Your impact on pathology's future – a junior pathologist's perspective

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Thanks

HUP
- K. Hirokawa
- C. Culin
- J. Dirienzi
- I. Tsimberg

PAH
- J. Milano
- I. Spector
- J. Immordino

- Department of Pathology and Laboratory Medicine

How I got interested

- Breast predictive marker panels – QA/QI
- CAP – standardization of ER/PR/Her-2 testing
  - Problems in Eastern Canada
  - Attempt to standardize testing and analysis

Cross-comparison methods

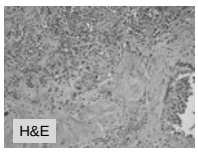
- ER+ breast carcinoma cases were subjected to commercial RT-PCR-based analysis of gene expression (mRNA) for assessment of treatment response
- Useful for validation of IHC and RT-PCR (and FISH)

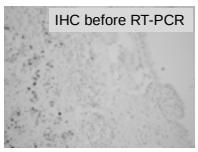
Comparison of IHC to commercial RT-PCR predictive marker testing

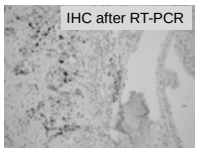
- ER 100% correlation
- PR 80% correlation
- Her-2 100% correlation

148 cases

IHC vs. RT-PCR discrepancy







IHC: Positive
RT-PCR result: Negative

Interpretation: mRNA degradation

What are the variables?

- Pre-analytical
  - Cold ischemia and fixation time
  - Early death of tumor cells
    - RNA degradation
    - Protein degradation
- Analytical sensitivity
- Post-analytical
  - Accurate scoring

Can we do a better job of determining the “truth” in a clinically meaningful context?

How else can we assess the “truth”?

- Genes
  - Microarray - based
    - Expression arrays for mRNA
    - miRNA array
    - Comparative genome hybridization
  - Sequencing - based
    - Genomic DNA sequence
    - Methylation/Epigenomics
    - Expressed gene seq.
- Other ‘omics’ (including IHC)
  - Proteins, lipids, sugars

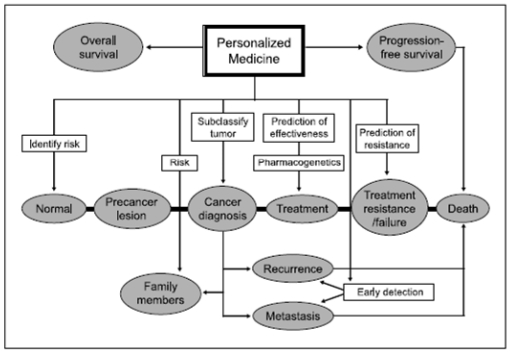
Personalized medicine

- Term coined in a WSJ article in 1998
- Inclusive of pharmacogenetic approaches already in practice
  - IHC/FISH for ER/PR/Her-2 for breast carcinomas are linked to clinical responses to medical therapies.
- Since, an explosion of new tests (CNS, sarcoma, carcinoma, melanoma, lymphoma, leukemia…)
- Non-neoplastic disease (HUGE!)

Definition – *personalized medicine*

- Identifiable target with a drug directed towards this target.

  Treatment can be individualized based on a "complete" molecular diagnostic profile.
- Some advocate the term *stratified medicine* since molecular testing to stratify the patients with shared biological characteristics to the best treatment.
- Distinct from *predictive medicine* already in practice mainly for *in utero* testing/family planning.



Personalized medicine - examples

Neoplastic

- Inhibitor used
  - B-raf in melanoma
  - *BCR-ABL* in CML
  - PDGFR in GIST
- K-ras+ in lung CA – avastin avoided
- Triage for BM transplant: Complex cytogenetics in AML

Personalized medicine - examples

Non-neoplastic

- Avoidance of side effects
- Factor V Leiden – thrombosis
- SNPs

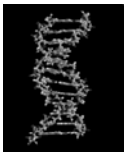
Table 1. A partial list of commercial tests currently available for PM in cancer patients

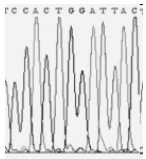
Test	Company	Cancer type	Test type	Technique	Application
Oncotype Dx Breast Cancer Assay	Genomic Health	Breast	Expression profile of a panel of 21 genes	RT-PCR	Predicts risk of recurrence and guides chemotherapy treatment decision
Oncotype Dx Colon Cancer Assay	Genomic Health	Colon	Expression profile of a panel of 12 genes	RT-PCR	Predicts recurrence and assists treatment decision in stage II colon cancer
MammaPrint	Agendia	Breast	Expression of a panel of 70 genes	Microarray	Predicts risk of recurrence
HercepTest	Dako	Breast	c-erbB-2 overexpression	IHC	Predicts response to trastuzumab (Herceptin)
Ventana Pathway	Ventana	Breast	c-erbB-2 overexpression	IHC	Predicts response to trastuzumab (Herceptin)
ThxaScreen: EGFR29	DxS	NSCLC	EGFR29 mutation	RT-PCR	Predicts response to gefitinib (Iressa) and erlotinib (Tarceva)
ThxaScreen: KRAS Mutation Kit	DxS	mCRC	KRAS mutation	RT-PCR	Predicts response to panitumumab (Vectibix) and cetuximab (Erbitux)
CYP450 Test	Amplichip	Breast	Identify CYP2D6 and CYP2C19 genotype	Microarray	Predicts response to tamoxifen and determines optimal treatment dose
BCR-ABL Mutation Analysis Test	Genzyme Genetics	CML	T315I mutation	RT-PCR	Predicts response to imatinib (Gleevec)
EGFR Amplification Test	Genzyme Genetics	CRC	EGFR amplification	FISH	Predicts response to cetuximab (Erbitux) and panitumumab (Vectibix)
LGFH Amplification Test	Genzyme Genetics	NSCLC	LGFH amplification	FISH	Predicts response to gefitinib (Iressa) and erlotinib (Tarceva)
ALK Gene Rearrangement Test	Genzyme Genetics	NSCLC	ALK gene rearrangement	FISH	Predicts response to crizotinib (Tarceva)
EGFR Mutation	Genzyme Genetics	NSCLC	EGFR Mutation	RT-PCR	Predicts response to tyrosine kinase inhibitors

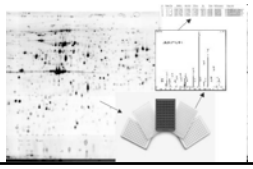
Abbreviations: RT-PCR, real-time PCR; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; mCRC, metastatic colorectal cancer; CML, chronic myelogenous leukemia; CRC, colorectal cancer; FISH, fluorescence in situ hybridization.

Analytes

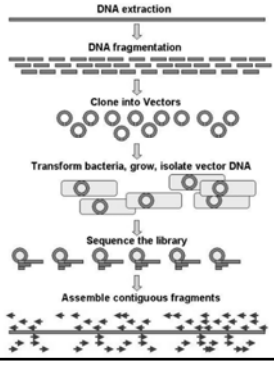
- DNA
- RNA
- Protein







Human genome sequencing (2003)
This took 13 years



For the last decade, my opinion was that genomic sequencing is too expensive, too limited in scope, and of uncertain value, for widespread use and it would be a long time before it is used routinely.

Recently, my viewpoint changed

- $
- Time
- Clinical utility

Cost for sequencing a genome

- 2003, Human Genome project $2.7 billion
- 2008, the $5,000 genome
- $1,700 genome Science 327(5961), 78-81 (2010)

- It may cost **$100,000** to analyze it!

Technological development has driven down the costs and increased the speed of sequence acquisition

Comparison to other sequencing methods

	Ion Torrent [11][12][13]	454 Sequencing [13]	Illumina [14]	SOLiD [15]
Sequencing Chemistry	Ion semiconductor sequencing	Pyrosequencing	Polymerase-based sequence-by-synthesis	Ligation-based sequencing
Amplification approach	Emulsion PCR	Emulsion PCR	Bridge amplification	Emulsion PCR
Mb per run	100 Mb	100 Mb	600 Gb	30000 Mb
Time per run	2 hours	7 hours	9 days	2 days
Read length	100 bp	400 bp	2x100 bp	35-50 bp
Cost per run	$ 500 USD	$ 8,438 USD	$ 20,000 USD	$ 17,447 USD
Cost per Mb	$ 5.00 USD	$ 84.38 USD	$ 0.03 USD	$ 5.81 USD
Cost per instrument	$ 50,000 USD	$ 500,000 USD	$ 500,000 USD	$ 591,000 USD

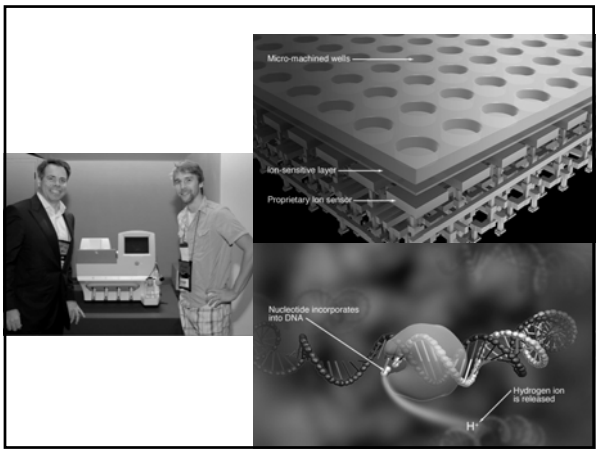
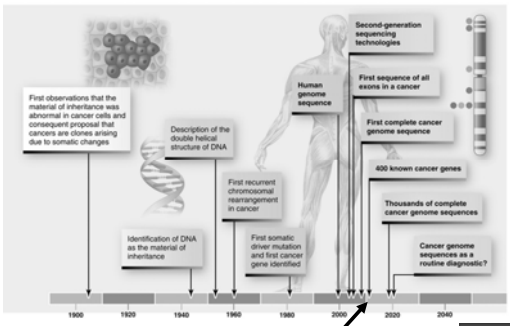


Fig. 1 Time line showing key events in the investigation of the cancer genome.



M R Stratton Science 2011;331:1553-1558

You are here!

Science AAAS

Published by AAAS

Deep or Second/Next generation sequencing

- Diagnosis
- Therapeutic decision-making
- Will add to current molecular and routine diagnostics

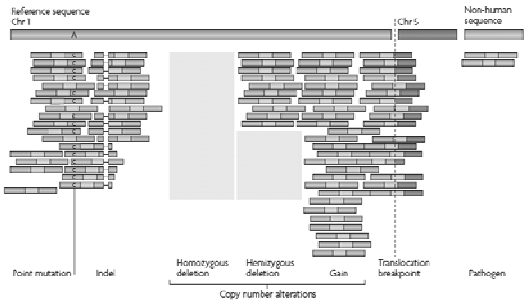


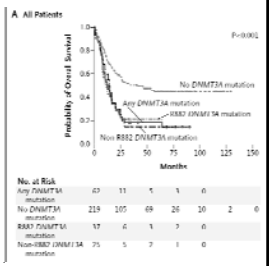
Figure 3 | **Types of genome alterations that can be detected by second-generation sequencing.** Sequenced

Currently, time and $ limit analysis, expect improvements in the near future

n engl j med 361;11 2009

Clinical utility

- Mutation in DNMT3A in AML
- Suggestion for early transplantation therapy



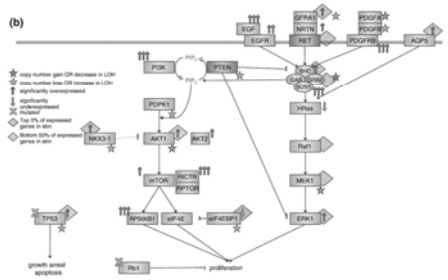
Initial genome sequencing and analysis of multiple myeloma

Michael A. Chapman[1¶], Michael S. Lawrence[2], Jonathan J. Keats[2,3], Kristian Cibulskis[2], Carrie Sougnez[2], Anna C. Schinzel[4], Christina L. Harview[4], Jean-Philippe Brunet[1], Gregory J. Ahmann[5,6], Mazhar Adli[5,6], Kenneth C. Anderson[5,6], Kristin G. Ardlie[2], Daniel Auclair[2,6], Angela Baker[2], P. Leif Bergsagel[5,6], Bradley E. Bernstein[2,7,8,9], Yotam Drier[2,10], Rafael Fonseca[5,11], Stacey B. Gabriel[2], Craig C. Hofmeister[5,12], Sundar Jagannath[5,13], Andrzej J. Jakubowiak[5,14], Amrita Krishnan[5,15], Joan Levy[5,6], Ted Liefeld[2], Sagar Lonial[5,13], Scott Mahan[2], Bunmi Mfuko[5,6], Stefano Monti[2], Louise M. Perkins[5,6], Robb Onofrio[2], Trevor J. Pugh[2], S. Vincent Rajkumar[5,16], Alex H. Ramos[2], David S. Siegel[5,17], Andrey Sivachenko[2], A. Keith Stewart[5,6], Suzanne Trudel[5,18], Ravi Vij[5,19], Douglas Voet[2], Wendy Winckler[2], Todd Zimmerman[2,20], John Carpten[4], Jeff Trent[4], William C. Hahn[7,8,9], Levi A. Garraway[7,8], Matthew Meyerson[1,7,8], Eric S. Lander[2,9,21], Gad Getz[2] & Todd R. Golub[1,7,8,9,*]

- "…activating mutations of the kinase BRAF were observed in 4% of patients, suggesting the evaluation of BRAF inhibitors in multiple myeloma clinical trials…"

Nature 471(7339):467-72 2011

Mutations and differences in gene expression before and after chemotherapy



Jones et al. Genome Biology 2010, 11:R82

Table 1. Selected details of cancer genome sequencing studies

Study	Cancer type	Total no. genes analyzed	No. mutated genes	Total no. mutations (nonsilent)	Average no. mutations per tumor
Sjoblom et al. (8) and Wood et al. (9)	Breast ($n = 11$)*	18,191	1,137	1,243	84
	Colorectal ($n = 11$)*		848	942	76
Greenman et al. (10)	Diverse ($n = 210$)	518	581	798	NA
Jones et al. (11)	Pancreatic ($n = 24$)	20,661	1,007	1,163	48
Parson et al. (12)	Glioblastoma ($n = 21$)†	20,661	685	748†	47
TCGA (13)	Glioblastoma ($n = 91$)	601	223	453	NA
Ding et al. (14)	Lung ($n = 188$)	623	348	1,013	NA
Ley et al. (15)	AML ($n = 1$)	Whole genome	10	500–1,000	NA

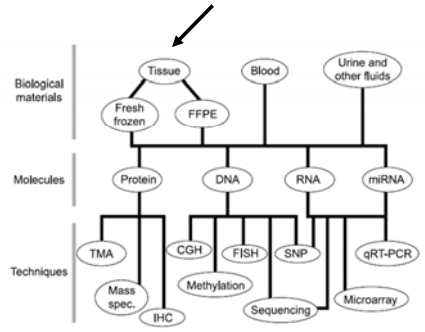
Abbreviation: NA, not applicable.
*Discovery phase of study only.
†Excludes posttherapy samples and mismatch repair–deficient samples.

The impact of “personalized medicine” approaches on anatomic pathology

Personalized Medicine

- Estimated market of $452B by 2015
  - Wall Street
    - Big and small pharma
    - Diagnostics companies
    - Informatics companies
  - Infrastructure (telemedicine, EMR, disease management services)

Pathology assistant + pathologist will have a primary role



Impact on you: Banking/tissue archiving will become more prevalent

- Critical selection of fresh disease and non-disease (reference specimen) tissues
- Need for access to patient specimens
- Effect on your time

Impact on you: Tissue collection

- Clean bench procedure to reduce cross contamination
- Fresh tissue is best for collecting DNA/RNA
- Need disease tissue!
  - ? frozen sections to identify adequacy of disease tissue
    - Many tumor tissues are "contaminated" with inflammatory cells
  - Minimal "guess-timate" - 0.25 gram of "pure" disease tissue (1.0 x 1.0 x 0.25 cm)
  - No standards established!
- Need for a reference sample (normal tissue) – your experience and skills will be helpful
  - ? frozen section
  - Especially for determination of adjacent "unremarkable" tissue
  - Others: skin, cheek swab, blood might be sufficient
- Many questions to be answered

Impacts

- How long before it makes a difference for patients?
- Clean areas for collecting tissue (contamination a problem for PCR)
- Huge institutional commitment (capital costs vs. loss of business)
- Costs - Who will pay? Insurance v. Medicare v. self v. institution

Some organizations are developing personalized medicine approaches

Change your future

- Get involved! Your opinions and thoughts will influence how it will or can be made to work
  - Hospital committees
  - Pathology group
    - Procedures
  - Education
    - Other staff
    - Pathologists
    - Other physicians