Pre-analytical variables affecting Fine Needle Aspiration Biopsies (FNAs) and their influence on clinical diagnosis

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Disclosure

Dr. Clark is entitled to a share of equity as a founder of BioMarker Strategies, LLC. He currently is an officer for the company. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

Objectives

- To understand the biospecimen challenges and opportunities presented by Fine Needle Aspiration Biopsies (FNAs) of solid tumors.

- To understand how FNAs can facilitate development and application of predictive markers for cancer therapy.

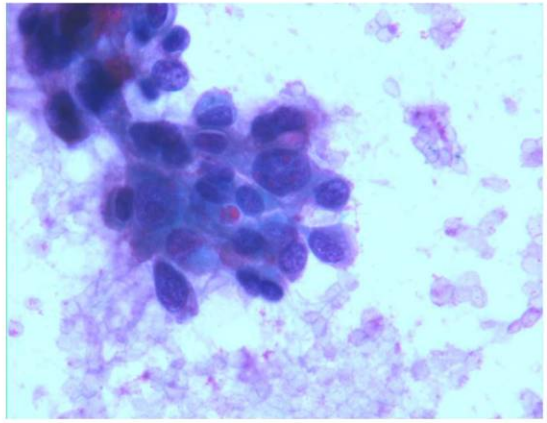
Personalized Cancer Treatment: A JH case study

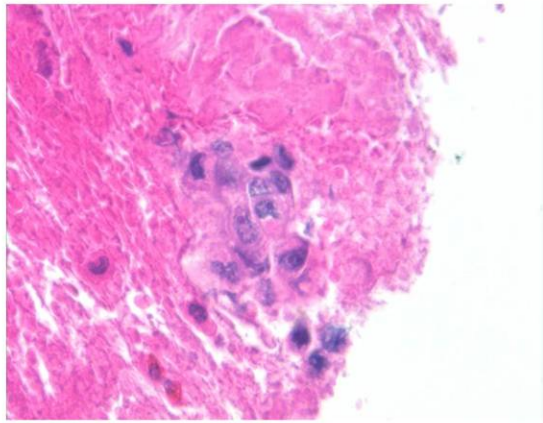
- 36 yr old female w/ history of breast carcinoma in 2004
- Mother died at young age of breast cancer
- Patient carries a germline *BRCA1* mutation
- Now presents with RUL lung mass, mediastinal adenopathy. R/O Lung primary.

Pre-carinal Lymph Node Transbronchial FNA

- Specimen handled by pulmonologist, processed using traditional methods

- Dx: Poorly-differentiated adenocarcinoma.





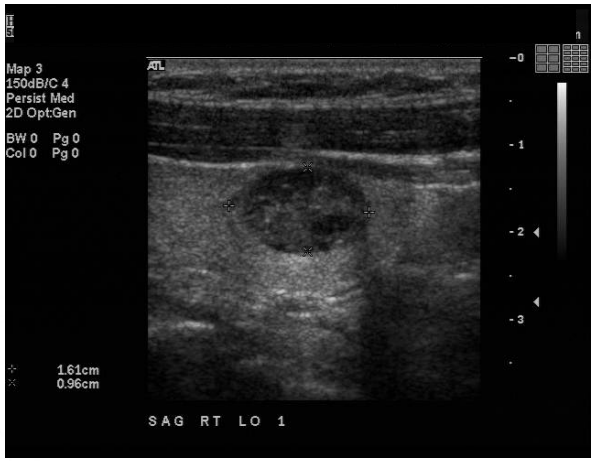
Clinician's response…

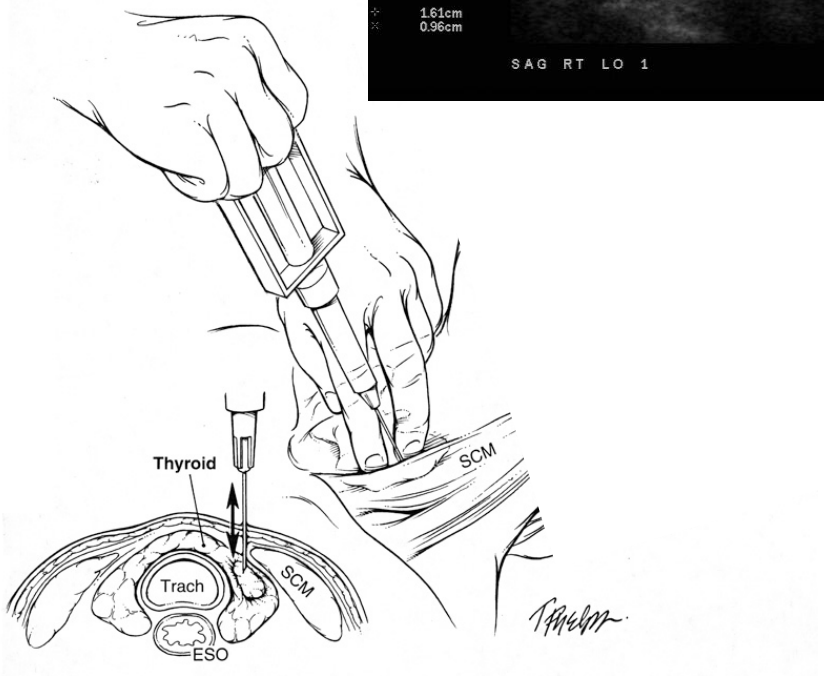
- The key issue in this case is to differentiate between breast and lung. If breast cancer, then predictive markers of response (i.e., ER/PR/HER2) are needed to decide if endocrine therapy or trastuzumab might be an option. If lung, molecular markers looking for k-ras and EGFR mutations might be useful as predictors of response to EGFR TKIs like erlotinib.

- Let's hope (if indeed breast) that she now has HER2-positive disease or (if lung) that it has EGFR mutation ...

Performing an FNA

- 25 gauge needle
- It's NOT an aspiration biopsy.
- Excursion of the needle through the lesion, NOT aspiration, is key to obtaining material.
- Ultrasound-guided

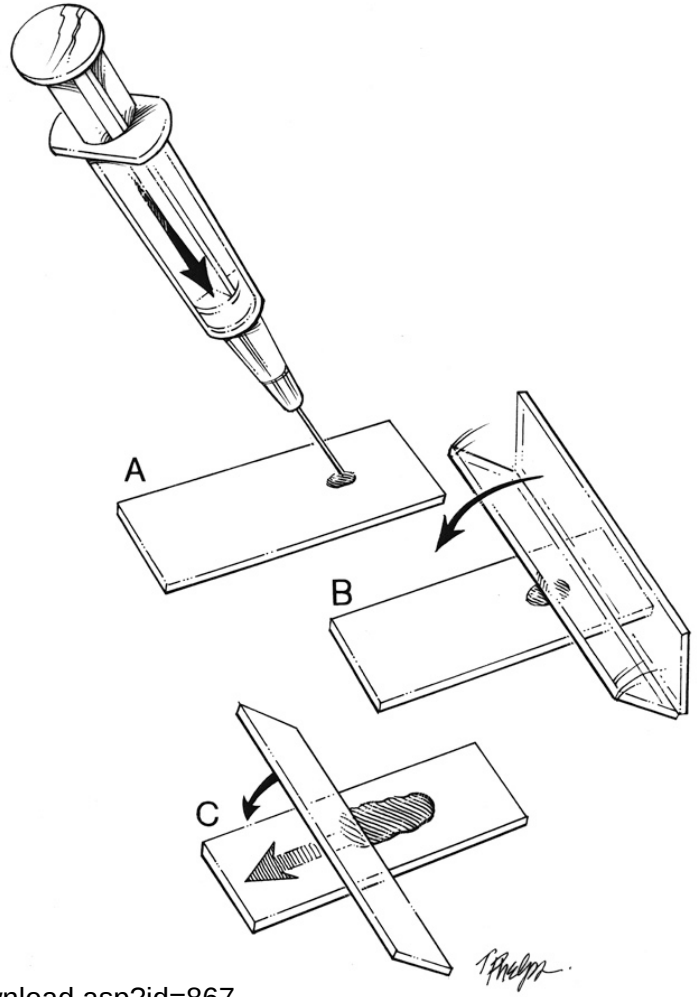




(Ljung) http://www.papsociety.org/fna.html

FNA Sample Processing: highly variable

- Direct Smears
  - Air-dried, methanol-fixed
  - EtOH-fixed
- Cytospins
- ThinPrep® (Hologic)
- PrepStain™ (BD)
- Cell block (FFPE)



Cytopreparation (Gary Gill) http://www.cytopathology.org:80/website/download.asp?id=867

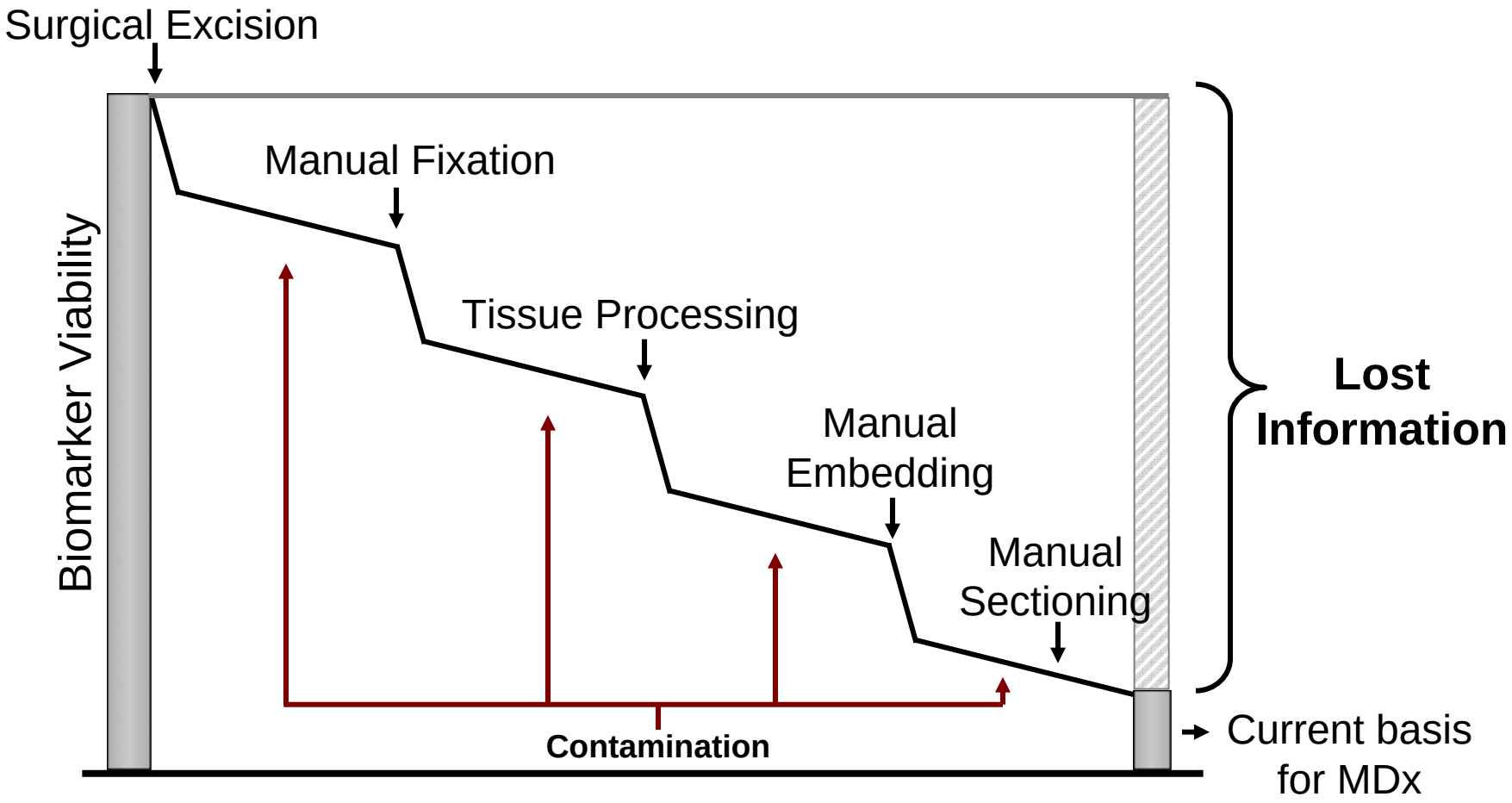
19th Century Processing vs. 21st Century Technology

- Tissue fixation with formaldehyde was discovered by Ferdinand Blum in **1863**.
- Paraffin wax embedding was described by Edward Klebs in **1869**.
- FNA processing methods were developed in the **1960's-1980's**.

Edward Klebs (1834-1913)

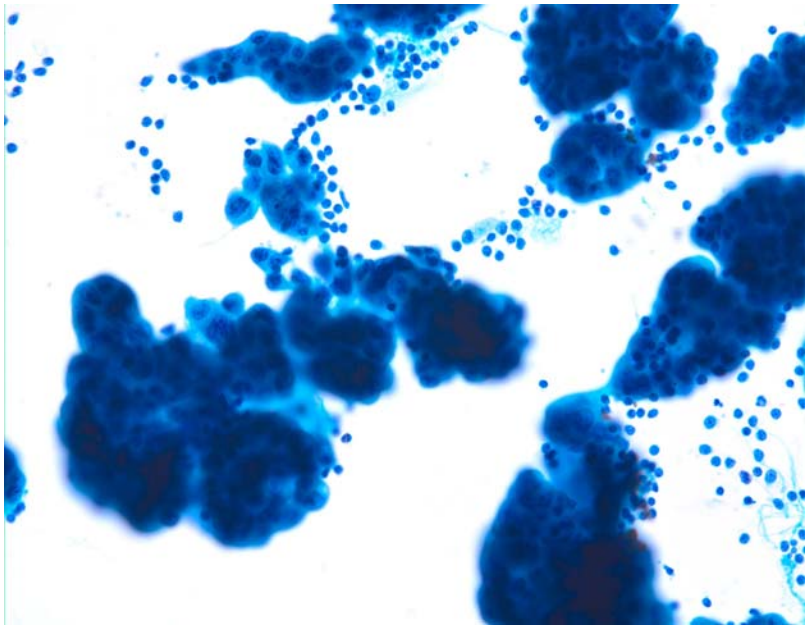
Gal AA. In search of the origins of modern surgical pathology. Adv Anat Pathol. 2001 Jan;8(1):1-13.

Biomarker information is lost during routine tissue processing



Biospecimen Challenges of FNAs for Molecular Diagnostics

- Relatively small sample size.
  - 200,000-10^6 cells
  - Representative of whole?
- Heterogeneous and variable cellular composition.
- Minimal, but unique pre-analytical variability.

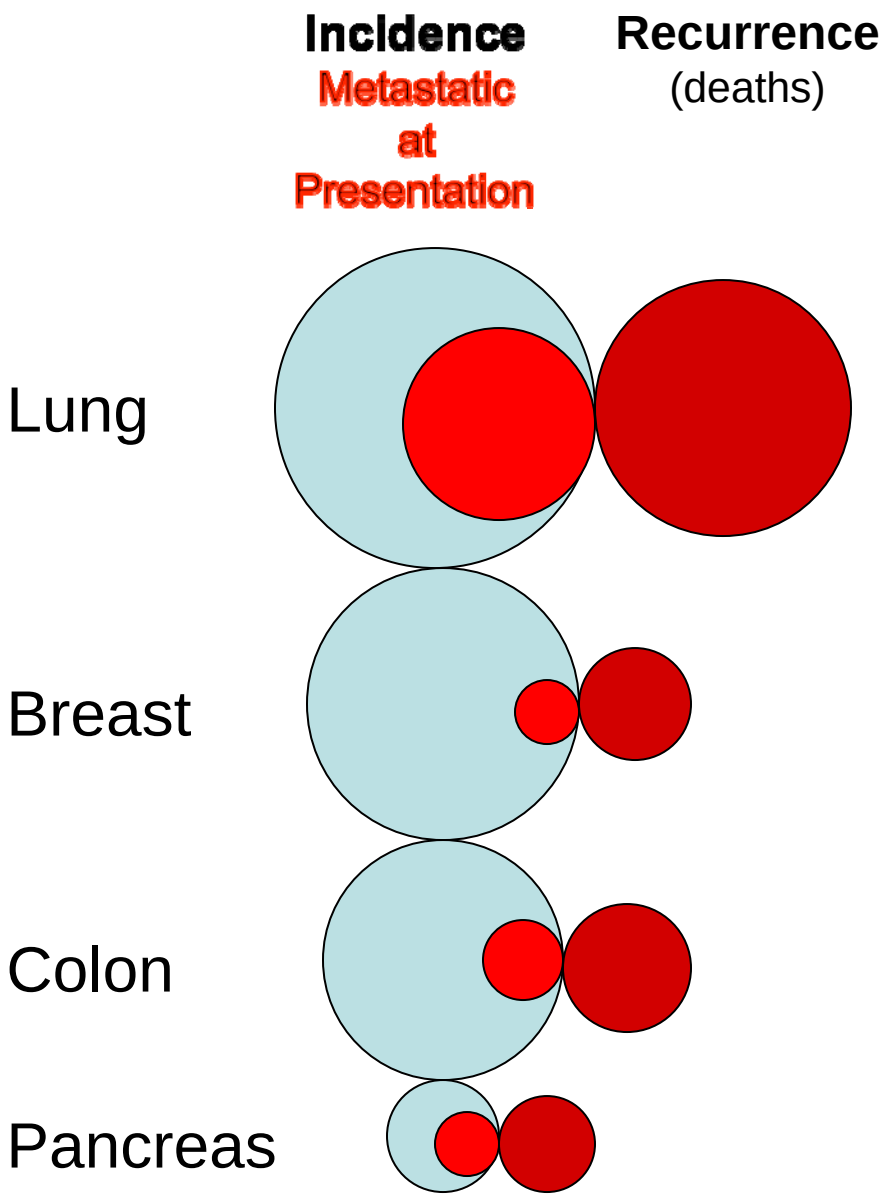


Biospecimen Advantages of FNA sampling:

- Avoids pre-analytical ischemia.
- Less invasive and more cost-effective than surgical excisional biopsies.
- Can obtain live cells.

There is a strong, but unmet, clinical need for MDx on FNAs

- Surgery is not indicated for most patients with advanced, metastatic disease.
- Neo-adjuvant treatment regimens will require pre-operative molecular characterization.
  - Tumor may be gone or altered after treatment.



- Approx. 50% of these cancer patients are NOT surgical candidates
- Tumor should be sampled via FNA

- Jemal, A., R. Siegel, et al. (2008). "Cancer statistics, 2008." CA Cancer J Clin **58**(2): 71-96.

Sample Size: Biomolecules in FNA samples

- Tumor cells
  - 3×10^4 - 1×10^6
  - >92% tumor cells (47%-98%)
- DNA
  - 10 ug
- RNA
  - > 1 ug (0.5-12 ug)
  - Breast FNA: 3.6 ug (2.8 ug in core biopsy)
- Protein
  - 200 ug

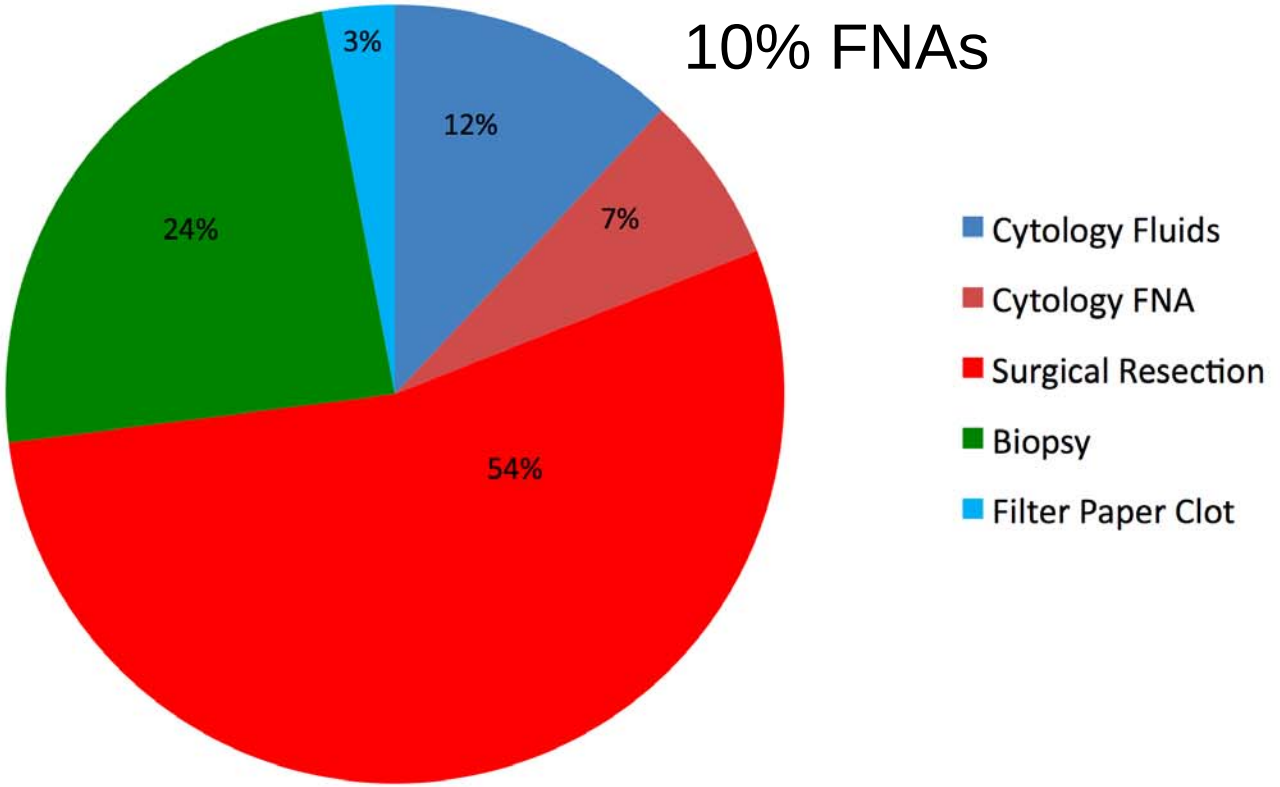
Centeno, et al. (2005). Cancer **105**(2): 101-9.; Symmans, et al. (2003). Cancer **97**(12): 2960-71.; Clark, et al (unpublished).

Successful Examples of Cytology Mdx

- DNA-based biomarkers
  - Cohen Y, et al. Mutational analysis of BRAF in fine needle aspiration biopsies of the thyroid: a potential application for the preoperative assessment of thyroid nodules. Clin Cancer Res. 2004 Apr 15;10(8):2761-5.
- DNA Methylation
  - Yang B, et al. Promoter methylation profiles of tumor suppressor genes in intrahepatic and extrahepatic cholangiocarcinoma. Mod Pathol. 2005 Mar;18(3):412-20.
- Proteomics
  - Rubio-Viqueira B, et al. Optimizing the development of targeted agents in pancreatic cancer: tumor fine-needle aspiration biopsy as a platform for novel prospective ex vivo drug sensitivity assays. Mol Cancer Ther. 2007 Feb;6(2):515-23.
- Quantitative RT-PCR/Microarrays
  - Jimeno A, et al. Dual EGFR and mTOR targeting in squamous cell carcinoma models, and development of early markers of efficacy. Br J Cancer. 2007 Mar 26;96(6):952-9.
  - Symmans, et al. Total RNA yield and microarray gene expression profiles from fine-needle aspiration biopsy and core-needle biopsy samples of breast carcinoma." Cancer **97**(12): 2960-71.
- Immunocytochemistry
  - Kelly D, et al. Detection of cervical high-grade squamous intraepithelial lesions from cytologic samples using a novel immunocytochemical assay (ProEx C). Cancer. 2006 Dec 25;108(6):494-500.

Krishnamurthy, S. (2007). "Applications of molecular techniques to fine-needle aspiration biopsy." Cancer **111**(2): 106-2

Summary of JH EGFR and KRAS Testing -2008



10% FNAs

5% FNAs at Brigham; Smouse, et al. Cancer 2009; 117:67-72

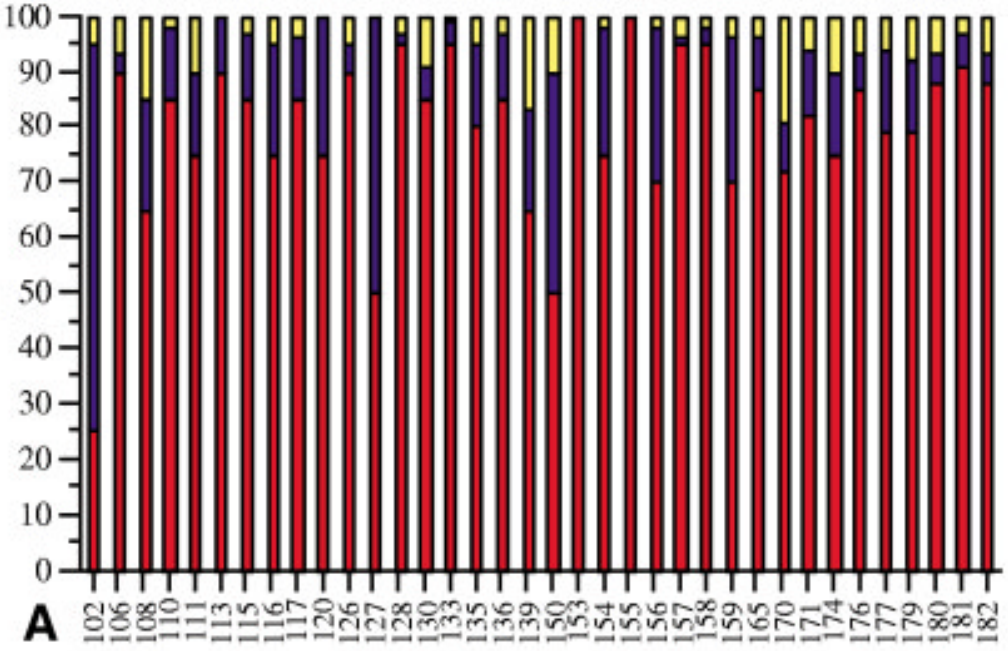
FNAs: Heterogeneous Cellular Composition

Lymphocytes

Stroma

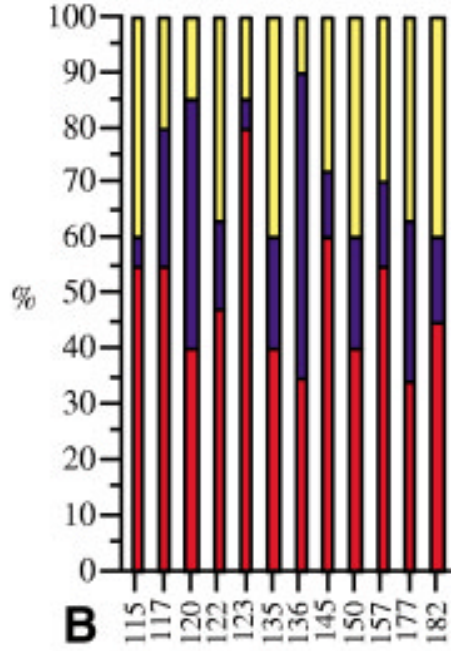
Tumor

Breast FNA



80% tumor; 15% lymphs; 5% stroma

Core Bx



50%/20%/30%

Symmans et al, Cancer, 97:12, 2003

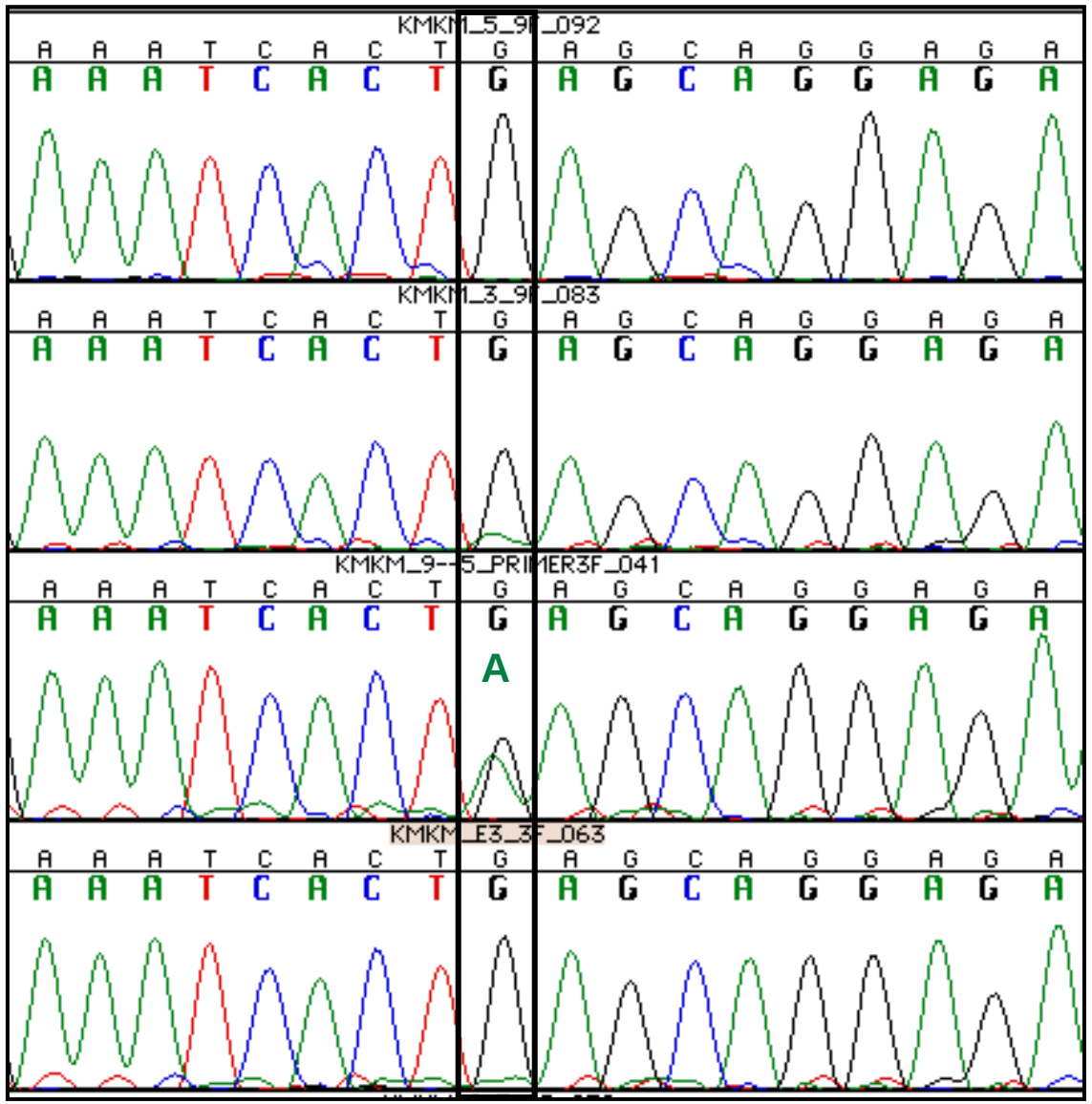
Impact of Specimen Heterogeneity on MDx: *PIK3CA* DNA Sequence

E545 Control

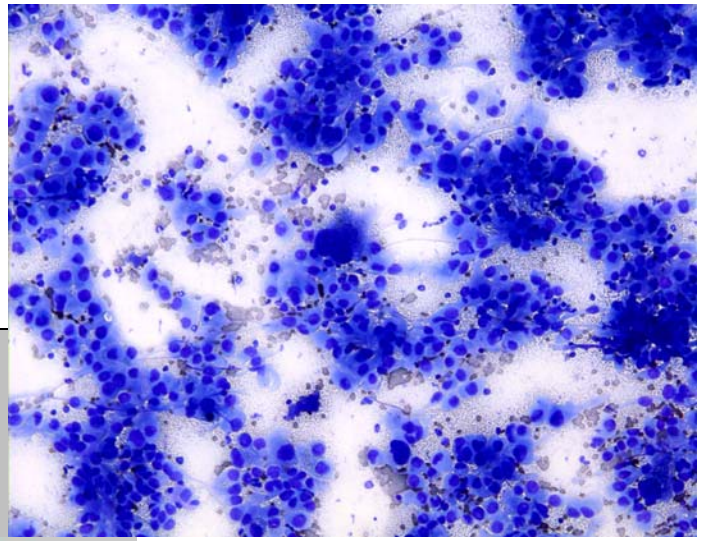
E545 Heterogeneous
False Negative

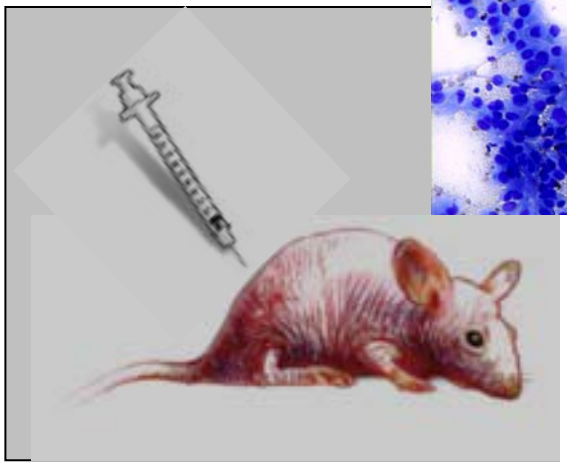
E545K Enriched

E545 Negative



Model systems for FNA Biospecimen Research: FNAs on murine xenografted human tumors





Molecular assays:

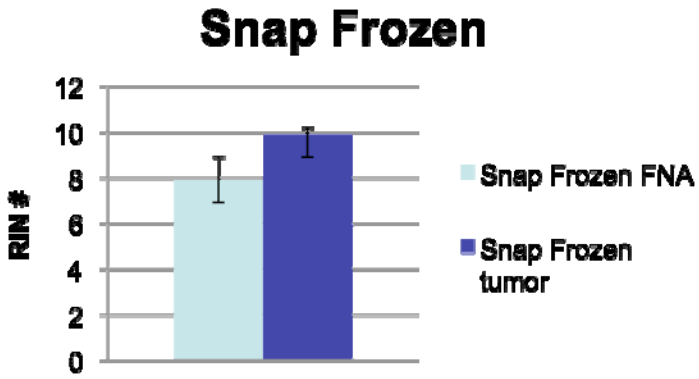
⇒mRNA levels to determine response to anti-EGFR therapy

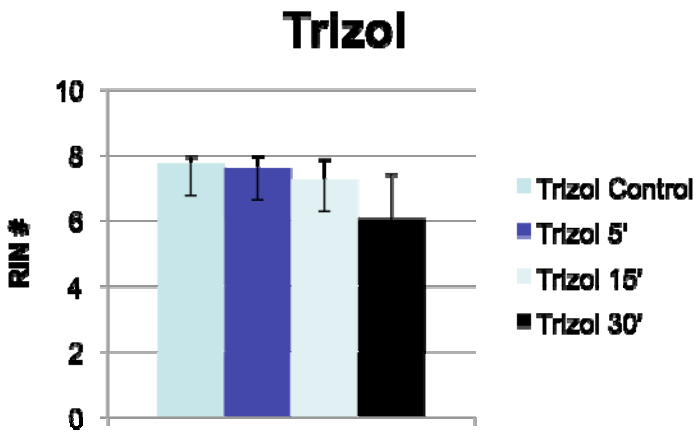
⇒Methylation-specific PCR to monitor treatments that modify chromatin structure

Jimeno A, et al. Cancer Res 2006, 66:2385-2390

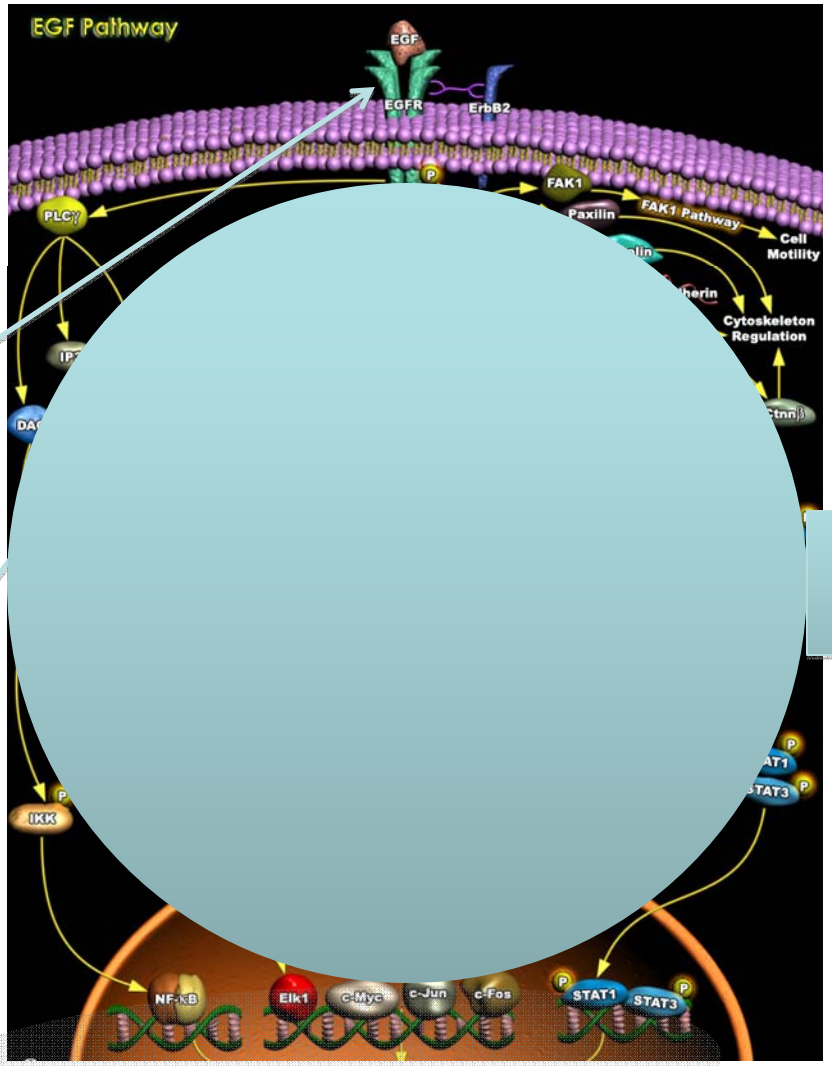
Slight Reduction in FNA RNA Integrity Numbers (RIN)

- RNA Integrity Numbers (RIN) lower in FNAs relative to frozen, excised tumor.
- FNA RINs decrease slightly over time at 37° C.
- Still fit-for-purpose.
- Higher than FFPE (RIN=2.5).
- Mechanism?
- Solutions?





Static vs. Dynamic Biomarkers



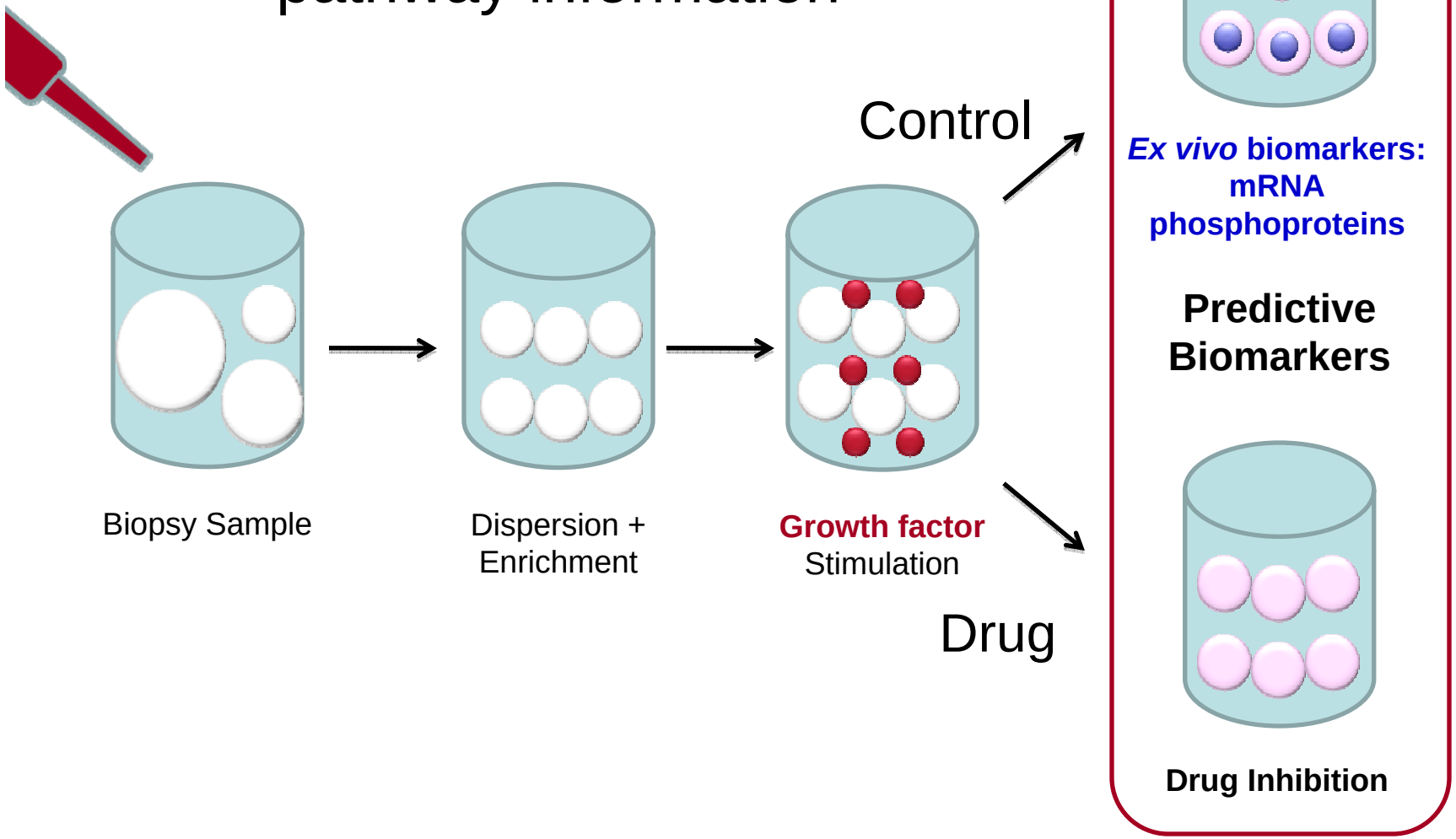
EGFR

KRAS

Functional Molecular Profile

Source: www.proteinlounge.com

The **live cell** opportunity: Ex vivo biomarkers provide <u>functional</u> pathway information



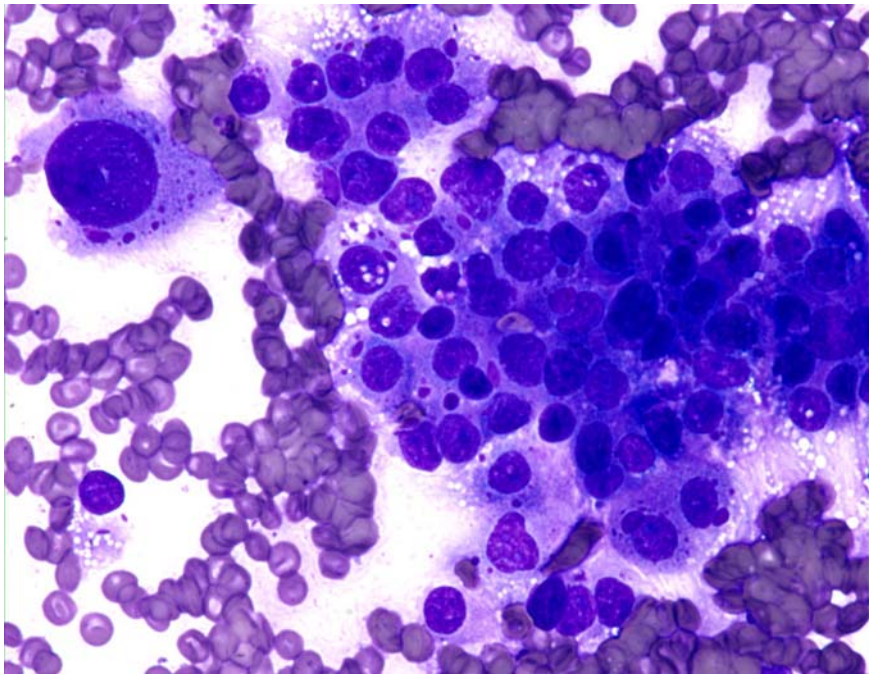
Ex vivo Biomarkers

Tissue	Stimulus	Inhibitor	Ex vivo Biomarker
AML	G-CSF	None	p-ERK; p-STAT3
Cholangio Ca	EGF	Erlotinib	c-FOS mRNA
Pancreatic Ca	FBS	Temsirolimus	p-S6-RB
Pancreatic Ca	FBS	ON 01910	Cyclin B1 mRNA

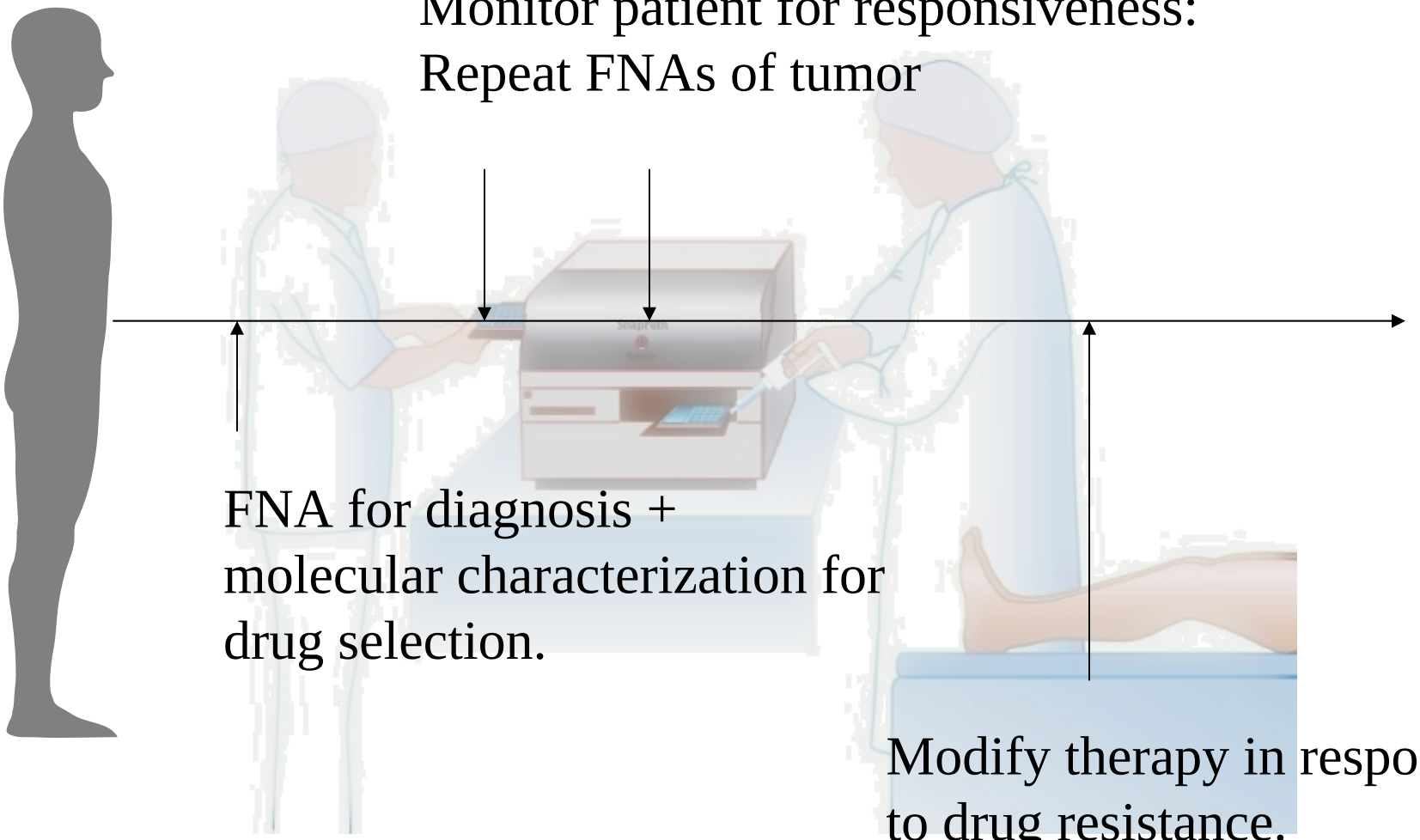
J. M. Irish *et al.*, *Cell* **118**, 217 ; A. Jimeno *et al.*, *Cancer Res* **66**, 2385; B. Rubio-Viqueira *et al.*, *Mol Cancer Ther* **6**, 515; A. Jimeno *et al.* 2008.

Question: This tumor is sensitive to which of the following drugs?

a. Iressa
b. Tarceva
c. Cetuximab
d. None of the above



Susceptibility testing for tumors?



Conclusions

- FNAs are clinically-important biospecimens.
- Molecular Diagnostic testing is possible on FNAs.
- FNAs present unique biospecimen challenges and opportunities.

- Thanks
  - Clark Lab: A. Polotsky, S. Kang., C. Adams
  - Adam Schayowitz, Ph.D., BioMarker Strategies.

- For further information:
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  - http://162.129.103.53/cytopath/