Cancer of Unknown Primary (CUP)

Four Decades of Evolution in Diagnostic Evaluation and Management

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Disclosure

- Dr. Greco is on the Speaker’s Bureau for bioTheranostics.

Four Decades of Changing Clinical Landscape In CUP

1. 1976-1986: Decade of Recognition of Favorable Clinicopathologic Subsets

2. 1986-1996: Decade of Improved Clinical Diagnostic Techniques/Testing

3. 1996-2006 : Decade of Empiric Chemotherapy

4. 2006-2014 :Decade of Improved Pathologic and Genetic Diagnostic Technologies and Better Outcomes for Many CUP patients

Agenda

- Cancer of Unknown Primary (CUP) Background
  - Historical Standard of Care: Recognition of favorable subsets; Empiric chemotherapy CUP Trials
  - Improving Clinical Evaluation and finding anatomical primary sites
- Immunohistochemical (IHC) and Gene Expression-Based Diagnostic Approaches
  - Overview of Clinical Data
  - Outcomes-Based Investigations
- A Prospective Outcomes Trial
- Summary

Background: Cancer of Unknown Primary

- Wide heterogeneity of clinical and pathologic presentations
- About 50,000 patients per year in USA
- Most patients have carcinoma and most of these adenocarcinomas
- Autopsy studies reveal small clinically undetectable primary tumor sites in 75% of patients (lung, pancreas, biliary tract, colorectal, kidney most common, but most tumors represented)
- Favorable subsets established; represent ~20% of CUP:
  - Squamous cell in the neck → Head & Neck Primary
  - Squamous cell in the inguinal region → Anal/Cervical Primary
  - Adenocarcinoma in the Axilla (women) → Breast Primary
  - Peritoneal carcinoma (women) → Ovary/fallopian tube/ primary peritoneal
  - Extragonadal Germ Cell Tumor Syndrome → Germ Cell
  - Neuroendocrine carcinoma → well or poorly differentiated- Many sites
  - Single metastasis → Many sites

Background

- Cancer of Unknown Primary (CUP) Definition
  - Metastatic cancer in the absence of a clinically-detectable anatomically-defined primary tumor site after an adequate diagnostic evaluation.

- CUP diagnosis can be considered a result of diagnostic failure.

- Improved clinical diagnostic techniques (CTs, MRI,PET, endoscopies) find anatomical primary sites more often then in the past.

- Many anatomical primary sites are too small to identify despite improved clinical diagnostic testing.

- The pathology (including modern IHC) and genetic testing of CUP biopsies has enabled a tissue of origin diagnosis in most patients despite an inability to identify the anatomical primary tumor site.

INITIAL DIAGNOSTIC EVALUATION

- Complete history: including detailed review of systems
- Complete physical examination: including pelvis examination, stool for occult blood
- Complete blood cell count, comprehensive metabolic panel, lactate dehydrogenase, urinalysis
- Computed tomography scans of chest, abdomen, and pelvis
- Mammography in women
- Serum prostate-specific antigen in men
- Positron emission tomography scan in selected patients
- Pathology-including screening immunohistochemistry marker stains (CK7, CK20, TTF-1, CDX2)
- Molecular Cancer Classifier as necessary

Background

- CUP patients within favorable subsets treated with “site-specifc” therapy have a better prognosis than the group as a whole.
- In the absence of a definitive diagnosis, 80% of patients (unfavorable prognosis group) with CUP traditionally have been treated as a single entity, usually with taxane/platinum or gemcitabine/platinum chemotherapy
- Patient prognosis is poor, with median survivals of approximately 9 months.

*Reference	Treatment	# of Patients	Median Survival
Greco et al., Oncologist. 2004;9(6):644-52.	Paclitaxel/Carboplatin/ Etoposide followed by Gemcitabine/Irinotecan	N=111	9.1 months
Greco et al., J Clin Oncol. 2002;20(6):1651-6.	Gemcitabine/Carboplatin/ Paclitaxel	N=113	9.0 months
Piga et al., Br J Cancer. 2004;90(10):1898-904.	Carboplatin/Doxorubicin /Etoposide	N=102	9.0 months
Hainsworth et al., Cancer J. 2010;16(1):70-5.	Paclitaxel/Carboplatin/ Etoposide vs Gemcitabine/Irinotecan	N=198	7.4 months 8.5 months

*CUP studies with patient populations greater than 100.

Evolving Role of IHC in Tissue of Origin Diagnosis in CUP

Lung, adenocarcinoma/large cell	CK7+, CK20-, TTF-1+
Lung, neuroendocrine (small cell/large cell)	Chromogranin+, Synaptophysin +, TTF-1+
Colorectal	CK7-, CK20+, CDX-2+
Breast	CK7+, ER+, GCDFP-2+, Mammoglobulin+
Prostate	CK7-, CK20-, PSA+
Ovary	CK7+, ER+, WT-1+
Melanoma	S100+, Melan-A+, HMB45+
Renal	RCC+, Vimentin+, CD10+, PAX-8+
Liver	Hepar 1+, CD10+, CD13+
Germ Cell	PLAP+ and/or OCT-4+
Adrenal	Alpha-inhibin+, Melan-A (A103)+
Thyroid (follicular/papillary)	TTF-1+, Thyroglobulin+

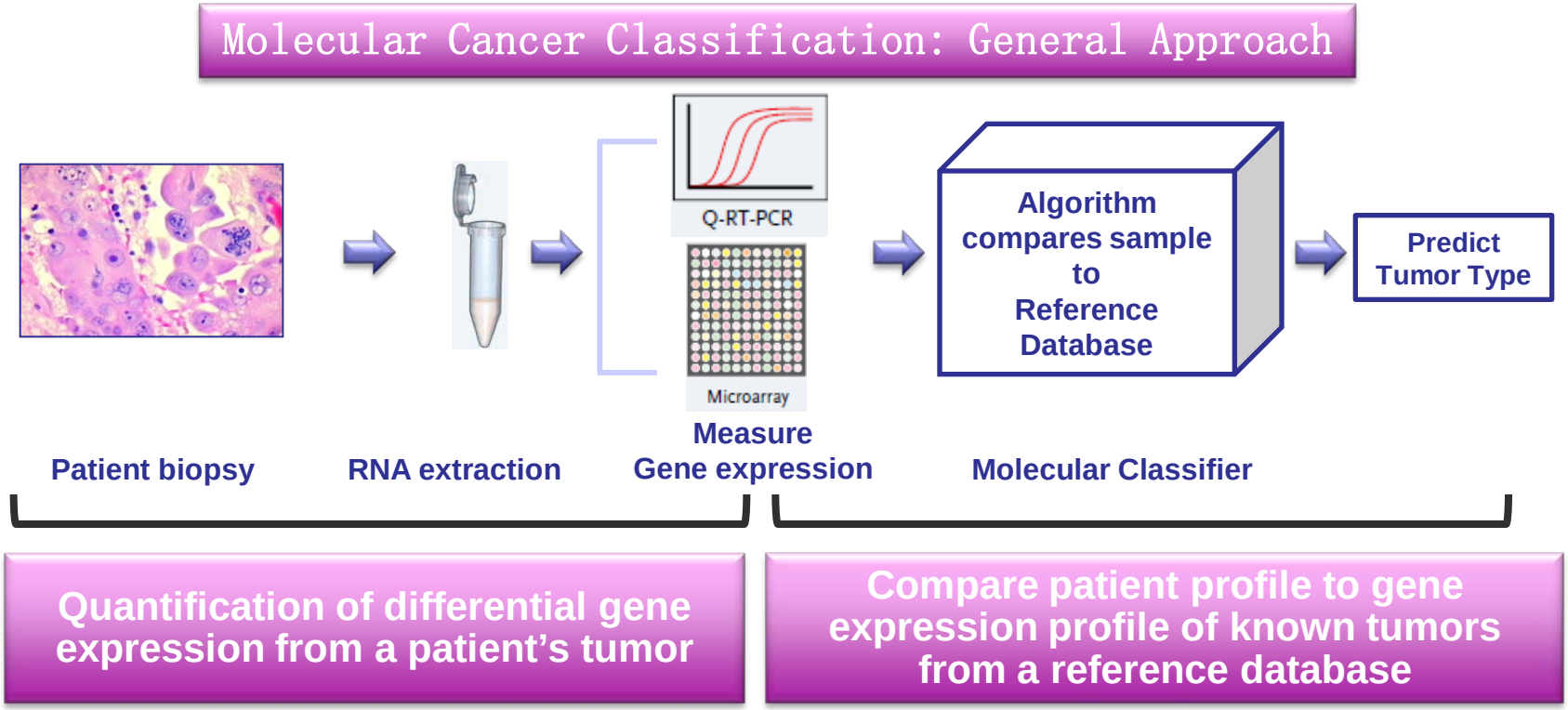
Single Tissue of Origin Diagnosed in 35% of CUP Cancers by IHC

Molecular Cancer Classifiers for CUP-Performed on Biopsies

	CancerTYPE ID	Tissue of Origin	Cancer Origin Test
Platform	Real-time RT-PCR mRNA	Microarray mRNA	Microarray miRNA
Tumor Types Classified	28 Main types, 50 Subtypes	15 types	42 types
Specimen Requirements	FFPE; Minimum 300-500 cells	FFPE; 6 slides 10 µM thick	FFPE; 3-10 slides 10 µM thick
Sensitivity	87%	88%	86%
Specificity	99%	99%	99%

Molecular Cancer Classification: General Approach

- In recent years, molecular cancer classification has emerged as a standardized, objective technique to help identify tumor type in patients with CUP
- Concept: neoplasms retain gene expression profile based on cellular origin; this profile can be exploited to identify tumor type



Major Questions regarding the 92-gene RT-PCR Assay

1) Accuracy in predicting primary tumor site?
2) What is the evidence that this assay can accurately identify tumor type in patients with CUP?
3) Will site-specific therapy based on the molecular assay diagnosis improve the outcome of patients with CUP?

Is the 92-gene RT-PCR assay accurate in predicting primary tumor site?

Published OnlineFirst on May 30, 2012; DOI:10.1158/1078-0432.CCR-12-0920

Clinical Cancer Research

Imaging, Diagnosis, Prognosis

Multisite Validation Study to Determine Performance Characteristics of a 92-Gene Molecular Cancer Classifier

Sarah E. Kerr[1], Catherine A. Schnabel[2], Peggy S. Sullivan[3], Yi Zhang[2], Veena Singh[2], Brittany Carey[4], Mark G. Erlander[2], W. Edward Highsmith[1], Sarah M. Dry[3], and Elena F. Brachtel[4]

- Sensitivity = 87% (95% CI, 0.84 to 0.89)
- Accuracy stable in metastatic tumors, high-grade tumors, and cases with limited tissue

This assay demonstrated excellent performance for classification of a diverse set of tumor histologies in known tumors

Major Questions regarding the 92-gene RT-PCR Assay

What is the evidence that this assay can accurately identify tumor type in patients with CUP?*

1. Evaluated biopsy specimens in patients found to have latent primary tumor sites months to years after initial presentation
2. Evaluated biopsy specimens in CUP patients with a single suspected diagnosis made by IHC
3. Evaluated directed IHC and clinical/histologic findings after molecular diagnosis known in attempt to confirm molecular diagnosis

*See – Journal of the National Cancer Institute – 2013, June 5; 105 (11); 782-90.

1. Accuracy of 92-gene RT-PCR assay in Patients with Latent Occult Primary Tumors

Molecular Profiling in Unknown Primary Cancer: Accuracy of Tissue of Origin Prediction
Oncologist 2010; 15 (5); 500-504

F. Anthony Greco, David R. Spigel, Denise A. Yardley, Mark G. Erlander, Xiao-Jun Ma, John D. Hainsworth

Molecular Tumor Profiling Diagnosis In Unknown Primary Cancer: Accuracy and Ability to Complement Standard Pathology
J Natl Cancer Insti. 2013;105(11):782-90

F. Anthony Greco, Wayne J. Lennington, David R. Spigel, John D. Hainsworth

- CUP patients that had latent primary tumors discovered during their follow-up
- The latent primary tumor site served as the reference known site of origin
- Original biopsy tissue tested by 92- gene assay (CancerTYPE ID)
- Molecular diagnosis was accurate in 18 of 24 cases
    - Sensitivity = 75%

This assay demonstrated high accuracy in CUP patients with latent primary tumors

2. 92-gene RT-PCR assay Concordance with suspected IHC diagnosis in CUP Patients

171 specimens from CUP patients referred to SCRI and tested by this assay

Single suspected diagnosis after initial IHC

- Molecular diagnosis concordant with IHC diagnosis in 40 of 52 cases (77%)

2 – 3 suspected diagnoses after initial IHC

43 of 97, assay prediction matched 1 of the Dx

- Clinical features validated molecular diagnosis prediction in 34 of 43 cases

54 of 97, assay prediction did not match the IHC Dx

- Molecular diagnosis consistent with clinical features in 41 of 54 cases
- Molecular diagnosis prediction validated with additional IHC and clinical findings in 26 of 35 cases (74%)

Correlative study demonstrated 92-gene RT-PCR had high concordance with suspected IHC diagnoses and provided additional objective diagnostic information when IHC was inconclusive. This molecular cancer classifier is about 80% accurate in determining the tissue of origin and complements standard pathology in CUP diagnosis.

3. 92-gene RT-PCR Assay Colorectal Diagnoses in CUP: Retrospective Outcomes

Carcinoma of Unknown Primary Site: Outcomes in Patients with a Colorectal Molecular Profile Treated with Site-Specific Chemotherapy
Jornal Cancer Therory 2012;3;37-43
F. Anthony Greco, Wayne J. Lennington, David R. Spigel, Gauri R. Varadhachary, John D. Hainsworth

- Retrospective analysis of 32 patients with CUP who were diagnosed by molecular assay with Colorectal Cancer (CRC).
- 29 patients received CRC-specific regimen.
- Median overall survival with CRC-specific regimens was 21 mo.

A Retrospective Study of Treatment Outcomes in Patients with Carcinoma of Unknown Primary Site and a Colorectal Cancer Molecular Profile
Clin Colorectal Cancer. 2012; 11(2):112-8
John D. Hainsworth, MD, Catherine A. Schnabel, PhD, Mark G. Erlander, PhD, David W. Haines III, BS, F. Anthony Greco, MD

- Retrospective analysis of 42 patients with CUP who were diagnosed by CancerTYPE ID with Colorectal Cancer.
- 50% of patients treated with CRC-specific regimen had objective response while only 17% of patients treated with empiric therapy had an objective response (p= 0.02).
- Median overall survival with CRC-specific regimens was 27 mo.

Responses and survivals are similar to known advanced CRC and compares favorably to empiric chemotherapy for CUP patients.

IHC Colorectal Diagnosis in CUP: Retrospective Outcomes

Carcinoma of unknown primary with gastrointestinal profile: immunohistochemistry and survival for this favorable subset. Int J CLIN Oncol 2013 June 28 (Epub ahead of print)

- Retrospective analysis of 74 CUP patients (2004-2010): all had CDX2+ stain with 34 CK20+,CK7- and 40 irrespective of CK7/CK20 stains.
- All patients received colorectal site-specific chemotherapy regimens.
- Liver, peritoneum and nodes were common metastatic sites.
- Median survivals exceeded 21 months (37 and 21 months for two groups).
- CUP patients should have “optimal” IHC to diagnose a colorectal profile (colorectal tissue of origin) as these CUP patients benefit substantially from colorectal site-specific therapy.

Responses and survivals are similar to known advanced CRC and compares favorably to empiric chemotherapy for CUP patients.

Prospective Outcomes Trial

JOURNAL OF CLINICAL ONCOLOGY
2013; 31(2): 217 – 223

ORIGINAL REPORT

Molecular Gene Expression Profiling to Predict the Tissue of Origin and Direct Site-Specific Therapy in Patients With Carcinoma of Unknown Primary Site: A Prospective Trial of the Sarah Cannon Research Institute

John D. Hainsworth, Mark S. Rubin, David R. Spigel, Ralph V. Boccia, Samuel Raby, Raven Quinn, and F. Anthony Greco

Prospective Outcomes with 92-gene RT-PCR assay: Background and Study Objective

- Objective
  - To evaluate the ability of gene expression-based classification with the 92-gene assay to render a tumor type diagnosis in patients with CUP
  - To determine the efficacy of treatment regimens based on molecular assay-predicted site of origin
- Endpoints
  - Further evaluation of the accuracy of the molecular assay to identify responsive vs non-responsive tumor types, and determine outcomes.
  - Improvement in overall survival of patients who received molecular assay-directed, site-specific therapy of at least 30% compared to previous trials from the same study group (9.1 months to at least 11.7 months). Overall survival compared to 396 patients from a compilation of 4 CUP trials with contemporary empiric chemotherapies performed by the same clinical trial network.

Study Design

- Design
  - Eligible patients had a diagnosis of CUP after diagnostic workup on initial presentation
  - Patients excluded if they had a treatable CUP syndrome
  - Patients were treated with standard first-line chemotherapeutic treatment regimens based on molecular results

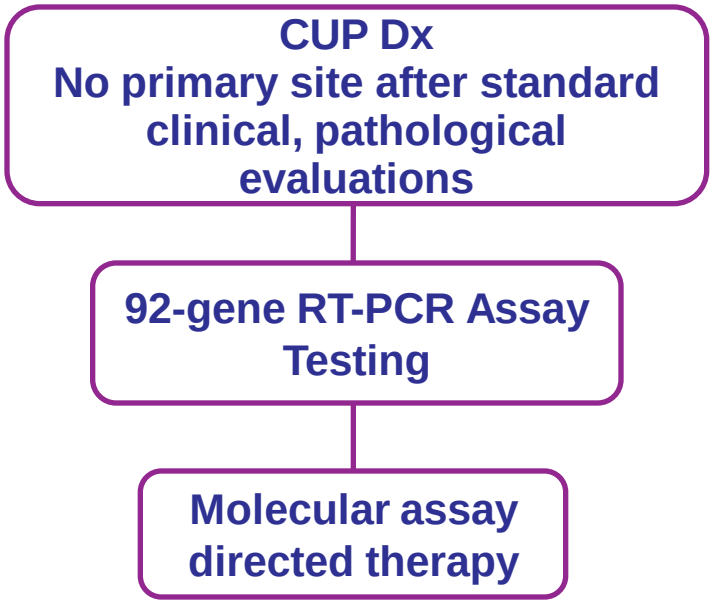


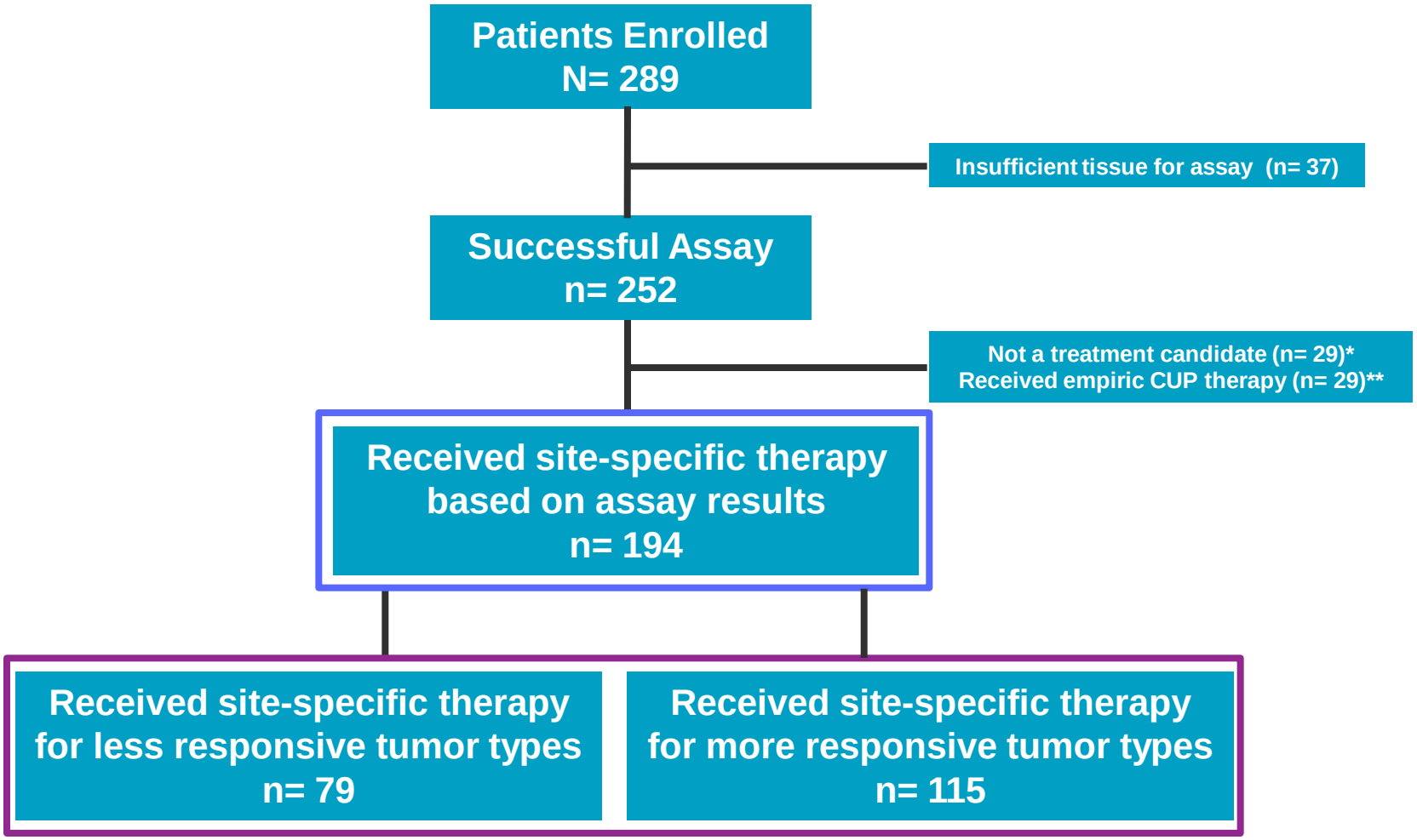
Table 1. Site-Specific Treatments

Predicted Tissue of Origin	Treatment*
Breast	Taxane/bevacizumab
Colorectal	FOLFOX (or variant) + bevacizumab, or FOLFIRI (or variant) + bevacizumab
Lung, non–small cell	Platinum-based doublet + bevacizumab
Ovary	Paclitaxel/carboplatin + bevacizumab
Pancreas	Gemcitabine/erlotinib
Prostate	Androgen ablation therapy
Renal	Sunitinib or bevacizumab ± interferon
Other diagnoses	Standard first-line treatment per guidelines

Abbreviations: FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin.
*Bevacizumab was omitted from the treatment regimen for patients with contraindications.

Hainsworth et al. J Clin Oncol. 2013;31(2):217-23

Patient Flow Diagram



* Declining performance status, brain metastasis, patient decision

**Unclassifiable result, physician chose to treat with CUP regimen, non-assay directed therapy

Hainsworth et al. J Clin Oncol. 2013;31(2):217-23

Tumor Classification Predicted by 92-gene RT-PCR Assay

Table 3. Tissue of Origin Predicted by Molecular Assay

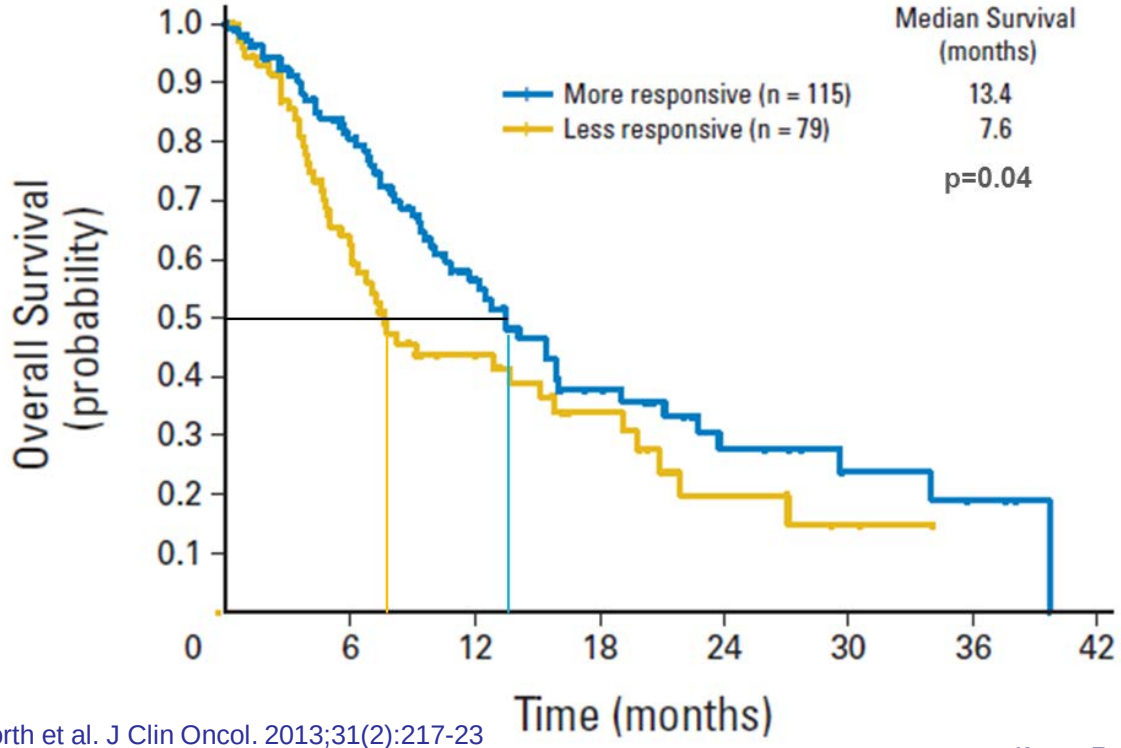
Predicted Tissue of Origin	No. of Patients (N = 252)	%
Biliary tract (gallbladder, bile ducts)	52	21
Urothelium	31	12
Colorectum	28	11
Non–small-cell lung	27	11
Pancreas	12	5
Breast	12	5
Ovary	11	4
Gastroesophageal	10	4
Kidney	9	4
Liver	8	3
Sarcoma	6	2
Cervix	6	2
Neuroendocrine	5	2
Prostate	4	2
Germ cell	4	2
Skin, squamous	4	2
Carcinoid, intestine	3	1
Mesothelioma	3	1
Thyroid	2	1
Endometrium	2	1
Melanoma	2	1
Skin, basal cell	2	1
Lung, small cell	1	1
Lymphoma	1	1
Head and neck	1	1
Adrenal	1	1
No prediction possible (unclassifiable)	5	2

- Molecular assay provided a primary site prediction in 98% of the cases
- 26 different tumor types predicted
  - Approximately 60% of patients had tumor types that are more likely to respond to site-directed chemotherapy (median survival >12 months)
  - 48% of identified tumors have indicated molecularly targeted therapies

Hainsworth et al. J Clin Oncol. 2013;31(2):217-23

Identification of Responsive Clinical Subsets

- **Patients identified by the 92-gene RT-PCR assay to have responsive tumor types had a statistically significant increase in overall survival compared to those with less responsive tumor types (p=0.04)**
- **Provides evidence that when more effective therapies are available, this molecular assay has an even greater impact on patient outcome**



Less Responsive Tumors*
- Biliary tract
- Pancreas
- Gastroesophageal
- Liver
- Sarcoma
- Cervix
- Carcinoid
- Endometrium
- Mesothelioma
- Melanoma
- Skin
- Thyroid
- Head and Neck
- Adrenal

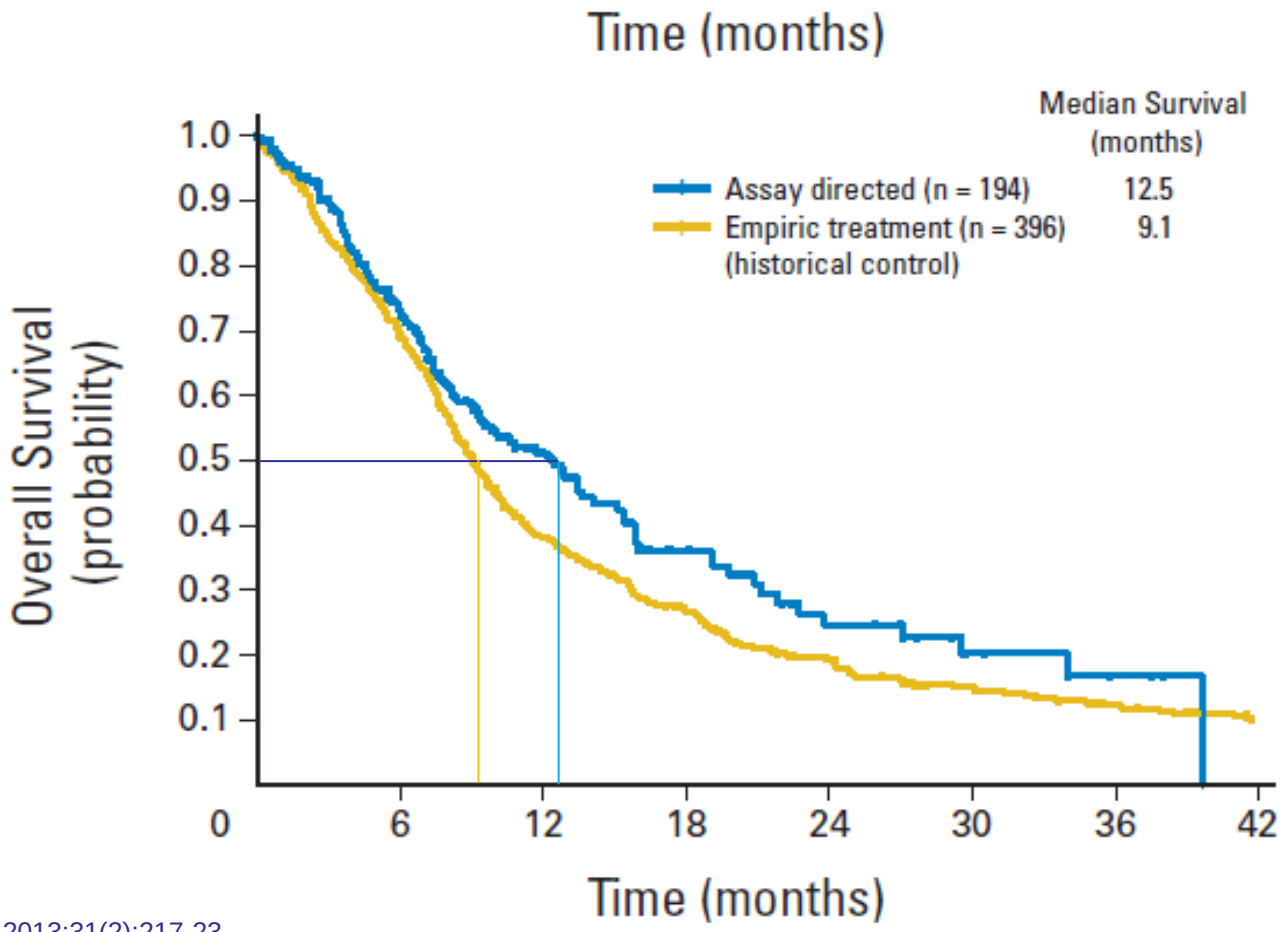
Responsive Tumors**
- Colorectal
- NSCLC
- Urothelial
- Breast
- Ovary
- Kidney
- Prostate
- Germ cell
- Lymphoma
- SCLC
- Neuroendocrine

Hainsworth et al. J Clin Oncol. 2013;31(2):217-23

***Less Responsive** (Median OS ≤12 mo with standard treatment)
****Responsive** (Median OS ≥12 mo with standard treatment)

Assay Directed Treatment vs. Empiric Treatment Historical Control

- **37% increase in overall survival with assay-directed therapy**



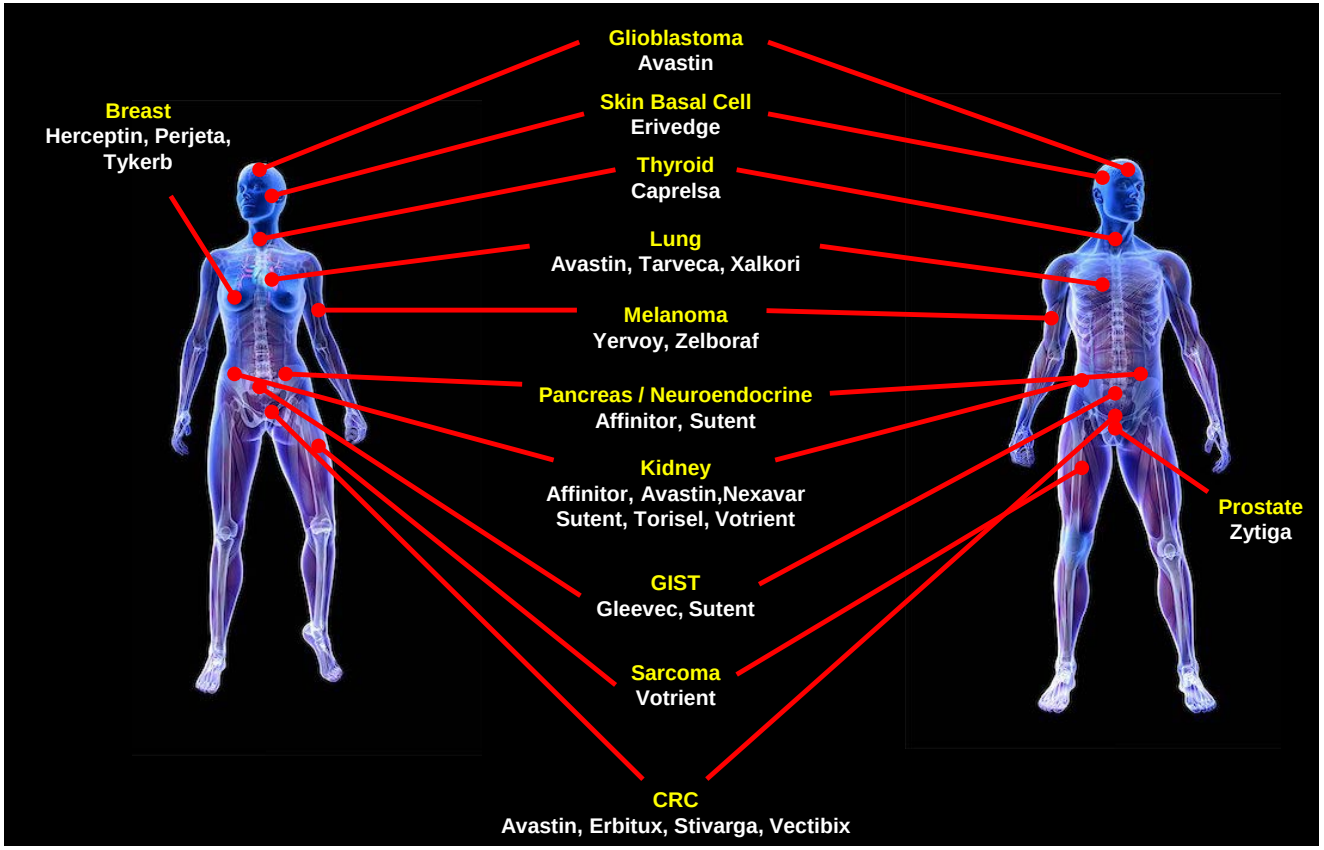
Hainsworth et al. J Clin Oncol. 2013;31(2):217-23

Summary

- First prospective trial in which molecular cancer classification has directed site-specific therapy.
- The molecular assay provided a primary site prediction in 98% of cases.
- Approximately 60% of patients were predicted to have responsive tumor types and as treatment options improve, molecular cancer classification may have an even greater impact on patient outcome.
- Even a correct diagnosis of a relatively unresponsive cancer type is now unlikely to provide much if any therapeutic benefit.
- In this study was there was a 37% increase in overall survival of the whole group receiving assay-directed therapy.
- Gene expression-based classification is recommended as part of the standard evaluation for selected patients with CUP.

CUP needs to be specifically diagnosed to offer the best therapy to patients

- Site of origin + Tumor subtype + Biomarker Profile =
  - Increasing ability to personalize cancer therapy with a combination of site-directed cytotoxic therapy and/or molecularly-targeted agents



Future Studies

- Future studies will concentrate on defining the genetic aberrations in CUP to explain the biology of these cancers and to test specific targeted drugs
- For example, in this study:

Tumor Type	Molecular Biomarker
Lung	EGFR mutations, EGFR expression, ALK rearrangement, ALK mutations, KRAS mutations, ROS1 rearrangement, c-MET amplification, c-MET expression, RRM1 expression, ERCC1 expression, DDR2 mutations, BRAF mutation, PTEN deletion, PIK3CA mutations
Breast	HER-2 expression, HER-2 amplification, ER/PR, FGFR1 amplification, PTEN deletion, PIK3CA mutations
Colorectal	KRAS mutations, BRAF mutation, NRAS mutations, ERCC1 expression, PTEN deletion, PIK3CA mutations
Gastric	HER-2 expression, HER-2 amplification, c-MET amplification, ERCC1 expression, PTEN deletion, PIK3CA mutations
Melanoma	BRAF mutation, C-kit mutation
Basal cell carcinoma	SMO mutation
Medullary Thyroid	RET rearrangement

When should a molecular assay be ordered in CUP?

- Any patient without IHC patterns diagnostic of a single primary site or tissue of origin.
- In patients with small biopsy specimens when sufficient IHC evaluation will not be feasible (e.g., FNAs, pleural effusions, small needle biopsies).
- In patients with metastasis and a history of 1 or more previous cancers, when IHC is inconclusive.
- In patients with atypical presentation / clinical presentation does not match pathologic characterization.
- In any tumor that is very poorly differentiated and there is question of lineage and/or tissue of origin from IHC.

Impact of Molecular Cancer Classification in CUP

- The integration of a molecular assay into the evaluation of CUP patients complements appropriate IHC/clinical findings and leads to the diagnosis of the tissue of origin in the majority (90%+) of patients, even though the anatomical primary site remains undetectable.
- Site-specific therapy is critical to give many of these patients the best outcome possible.
- As therapy improves for solid tumors of many types these therapies may be administered to CUP patients provided their primary tumor sites or tissues of origin are recognized.

Changing Clinical Landscape of CUP over the Decades

	CUP in 1976	CUP in 1996	CUP in 2013
Clinical Evaluation	• Rudimentary • CT not yet available	• CT scans • Endoscopies	• Can be extensive • CT, PET, MRI, endoscopy, ultrasound, etc
Pathology	• H&E • No IHC	• Limited IHC	• Evolving IHC, useful panels • Molecular diagnosis very useful
Favorable Subsets	• NOT appreciated	• Multiple subsets appreciated with specific therapy (20% of all CUP)	• Specific IHC and molecular diagnosis • Outcome improved with site-specific therapy
Treatment	• Symptomatic/supportive • No effective therapies • Empiric regimens	• Treatment helpful in favorable subsets • Empiric regimens	• Site-specific therapy • Most CUP patients can have primary site diagnosed by molecular dx
Prognosis	• Very poor • All patients lumped together • Only a few known solid tumors had useful therapy	• Good for favorable subsets • Empiric regimens helpful for some tumors	• Improved with site-specific therapy based upon an accurate diagnosis of the primary site • Poor for specific tumors with ineffective therapy

Evaluation and Management of Possible CUP Patient