, expensive and specific,"one at a time")
- RNA – cheap and all inclusive

Structural Alterations of CDKN2A by RNA

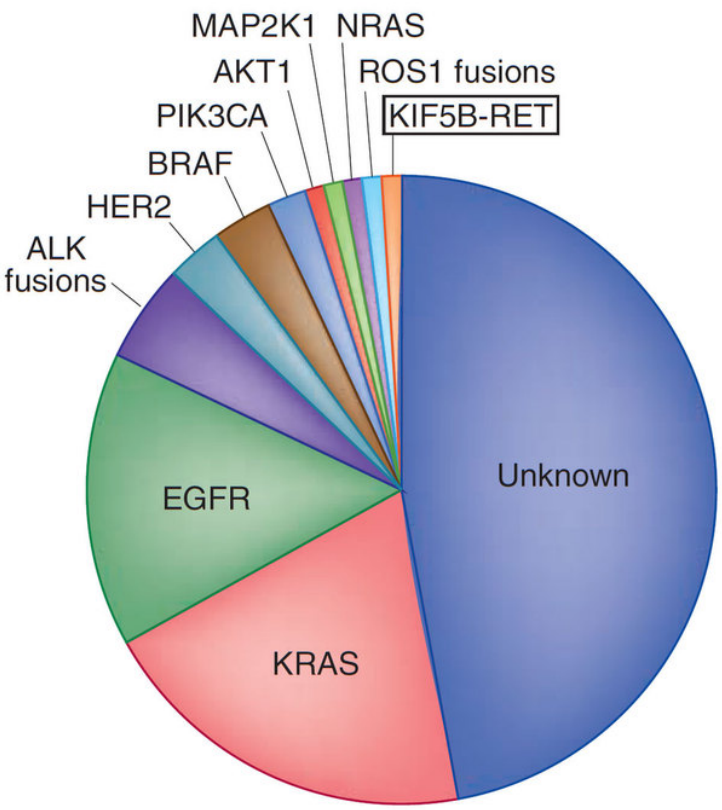




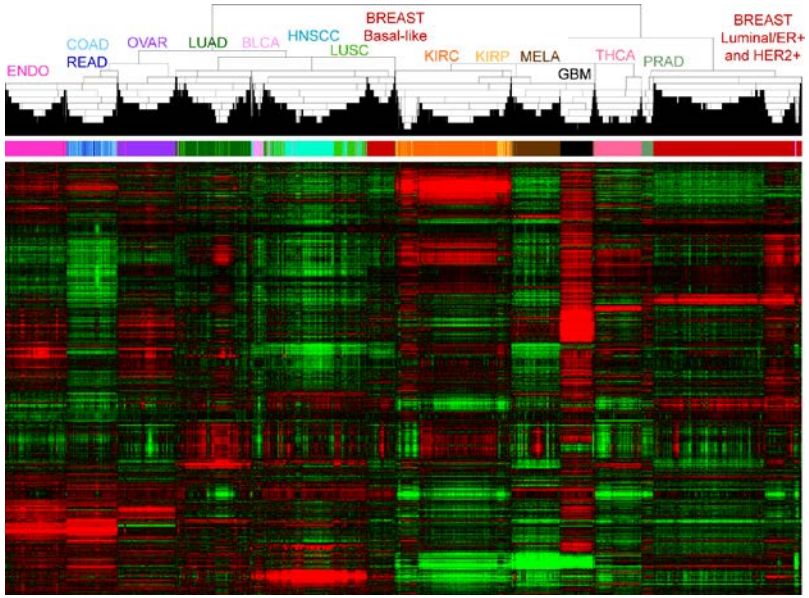
Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012

Integrated classification beyond mutation

RNA



DNA alterations



Immune System?

Pao, Nature Medicine 2012

Clinical Validation

- Hypotheses generated (validated) in convenience datasets
- Clinical validation needs to happen in clinical trials datasets
  - Largely absent or unavailable ($)
  - Generation of new data prohibitive ($$$)

Science

Platform

Tissue Requirements and Quality: Lots of opinions, lots of experience, few published data

- % tumor
  - min and max
- Enrichment
  - macro, micro dissection
  - other
- Total amounts
  - Amplification
- Frozen vs paraffin

- Lower amounts and % tumor are useful for finding known variants (1% tumor) and signatures

- More tumor helps find new variants and signatures

Research Frozen vs Clinical Paraffin

- Classical teaching
  - RNA degrades quickly
  - Assays on frozen tissue
- Recent experience
  - Intact 200-300 bp RNA fragments remain
  - Technologies targeting 300 bp robust

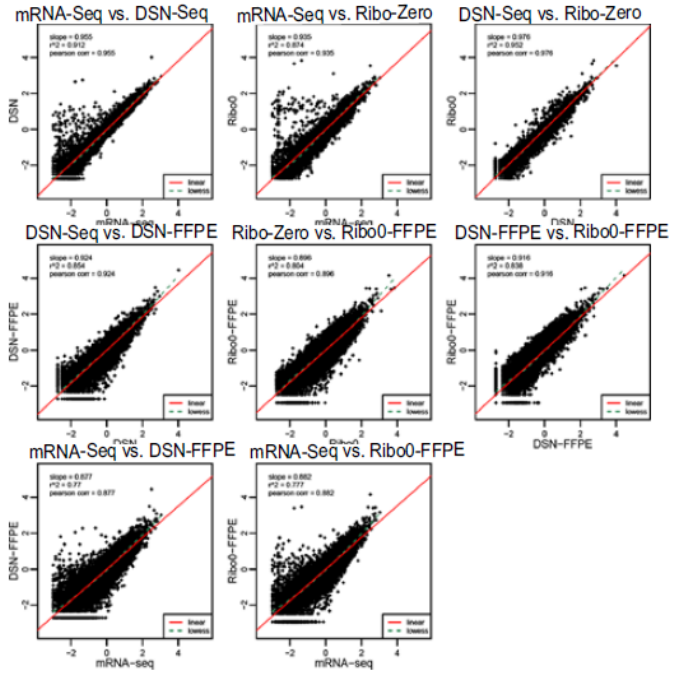
Zhao et al. BMC Genomics 2014, 15:419
http://www.biomedcentral.com/1471-2164/15/419

BMC Genomics

RESEARCH ARTICLE Open Access

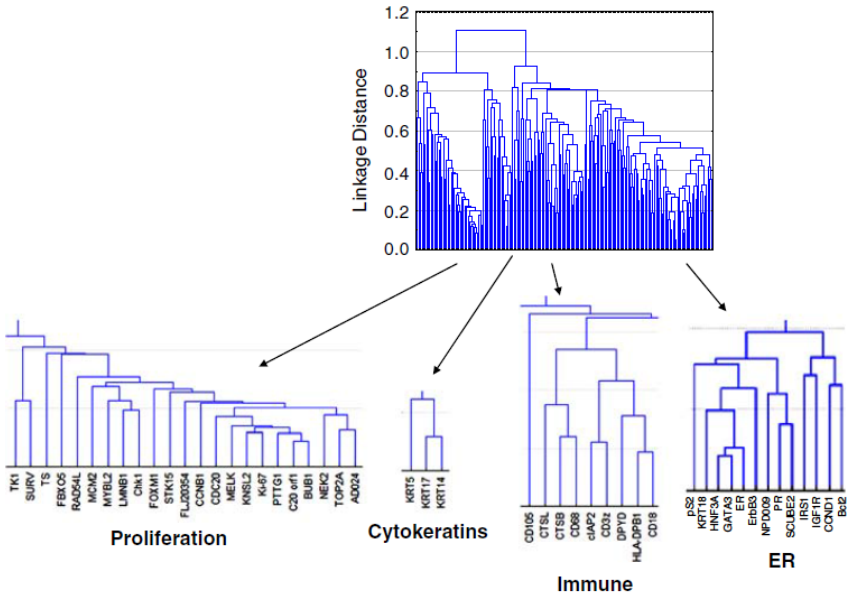
Comparison of RNA-Seq by poly (A) capture, ribosomal RNA depletion, and DNA microarray for expression profiling

Wei Zhao[1,2], Xiaping He[2,3], Katherine A Hoadley[2,3], Joel S Parker[2,3], David Neil Hayes[3,5] and Charles M Perou[1,2,3,4*]

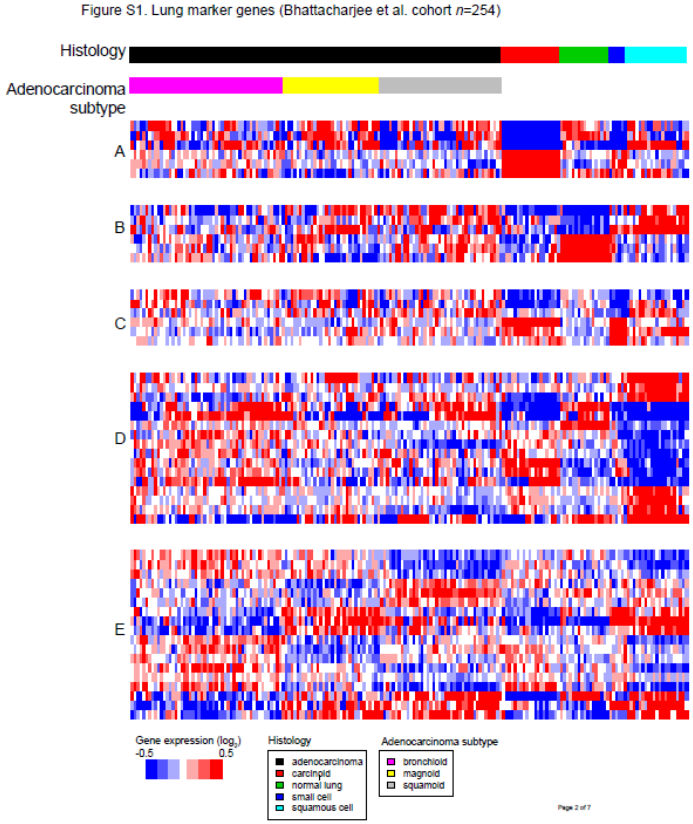


Paraffin Diagnostics

Genomic Health: Oncotype DX, breast Cancer



LabCorps: HistoPlus, lung Cancer

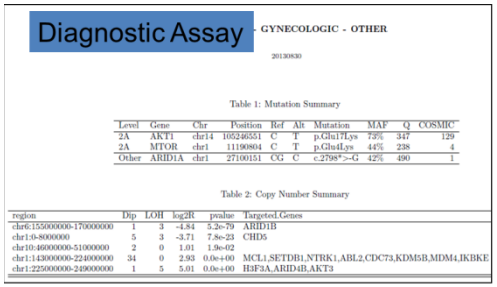


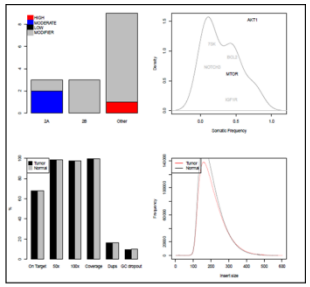
J Mol Diagn. 15(4), 485-97. 2012

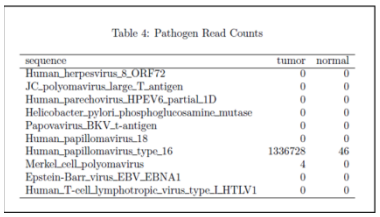
UNC Experience = UNCSeq: LCCC1108

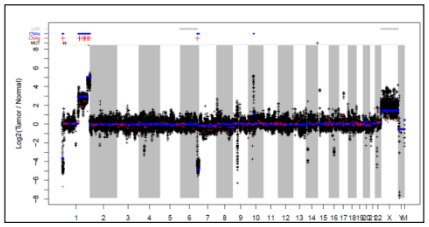
- DNA and RNA assays (capture)
- 1400 patients
- 10 microns tissue (500-1000 ug)
- Variety sources
  - Biopsy (core)
  - Gross resection

- FNA (no quantification)
  - TruSite









Platforms (predicate instrument)

Issues

- Regulatory clearance for RNA? Mostly no.
- Cost
- Throughput
- Availability to small and large diagnostics labs
- Bridging of “evidence” to commercial assay

Examples

- Roche LightCycler or similar
- Roche Life Technologies sequencers
- Illumina sequencers
  - miSeq, HiSeq (multiple formats), NextSeq 500
- Nanostring (FDA)

Regulatory

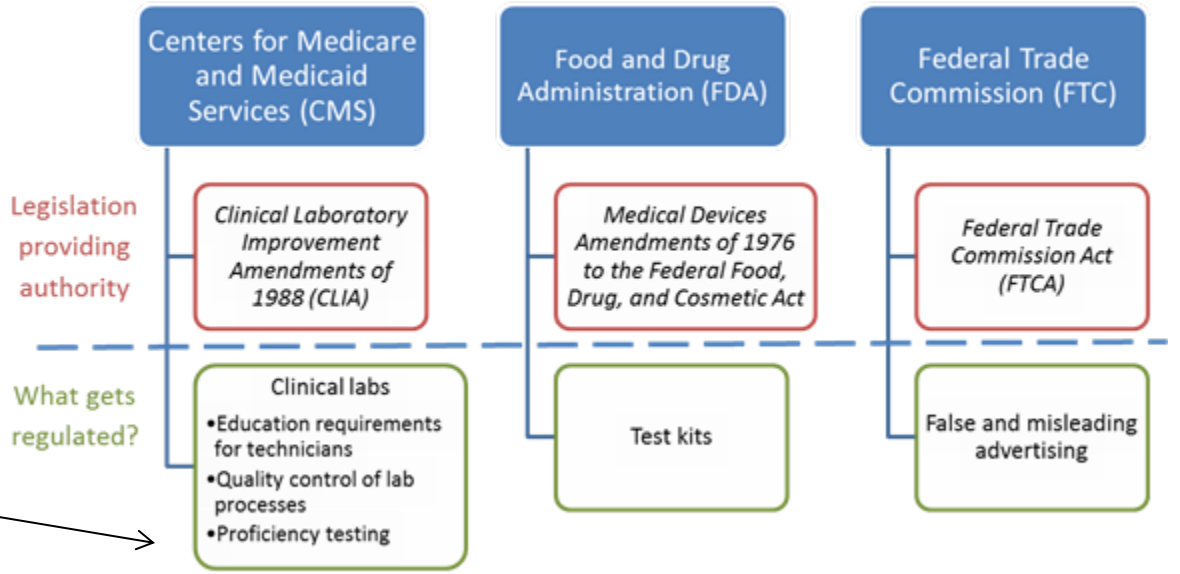
Tests need to be compliant

Compliance is complicated and expensive

Diagnostic tests may often require private sector development

Federal Regulation of Genetic Tests

LDT = Largely unregulated, although the lab itself is regulated



FDA regulates a test is determined by how it comes to market. A test may be marketed as a commercial test "kit," a group of reagents used in the processing of genetic samples that are packaged together and sold to multiple labs. More commonly, a test comes to market as a laboratory-developed test (LDT), where the test is developed and performed by a single laboratory, and where specimen samples are sent to that laboratory to be tested. The FDA regulates only tests sold as kits and, to date, has practiced "enforcement discretion" for LDTs.

http://www.genome.gov/10002335

Take Home: 2 strategies

- LDT
  - Potentially cheap
    - IHC
    - Foundation One, Genomic Health
  - Regulatory status is unclear
- FDA
  - 510k – <$10 million (but >any R01)
  - PMA - >$10 million

Laboratory-developed test (LDT)

Intended

- Diagnostics (IVDs) manufactured. Developed, validated, and offered, within a single laboratory.
- Simple, well-understood pathology tests or
- Diagnosed rare diseases
- Used in a single institution as part of patient care
- Testing outside the institution would be prohibitive to patient care (due to timing between test need and result delivery)

Actual

- Delivery often is by large corporations
- Test is not simple or well understood
- Disease are common (breast cancer)
- Use in patient care not always clear
- Test is not intended for a single institution but rather reference lab strategy where entire country sends test to the lab
- Common use of an LDT in place of an FDA approved assay

- Examles
  - Genomic health
  - Foundation medicine
  - Labcorp (many LDT's) including GeneCentric

Payment

1. Cost of assay
2. Investment of development for private sector partners

Intellectual Property Uncertainty

- “Mayo vs Prometheus”
- “Association for Molecular Pathology v. Myriad Genetics”
- Patent office struggling in light of these decisions, and by extension those wish to develop novel tests

Test Reimbursement

- Medicare
  - "Medicare Has Stopped Paying Bills For Medical Diagnostic Tests. Patients Will Feel The Effects" *Forbes. 3/27/2013*
- State by State
- Insurer by insurer
- Self pay

Practice

Adoption in clinical practice

Impact on clinical workflow

Changing Provider Behavior

- Difficult even when evidence suggests a superior test
- Cancer - multiple physicians involved
  - Subspecialists (biopsy)
  - Surgeons (biopsy and definitive surgery)
  - Med onc
    - User of diagnostic
    - Involved after biopsy / tissue processed

Pathology Workflow

- Anatomic pathologist diagnosis of cancer have short timeline
- Special tests outside workflow
  - Send out LDT
  - Molecular tests in molecular path lab
  - Default IHC (even if test is inferior)
- Lack of coordination in information management

Diagnostic Test Adoption

- Science
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Carcinoma of Unknown Primary (CUP) Personal experience

- Challenging diagnosis
- Extensive IHC evaluation
- Multiple LDT RNA assays
  - bioTheranostics, Rosetta, others
  - Send out
- Frequently desired by physicians
- Never sent voluntarily by our path department
  - Lack of knowledge about the CUP assays
  - Discussed largely in negative

Blinded Comparator Study of Immunohistochemical Analysis versus a 92-Gene Cancer Classifier in the Diagnosis of the Primary Site in Metastatic Tumors

Lawrence M. Weiss,* Peiguo Chu,† Brock E. Schroeder,† Veena Singh,† Yi Zhang,† Mark G. Erlander,† and Catherine A. Schnabel†

Table 2 Sensitivity and Specificity of the 92-Gene Assay and IHC/Morphology Analysis at the Main Type Level and for the Colon/Appendix Subtype

		IHC/morphology analysis		92-gene assay	
Main type	No.	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Melanoma	2	0 (0—66)	100 (97—100)	0 (0—66)	99 (95—100)
Sarcoma	7	86 (49—97)	98 (94—100)	100 (65—100)	98 (94—100)
Mesothelioma	1	0 (0—79)	100 (97—100)	100 (21—100)	99 (95—100)
Lung	24	67 (47—82)	94 (87—97)	75 (55—88)	95 (89—98)
Gynecologic	8	88 (53—98)	98 (94—100)	88 (53—98)	96 (91—99)
Gastrointestinal	26	92 (76—98)	93 (86—96)	92 (76—98)	97 (91—99)
Colon/appendix subtype	17	94 (73—99)	97 (92—99)	94 (73—99)	99 (95—100)
Urinary bladder	11	45 (21—72)	99 (95—100)	82 (52—95)	99 (95—100)
Kidney	13	77 (50—92)	100 (97—100)	77 (50—92)	99 (95—100)
Endocrine	9	56 (27—81)	99 (95—100)	56 (27—81)	99 (95—100)
Hepatocellular	1	100 (21—100)	100 (97—100)	100 (21—100)	100 (97—100)
Head and neck/esophageal squamous	3	67 (21—94)	98 (94—100)	67 (21—94)	97 (92—99)
Salivary gland	1	0 (0—79)	100 (97—100)	0 (0—79)	98 (93—99)
Prostate	4	50 (15—85)	100 (97—100)	100 (51—100)	100 (97—100)
Breast	11	55 (28—79)	97 (92—99)	73 (43—90)	100 (97—100)
Skin basal cell	1	0 (0—79)	100 (97—100)	0 (0—79)	100 (97—100)
Total main type	122	69 (60—76)	99 (98—99)	79 (71—85)	99 (98—99)

The 95% CIs are provided in parentheses.

The Journal of Molecular Diagnostics, Vol. 15, No. 2, March 2013