Molecular Testing and Targeted Therapy

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Chair of Anatomical Pathology and Executive Director of Esoteric Laboratory Services
H. Lee Moffitt Cancer Center

Feb 6 2013,

Protocol Support Committee Workshop

MOFFITT CANCER CENTER
25 A Bold Beginning · A Boundless Future 1986-2011

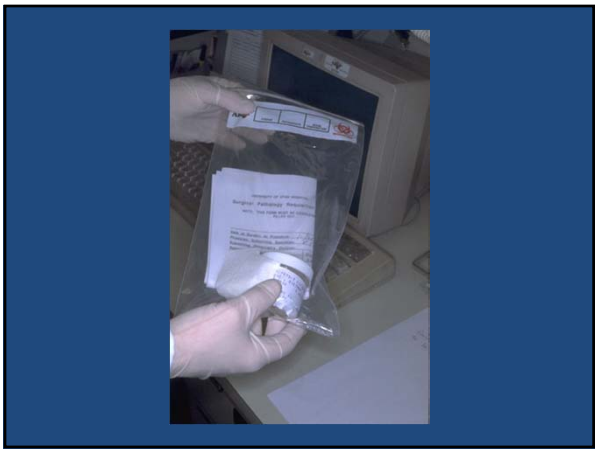
Molecular Testing

- Specimen Handling
- Technologies
  - IHC
  - FISH
  - DNA Sequencing
  - Next Generation technologies
  - Circulating Tumor Cells and CF DNA analysis
- Challenges

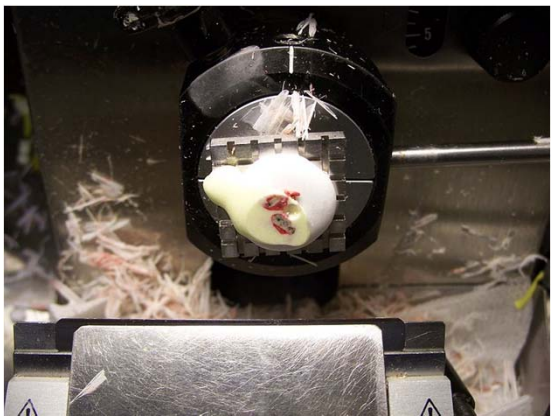
What happens to the specimen?

The current state of pathology

Pathology Tour

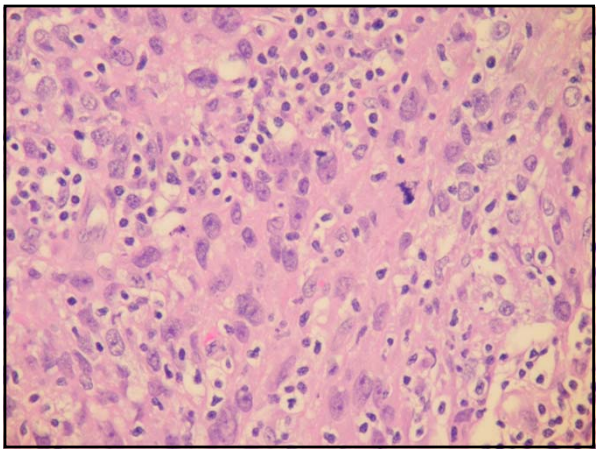


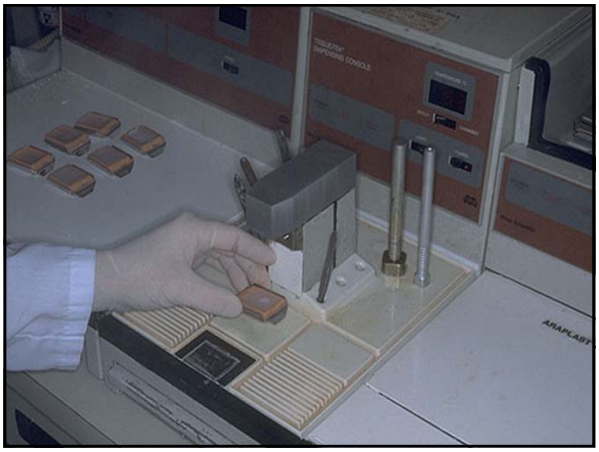

HEDY



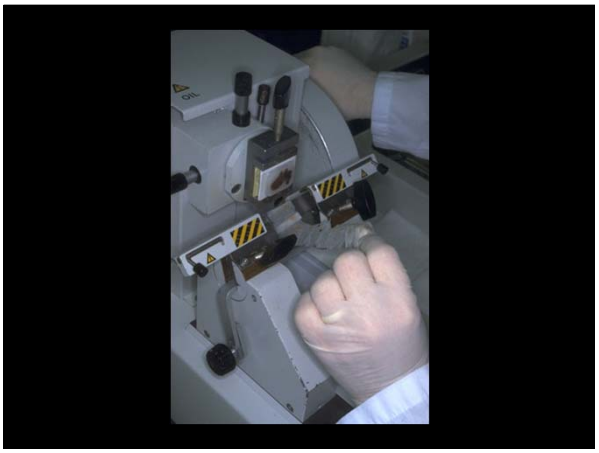

Product Name: Hematoxylin
Sept 27/01
Storage:
Prepared By:
Refer to MSDS

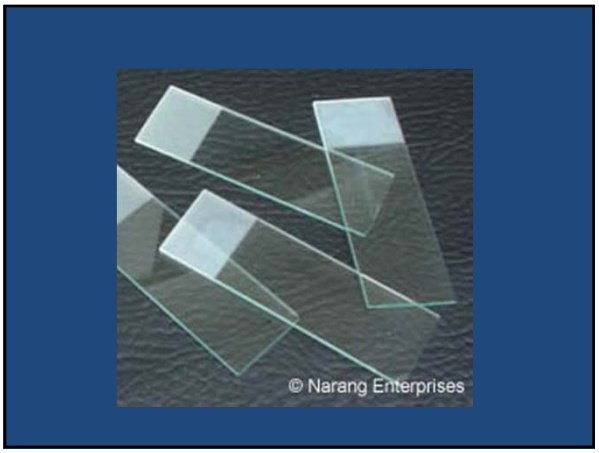

Product Name: EoSin
01/09/14
Storage:
Prepared By:
Refer to MSDS

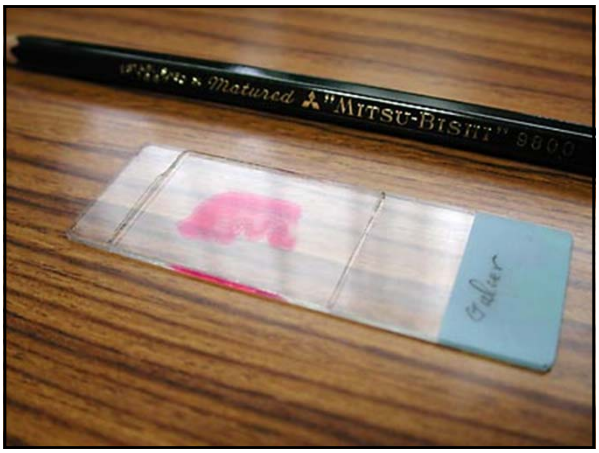


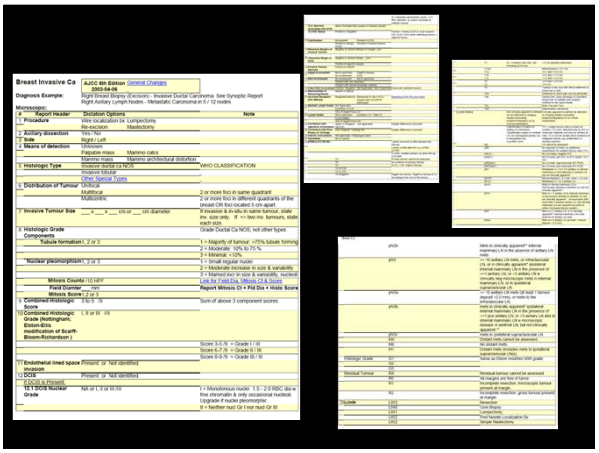



2040




© Narang Enterprises

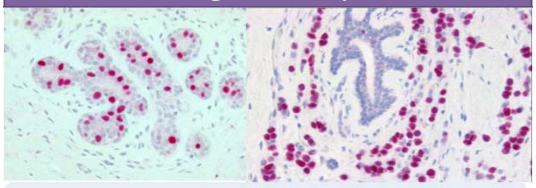

Matured "MITSU-BISHI"
Gdar


Breast Invasive Ca
AJCC 6th Edition
2003-04-06
Diagnosis Example:
Microscopic:
Report Header
Dictation Options
Note
Procedure
Axillary dissection
Side
Means of detection
Histologic Type
Distribution of Tumour
Invasive Tumour Size
Histologic Grade
Components
Tubule formation
Nuclear pleomorphism
Mitosis Count
Field Diameter
Mitosis Score
Combined Histologic Score
Combined Histologic Grade (Nottingham; Elston-Ellis modification of Scarff-Bloom-Richardson)
Endothelial lined space invasion
DCIS
LCIS is Present
Nuclear Grade

Predictive Factors

- What therapy to use??

Estrogen Receptor

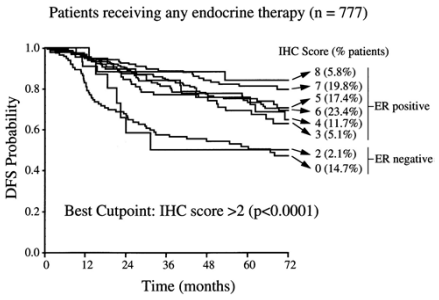


*Allred score for ER status (0-8)**

% Staining Score	Proportion of positive staining cells	Intensity Score	Average intensity of positively stained cells
0	none	0	none
1	< 1/100	1	weak
2	1/100 to 1/10	2	intermediate
3	1/10 to 1/3	3	strong
4	1/3 to 2/3		
5	> 2/3		

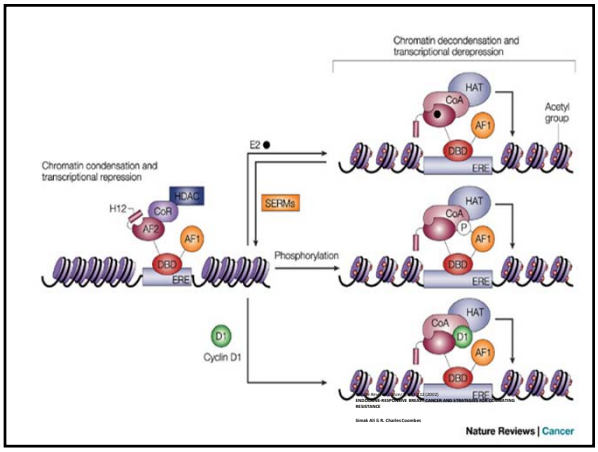
**Allred Score = % Staining Score + Intensity Score*
Allred DC et al. *Mod Pathol* 1998;11:155-68.

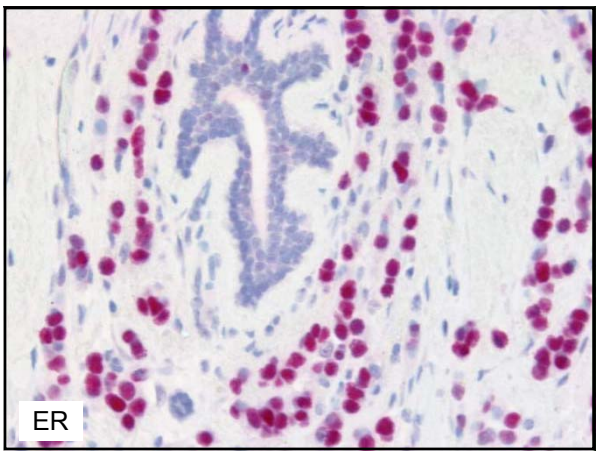
Fig 2. Univariate DFS curves for all possible total IHC scores in patients receiving any adjuvant endocrine therapy (almost always tamoxifen)

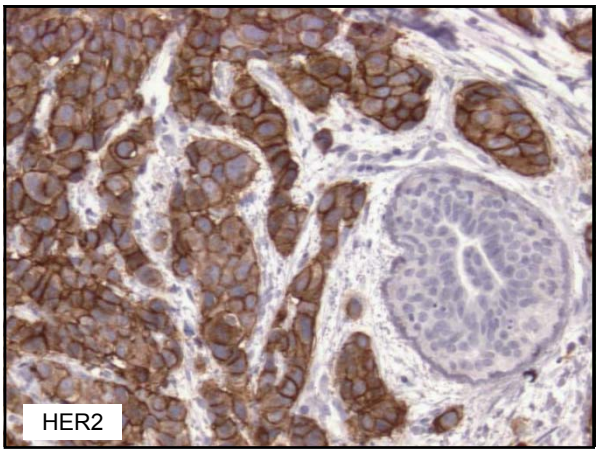


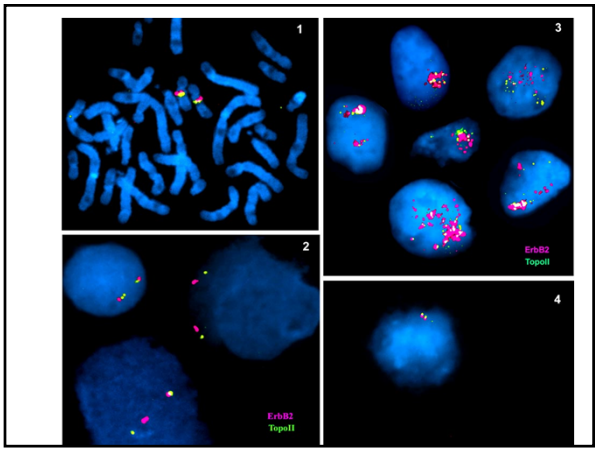
Harvey, J. M. et al. J Clin Oncol; 17:1474 1999

JOURNAL OF CLINICAL ONCOLOGY









HER2 In Situ Hybridization

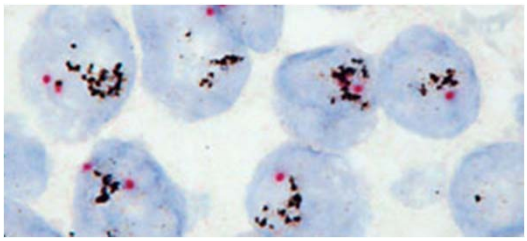
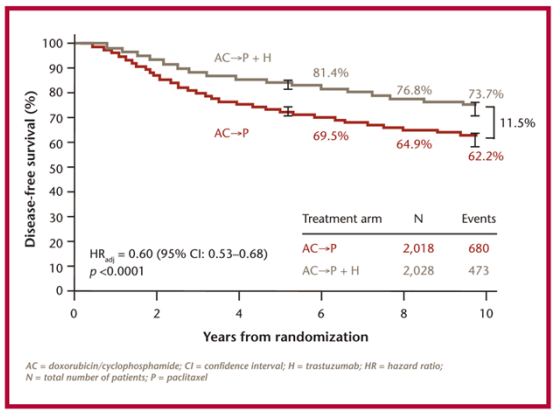
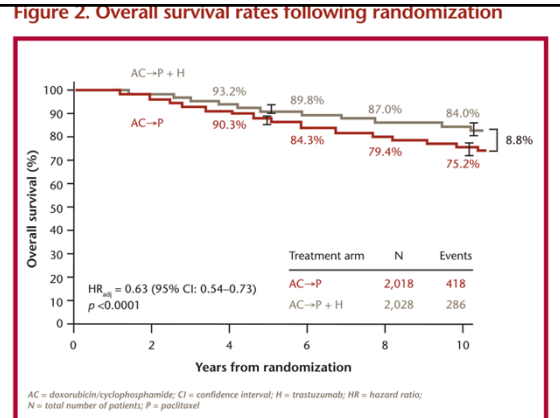


Figure 1. Disease-free survival rates following randomization



AC = doxorubicin/cyclophosphamide; CI = confidence interval; H = trastuzumab; HR = hazard ratio; N = total number of patients; P = paclitaxel

Figure 2. Overall survival rates following randomization



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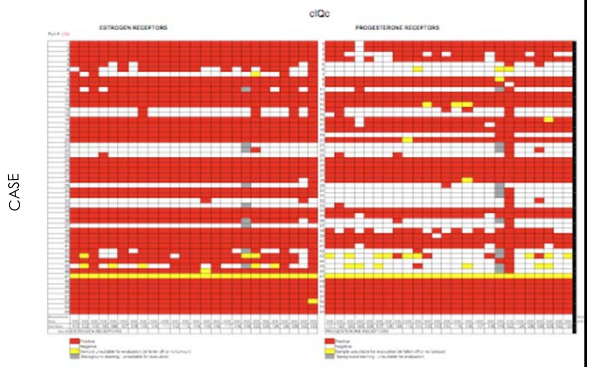
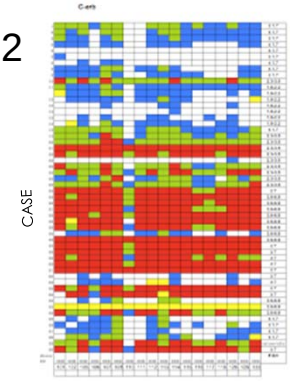
THE GLOBE AND MAIL
CANADA'S NATIONAL NEWSPAPER
WEEKEND SPECIAL | PAINFUL MISTAKES
Botched tests cast doubts on cancer screening
Thousands at risk across Canada as Newfoundland errors highlight deficiencies, pathologists caution

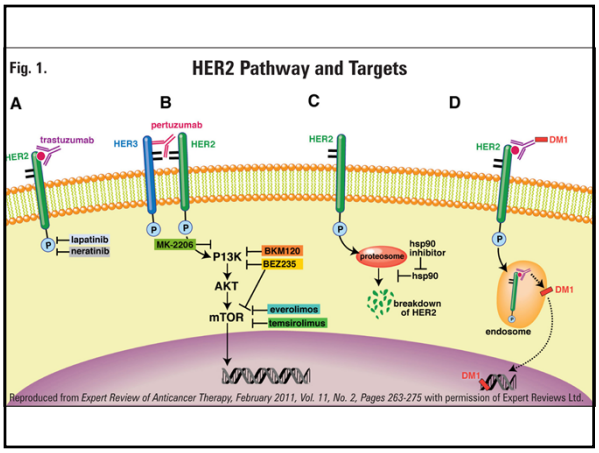
Quebec probes flawed cancer tests
Health officials compare faulty breast exam results to problems in Newfoundland, promise fast action

National Standards Immuno



HER-2





Need for HER2 testing in Gastric Cancer

- HER2 gene amplification and HER2 protein overexpression have been observed in various solid tumors other than breast, including gastric carcinomas
- The rate of HER2 positivity in advanced gastric cancer is comparable to that seen in locally advanced and metastatic breast cancer
  - using validated methodology, the large sample set from the ToGA trial revealed a HER2-positivity rate of 22% in advanced GC [1]
- HER2 has predictive value in gastric cancer [2]

As in breast cancer, accurate HER2 testing is essential to identify patients who may benefit from treatment with HER2 targeted therapy

1. Bang YJ, *et al. J Clin Oncol* 2009; 27:Abstract 4556.
2. van Cutsem E, *et al. J Clin Oncol* 2009; 27:Abstract LBA4509.

ToGA, Design
the first randomized trial investigating a targeted treatment for gastric cancer (anti-HER2)

- Design: Phase III, randomized, open-label, international, multicentre study
- Population: patient with HER2-positive locally advanced or metastatic gastric cancer (stomach or GEJ)

3807 patients screened
810 HER2-positive* → HER2-positive advanced GC (n=584)

*22.1% HER2 positivity

- Treatment:

5-FU / capecitabine† + cisplatin + trastuzumab (n=294) vs 5-FU / capecitabine† + cisplatin (n=290)

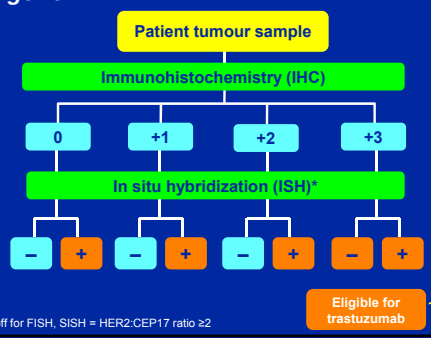
†89% of patients received capecitabine

- Primary endpoint: overall survival (OS)

GEJ: gastroesophageal junction

[1]Van Cutsem et al 2009; [2]Bang et al 2009

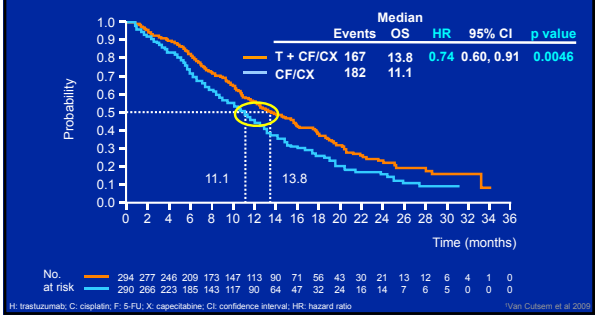
ToGA, Design
identification of HER2 positivity, algorithm



*cut off for FISH, SISH = HER2:CEP17 ratio ≥2

[1]Van Cutsem et al 2009

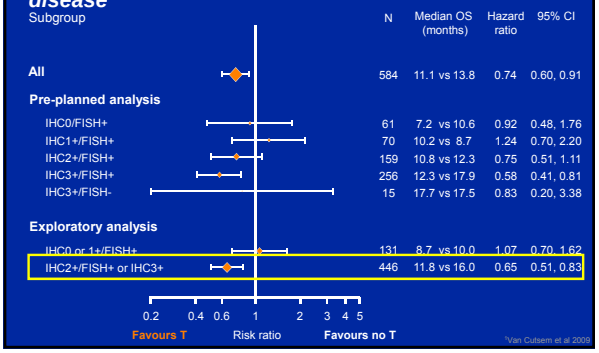
ToGA, Efficacy
Trastuzumab provides unprecedented survival benefit



H: trastuzumab; C: cisplatin; F: 5-FU; X: capecitabine; CI: confidence interval; HR: hazard ratio

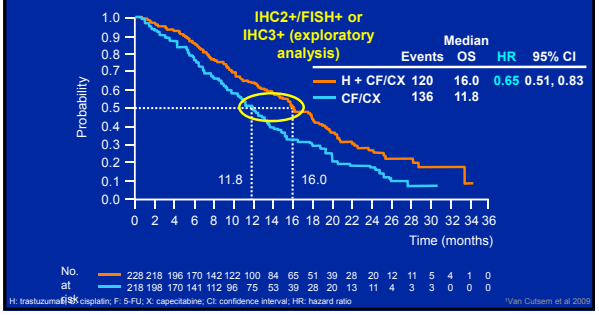
[1]Van Cutsem et al 2009

ToGA, Efficacy
Greater OS benefit in IHC 2+/FISH+ or IHC 3+ disease



[1]Van Cutsem et al 2009

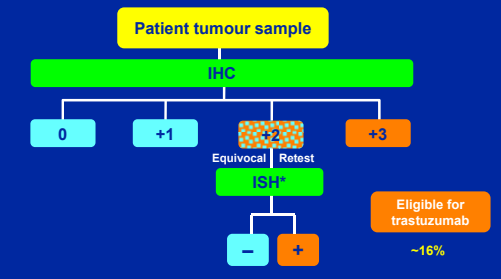
Patients with IHC 2+/FISH+ or IHC 3+ disease derive greater benefit



H: trastuzumab; C: cisplatin; F: 5-FU; X: capecitabine; CI: confidence interval; HR: hazard ratio

[1]Van Cutsem et al 2009

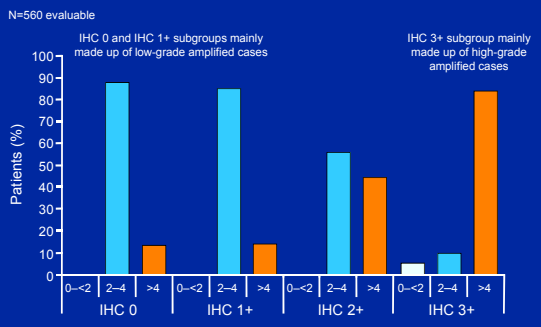
ToGA, Outcomes
suggested testing algorithm in clinical practice



*cut off for FISH, SISH = HER2:CEP17 ratio ≥2

[1]Van Cutsem et al 2009

FISH ratios within IHC subgroups

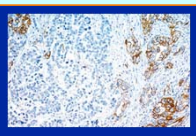


FISH Ratio: 0–<2 = no amplification; 2–4 = low-grade amplified; >4 = high-grade amplified

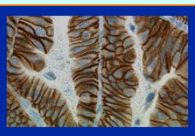
Differences in HER2 testing in breast and gastric cancers

- Pre-ToGA international validation study investigated HER2 testing of 168 gastric cancer samples
- Histological differences between gastric and breast cancers necessitate modifications to the HER2 scoring criteria for gastric cancer

Tumour heterogeneity is more common in gastric cancer

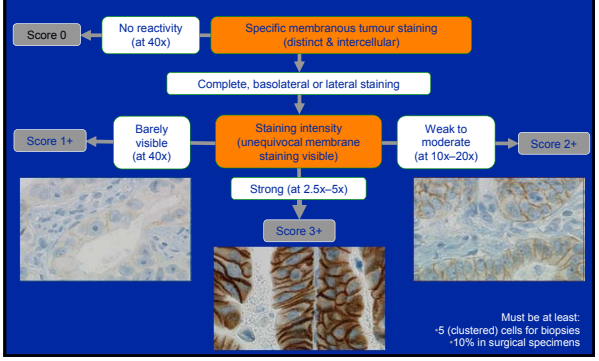


Incomplete membrane staining with IHC is more common in gastric cancer



Hofmann M, *et al. Histopathology* 2008; **52**:797–805.

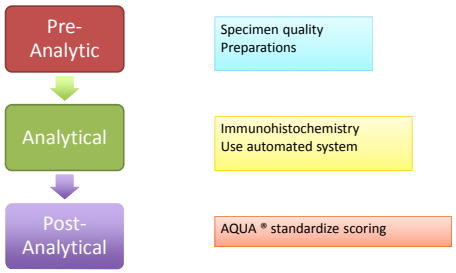
IHC: scoring criteria



Interpretation of IHC scoring in gastric cancer

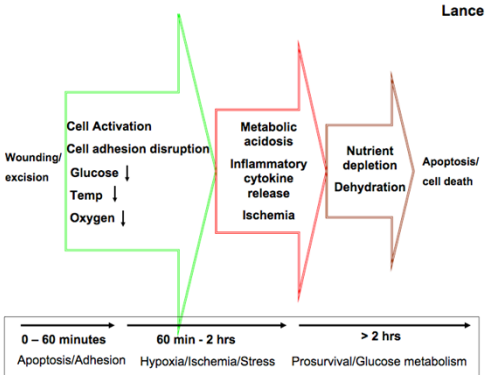
- Tumour cells showing complete, basolateral or lateral membrane staining should be scored
- Cytoplasmic staining should not be included when interpreting results
- Normal epithelial cells should not be scored
- Artefacts may lead to false positive interpretation

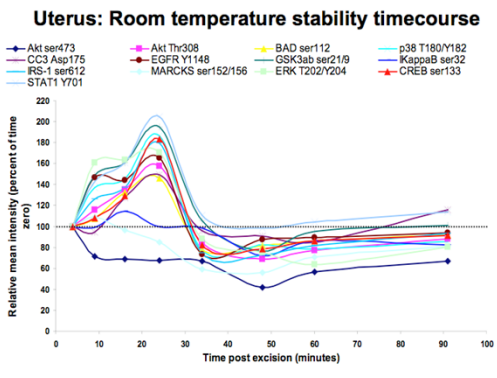
Understanding Variation in Biomarker Analysis



http://news.nationalgeographic.co.uk/news/2009/06/photogalleries/fathers-day-2009-animal-dads-pictures/images/primary/090618-07-greatest-animal-dads-emperor-penguin_big.jpg

http://www.nasa.gov/images/content/235791main_image_1098_946-710.jpg

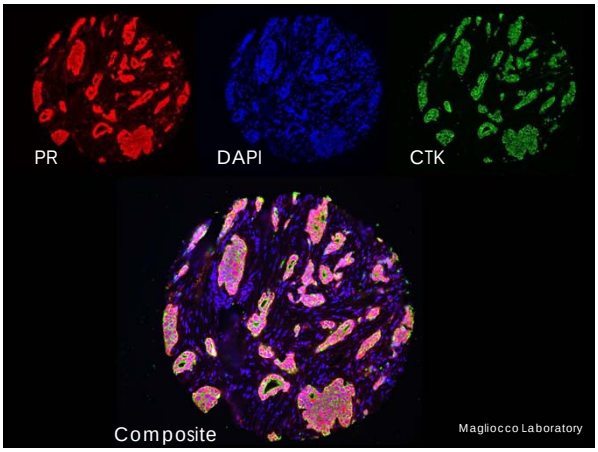




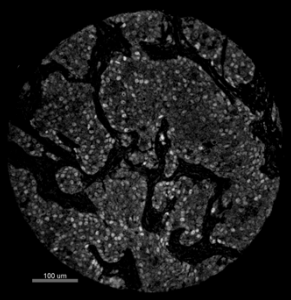
HistoRx™ AQUA System

Quantify protein expression

- Immunohistochemistry
- Automated image analysis (AQUA)

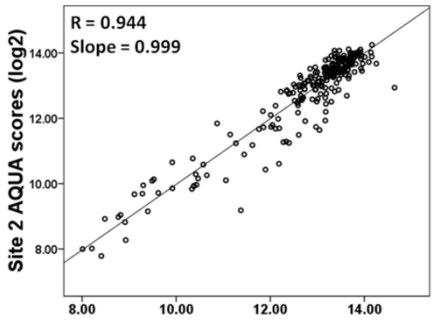


DAB-IHC vs IF-IHC



AQUA = Average target pixel intensity/Area of the defined compartment

ER AQUA interlab



ER: Pathologist vs AQUA (All Patients)

DAB-IHC **vs** **AQUA-IHC**

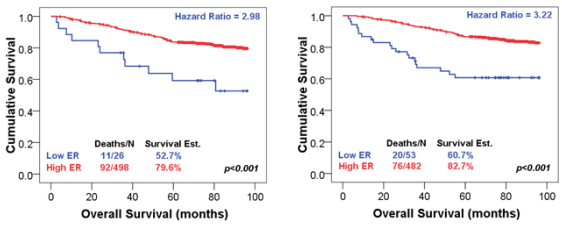
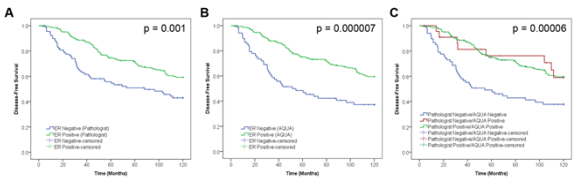
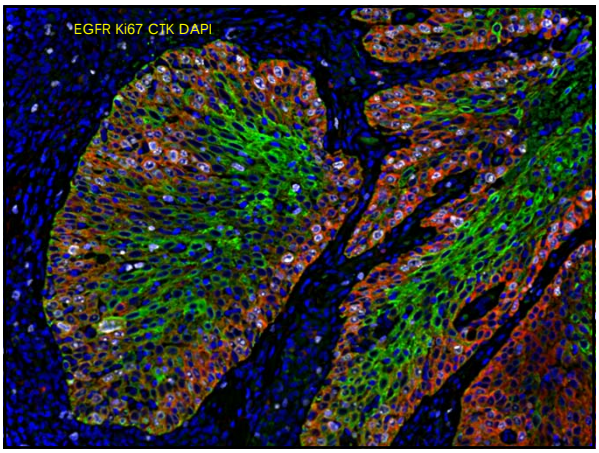
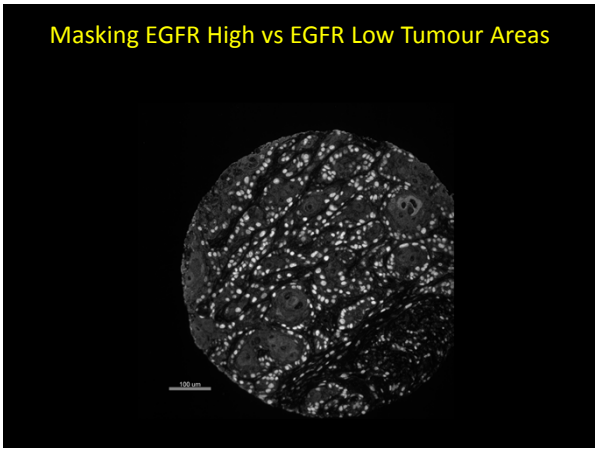


Figure 4

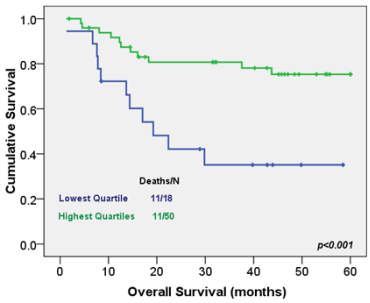


Magliocco Laboratories

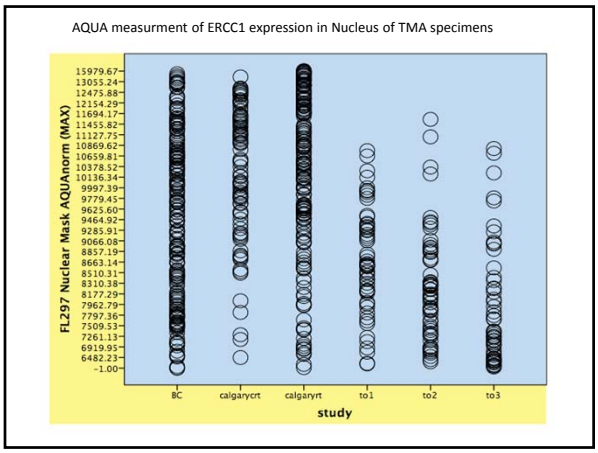




Low EGFR/Ki67 Ratio Predicts Poor Survival



Kaplan-Meier survival curves measuring the overall disease specific survival based on stratification by EGFR/Ki67 Ratio. The average ratio for each patient (from replicate histospots) was used and patients were categorized as having a high ratio if they fell within the top 3 quartiles of expressers (n=68). Five year estimates for overall survival are 78% for high Ratio patients and 39% for low Ratio patients.


AQUA measurment of ERCC1 expression in Nucleus of TMA specimens
FL297 Nuclear Mask AQUAnorm (MAX)
15979.67
13055.24
12475.88
12154.29
11694.17
11455.82
11127.75
10869.62
10659.81
10378.52
10136.34
9997.39
9779.45
9625.60
9464.92
9285.91
9066.08
8857.19
8663.14
8510.31
8310.38
8177.29
7962.79
7797.36
7509.53
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6919.95
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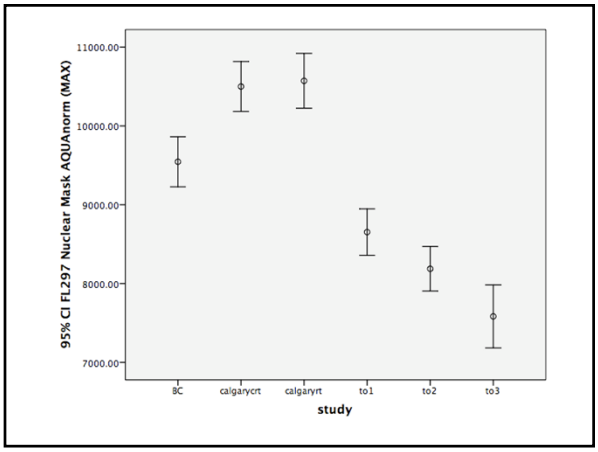
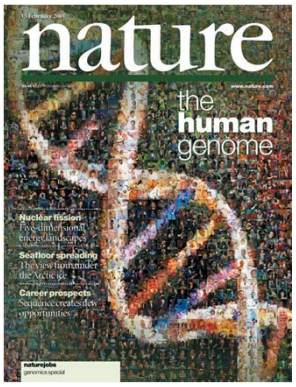
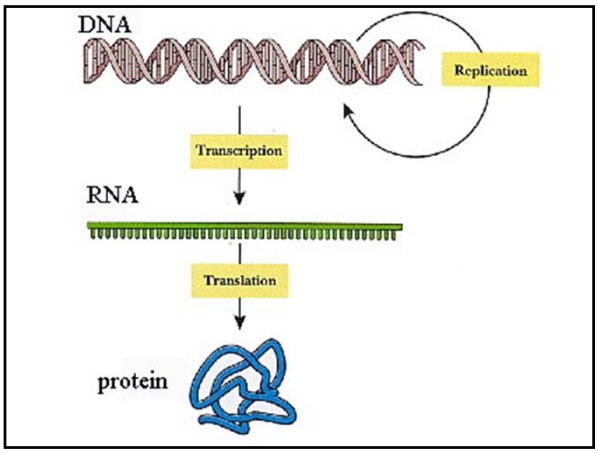
-1.00
BC
calgarycrt
calgaryrt
to1
to2
to3
study

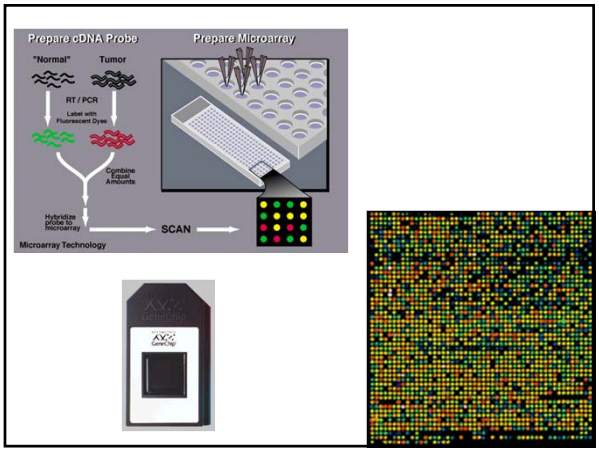

95% CI FL297 Nuclear Mask AQUAnorm (MAX)
11000.00
10000.00
9000.00
8000.00
7000.00
BC
calgarycrt
calgaryrt
to1
to2
to3
study

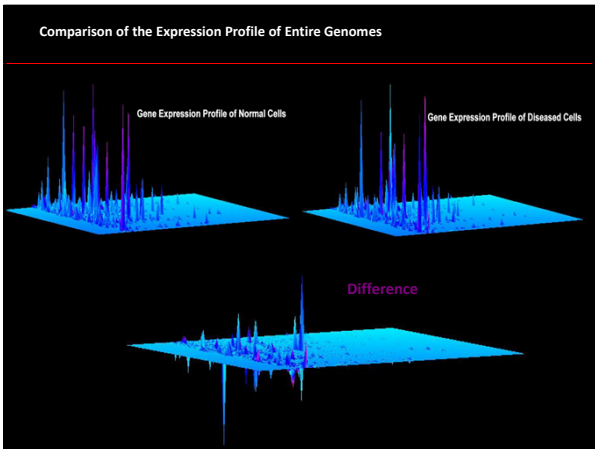
Molecular Classification
and
Personalized Medicine

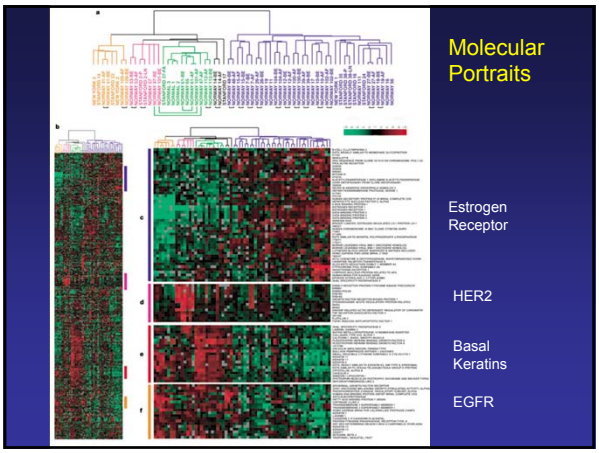
'OMIC Revolution




DNA
Replication
Transcription
RNA
Translation
protein


Prepare cDNA Probe
Prepare Microarray
"Normal"
Tumor
RT / PCR
Label with Fluorescent Dyes
Combine Equal Amounts
Hybridize probe to microarray
SCAN
Microarray Technology


Comparison of the Expression Profile of Entire Genomes
Gene Expression Profile of Normal Cells
Gene Expression Profile of Diseased Cells
Difference

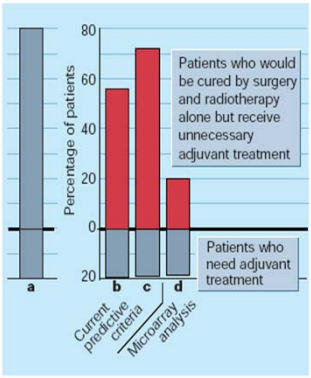


Gene expression profiling predicts clinical outcome of breast cancer

Laura J. van 't Veer*†, Hongyue Dai†‡, Marc J. van de Vijver*†, Yudong D. He‡, Augustinus A. M. Hart*, Mao Mao‡, Hans L. Peterse*, Karin van der Kooy*, Matthew J. Marton‡, Anke T. Witteveen*, George J. Schreiber‡, Ron M. Kerkhoven*, Chris Roberts‡, Peter S. Linsley‡, René Bernards* & Stephen H. Friend‡

** Divisions of Diagnostic Oncology, Radiotherapy and Molecular Carcinogenesis and Center for Biomedical Genetics, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands*
‡ Rosetta Inpharmatics, 12040 115th Avenue NE, Kirkland, Washington 98034, USA
† These authors contributed equally to this work

Who does not need treatment?



Oncotype DX 21 Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

PROLIFERATION
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN
ER
PR
Bcl2
SCUBE2

GSTM1 **BAG1**

INVASION
Stromelysin 3
Cathepsin L2

CD68

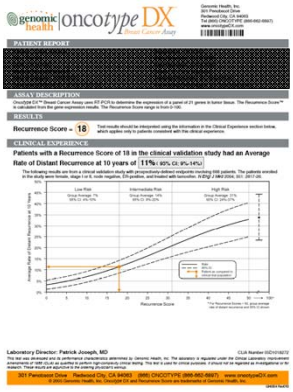
HER2
GRB7
HER2

REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

RS = + 0.47 x HER2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

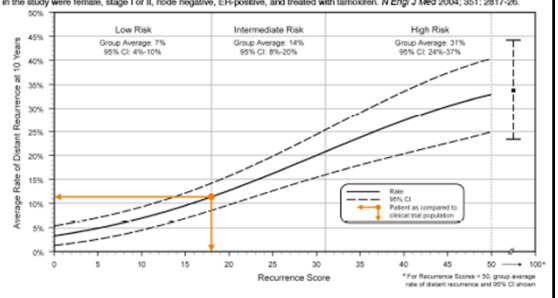
Category	RS (0 – 100)
Low risk	RS < 18
Int risk	RS ≥ 18 and < 31
High risk	RS ≥ 31

5



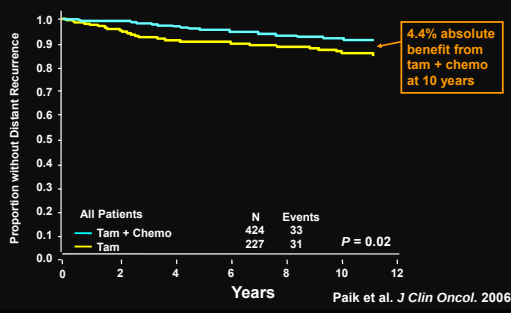
Patients with a Recurrence Score of 18 in the clinical validation study had an Average Rate of Distant Recurrence at 10 years of 11% (95% CI: 9%-14%)

The following results are from a clinical validation study with prospectively-defined endpoints involving 668 patients. The patients enrolled in the study were female, stage I or II, node negative, ER-positive, and treated with tamoxifen. *N Engl J Med* 2004; 351: 2817-26.



B-20 Results: 4% benefit from addition of chemotherapy

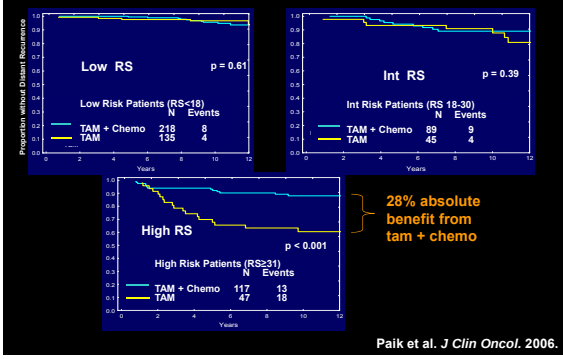
Tam vs Tam + Chemo – All 651 Patients



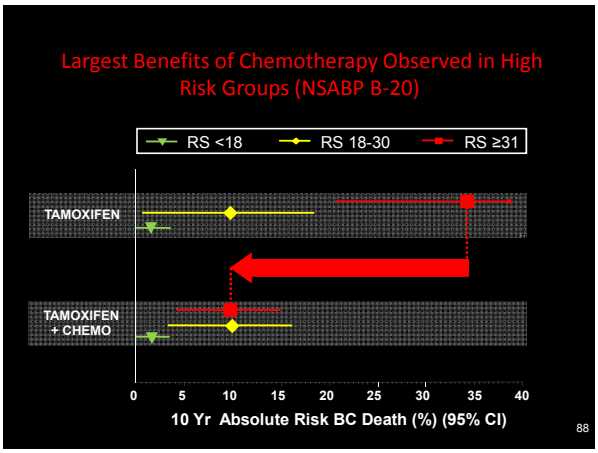
Paik et al. *J Clin Oncol.* 2006.

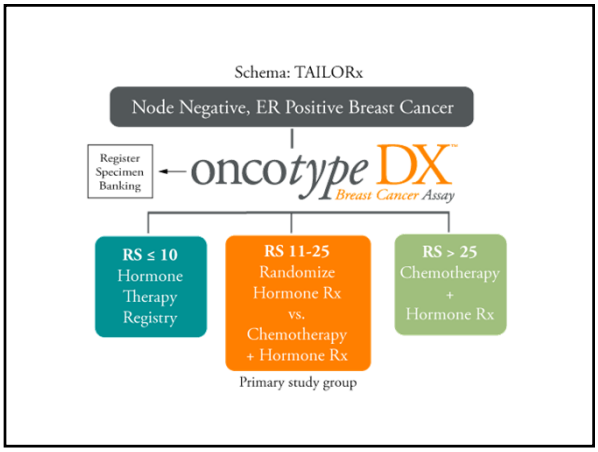
86

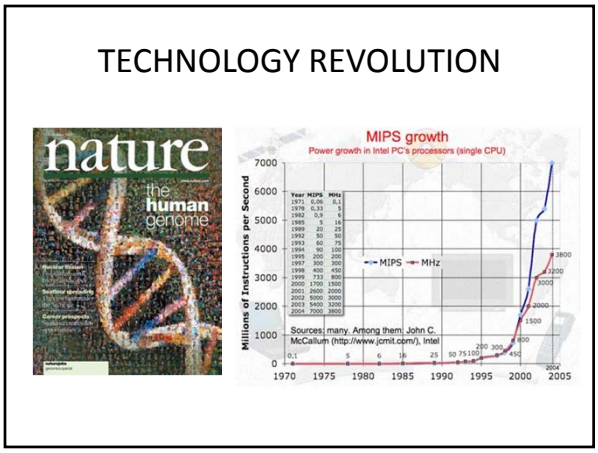
Oncotype DX B-20 Results: Only high RS patients show large benefit from chemotherapy



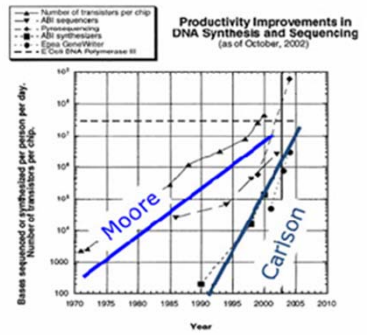
Paik et al. *J Clin Oncol.* 2006.


Largest Benefits of Chemotherapy Observed in High Risk Groups (NSABP B-20)
RS <18
RS 18-30
RS ≥31
TAMOXIFEN
TAMOXIFEN + CHEMO
0 5 10 15 20 25 30 35 40
10 Yr Absolute Risk BC Death (%) (95% CI)
88


Schema: TAILORx
Node Negative, ER Positive Breast Cancer
Register Specimen Banking
oncotype DX Breast Cancer Assay
RS ≤ 10 Hormone Therapy Registry
RS 11-25 Randomize Hormone Rx vs. Chemotherapy + Hormone Rx
RS > 25 Chemotherapy + Hormone Rx
Primary study group


TECHNOLOGY REVOLUTION
nature
the human genome
MIPS growth
Power growth in Intel PC's processors (single CPU)
Millions of Instructions per Second
MIPS
MHz
Sources: many. Among them: John C. McCallum (http://www.jcmit.com/), Intel
1970 1975 1980 1985 1990 1995 2000 2005

CARLSON CURVE



Technology Platforms

- Sequenom
- NextGen Seq (massive parallel, NGS)
- CTC
- NanoString
- Array systems
- Digital Image Analysis (AQUA)

FDA, CLIA, and Assays

- FDA Approved
- FDA Cleared

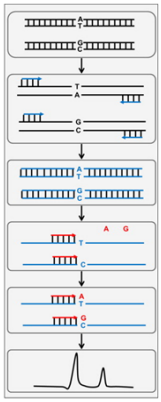
- LDTs (Laboratory Developed Assays)

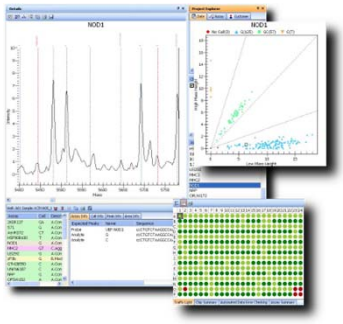
Clinical Technologies

SEQUENOM

SEQUENOM – MASS SPEC for DNA

SEQUENOM





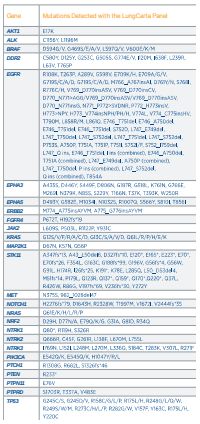
LungCarta™

26 Oncogenes
214 Selected Mutations

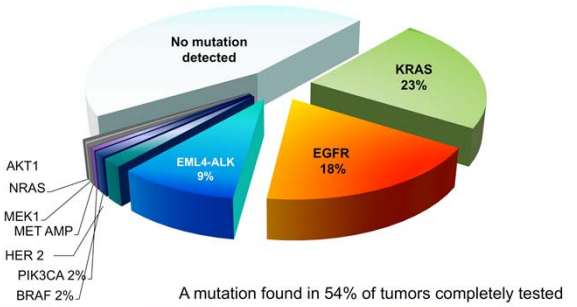
Genes Included in the LungCarta Panel:

AKT1	*JAK2*	*NTRK3*
ALK	*KRAS*	*PIK3CA*
BRAF	*MAP2K1*	*PTCH1*
DDR2	*MET*	*PTEN*
EGFR	*NOTCH1*	*PTPN11*
EPHA3	*NRAS*	*PTPRD*
EPHA5	*NRF2*	*STK11*
ERBB2	*NTRK1*	*TP53*
FGFR4	*NTRK2*	

SEQUENOM



LCMC: incidence of mutations detected

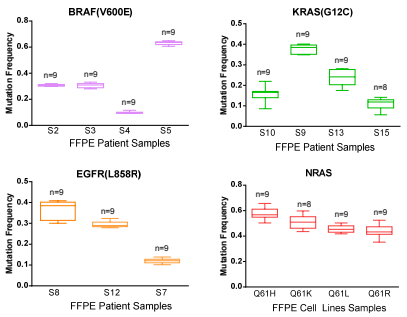


A mutation found in 54% of tumors completely tested

CLIA REQUIREMENTS FOR LDT

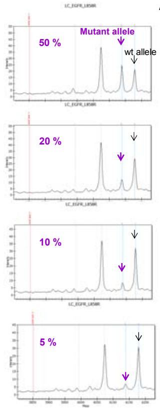
- ACCURACY
- PRECISION
- Reportable Range
- Reference Range
- Repeatability
- Analytical Sensitivity
- Analytical Specificity

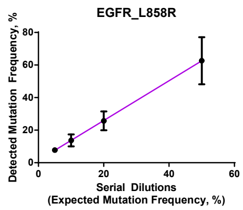
Precision/Reproducibility Validation Summary



Each sample has been run in triplicates in three independent tests performed on different days. The descriptive statistics for combined data (n=9) is shown.

Analytical Sensitivity/ Lower Limit of Detection: EGFR





FFPE Reference Standards containing defined ratio of mutant alleles (Horizon Diagnostics) were diluted with WT FFPE Reference Standards. Samples with mutation frequencies of 50%, 20%, 10%, and 5 % were run on LungCarta panel in 4 independent tests.

(Spectra from a representative run + chart with average+-STD on 4 runs)

LungCarta™

26 Oncogenes
214 Selected Mutations

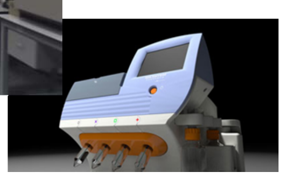
Genes Included in the LungCarta Panel:

AKT1	*JAK2*	*NTRK3*
ALK	*KRAS*	*PIK3CA*
BRAF	*MAP2K1*	*PTCH1*
DDR2	*MET*	*PTEN*
EGFR	*NOTCH1*	*PTPN11*
EPHA3	*NRAS*	*PTPRD*
EPHA5	*NRF2*	*STK11*
ERBB2	*NTRK1*	*TP53*
FGFR4	*NTRK2*	

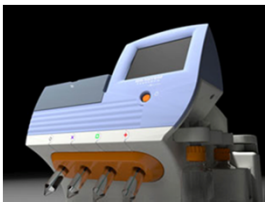
SEQUENOM

Gene	Mutations Detected with the LungCarta Panel
AKT1	E17K
ALK	C1156Y, L1196M
BRAF	D594G/V, G469S/E/A/V, L597Q/V, V600E/K/M
DDR2	C580Y, D125Y, G253C, G505S, G774E/V, I120M, I638F, L239R, L63V, T765P
EGFR	R108K, T263P, A289V, G598V, E709K/H, E709A/G/V, G719S/C/A/D, G719S/C/A/D, M766_A767insAI, D761Y/N, S768I, R776C/H, V769_D770insASV, V769_D770insCV, D770_N771>AGG/V769_D770insASV/V769_D770insASV, D770_N771insG, N771_P772>SVDNR, P772_H773insV, H773>NPY, H773_V774insNPH/PH/H, V774L, V774_C775insHV, T790M, L858R/M, L861Q, E746_T751del, E746_A750del, E746_T751del, E746_T751del, S752D, L747_E749del, L747_T750del, L747_S752del, L747_T751del, L747_S752del, P753S, A750P, T751A, T751P, T751I, S752I/F, S752_I759del, L747_Q ins, E746_T751del, I ins (combined), E746_A750del, T751A (combined), L747_E749del, A750P (combined), L747_T750del, P ins (combined), L747_S752del, Q ins (combined), T854A
EPHA3	A435S, D446Y, S449F, D806N, G187R, G518L, K761N, G766E, M269I, N379K, N85S, S229Y, T166N, T37K, T393K, W250R
EPHA5	D493Y, G582E, M1034I, N1032S, R1007Q, S566Y, S810I, T856I
ERBB2	M774_A775insAYVM, A775_G776insAYVM
FGFR4	P672T, H192fs*19
JAK2	L609S, P503L, R1122P, Y931C
KRAS	G12S/V/F/R/A/C/D, G13C/S/A/V/D, Q61L/R/P/H/E/K
MAP2K1	D67N, K57N, Q56P
STK11	A347fs*13, A43_L50del6, D327fs*10, E120*, E165*, E223*, E70*, E70fs*26, F354L, G163C, G188fs*99, G196V, G56fs*4, G56W, G91L, H174R, I26fs*25, K191*, K78E, L285Q, L50_D53del4, M51fs*14, P179L, Q123R, Q137*, Q159*, Q170*,Q220*, Q37L, R426W, R86G, V197fs*69, V236fs*30, Y272Y
MET	N375S, 982_1028del47
NOTCH1	H2276fs*79, D1643H, R2328W, T1997M, V1672I, V2444fs*35
NRAS	Q61E/K/H/L/R/P
NRF2	D29H, D77N/A, E79Q/K/G, G31A, G81D, R34Q
NTRK1	Q80*, R119H, S326R
NTRK2	Q666R, C45F, G261R, L138F, L670M, L755L
NTRK3	I769N, L152I, L248M, L270M, L336Q, S184C, T283K, V307L, R271F
PIK3CA	E542Q/K, E545Q/K, H1047Y/R/L
PTCH1	R1308G, R682L, S1326fs*46
PTEN	R233*
PTPN11	E76V
PTPRD	S1703R, T337A, V483E
TP53	G245C/S, G245D/V, R158C/G/L/P, R175L/H, R248G/L/Q/W, R249S/W/M, R273C/H/L/P, R282G/W, V157F, Y163C, R175L/H, Y220C

Next Generation Sequencing



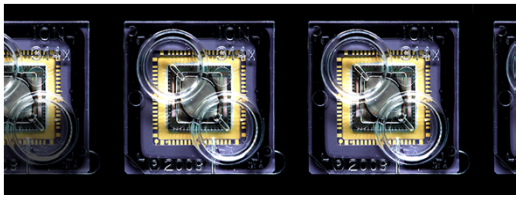
Next Generation Sequencing



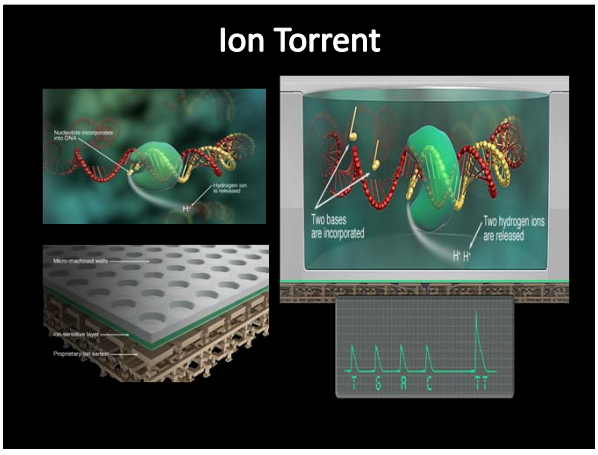
Ion Torrent

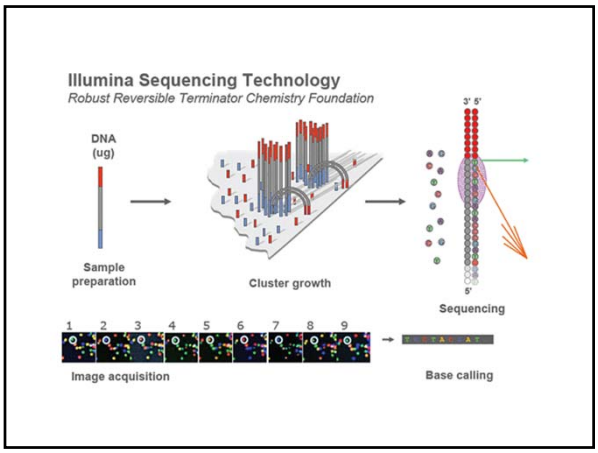
MiSEQ

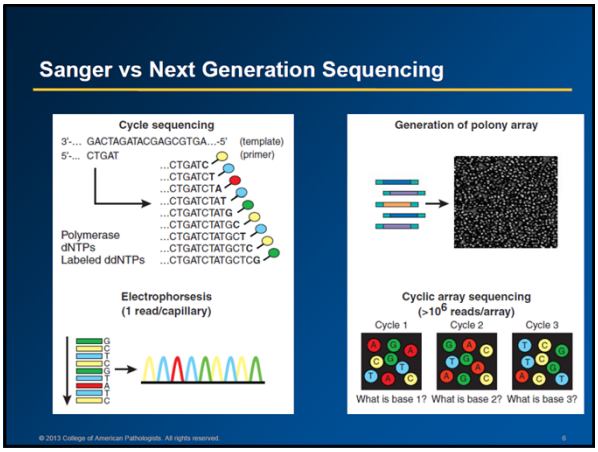
Ion Torrent



Ion Torrent




Illumina Sequencing Technology
Robust Reversible Terminator Chemistry Foundation
DNA (ug)
Sample preparation
Cluster growth
3' 5'
5'
Sequencing
1 2 3 4 5 6 7 8 9
Image acquisition
Base calling


Sanger vs Next Generation Sequencing
Cycle sequencing
3'-... GACTAGATACGAGCGTGA...-5' (template)
5'-... CTGAT (primer)
...CTGATC
...CTGATCT
...CTGATCTA
...CTGATCTAT
...CTGATCTATG
...CTGATCTATGC
...CTGATCTATGCT
...CTGATCTATGCTC
...CTGATCTATGCTCG
Polymerase
dNTPs
Labeled ddNTPs
Electrophorsesis
(1 read/capillary)
Generation of polony array
Cyclic array sequencing
(>10^6 reads/array)
Cycle 1
Cycle 2
Cycle 3
What is base 1? What is base 2? What is base 3?
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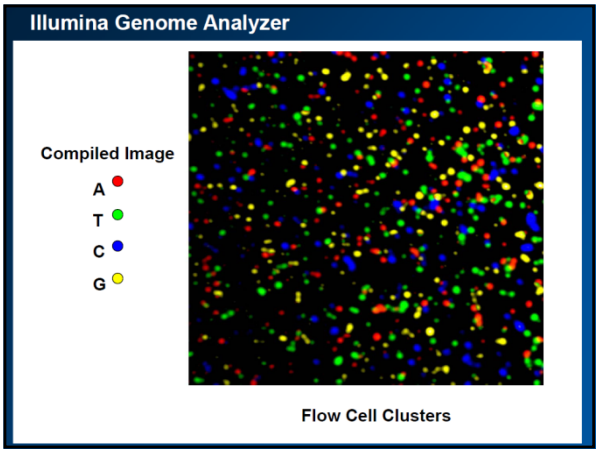
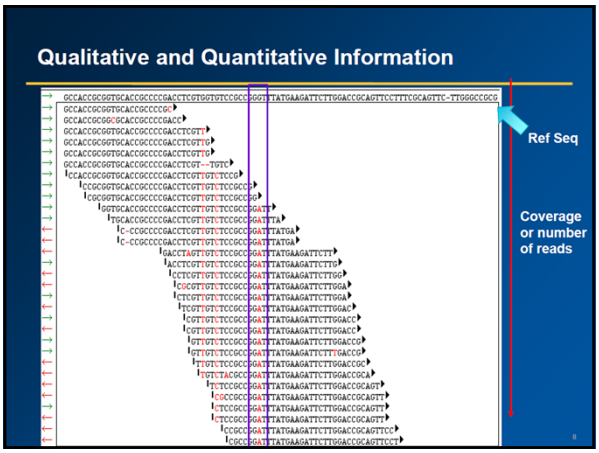
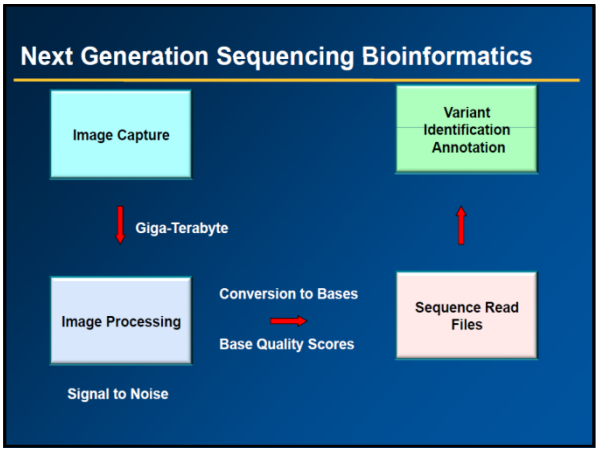
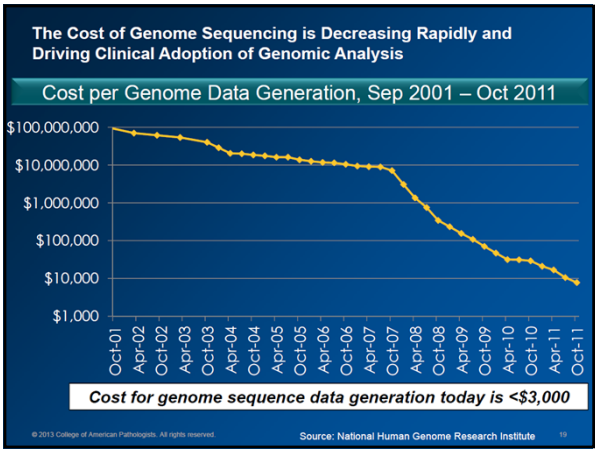
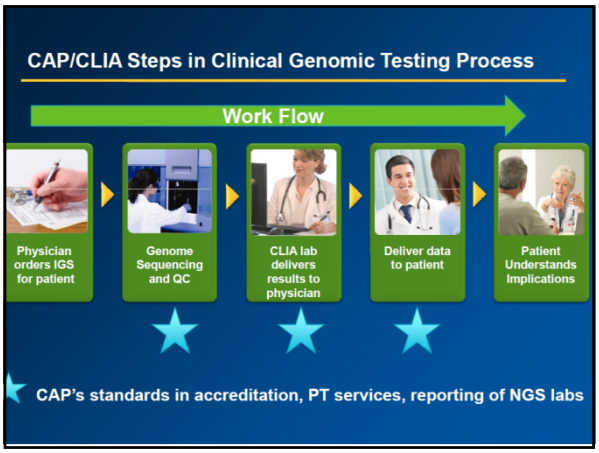
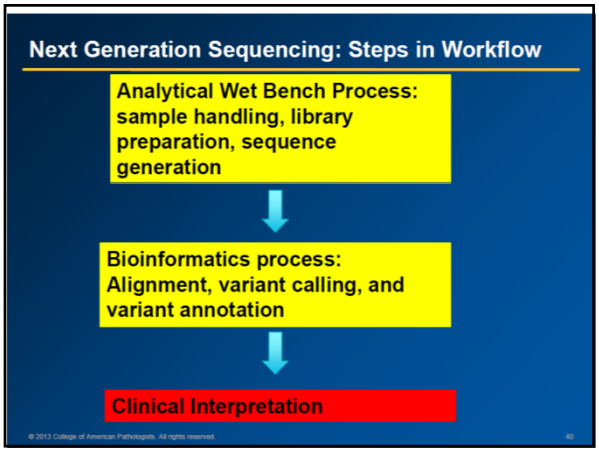

Illumina Genome Analyzer
Compiled Image
A
T
C
G
Flow Cell Clusters


Qualitative and Quantitative Information
Ref Seq
Coverage or number of reads


Next Generation Sequencing Bioinformatics
Image Capture
Variant Identification Annotation
Giga-Terabyte
Image Processing
Conversion to Bases
Base Quality Scores
Sequence Read Files
Signal to Noise


The Cost of Genome Sequencing is Decreasing Rapidly and Driving Clinical Adoption of Genomic Analysis
Cost per Genome Data Generation, Sep 2001 – Oct 2011
$100,000,000
$10,000,000
$1,000,000
$100,000
$10,000
$1,000
Oct-01
Apr-02
Oct-02
Apr-03
Oct-03
Apr-04
Oct-04
Apr-05
Oct-05
Apr-06
Oct-06
Apr-07
Oct-07
Apr-08
Oct-08
Apr-09
Oct-09
Apr-10
Oct-10
Apr-11
Oct-11
Cost for genome sequence data generation today is <$3,000
Source: National Human Genome Research Institute


CAP/CLIA Steps in Clinical Genomic Testing Process
Work Flow
Physician orders IGS for patient
Genome Sequencing and QC
CLIA lab delivers results to physician
Deliver data to patient
Patient Understands Implications
CAP's standards in accreditation, PT services, reporting of NGS labs


Next Generation Sequencing: Steps in Workflow
Analytical Wet Bench Process: sample handling, library preparation, sequence generation
Bioinformatics process: Alignment, variant calling, and variant annotation
Clinical Interpretation
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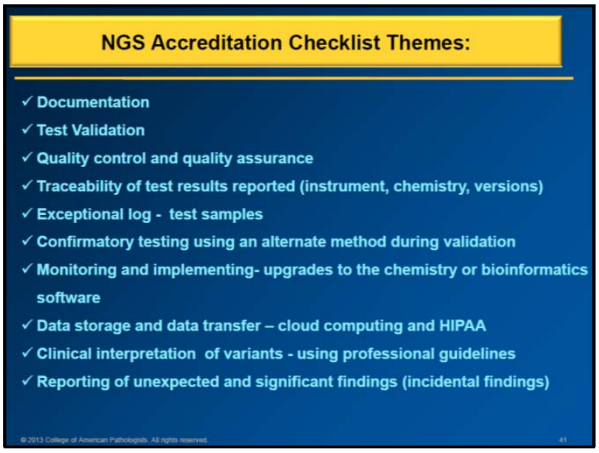
NGS Accreditation Checklist Themes:
✓ Documentation
✓ Test Validation
✓ Quality control and quality assurance
✓ Traceability of test results reported (instrument, chemistry, versions)
✓ Exceptional log - test samples
✓ Confirmatory testing using an alternate method during validation
✓ Monitoring and implementing- upgrades to the chemistry or bioinformatics software
✓ Data storage and data transfer – cloud computing and HIPAA
✓ Clinical interpretation of variants - using professional guidelines
✓ Reporting of unexpected and significant findings (incidental findings)
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MOL.34936: Validation - Wet Bench Analytical

The laboratory validates the analytical wet bench process and revalidates after changes or upgrades to any components used to generate next generation sequencing data

- *Validations must determine and document analytical sensitivity, specificity, reproducibility, repeatability and precision for the types of variants assayed (e.g. single nucleotide variants, insertions and deletions, homopolymer or repetitive sequences).*
- *Interference by clinically relevant pseudogenes and other sequences highly homologous to the target must be determined and documented.*
- *Sequencing error rates (i.e. false positives and false negatives) for variants assayed must be determined and documented using an alternative method which may include an alternate NGS chemistry.*
- *Indexing (barcoding) and sample pooling methods must be validated to ensure that individual sample identity is maintained throughout the analytical wet bench process.*

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MOL.34940: Confirmatory Testing

The laboratory has a policy for when confirmatory testing of identified or reported variants will be determined by an alternative method.

The laboratory maintains an ongoing record of the sensitivity, specificity, false positives, false negatives, reproducibility and repeatability of results and compares these with data obtained during the validation process.

Evidence of Compliance:
Policy or procedure that describes the indications for confirmatory testing.

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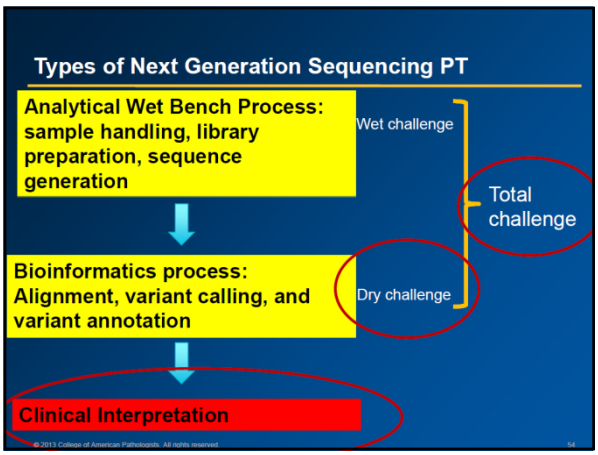
Types of Next Generation Sequencing PT



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Genes targeted in the TruSight Tumor Panel

AKT 1	**EGFR**	GNAS	**NRAS**	**STK 11**
ALK	ERBB2	KIT	PDGFRA	TP53
APC	FBXW7	**KRAS**	**PIK3CA**	
BRAF	FGFR2	MAP2K1	**PTEN**	
CDH1	FOXL2	MET	SMAD4	
CTNNB1	GNAQ	**MSH6**	SRC	

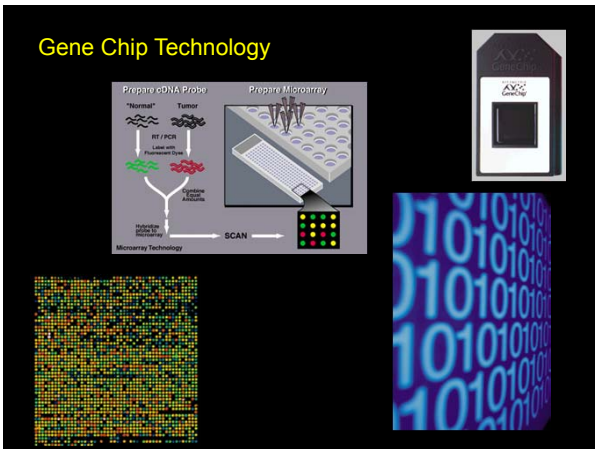
Genes selected from NCCN and CAP guidelines, late-stage clinical trails and relevant publications for lung, colon, melanoma, gastric and Ovarian

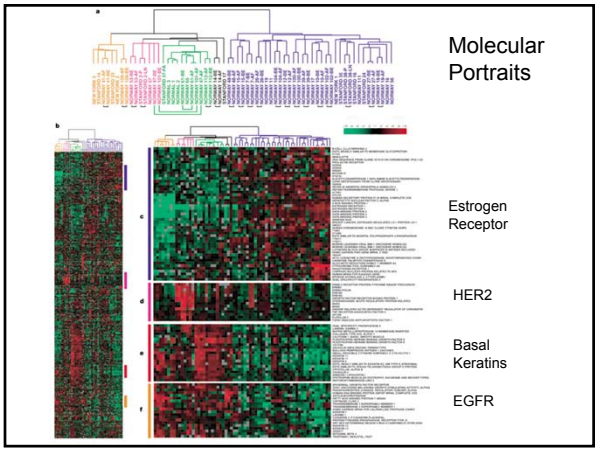
Mutations in genes sequenced (bold and Blue) by Sequenom in our lab.

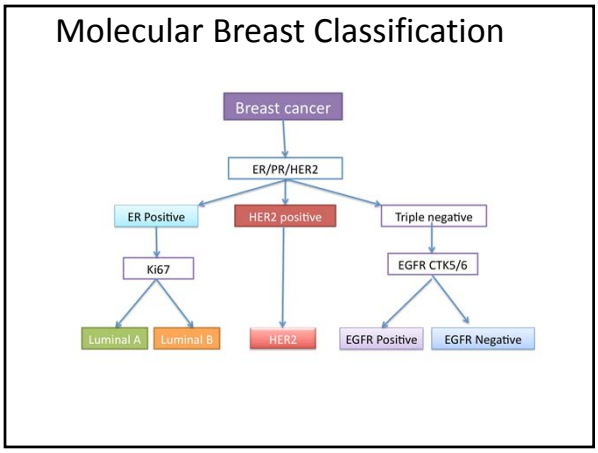
NGS

- Can sequence hundreds of targets to deep depths
- Can be applied to FFPE
- Can detect subclones
- Sensitivity based on depth of sequencing
- Can also be used to study expression, methylation, copy number, and translocations

Array Based Analysis


Gene Chip Technology
Prepare cDNA Probe
Prepare Microarray
"Normal"
Tumor
RT / PCR
Label with Fluorescent Dyes
Combine Cancer & Normal
Hybridize probe to microarray
SCAN
Microarray Technology


Molecular Portraits
Estrogen Receptor
HER2
Basal Keratins
EGFR


Molecular Breast Classification
Breast cancer
ER/PR/HER2
ER Positive
HER2 positive
Triple negative
Ki67
EGFR CTK5/6
Luminal A
Luminal B
HER2
EGFR Positive
EGFR Negative

Oncotype DX® 21-Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

PROLIFERATION
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN
ER
PR
Bcl2
SCUBE2

GSTM1 **BAG1**

INVASION
Stromelysin 3
Cathepsin L2

CD68

Category | RS (0 -100)

HER2
GRB7
HER2

REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

Whole Genome View

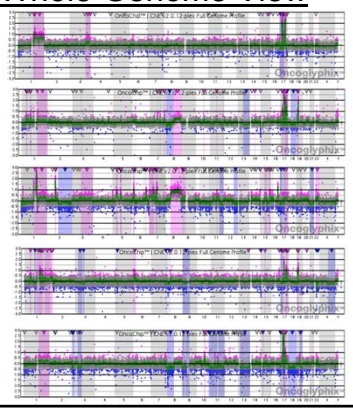
Moffitt 5770

Moffitt 2463

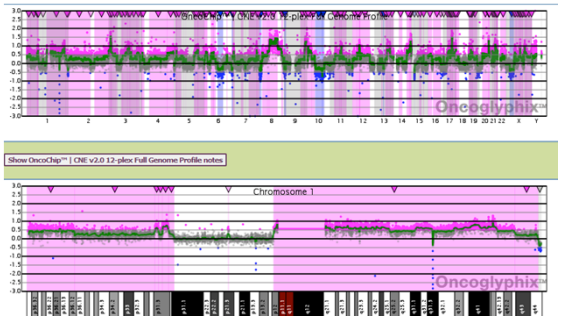
Moffitt 2464

Moffitt 2436

Moffitt 2437



Solid Tumor Microarray Analysis (FFPE: Breast Cancer #2448)

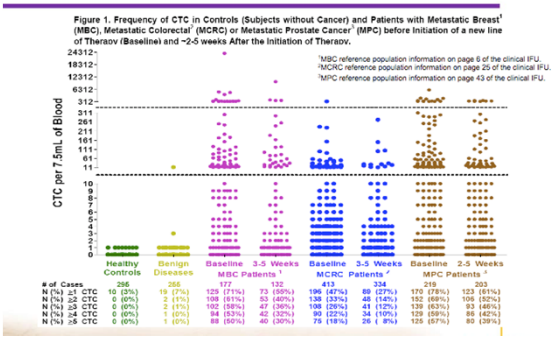


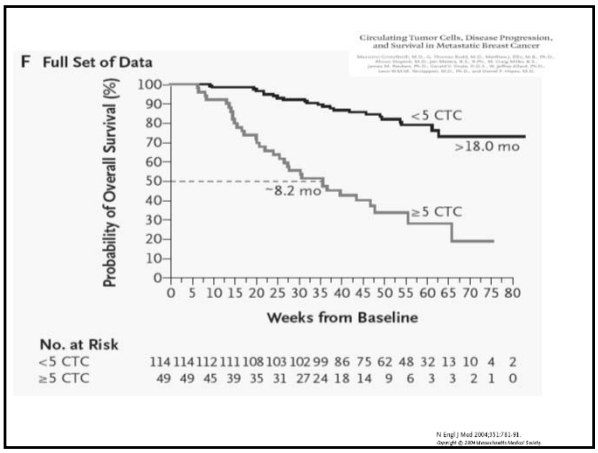
Array Based Method Applications

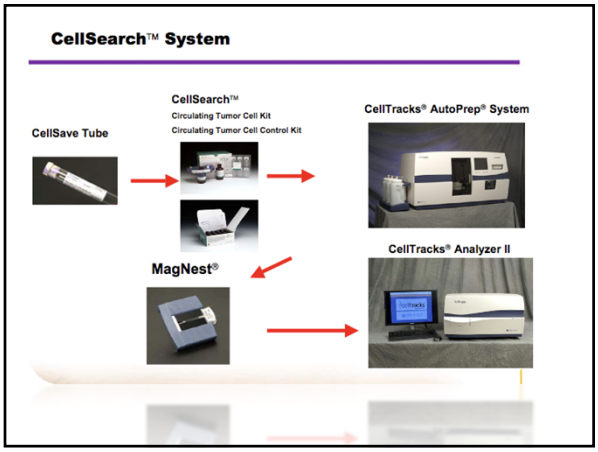
- Transcription analysis
  - Breast Cancer Classification
    - AGENDIA
    - NanoString PAM50
- Comaprative Genomic Hybridization
  - LOH
  - CNV
  - AMplifications

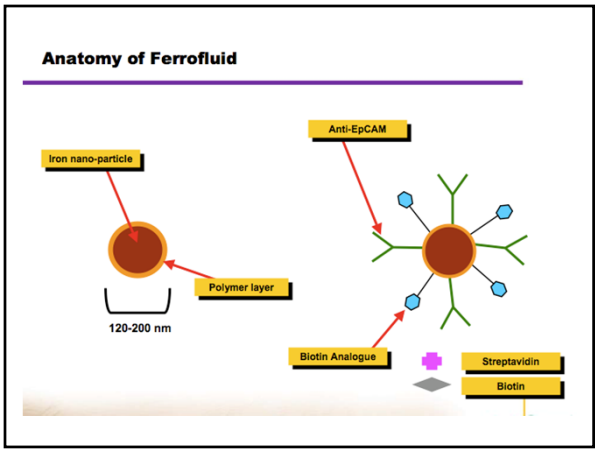
CIRCULATING TUMOR CELLS

Frequency of CTC




Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer
F Full Set of Data
Probability of Overall Survival (%)
100
90
80
70
60
50
40
30
20
10
0
<5 CTC
>18.0 mo
~8.2 mo
≥5 CTC
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Weeks from Baseline
No. at Risk
<5 CTC 114 114 112 111 108 103 102 99 86 75 62 48 32 13 10 4 2
≥5 CTC 49 49 45 39 35 31 27 24 18 14 9 6 3 3 2 1 0


CellSearch™ System
CellSave Tube
CellSearch™
Circulating Tumor Cell Kit
Circulating Tumor Cell Control Kit
CellTracks® AutoPrep® System
MagNest®
CellTracks® Analyzer II

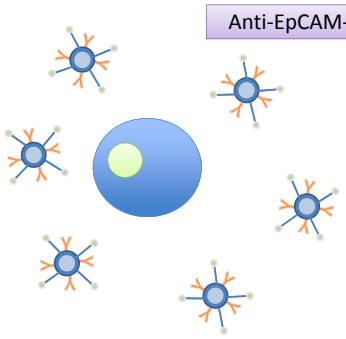

Anatomy of Ferrofluid
Iron nano-particle
Polymer layer
120-200 nm
Anti-EpCAM
Biotin Analogue
Streptavidin
Biotin

Automated Optimization of Ferrofluid Binding Activity

Circulating Tumor Cell

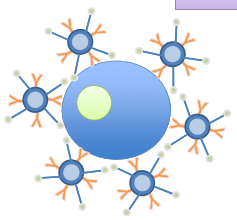
Automated Optimization of Ferrofluid Binding Activity

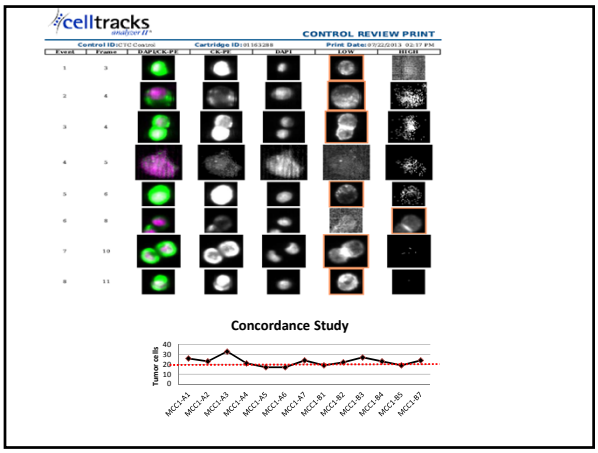
Anti-EpCAM-Ferrofluid

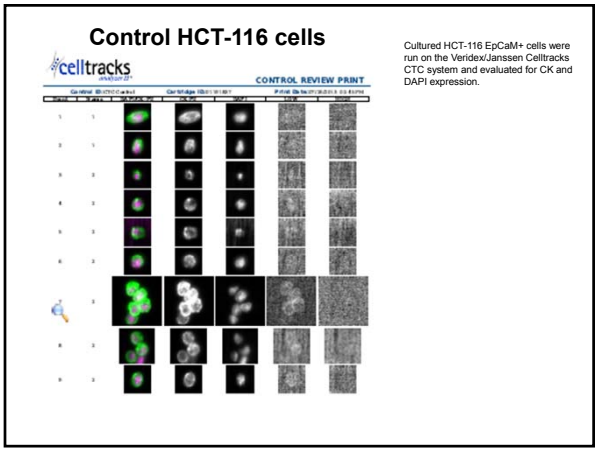


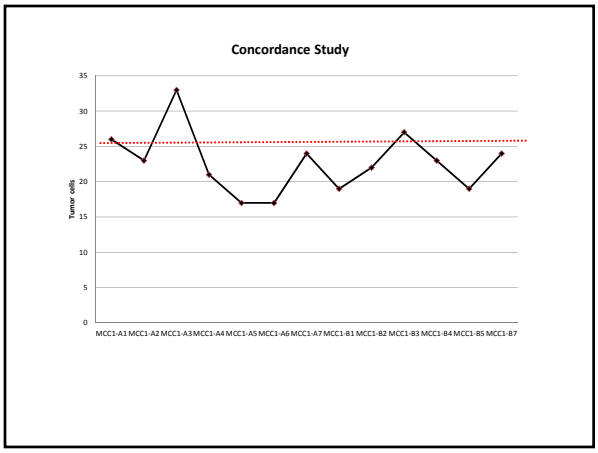
Automated Optimization of Ferrofluid Binding Activity

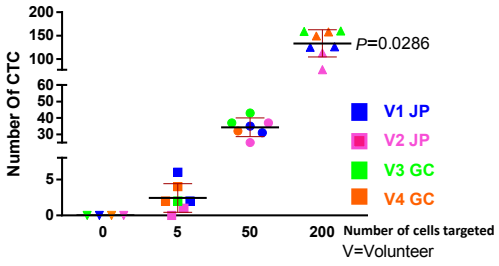
Anti-EpCAM-Ferrofluid binds to CTCs




celltracks
CONTROL REVIEW PRINT
Concordance Study
Tumor cells
40
30
20
10
0
MCC1-A1
MCC1-A2
MCC1-A3
MCC1-A4
MCC1-A5
MCC1-A6
MCC1-A7
MCC1-B1
MCC1-B2
MCC1-B3
MCC1-B4
MCC1-B5
MCC1-B7


Control HCT-116 cells
celltracks
CONTROL REVIEW PRINT
Cultured HCT-116 EpCaM+ cells were run on the Veridex/Janssen Celltracks CTC system and evaluated for CK and DAPI expression.


Concordance Study
Tumor cells
35
30
25
20
15
10
5
0
MCC1-A1 MCC1-A2 MCC1-A3 MCC1-A4 MCC1-A5 MCC1-A6 MCC1-A7 MCC1-B1 MCC1-B2 MCC1-B3 MCC1-B4 MCC1-B5 MCC1-B7



Variability between technologists is minimal and the CellTracker® system can detect CTCs in human blood as low as 5 cells. Blood was drawn from 4 volunteers and 200, 50, 5, and zero cultured and fixed SK-BR-3 breast cancer cells were placed in 6.5mls of dilution buffer. Then 7.5ml of blood from each donor was added and run as per the normal protocol. SK-BR-3 cells were placed in a CellSearch Preservative Tube to more closely mimic how patient samples will be treated and then counted. Extra cells in the CellSearch Preservative Tube were placed at RT and used for the second day. The cells were diluted to a concentration of 200 cells/6.5mls of dilution buffer (approximately 30mls). This stock was serially diluted in dilution buffer to evaluate 50 cells/6.5mls buffer, and 5 cells/6.5mls buffer. The dilution buffers containing CTCs were then added to 7.5mls of human blood in duplicate and processed. The experiment was repeated by both John Puskas Ph.D. and Gisela Caceres Ph.D. on two consecutive days using 1 CTC kit.

Frequency of CTC

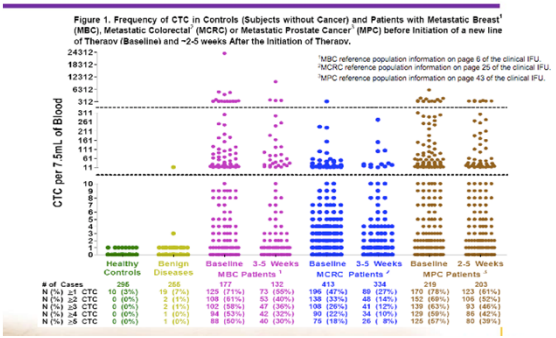


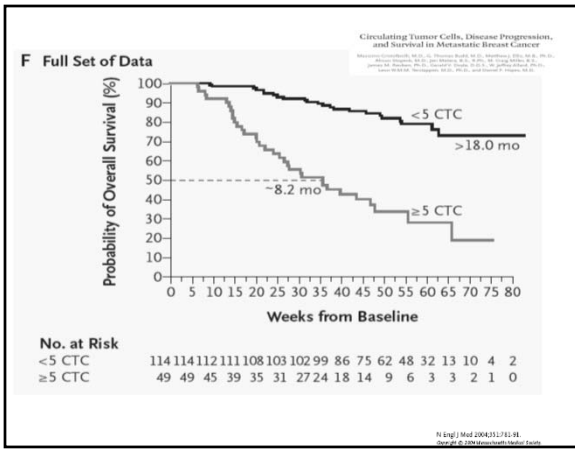
Figure 1. Frequency of CTC in Controls (Subjects without Cancer) and Patients with Metastatic Breast[1] (MBC), Metastatic Colorectal[2] (MCRC) or Metastatic Prostate Cancer[3] (MPC) before Initiation of a new line of Therapy (Baseline) and ~2-5 weeks After the Initiation of Therapy.

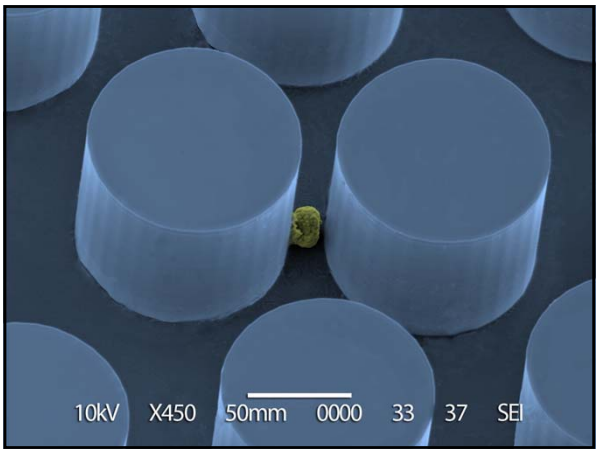
[1]MBC reference population information on page 6 of the clinical IFU.
[2]MCRC reference population information on page 25 of the clinical IFU.
[3]MPC reference population information on page 43 of the clinical IFU.

CTC per 7.5mL of Blood

Healthy Controls | Benign Diseases | Baseline MBC Patients [1] | 3-5 Weeks MBC Patients [1] | Baseline MCRC Patients [2] | 3-5 Weeks MCRC Patients [2] | Baseline MPC Patients [3] | 2-5 Weeks MPC Patients [3]

# of Cases	296	266	177	132	413	334	219	203
N (%) ≥1 CTC	10 (3%)	19 (7%)	125 (71%)	73 (55%)	196 (47%)	89 (27%)	170 (78%)	123 (61%)
N (%) ≥2 CTC	0 (0%)	2 (1%)	108 (61%)	53 (40%)	138 (33%)	48 (14%)	152 (69%)	106 (52%)
N (%) ≥3 CTC	0 (0%)	2 (1%)	102 (58%)	47 (36%)	106 (26%)	41 (12%)	139 (63%)	93 (46%)
N (%) ≥4 CTC	0 (0%)	1 (0%)	94 (53%)	42 (32%)	90 (22%)	34 (10%)	129 (59%)	86 (42%)
N (%) ≥5 CTC	0 (0%)	1 (0%)	88 (50%)	40 (30%)	75 (18%)	26 (8%)	125 (57%)	80 (39%)

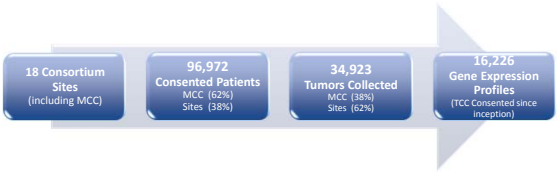




CTCs

- FDA approved for Breast, Colon, Prostate
- Must be analyzed within 48 hrs
- Monitor response to therapy / early recurrence in metastatic setting
- Opportunity to use open channel- other antibody, FISH
- May be complementary to cell free serum circulating DNA studies

M2Gen currently partners with several Consortium Sites (including Moffitt) to collect patient tissue and data

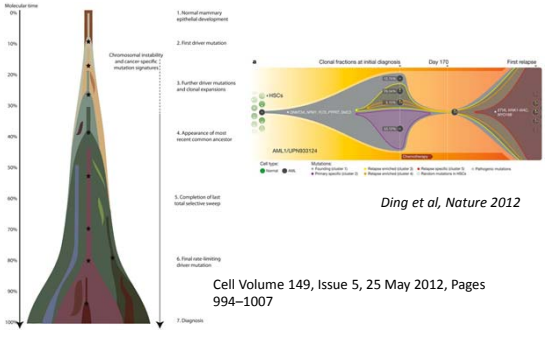


Data Generated from Specimens	
CEL Files (Gene Expression Data)	16,226 files
Targeted Exome Sequencing	4,016 samples
Whole Exome Sequencing (Ovary, Lung, Colon)	535 samples
Whole Genome Sequencing (Melanoma)	13 samples with normal pairs
SNP/CNV (Lung, Breast, Colon)	559 samples

Disease Site	Patients Consented to TCC		Cancer Registry Data Collected		Gene Expression Data Available		Sequencing Data Available	
	All	Alive	All	Alive	All	Alive	All	Alive
Breast	15,932	14,496	15,182	13,734	3,268	2,868	544	467
Prostate	8,998	8,018	8,203	7,219	316	289	57	53
Lung	7,814	4,669	7,397	4,340	2,348	1,296	687	421
Head-Neck	7,129	5,463	6,580	4,920	530	267	186	90
Colorectal	6,434	4,872	5,940	4,412	1,848	1,261	662	441
Kidney	3,294	2,634	2,999	2,345	752	561	231	175
Bladder	2,228	1,475	2,197	1,444	197	81	8	0
Pancreas	2,103	1,060	1,923	904	452	209	188	105
Ovary	1,837	1,369	1,573	1,120	546	315	259	159
Brain	1,553	937	1,396	807	438	206	107	49
Liver	1,125	610	1,027	529	93	47	39	19
Melanoma	685	575	578	470	27	15	10	2

146

Heterogeneity and tumor evolution



Ding et al, Nature 2012

Cell Volume 149, Issue 5, 25 May 2012, Pages 994–1007

Whole Genome View

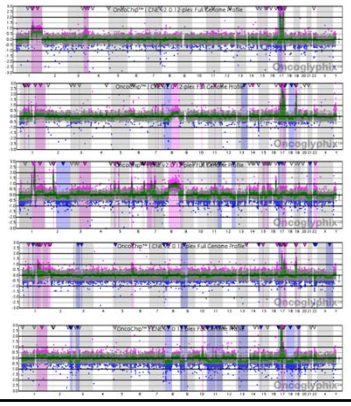
Moffitt 5770

Moffitt 2463

Moffitt 2464

Moffitt 2436

Moffitt 2437



Solid Tumor Microarray Analysis (FFPE: Breast Cancer #2448)

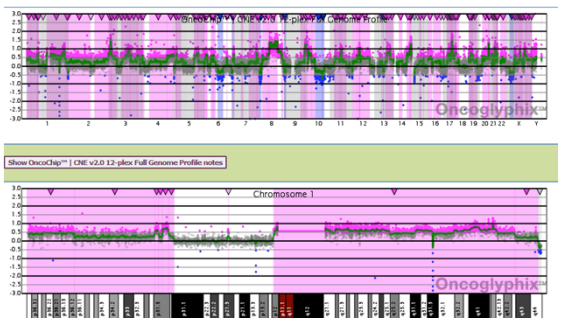
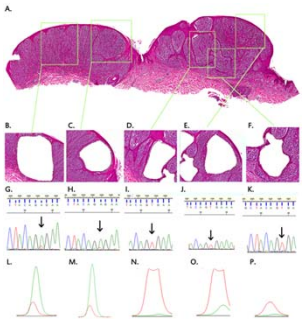


Figure 1. Representative Laser microdissection of a primary melanoma demonstrating intratumor heterogeneity of BRAF mutations.



Yancovitz M, Litterman A, Yoon J, Ng E, et al. (2012) Intra- and Inter-Tumor Heterogeneity of BRAFV600EMutations in Primary and Metastatic Melanoma. PLoS ONE 7(1): e29336. doi:10.1371/journal.pone.0029336
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0029336

PLOS | ONE

Types of Biomarkers

Prognosis	Who needs extra treatment ?
Prediction	What Treatment ?
Toxicity	Who should avoid Treatment ?

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Collaborators

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- Martin Kobel

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Alberta Cancer Board | Alberta Cancer Foundation

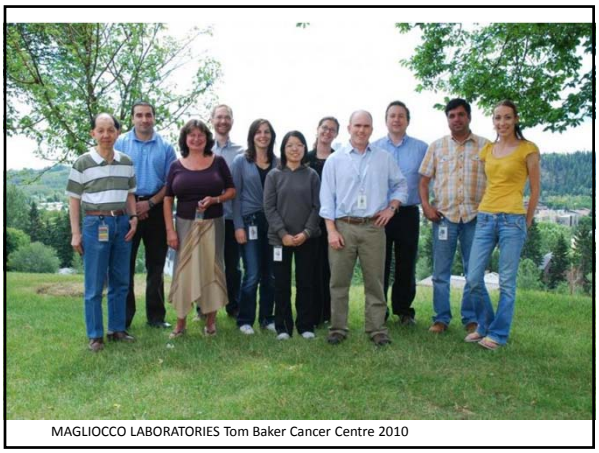
Canadian Breast Cancer Foundation | Fondation canadienne du cancer du sein

The Terry Fox Research Institute

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

National Cancer Institute of Canada | Institut national du cancer du Canada

RTOG Radiation Therapy Oncology Group



MAGLIOCCO LABORATORIES Tom Baker Cancer Centre 2010