
Tissue Collection Challenges in Co-Development Clinical Trials

Eric Walk, MD FCAP

Chief Medical Officer, Ventana Medical Systems/Roche Tissue Diagnostics

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caHUB Biospecimen-Based Reference Sets for Drug-Diagnostic Codevelopment Workshop



Biospecimens for Diagnostic Assay Development and Validation

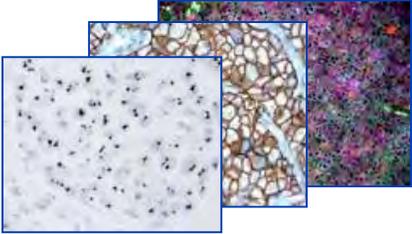
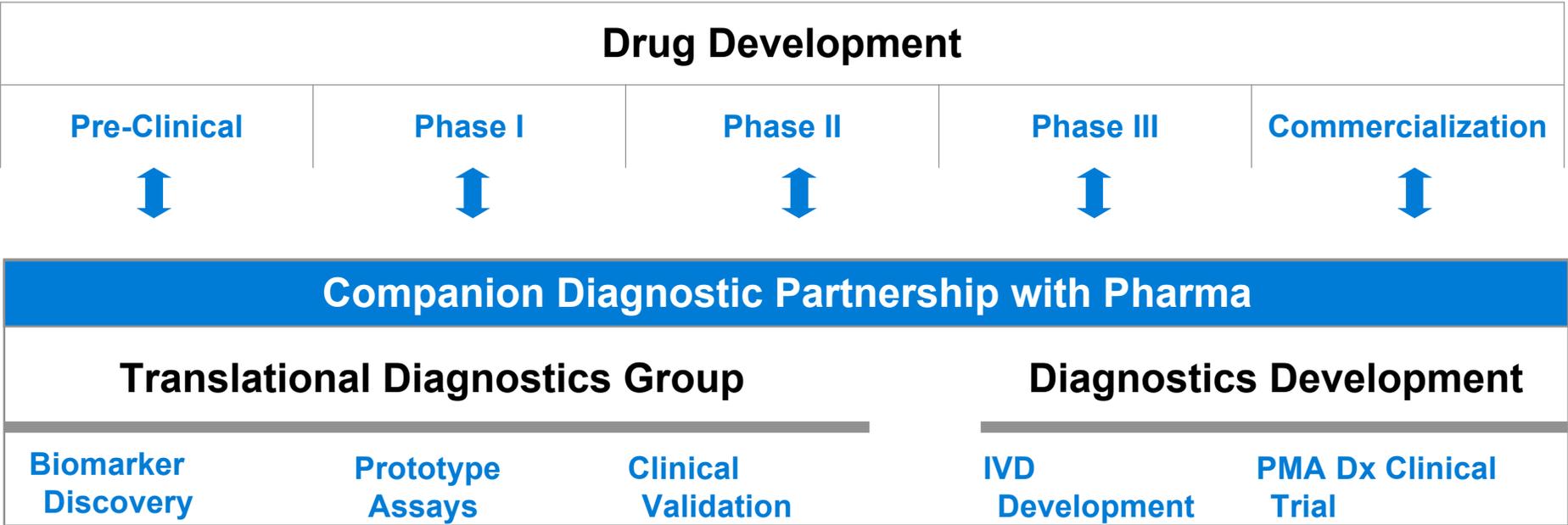
Annotation Needs Depend on Dx Intended Use

- **Routine Diagnostic Assay (e.g. cytokeratin, p63)**
 - Anatomic pathology diagnostic information (e.g. subtype, invasive vs. in situ, etc.)
 - Characterization data, especially with comparative assays/technologies is 'nice to have'
 - Typical sources: public/private tissue providers
- **Prognostic Assay (e.g. Oncotype Dx, TMPRSS/ERG)**
 - Dependent on patient outcome data (overall survival, progression-free survival)
 - Typical sources: cooperative groups (NSABP, ECOG, RTOG)
- **Companion Diagnostic Assay (e.g. HER2, EGFR mut)**
 - Need biomarker positive and negative samples
 - Need drug treated and non-drug treated (CDx vs. prognostic)
 - Need drug response and outcome data
 - Typical sources: Pharma clinical trials

Quality fit-for-purpose
Standardization of Pre-
Analytical Factors

Companion Diagnostics Development

Ventana Translational Diagnostics Group



Challenges in Companion Diagnostic Co-Development

Logistic → Sample Collection

Technical → Pre-Analytical Variables
Primary Ab Selection
Sample Limitations

Conceptual /Scientific → Primary vs. Metastasis
Single vs. Multiple
Biomarkers

Iressa Survival Evaluation in Lung Cancer (ISEL) Trial

- **Phase III trial compared gefitinib with placebo in 1,692 patients with refractory advanced NSCLC**
- **Biomarkers**
 - EGFR IHC (n=379)
 - EGFR FISH (n=370)
 - p-Akt expression (n=382)
 - Mutations in EGFR (n=215), KRAS (n=152), BRAF (n=118)
- **Availability of tumor samples remains a challenge**
 - 460/1,692 (27.2%) patients with assessable tissue samples
 - Only 91/1,692 (5.4%) patients were assessable for all biomarkers

BR21 Sample Collection Results

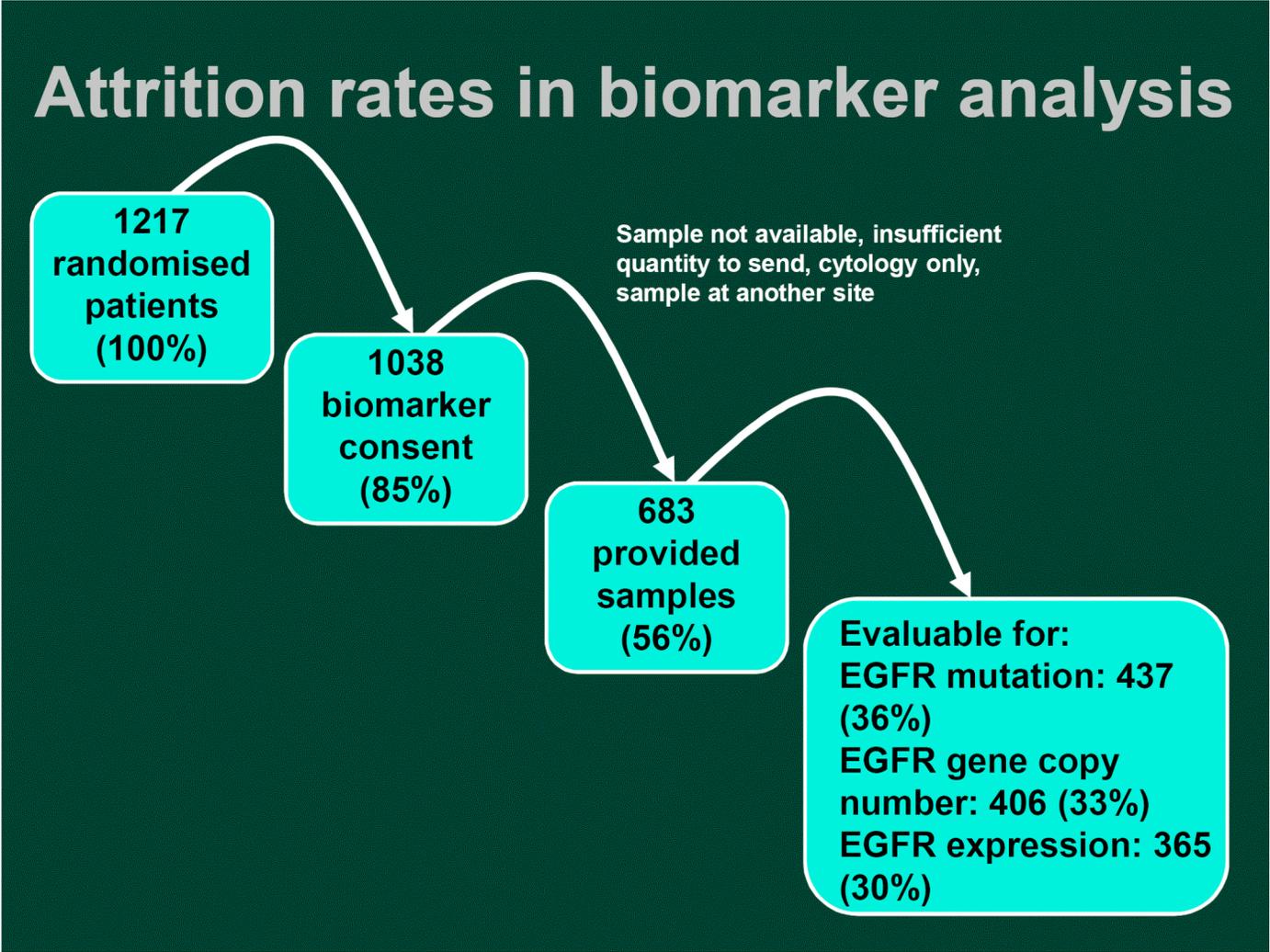
Supplemental information for on-line publication only

Supplemental Table 1. Summary of Sample Collection and Laboratory Testing by Treatment Arm

	Erlotinib	Control	Total
Number of patients in trial	488	243	731 (100%)
Number of patients who consented to EGFR testing	313	159	472 (65%)
Number of patients with usable slides for IHC	242	133	325 (44%)
Number of patients with usable tissue for sequencing or FISH	148	78	226 (31%)
Number of patients with successful IHC analysis	210	115	325 (44%)
Number of patients with successful FISH analysis	77	48	125 (17%)
Number of patients with successful molecular sequencing	114	63	177 (24%)

IHC: immunohistochemistry; FISH: fluorescence in-situ hybridization

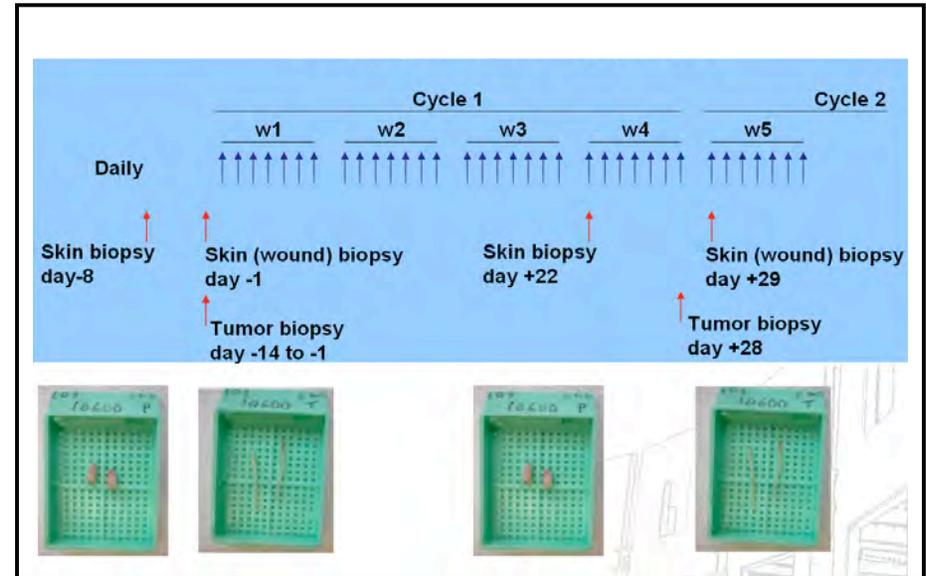
IPASS Trial: Sample Collection



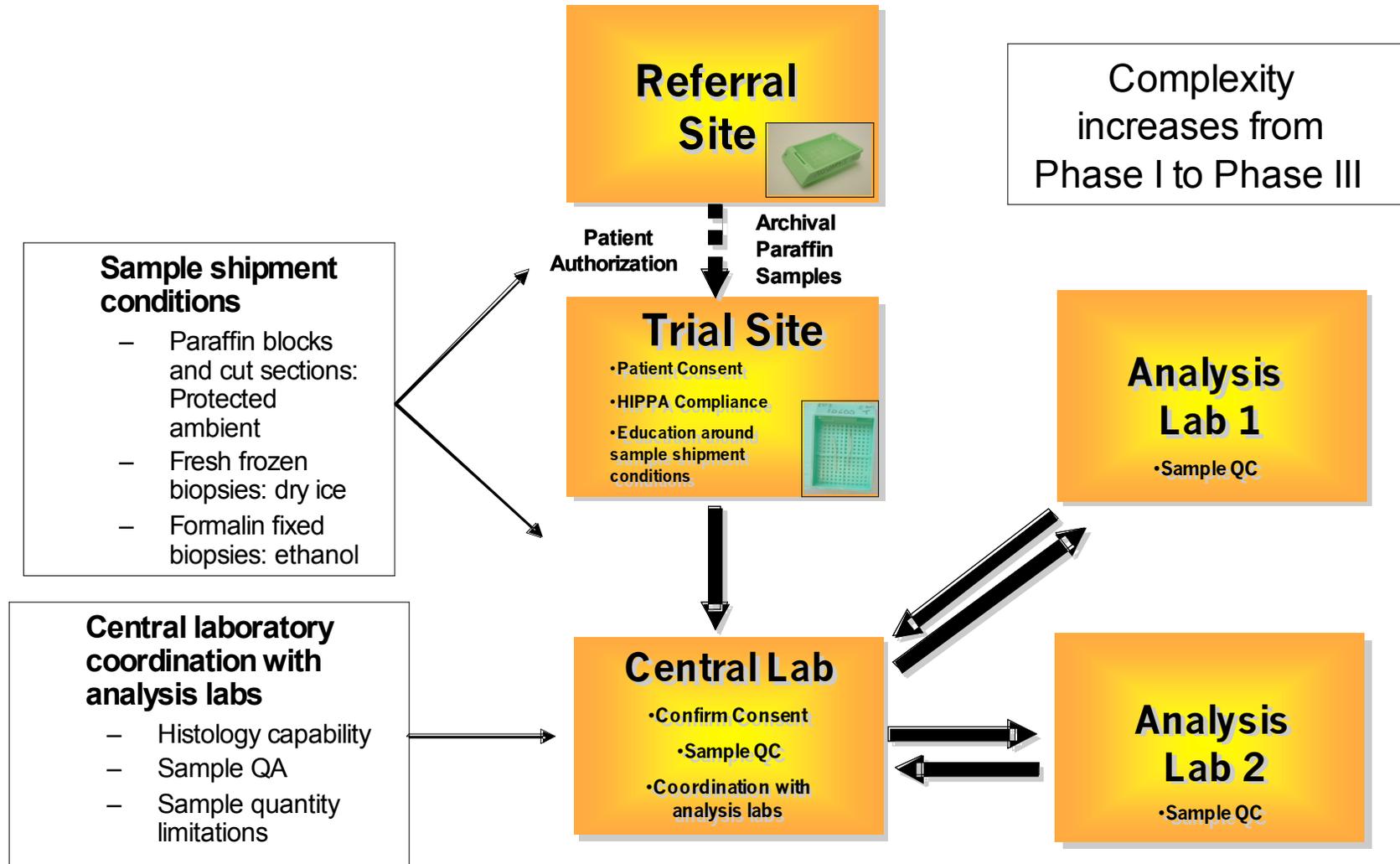
Mok et al. Phase III, randomised, open-label, first-line study of gefitinib vs carboplatin / paclitaxel in clinically selected patients with advanced NSCLC

Tissue Collection Remains Challenging in Oncology Clinical Trials

- **Inclusion of sample collection in a clinical trial design**
 - Increases logistic complexity
 - Potential IRB issues
 - Has the potential to slow enrollment
 - Increases cost
 - Mandatory tissue collection is becoming more common but is not standard
 - Enrollment and sample collection of biomarker negative patients is critical for diagnostic regulatory approval
- **Prospective biopsies**
 - Most control over pre-analytical variables
 - Adds the most logistic complexity and cost
 - Limited tissue
- **Archival paraffin blocks**
 - Relatively “easy” to collect
 - No control over pre-analytical variables
 - Typically represent primary tumor at time of initial diagnosis



Logistic Challenges: Sample Management and Disposition

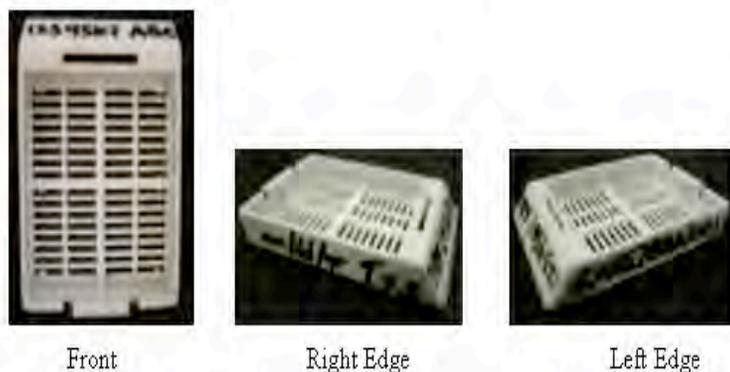


Logistic Challenges

Trial Site Compliance with Sample Procedures

Protocol Lab Manual

Figure 4-2 Example of cassette labelling for tumor biopsies



4. A standard plastic tissue cassette (e.g. Sakura Tissue Tek® Uni-Cassette® or equivalent) for each first pass tumor biopsy should be labelled in the following manner using a solvent-resistant marking pen (e.g. Precision Dynamics Corp. Secureline® MarkerII/Superfrost® or equivalent) (see Figure 4-2):

- Write the 7-digit site/subject number and patient initials across front of cassette.
- Write the date of collection (mm/dd/yr) and the letter “T” (for tumor) followed by the biopsy time point (e.g. “TBL” for baseline, “T28” for day 28) along the right edge of cassette.
- Write the protocol number along left edge of cassette.

Note: If the tissue cassette holes are large enough to allow the biopsy to escape, have filter paper or cassette biopsy sponges available in order to secure the specimen.

Received from Trial Site



- Protocol and lab manual instruction is not sufficient
- Trial site education including study nurses/coordinators and sponsor CRAs/site monitors is critical

Issues Related to Sample Collection

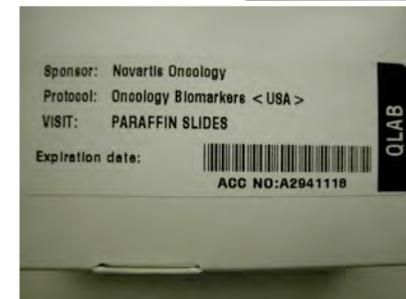
- **Samples delivered to wrong location/laboratory**
- **Samples shipped under wrong conditions**
- **Unstained tissue slide on wrong slide type (not charged/superfrost plus)**
- **Sample transport and tracking issues**
- **Ensuring/tracking appropriate informed consent: eCRF**
- **Sample quantity insufficient (e.g. no tumor found in sample)**



PK Samples sent to
Central IHC Testing
Lab

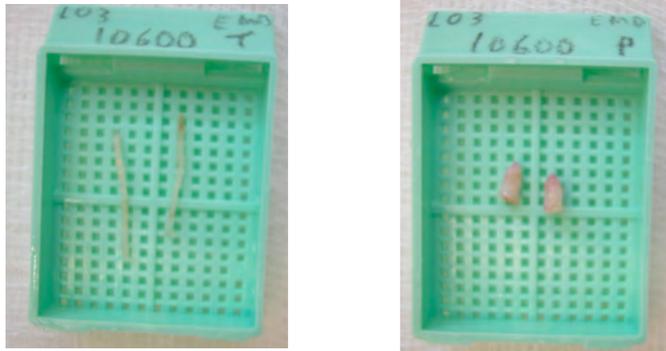
Sample Collection Lessons Learned *Solutions*

- **Creation of a clear laboratory manual and/or protocol sections**
- **Creation of appropriate sample collection kits**
 - 10% NBF, charged slides, etc.
- **Education of trial site and central lab staff**
 - Investigators meeting on-site, web-ex, telecon
- **Qualification of tumor on site**
 - Touch prep

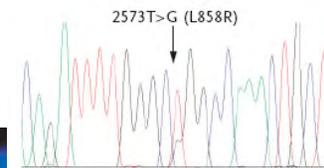
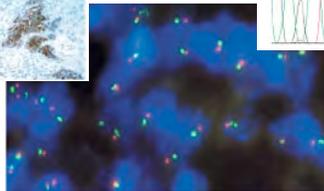
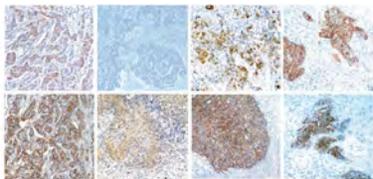


Sample Collection Kits

The Reality of Limited Sample Quantity



IHC, FISH, genotyping, etc.



- Keep trimming of paraffin block to a minimum
- Use central lab with histology services and coordinate downstream analyses with sectioning to minimize microtome visits
- Multiplex assays when possible
- Sites reluctant to send paraffin block
 - Maximum number of unstained slides achievable = 20

Tissue Collection Technical Challenges

Controlling Pre-analytical Variables

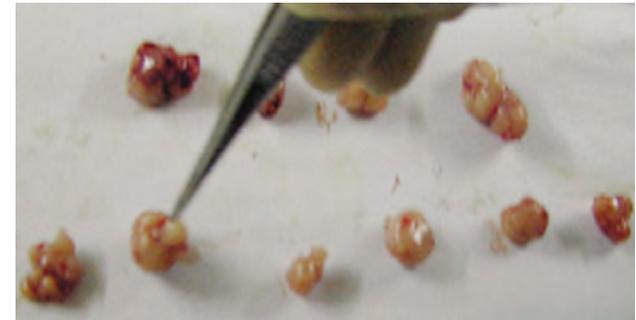
- Time to fixation
- Time of fixation
- Type of fixative
- Age of cut sections at time of analysis
- Use of phosphatase inhibitors
- Tissue processing protocol
- Embedding: Paraffin temp. (<math><60^{\circ}\text{C}</math>)
- Type of glass slides (e.g. Superfrost plus)
- Use of tape transfer system
- Thoroughness of deparaffinization



Ventana Study: Impact of Tissue Fixation on ISH Assay Performance

Study Materials

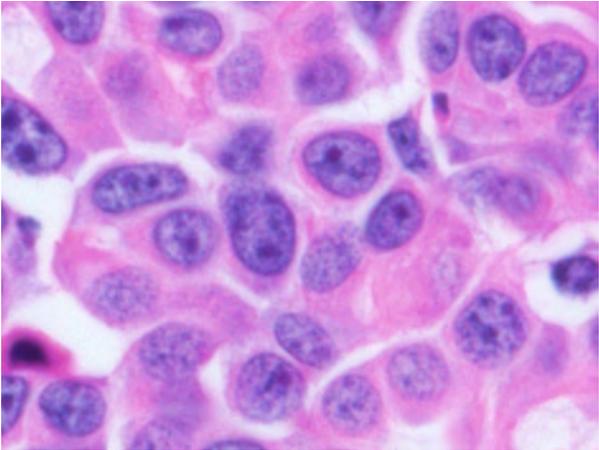
- Model System: MCF7 Xenograft tumors
- Assays Tested
 - **Ventana HER2 Dual ISH, Vysis HER2 FISH**
- Fixatives Tested
 - **10% NBF, Davidson's AFA (Alcohol-Formalin-Acetic Acid), Alcoholic NBF, Bouin's, Prefer, Zinc Formalin**
- MCF7 tumor cut into pieces of uniform size
- Fixed using the 6 fixatives at 6 different time points
 - **1, 3, 6, 12, 24 and 48 hours**
- Standard paraffin embedding and sectioning
- Ventana HER2 Dual ISH and Vysis HER2 FISH performed using standard protocols



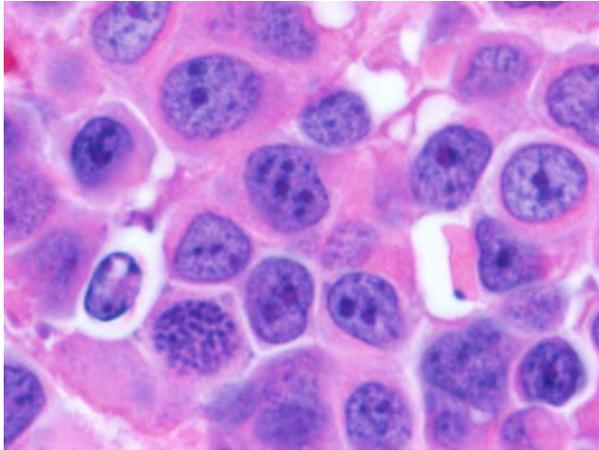
Study Results

10% Neutral Buffered Formalin – H&E

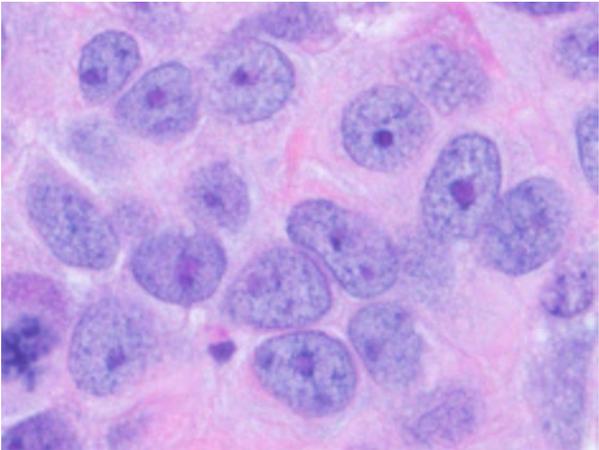
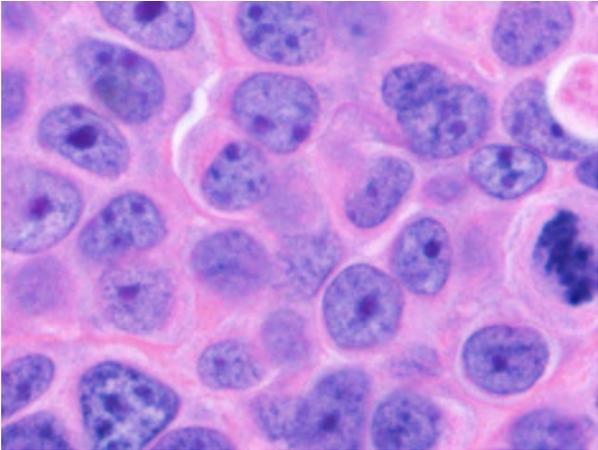
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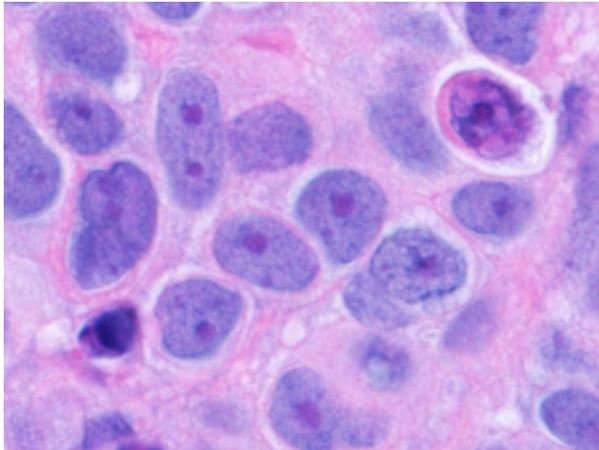
3 hours



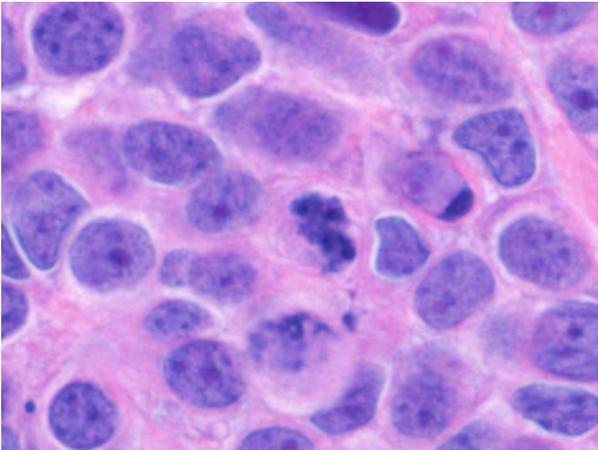
6 hours



48 hours



24 hours



12 hours

Study Results

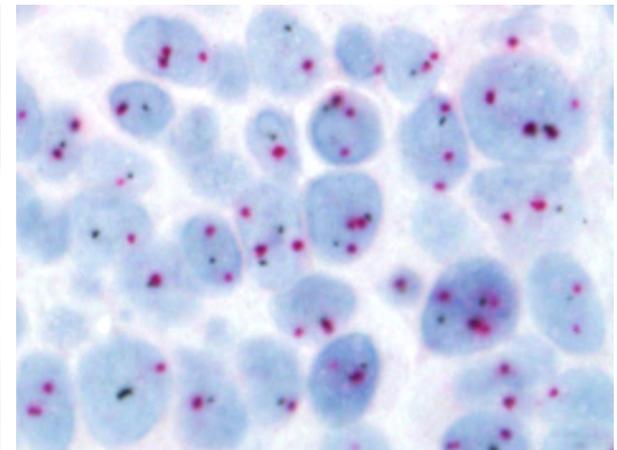
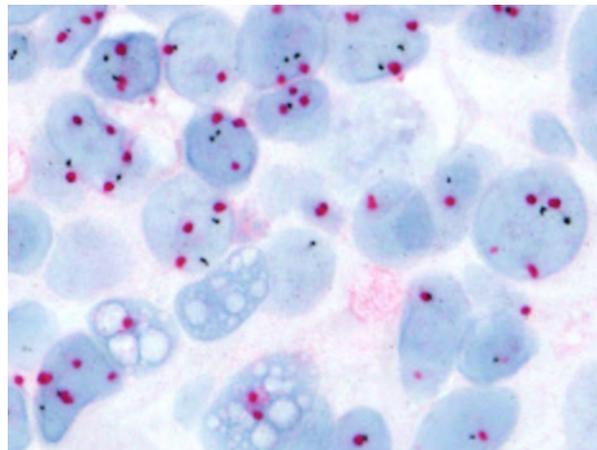
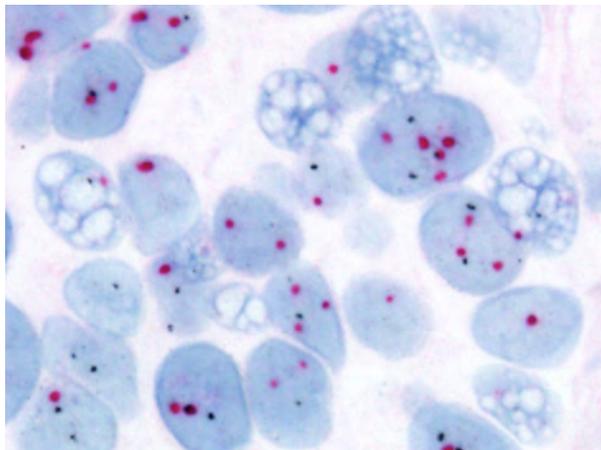
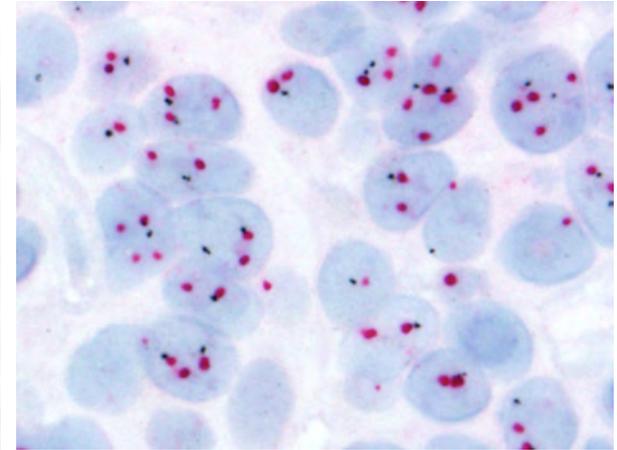
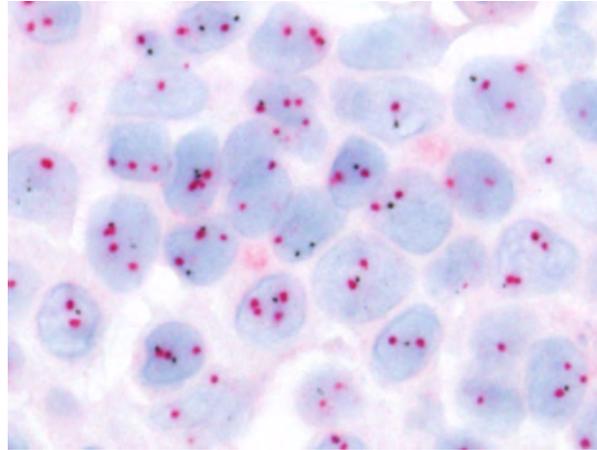
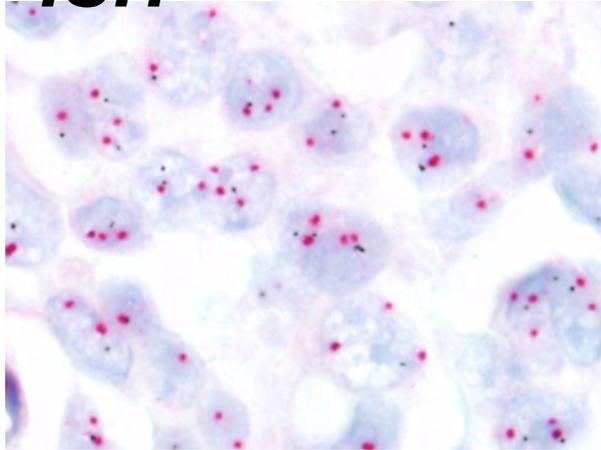
10% Neutral Buffered Formalin – Dual

ISH

1 hour

3 hours

6 hours



48 hours

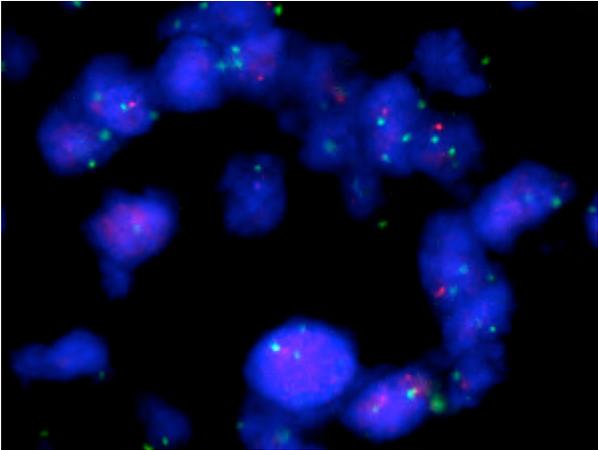
24 hours

12 hours

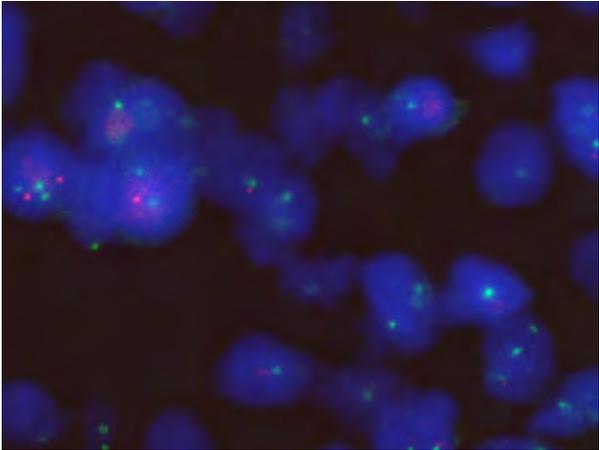
Study Results

10% Neutral Buffered Formalin – FISH

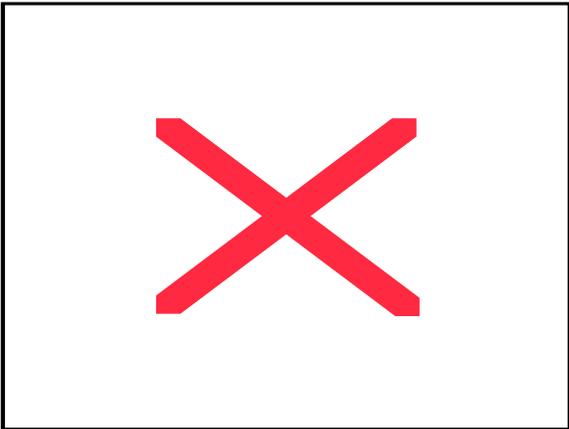
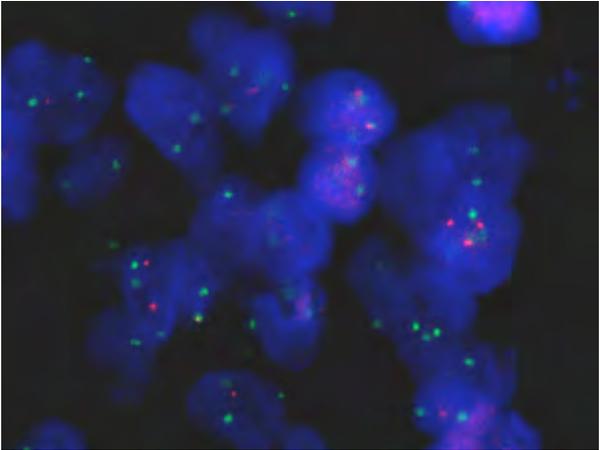
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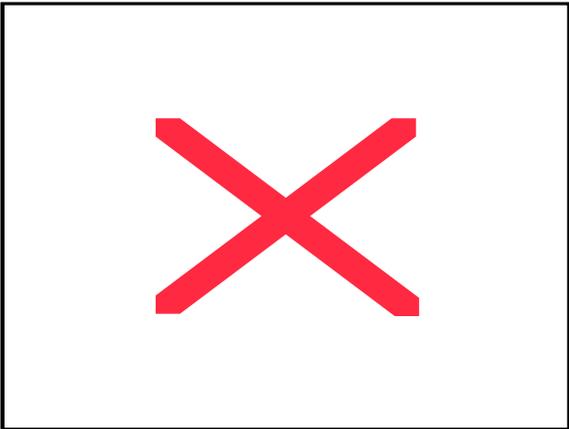
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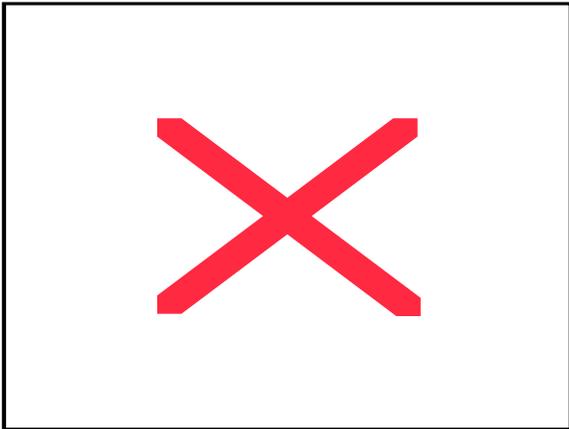
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48 hours



24 hours

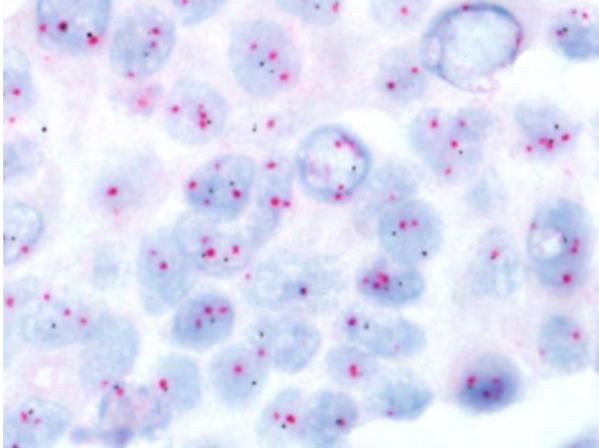


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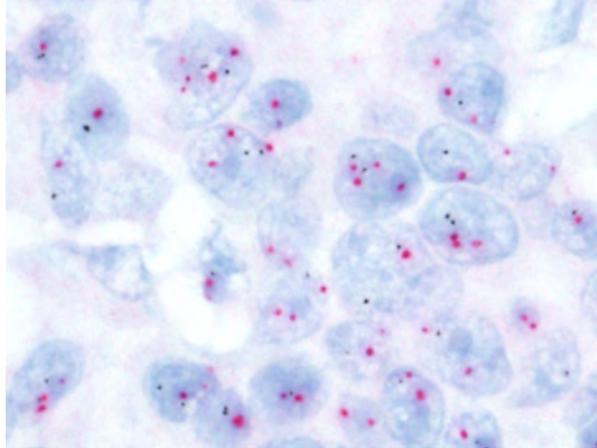
Study Results

AFA – Dual ISH

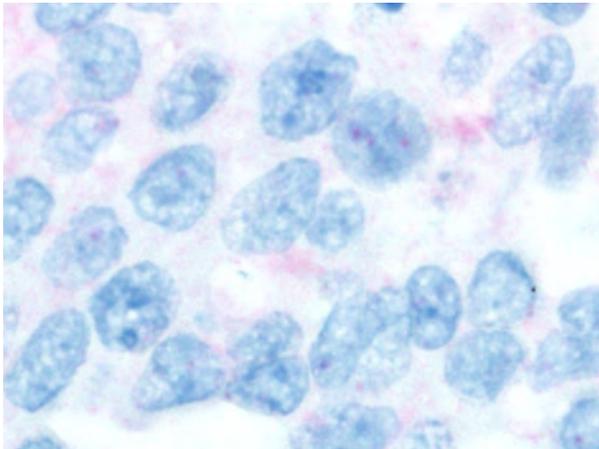
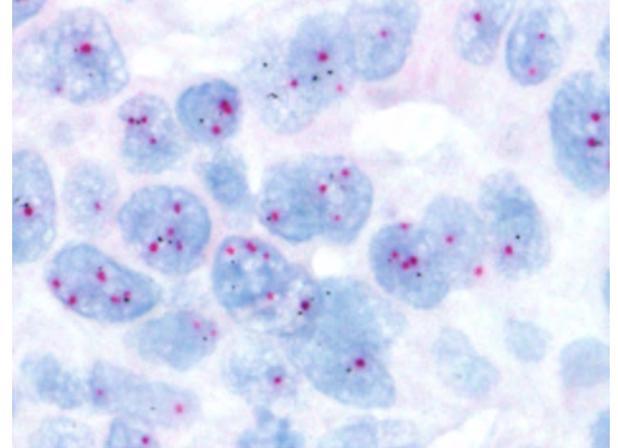
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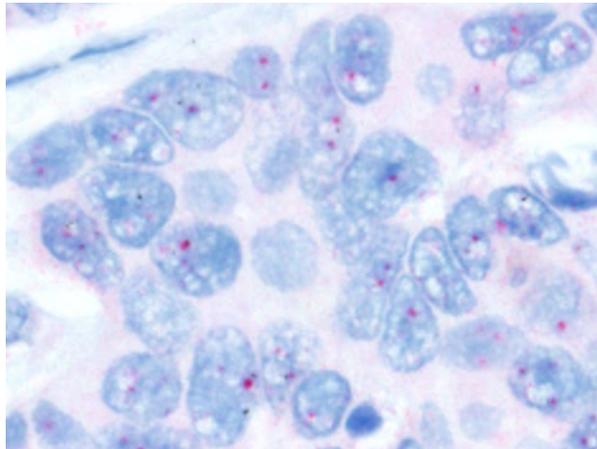
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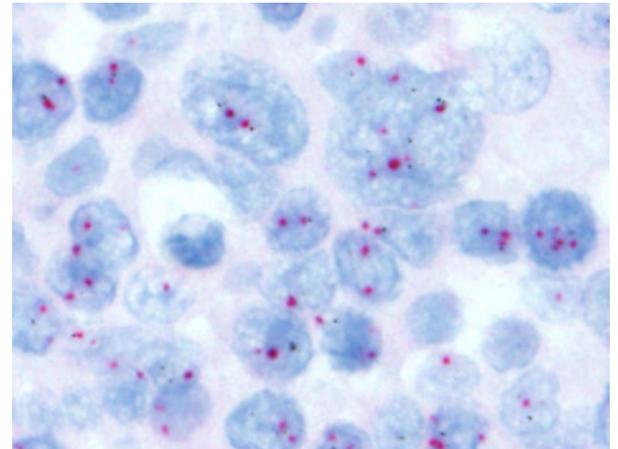
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48 hours



24 hours

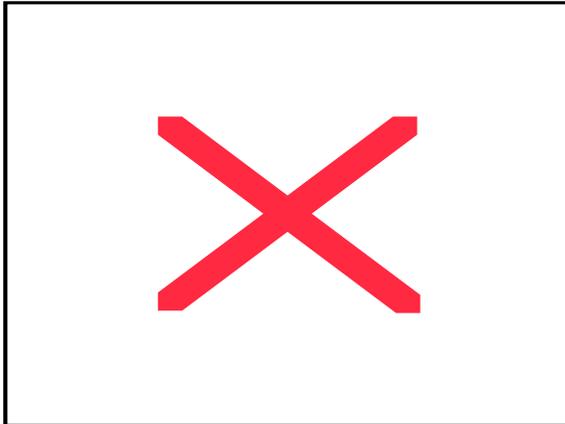


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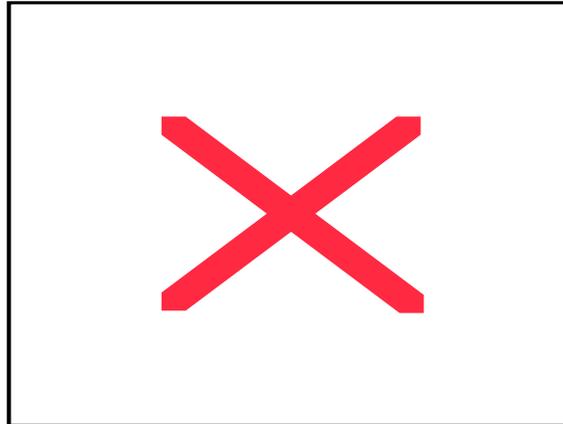
Study Results

AFA – FISH

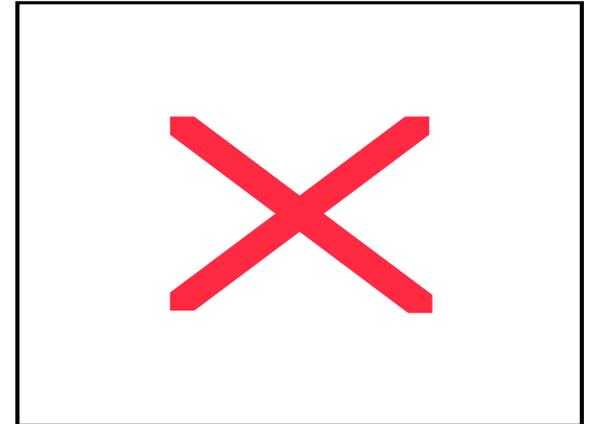
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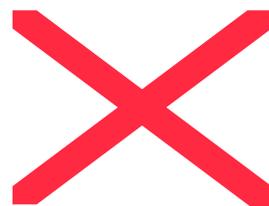
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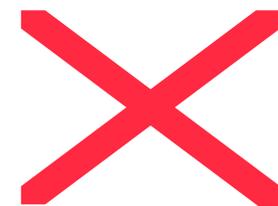
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24 hours

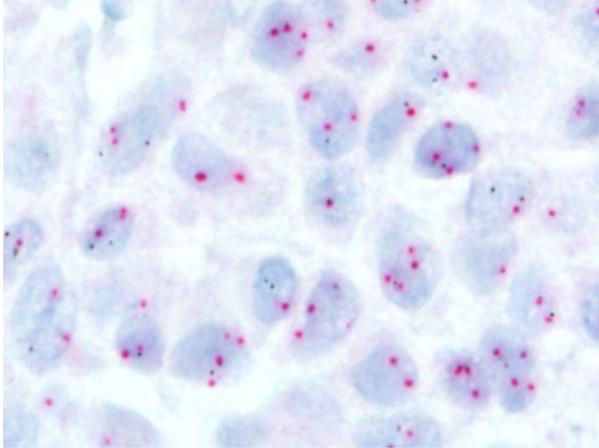


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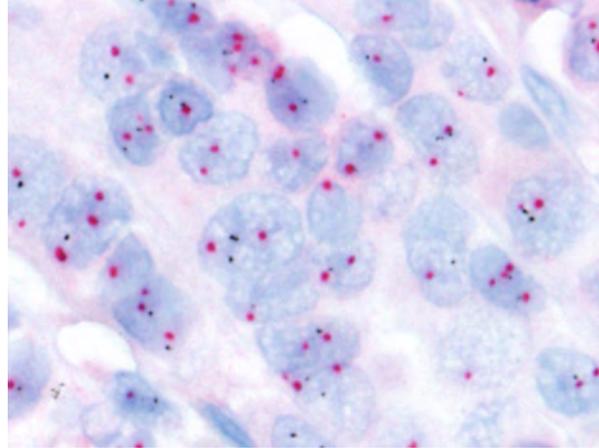
Study Results

Bouin's – Dual ISH

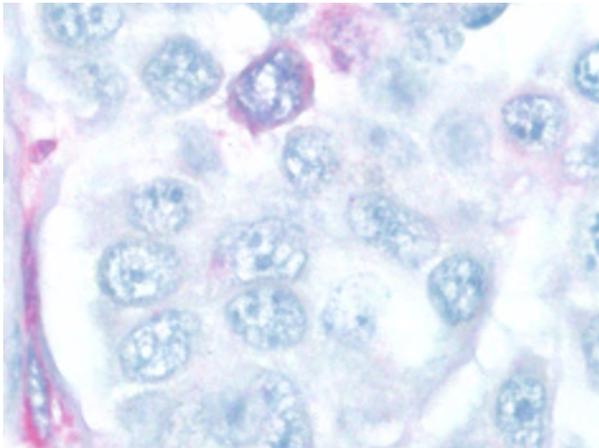
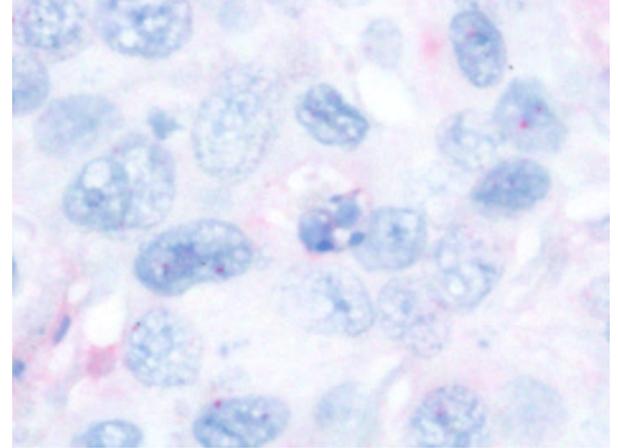
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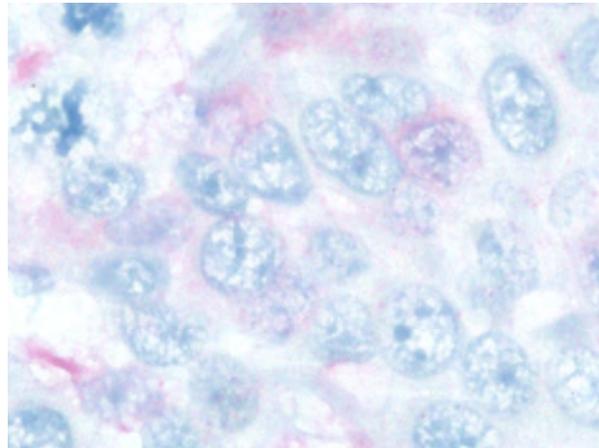
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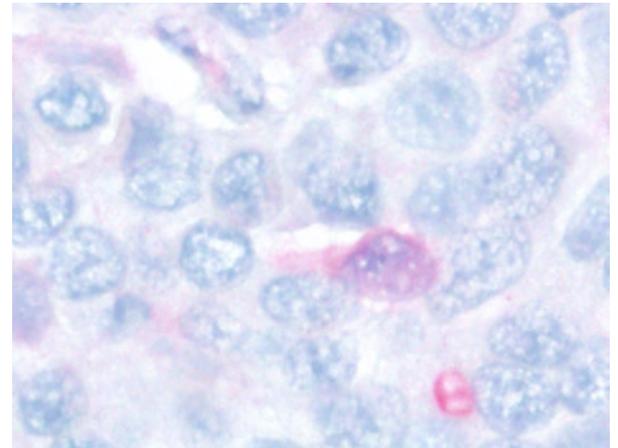
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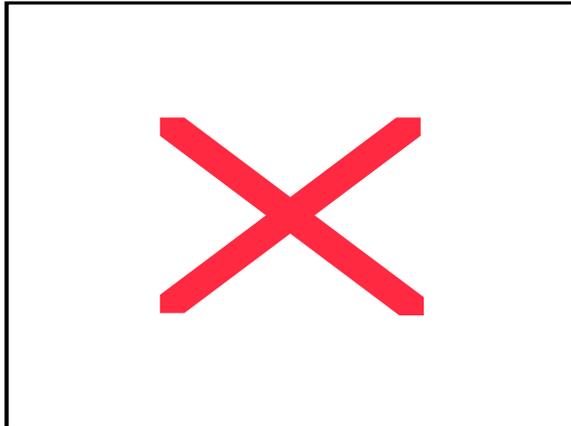


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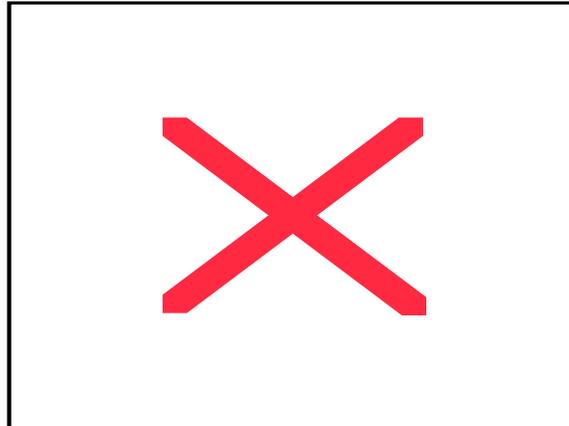
Study Results

Bouin's – FISH

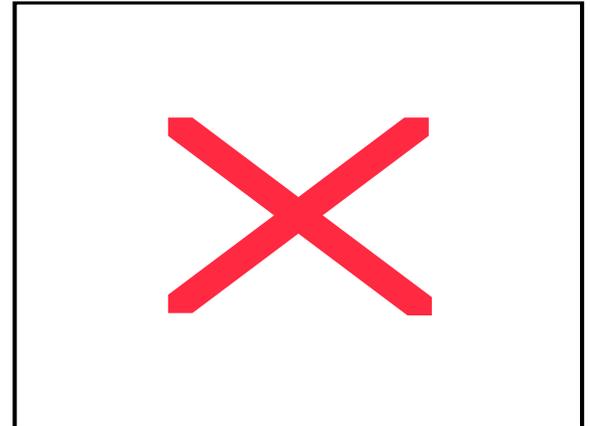
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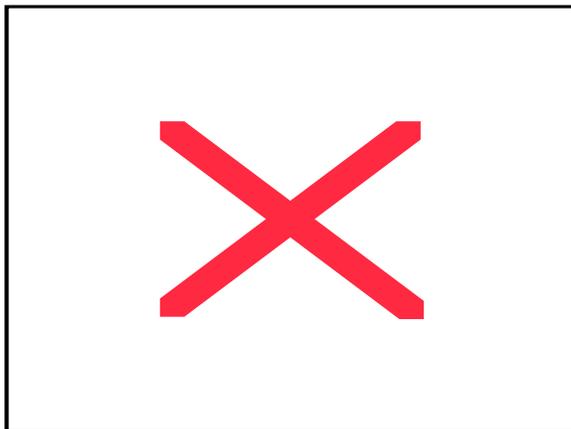
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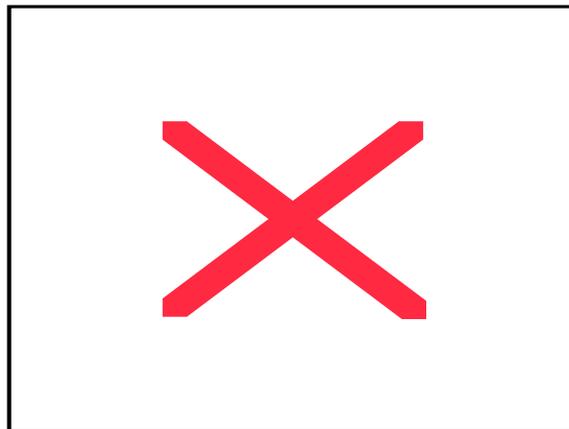
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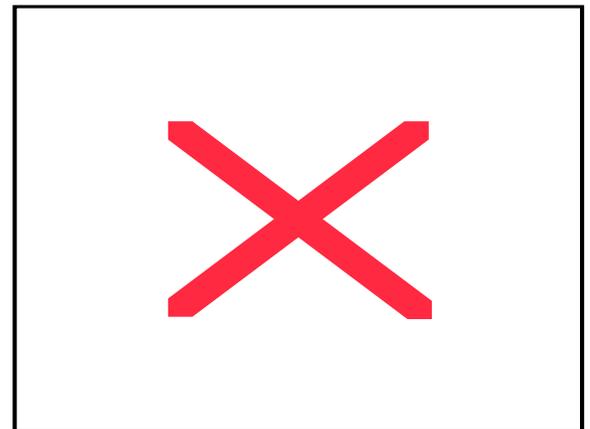
48 hours



24 hours



12 hours



Fixation Study Results Summary

- 10% Neutral Buffered Formalin (NBF)
 - FISH: 6-24 hours provides optimal staining
 - Dual ISH: 6-24 hours provides optimal staining
- AFA
 - FISH: Significant issues with morphology and background
 - Dual ISH: 1-24 hours demonstrated staining, but no timepoint delivered optimal results
- Bouin's
 - FISH: No staining possible
 - Dual ISH: 3 hours fixation provided adequate staining



High quality tissue biobanking – a major challenge for improving cancer drug and companion diagnostic development

Kerstin A. David, Jörg Spangenberg, Annika Sprüssel, Kevin Sullivan, Heinz Joachim List, Carsten Zornig*, Hartmut Juhl

Indivumed, Center for Cancer Research at the Israelitiches Krankenhaus, Hamburg, Germany;
*Department of Surgery, Israelitiches Krankenhaus, Hamburg, Germany

Introduction

Molecular tissue data in target discovery and validation highly depend on tissue quality and the capability to correlate molecular data with clinical variables. Studies are missing which define standards for science guided biobanking and which assure that experimental data represent molecular reality in patient tissues. Indivumed's special infrastructure with eight clinical cancer centers allows SOP-guided tissue biobanking, documentation of all surgical and clinical variables possibly affecting molecular data.

We have studied variables such as intrasurgical factors (ligation of main arteries, application of narcotic drugs), processing factors (e.g. time interval between surgical tumor removal and tissue fixation) and molecular differences among tumor regions (center vs. periphery). Using cDNA microarray technique, real-time RT-PCR, protein mass-spectroscopy and immunostaining, we found significant differences in the molecular pattern of tissue. In particular, cold ischemia has a high impact, affecting within minutes the expression of up to 20-30% of all cellular molecules. This dramatic variability is also reflected by immunostaining studies in particular for analyzing phosphoproteins. For example, staining intensity of phospho-m-TOR significantly increases after 10-15 min of cold ischemia time.

The availability of a comprehensive data base and tumor biobank with high quality has a significant impact on development of cancer drugs and companion diagnostics.

Methods

Tissue biobanking

- Availability of all major tumor entities
- Tissues are collected according to Indivumed Standard (e.g., shortest possible ischemia time, fixation of similar sized blocks, full documentation of pre-, intra- and post surgery factors)
- Comprehensive clinical data including follow-up information are available
- In some studies, the collection of tissues was changed according to experimental design (e.g., impact of cold ischemia on gene expression).

Immunohistochemistry (IHC)

Ischemia-dependent change of phosphorylation of mTOR and MAPK was analyzed by immunohistochemistry using the Ventana Technology. Both antibodies (anti-p-mTOR and anti-pMAPK) were from Cell Signalling (Danvers, MA). For detection, the UltraMap™ HRP Kit from Ventana was used.

RNA and DNA Extraction

Total RNA was extracted from freshly frozen tissue (normal colon tissue, tumor tissue) with the RNeasy Mini Kit (Qiagen). RNA quality and quantity was assessed using the RNeasy Nano assay kit (Agilent) and the Agilent 2100 Bioanalyzer.

Real-time PCR

Quantitative RT-PCR was performed on an iCycler (BioRad) using TaqMan® probes. The expression of the housekeeping genes GAPDH and Cyclophilin A was used for normalization.

Microarray

Analysis of gene expression was performed using the HG-U133A GeneChip® (Affymetrix). For data analysis, GeneSpring software (Affymetrix) was used.

Results

Overview of exogenous factors affecting molecular data



Figure 1: Overview of exogenous factors affecting molecular data. Tissue used for analytical and diagnostic purposes is alive and reacts to its environment on the molecular level (e.g., changes of gene expression, degradation of proteins, changes in phosphorylation status). SOP-guided biobanking is necessary to reflect molecular reality in patient tissues. Indivumed's SOP-guided sample collection process includes standardized tissue processing in the operation room resulting in short ischemia time (<10 min), controlled storage and transportation of tissues as well as standardized handling and processing. Experimental data from individual cases can be related to comprehensive clinical data obtained from all patients by Indivumed study network.

Impact of tumor region

Tumor tissue varies in center and peripheral areas

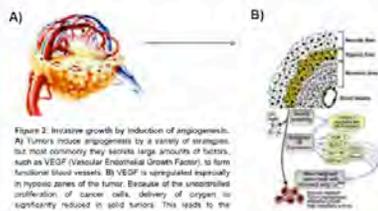


Figure 2: Invasive growth by induction of angiogenesis. A) Tumors induce angiogenesis by a variety of strategies, but most commonly they secrete large amounts of factors, such as VEGF (Vascular Endothelial Growth Factor) to form functional blood vessels. B) VEGF is secreted especially in hypoxic zones of the tumor. Escape of the underoxygenated proliferation of cancer cells, delivery of oxygen to significantly reduced in solid tumors. This leads to the development of cancer cells which acquire constitutive expression of HIF-1 alpha (Hypoxia Inducible Factor) a transcription factor stimulating the expression of VEGF.

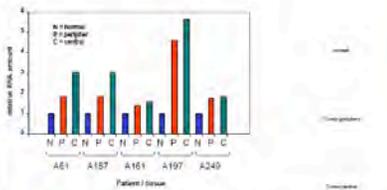


Figure 3: Analysis of VEGF expression in colon tissue of different regions (normal - tumor periphery, tumor center). Real-time RT-PCR to amplify VEGF (TaqMan® approach) in tissue from five different patients.

Impact of cold ischemia on gene expression

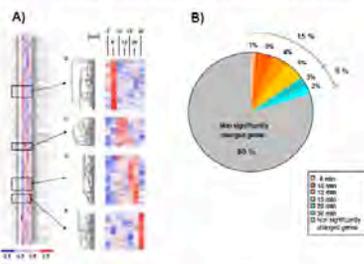


Figure 4: A) Variation in gene expression in seven samples of normal human colon tissue, frozen at different time points after colon resection. In reference to the housekeeping genes blue bands represent lower-expressed genes, red bands, higher-expressed genes. B) C, D, E: Dendrogram, heatmaps representing the expression patterns between experimental samples.

Figure 5: Analysis of normal colon tissue by cDNA microarray technology. Determination of differentially expressed genes (p < 2e-16, adjusted p < 0.05) at various time points following tissue resection (HG-U133A-mp, Affymetrix).

Real-time PCR and Microarray analysis of HIF-1α in normal human colon tissue

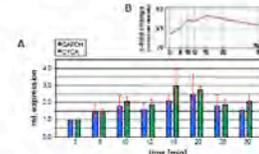


Figure 5: Analysis of HIF-1α (Hypoxia Inducible Factor) expression in normal colon tissue at different time points after colon resection. A) Real-time RT-PCR to amplify HIF-1α (TaqMan® approach). The expression of the housekeeping genes GAPDH and Cyclophilin A (CYCA) were used for normalization. B) Corresponding findings for HIF-1α from Microarray analysis (HG-U133A-mp, Affymetrix).

Real-time PCR and Microarray analysis of CEA in normal human colon tissue

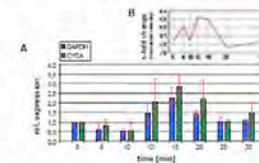


Figure 6: Analysis of CEA (Carcino Embryonic Antigen) expression in normal colon tissue at different time points after colon resection. A) Real-time RT-PCR to amplify CEA (TaqMan® approach). The expression of the housekeeping genes GAPDH and cyclophilin A (CYCA) were used for normalization. B) Corresponding findings for CEA from Microarray analysis (HG-U133A-mp, Affymetrix).

Results

Impact of ischemia on phosphoprotein expression

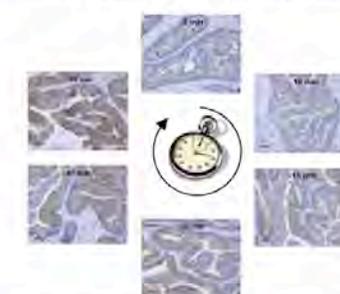


Figure 7: Immunostaining of colorectal cancer tissue with anti-phospho-mTOR. Phosphoproteins are extremely sensitive to exogenous variables. Shown is an immunostaining for phospho-mTOR of colon cancer tissue that has been fixed at various time points after surgical tumor removal. Ischemia dependent change of phosphorylation becomes obvious after 10-15 minutes, it demonstrates the importance of its tissue rapidly after surgical removal for obtaining clinically meaningful data.



Figure 8: Immunostaining of colorectal cancer tissue with anti-phospho-MAPK. Shown is the difference in immunostaining for phospho-MAPK in colon cancer tissue after 10 min and 60 min of fixation.

Summary

Molecular data used to bring cancer drugs and companion diagnostics to market depend highly on tissue quality and the capability to correlate molecular data with clinical variables. The studies presented here illustrate how important it is that experimental data reflect the molecular reality in patient tissue. Variables such as intrasurgical factors (ligation of main arteries, narcotic drugs applied), processing factors (time interval between surgical removal of tissue and tissue freezing/fixation) and molecular differences between tumor regions (center vs. periphery) create significant differences in the molecular pattern of the tissue. In particular, cold ischemia variability affects within minutes the expression of up to 30% of all cellular molecules. This dramatic variability is also reflected by immunostaining studies, especially for analyzing phosphoproteins. Ischemia-related phosphorylation changes after just 10-15 minutes. The availability of a tumor biobank collected under highly standardized conditions with a corresponding, comprehensive database is critical in the development of cancer drugs and companion diagnostics.

Time to Fixation Effect on p-mTOR (CST 49F9) Expression

Impact of ischemia on phosphoprotein expression

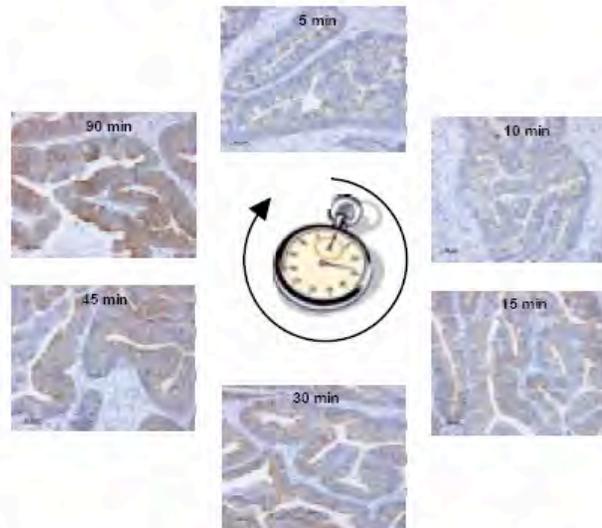


Figure 7: Immunostaining of colorectal cancer tissue with anti-phospho-mTOR. Phosphoproteins are extremely sensitive to exogenous variables. Shown is an immunostaining for phospho-mTOR of colon cancer tissue that has been fixed at various time points after surgical cancer removal. Ischemia-dependent change of phosphorylation becomes obvious after 10-15 minutes. It demonstrates the importance to fix tissue rapidly after surgical removal for obtaining clinically meaningful data.



Figure 8: Immunostaining of colorectal cancer tissue with anti-phospho-MAPK. Shown is the difference in immunostaining for phospho-MAPK in colon cancer tissue after 10 min and 90 min of fixation.

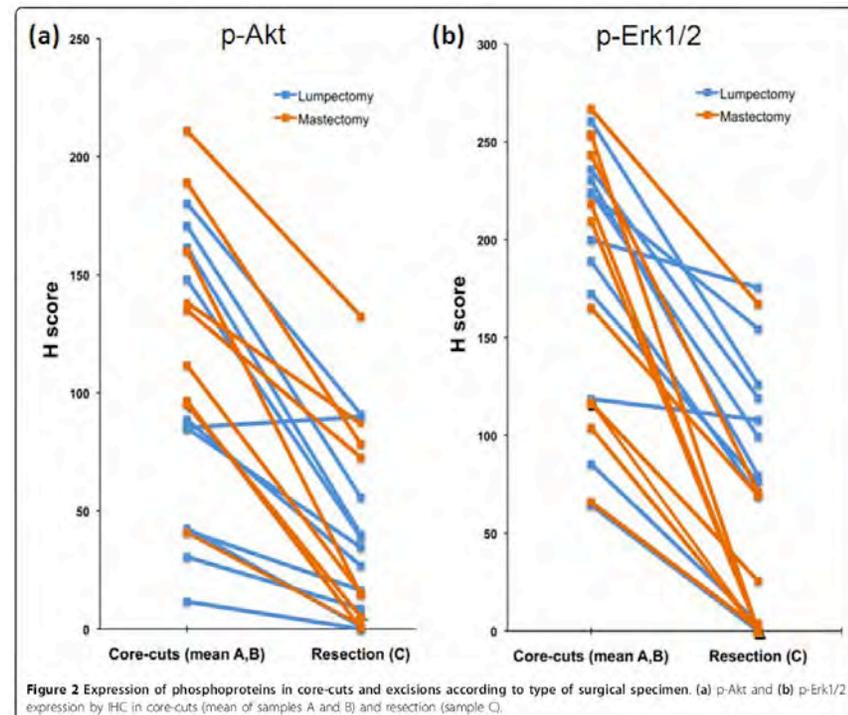
RESEARCH ARTICLE

Open Access

Extreme loss of immunoreactive p-Akt and p-Erk1/2 during routine fixation of primary breast cancer

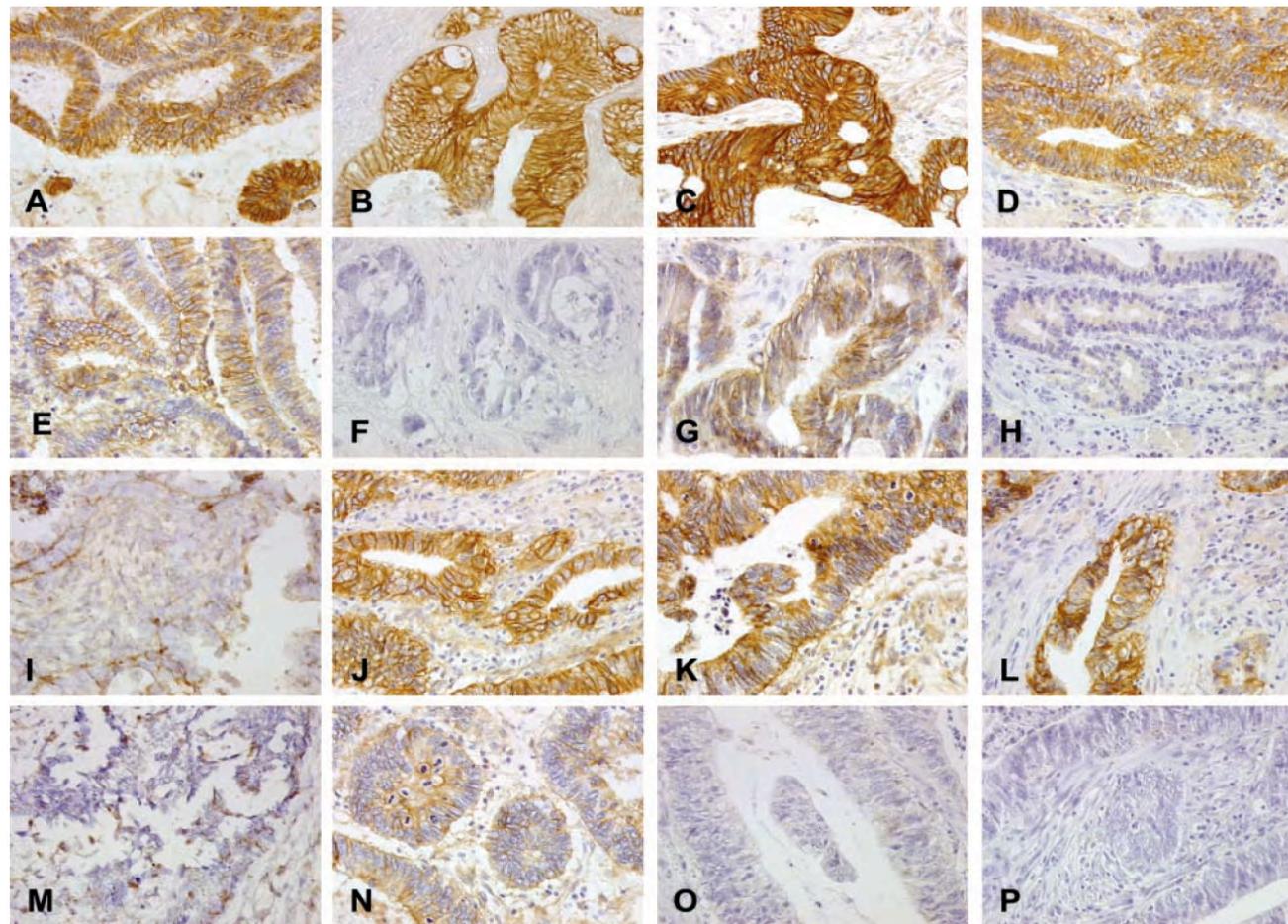
Isabel F Pinhel^{1*}, Fiona A MacNeill², Margaret J Hills³, Janine Salter^{3,4}, Simone Detre³, Roger A'Hern⁵, Ashutosh Nerurkar⁶, Peter Osin⁶, Ian E Smith², Mitch Dowsett³

- Examined immunoreactivity for Ki67, ER, PgR, HER2 p-Akt and p-Erk1/2 in immediate core bx, delayed (30 mins. average) core bx, resection specimen
- None of the markers showed significant differences between immediate and delayed core bx
- Ki67, ER, PgR and HER2 did not differ significantly between core-cuts and main resection specimen
- p-Akt and p-Erk1/2 were markedly lower in resections than core-cuts (median, 27 versus 101 and 69 versus 193, H-score, respectively; both $P < 0.0001$ [two-sided])



Immunohistochemical Detection of EGFR in Paraffin-embedded Tumor Tissues: Variation in Staining Intensity Due to Choice of Fixative and Storage Time of Tissue Sections

Derek Atkins, Karl-August Reiffen, Conny Lund Tegmeier, Henrik Winther, Marcellus S. Bonato, and Stephan Störkel



Atkins et al. J Histo Cytochem 52: 893-901

Long-term preservation of antigenicity on tissue microarrays

Kyle A DiVito*, Lori A Charette*, David L Rimm and Robert L Camp

Department of Pathology, Yale University, New Haven, CT, USA



a member of the Roche group

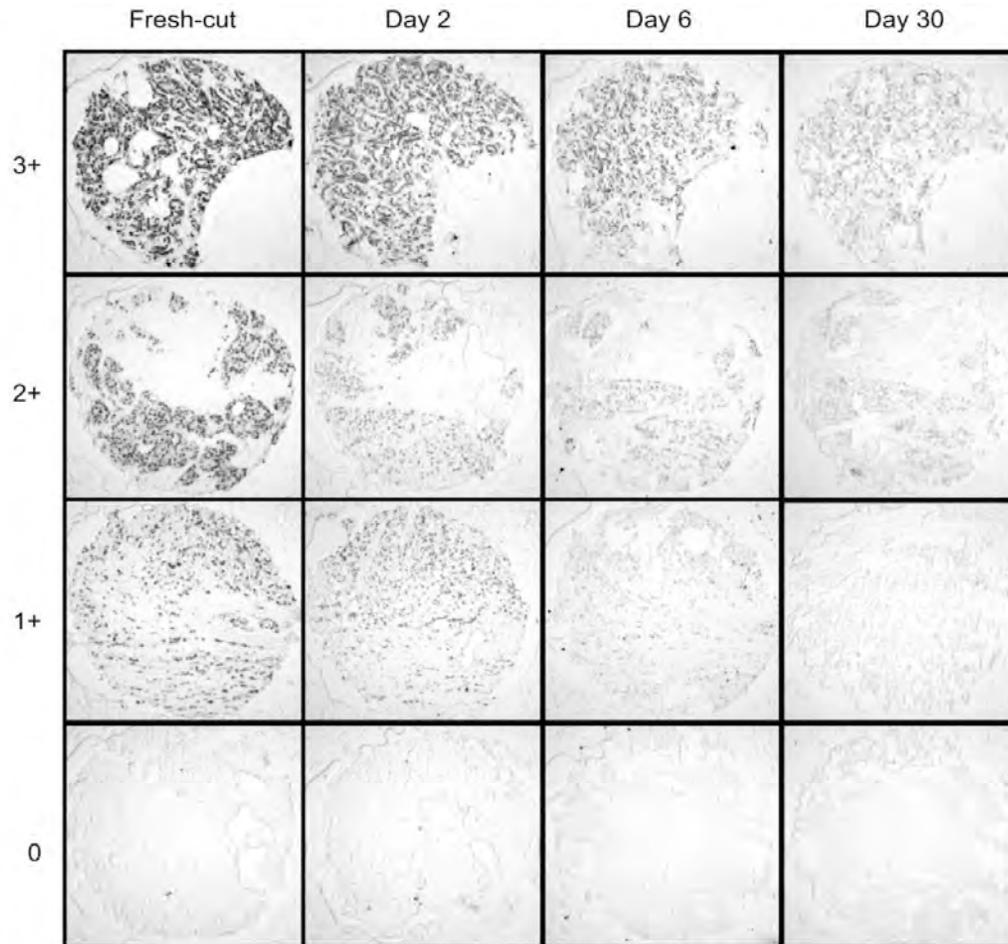


Table 3 Paired T-test of mean cytokeratin scores of stored slides stored for 3 months with nitrogen desiccation and/or paraffin coating

Condition	Automated score	
	% Fresh	P-value
Cytokeratin		
Fresh	100	
Uncoated, room-air	12	<0.0001
Uncoated, nitrogen	54	<0.0001
Paraffin coated, room-air	69	<0.0001
→ Paraffin coated, nitrogen	96	0.4698

Table 4 Paired T-test of mean Ki-67 scores of stored slides stored for 3 months with nitrogen desiccation and/or paraffin coating

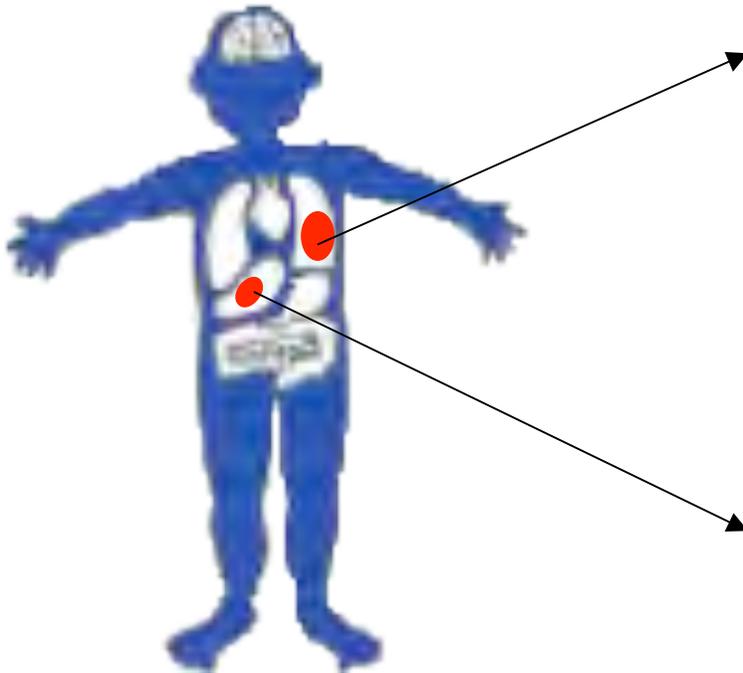
Condition	Manual score	
	% Fresh	P-value
Ki-67		
Fresh	100	
Uncoated, room-air	48	<0.0001
Uncoated, nitrogen	59	<0.0001
Paraffin coated, room-air	48	<0.0001
→ Paraffin coated, nitrogen	72	<0.0001

Table 5 Paired T-test of mean ER scores of stored slides stored for 5 months under nitrogen, coated with paraffin containing various antioxidants

Condition	Automated score	
	% Fresh	P-value
ER		
Fresh	100	
No additive	80	<0.0001
1% BHT	62	<0.0001
1% BHA	67	<0.0001
10% BHT	69	<0.0001
10% BHA	68	<0.0001

Figure 2 Estrogen receptor immunohistochemistry is abrogated by storage under ambient conditions. Representative, matched histospots (0-3+) from freshly-cut slides as well as slides stored for 2-, 6-, or 30 days are shown. Slides were stained for ER and visualized with DAB. For clarity, histospots were not counterstained; however, each histospot presented is covered with greater than 50% tumor. Staining is localized to the tumor nuclei when assayed under high power.

Does The Biomarker Readout From The Primary Tumor Accurately Reflect Metastatic Disease?



Primary Tumor

- Basis for diagnosis
- Paraffin embedded archival tumor available
- Usual sample used for biomarker assessment

Metastatic Tumor

- Target of investigational therapy
- Tissue sample usually not available
- Additional biopsy required

Epidermal Growth Factor Receptor (EGFR) Status in Primary Colorectal Tumors Does Not Correlate With EGFR Expression in Related Metastatic Sites: Implications for Treatment With EGFR-Targeted Monoclonal Antibodies

Mario Scartozzi, Italo Bearzi, Rossana Berardi, Alessandra Mandolesi, Guidalberto Fabris, and Stefano Cascinu

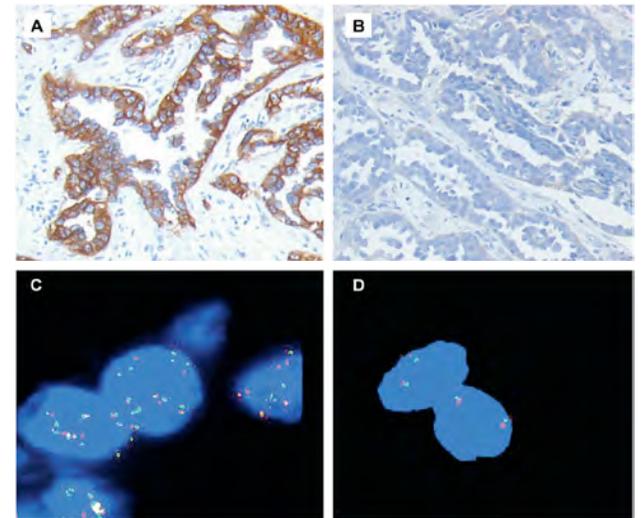


- **Evaluated EGFR IHC primary vs. metastatic site in 99 cases of CRC**
- **19/53 (36%) primary tumors EGFR IHC+ had corresponding metastatic tumors EGFR IHC-**
- **7/47 (15%) primary tumors EGFR IHC- had corresponding metastatic tumors EGFR IHC+**
- **Detection of EGFR in primary CRC could be inadequate for planning therapy with EGFR-targeted therapy**

Comparison of the epidermal growth factor receptor gene and protein in primary non-small-cell-lung cancer and metastatic sites: implications for treatment with EGFR-inhibitors

A. Italiano^{1,2*}, F. Burel Vandembos³, J. Otto¹, J. Mouroux⁴, D. Fontaine⁵, P.-Y. Marcy¹, N. Cardot³, A. Thyss¹ & F. Pedeutour²

- **EGFR status analyzed by IHC and FISH in primary tumor and matched metastatic lesion**
- **IHC**
 - **10/30 (33.3%) cases showed discordance (P=0.0074)**
 - 7/10: Primary EGFR+/Metastasis EGFR-
 - 3/10: Primary EGFR-/Metastasis EGFR+
- **FISH**
 - **7/26 (27%) cases showed discordance (P=0.007)**
 - 6/7: Primary FISH+/Metastasis FISH-
 - 1/7: Primary FISH-/Metastasis FISH+
 - EGFR protein level and gene copy number are not stable during metastatic progression in a significant proportion of NSCLC



Stability of HER2-positive status in breast carcinoma: a comparison between primary and paired metastatic tumors with regard to the possible impact of intervening trastuzumab treatment



C. Xiao^{1,2†}, Y. Gong^{1*†}, E. Y. Han¹, A. M. Gonzalez-Angulo^{3,4} & N. Sneige¹

¹Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, USA; ²Department of Breast Oncology, Tianjin Medical University Cancer Hospital, Tianjin, China; ³Departments of Breast Medical Oncology; ⁴Department of System Biology, The University of Texas MD Anderson Cancer Center, Houston, USA

- 56/66 (84.9%) patients had HER2 status agreement between paired primary and metastatic tumors
- 10 patients had HER2- positive-to-negative conversion. The agreement rate in the trastuzumab-treated group and in the control group was comparable (86.8% versus 82.1%) (P = 0.858).

Table 4. Details of HER2 result for 10 patients with HER2-positive primary breast carcinoma but HER2-negative metastatic carcinoma

Patient	Primary breast carcinoma			Metastatic carcinoma				
	IHC	FISH	Testing site	IHC	FISH	Location of tumor	Testing site	Trastuzumab treatment
1	Positive	NA	OS, without MDA review	NA	1.11	Supraclavicular lymph node	MDA	Yes
2	2+	2.40, 0.96 ^a	MDA	0	NA	Ovary	MDA	Yes
3	Positive	NA	OS, without MDA review	NA	0.94	Soft tissue, arm	MDA	Yes
4	Positive	NA	OS, without MDA review	NA	1.03	Pleural fluid	MDA	Yes
5	3+	1.21	OS and MDA ^b	NA	1.30	Soft tissue, chest wall	MDA	Yes
6	3+	7.02	MDA	NA	1.05	Lung	MDA	No
7	2+	2.31	MDA	NA	1.07	Lung	MDA	No
8	3+	7.85	MDA	NA	1.23	Axillary lymph node	MDA	No
9	NA	9.00	MDA	NA	1.01	Supraclavicular lymph node	MDA	No
10	3+	7.77	MDA	NA	0.53	Liver	MDA	No

Stability of Estrogen Receptor Status in Breast Carcinoma



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A Comparison Between Primary and Metastatic Tumors With Regard to Disease Course and Intervening Systemic Therapy

Yun Gong, MD¹; Eric Yulong Han, MD¹; Ming Guo, MD¹; Lajos Pusztai, MD, PhD²; and Nour Sneige, MD¹

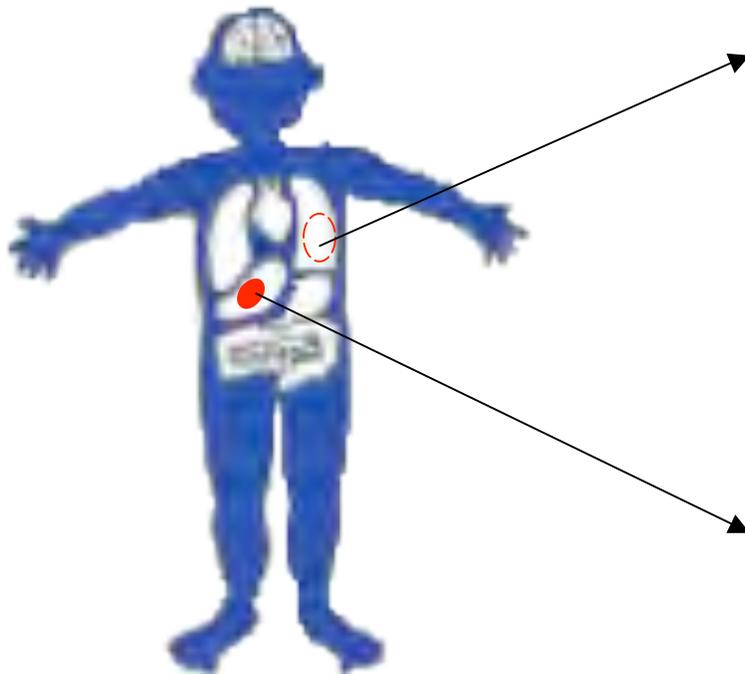
- **ER status agreed in 210/227 (92.5%) patients, including 147 positive and 63 negative.**
- Of the 17 patients (7.5%) with discordant ER status, both negative to positive conversion (n ¼ 7) and positive to negative conversion (n ¼ 10) were observed.
- ER discordance was not significantly associated with metastatic site (locoregional vs distant), time interval between assays (<5 years vs. 5 years), or intervening chemotherapy and endocrine therapy

Table 4. Information on 17 Patients With Discordant ER Status in Primary and Paired Metastatic Tumors

	Primary Carcinoma				Metastatic Carcinoma			
	ER %	No. of Tumors	Sample Type	Testing Site	ER %	Metastatic Site	Sample Type	Testing Site
Negative to Positive Conversion								
Case 1	Negative	1	Tissue	OS	10	LN, axillary	FNA	MDA
Case 2	Negative	2	Tissue	OS	10	LN, supraclavicular	FNA	MDA
Case 3	5	1	Tissue	MDA	10	bone	FNA	MDA
Case 4	Negative	1	Tissue	OS	50	LN, supraclavicular	FNA	MDA
Case 5	0	2	Tissue	OS	12	chest wall	FNA	MDA
Case 6	Negative	1	Tissue	OS	10	breast	FNA	MDA
Case 7	5.5	1	Tissue	OS	100	LN; supraclavicular	FNA	MDA
Positive to Negative Conversion								
Case 8	Positive	1	Tissue	OS	0	LN, pelvic	FNA	MDA
Case 9	Positive	2	Tissue	OS	0	chest wall	FNA	MDA
Case 10	Positive	2	Tissue	OS	0	LN, axillary	FNA	MDA
Case 11	85	1	Tissue	OS	<5	LN, axillary	FNA	MDA
Case 12	80	1	Tissue	OS	0	Breast	FNA	MDA
Case 13	Positive	1	Tissue	OS	0	Liver	FNA	MDA
Case 14	15	1	Tissue	MDA	Negative	LN, infraclavicular	FNA	MDA
Case 15	Positive	1	Tissue	OS	Negative	LN, supraclavicular	FNA	MDA
Case 16	90	1	Tissue	OS	Negative	Liver	FNA	MDA
Case 17	10	1	Tissue	OS	Negative	Lung	FNA	MDA

OS indicates outside hospital; MDA, M. D. Anderson Cancer Center; LN, lymph node; FNA, fine-needle aspiration.

Does The Biomarker Readout From The Primary Tumor Accurately Reflect Metastatic Disease?



Primary Tumor

Positive for
Companion
Diagnostic

Metastasis

Negative for
Companion
Diagnostic

No
Response to
Targeted
Therapy

Biomarker Status in Primary vs. Metastasis

Implications for Clinical Trial Design



- **Assessment of putative predictive biomarkers needs to be done with knowledge of whether the primary or metastatic sample was procured and analyzed**
- **All samples collected in clinical trials need to be annotated with anatomic site and identity; ‘primary’ or ‘metastasis’ (including multiple metastases)**
- **Ideally, both the primary tumor (archival paraffin) and the metastatic sample (prospective biopsy) should be collected and analyzed**

Tissue Collection Challenges in Co-Development Clinical Trials

Wish List

- **Standardized procedures/methods/technology for sample collection that reduce pre-analytical variability or at least document pre-analytical status**
 - Set of tissue quality metrics/assays
 - Fit-for-purpose grading approach
 - Example: Low-grade: morphology, Medium-grade: routine IHC/ISH, High-grade: sequencing, phospho-protein IHC
- **Widespread availability of materials to enable IHC assay development**
 - Clinical samples sets pre-characterized for known markers of interest
 - Example: NSCLC- EGFR L858R, T790M, EML4-ALK, etc.
 - Characterized cell lines and xenografts for use as assay controls
- **Global central laboratories/CROs capable of routinely handling and processing tissue samples for molecular pathology applications**

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