Molecular Testing and Targeted Therapy

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H. Lee Moffitt Cancer Center

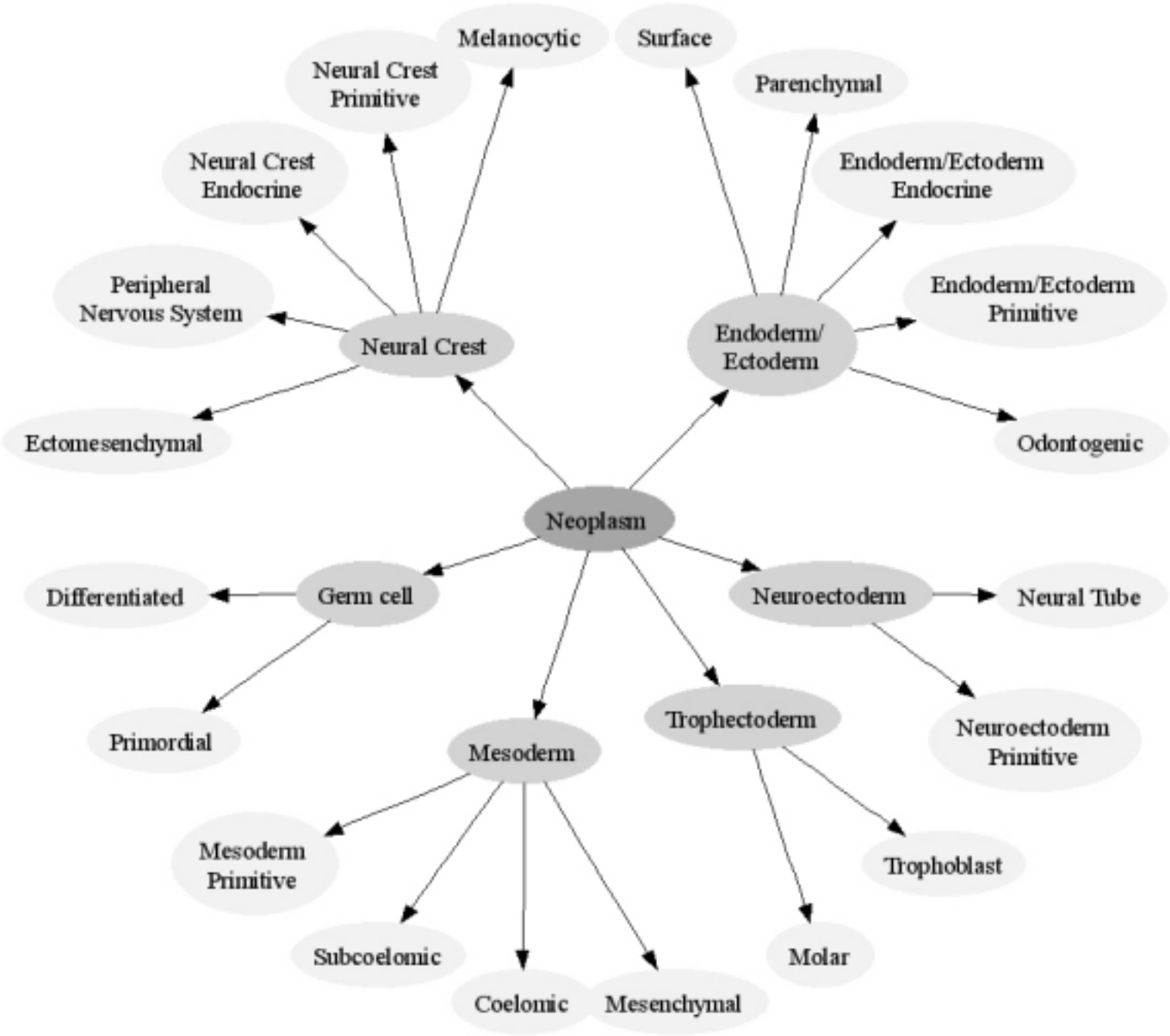
July 10, 2014,

Protocol Support Committee Workshop

Molecular Testing

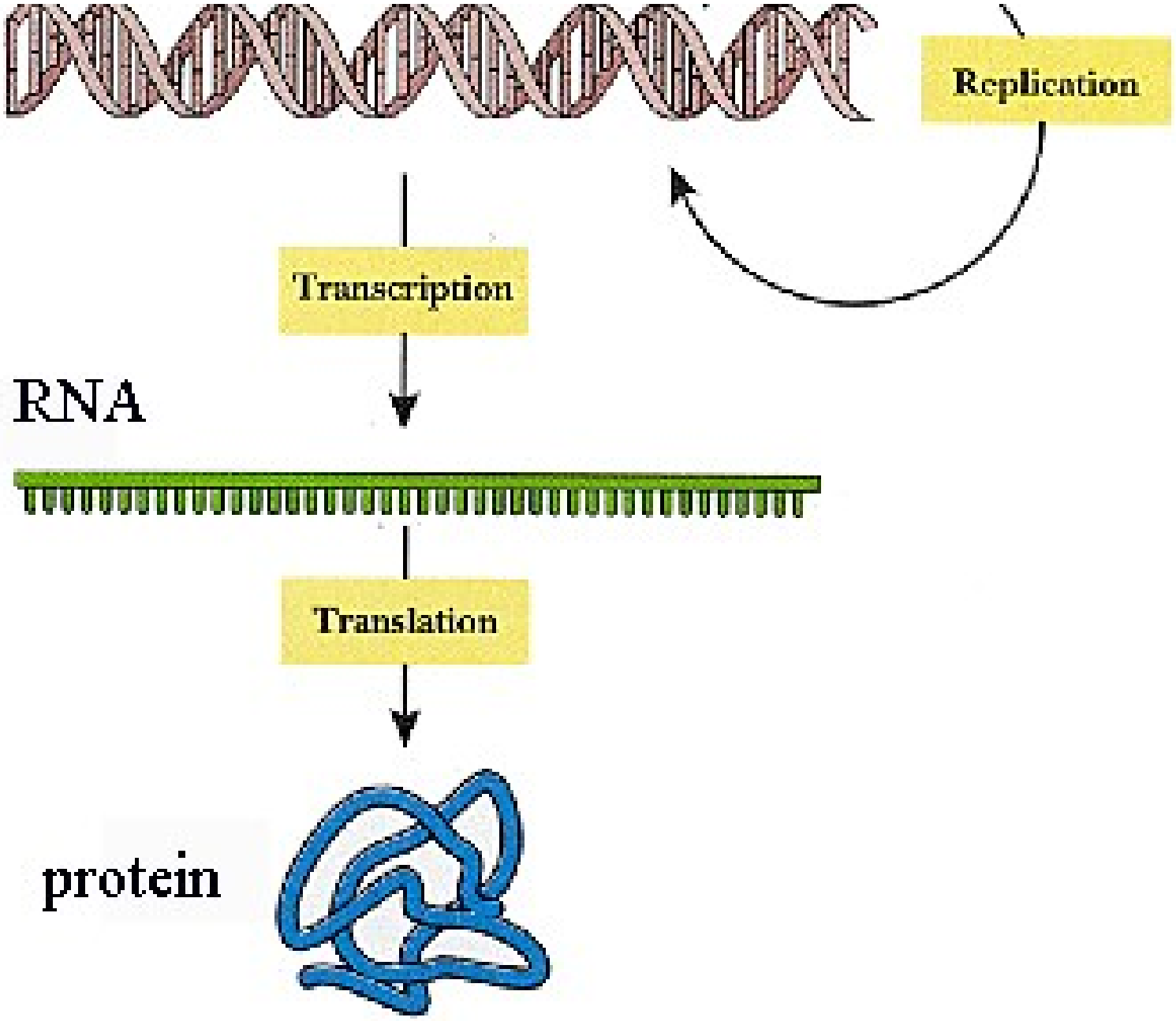
- Technologies
  - IHC
  - FISH
  - DNA / Next Generation technologies
  - Circulating Tumor Cells and CF DNA analysis
- Role in clinical trials
  - Molecular classification of cancer
  - Targeted therapies


Nikon

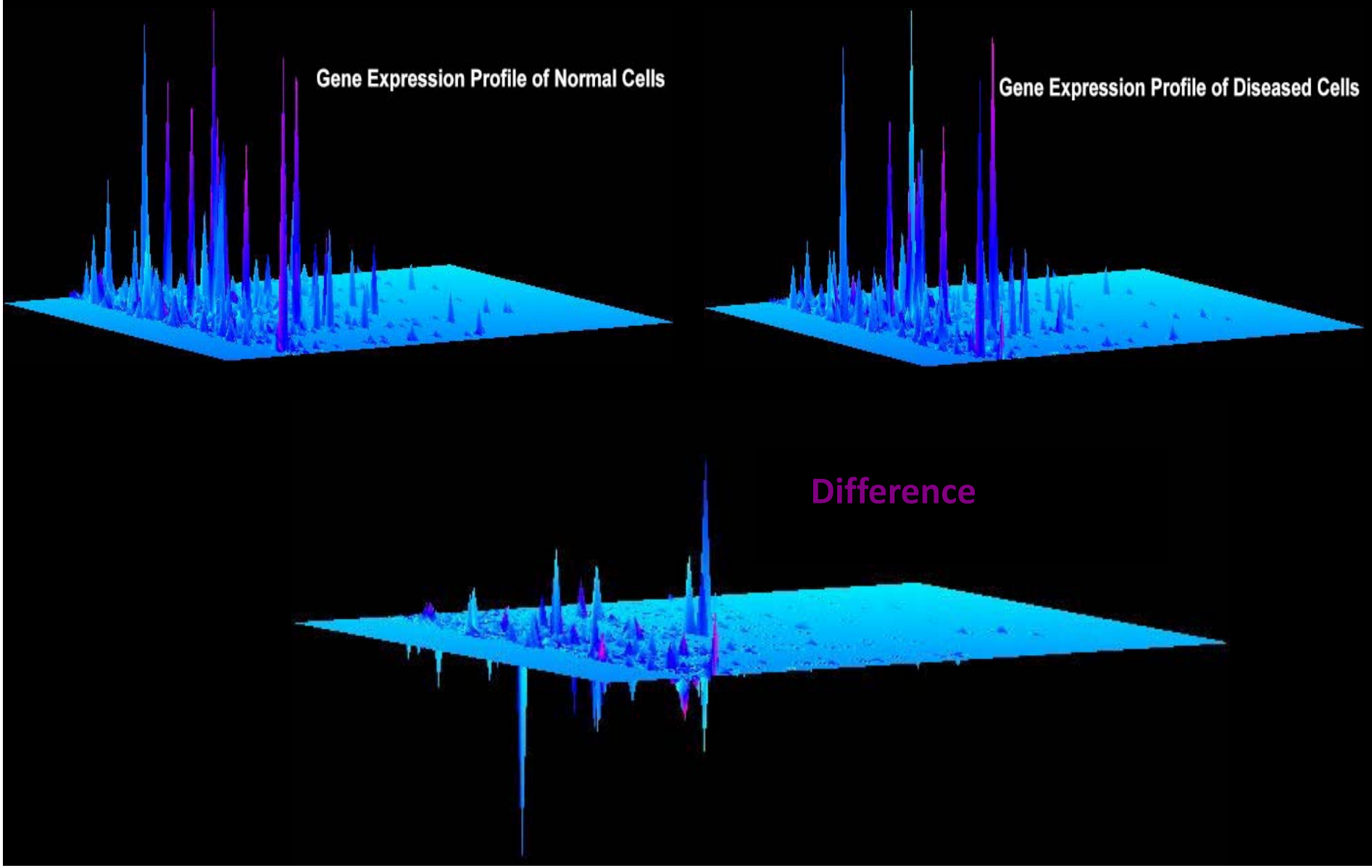

Melanocytic
Surface
Neural Crest Primitive
Parenchymal
Neural Crest Endocrine
Endoderm/Ectoderm Endocrine
Peripheral Nervous System
Endoderm/Ectoderm Primitive
Neural Crest
Endoderm/ Ectoderm
Ectomesenchymal
Odontogenic
Neoplasm
Differentiated
Germ cell
Neuroectoderm
Neural Tube
Primordial
Trophectoderm
Neuroectoderm Primitive
Mesoderm
Mesoderm Primitive
Trophoblast
Subcoelomic
Molar
Coelomic
Mesenchymal

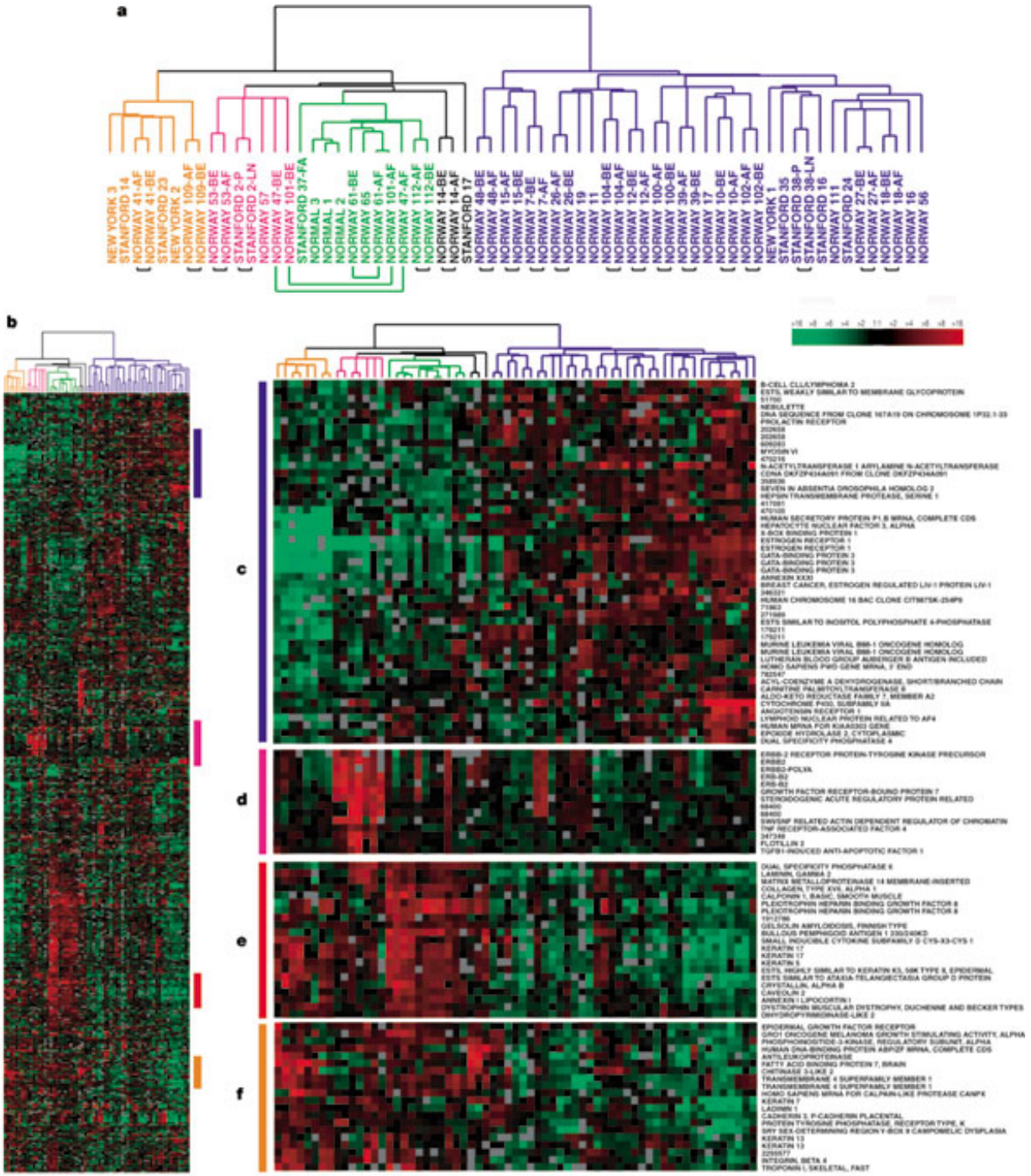
Microscopes

Antony van Leewenhoek (1632-1723).


Replication
Transcription
RNA
Translation
protein

Comparison of the Expression Profile of Entire Genomes





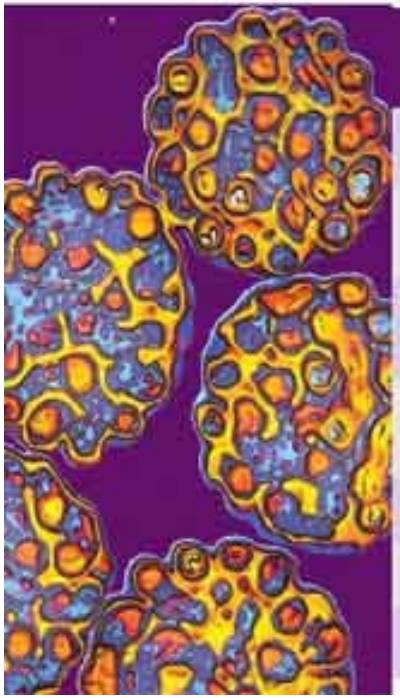
Molecular Portraits

Estrogen Receptor

HER2

Basal Keratins

EGFR



	HPV-Positive HNSCC	HPV-Negative HNSCC
Age of patient	Younger	Older
Socioeconomic status of patient	Higher	Lower
Risk factors	High-risk sexual practices Marijuana exposure	Tobacco and alcohol exposure*
Location of primary tumor	Oropharynx (palatal and lingual tonsils)	All head and neck sites
Survival	Better	Worse
Response to chemoradiation	Better	Worse
Tumor recurrence	Lower risk	Higher risk

Table 1 Comparison of HPV-positive and HPV-negative head and neck cancers

	HPV-positive	HPV-negative
Incidence	Increasing	Decreasing
Age	Younger	Older
Gender	3:1 men	3:1 men
Risk factors	Sexual behavior	Tobacco, alcohol
Cofactors	Marijuana, immunosuppression	Diet, oral hygiene
Molecular genetics findings	P16 ↑	P16 ↓
	Rb ↓	Rb ↑
	P53 wild-type	P53 mutated
Anatomic site	Lingual and palatine tonsils	All sites
Pathologic findings		
Primary	Basaloid	Keratinized
Lymph node metastasis	Cystic	Solid
Survival	Better	Worse

High-Risk Human Papillomavirus Affects Prognosis in Patients With Surgically Treated Oropharyngeal Squamous Cell Carcinoma

Lisa Licitra, Federica Perrone, Paolo Bossi, Simona Suardi, Luigi Mariani, Raffaella Artusi, Maria Oggionni, Chiara Rossini, Giulio Cantù, Massimo Squadrelli, Pasquale Quattrone, Laura D. Locati, Cristiana Bergamini, Patrizia Olmi, Marco A. Pierotti, and Silvana Pilotti

J Clin Oncol 24:5630-5636. © 2006 by American Society of Clinical Oncology

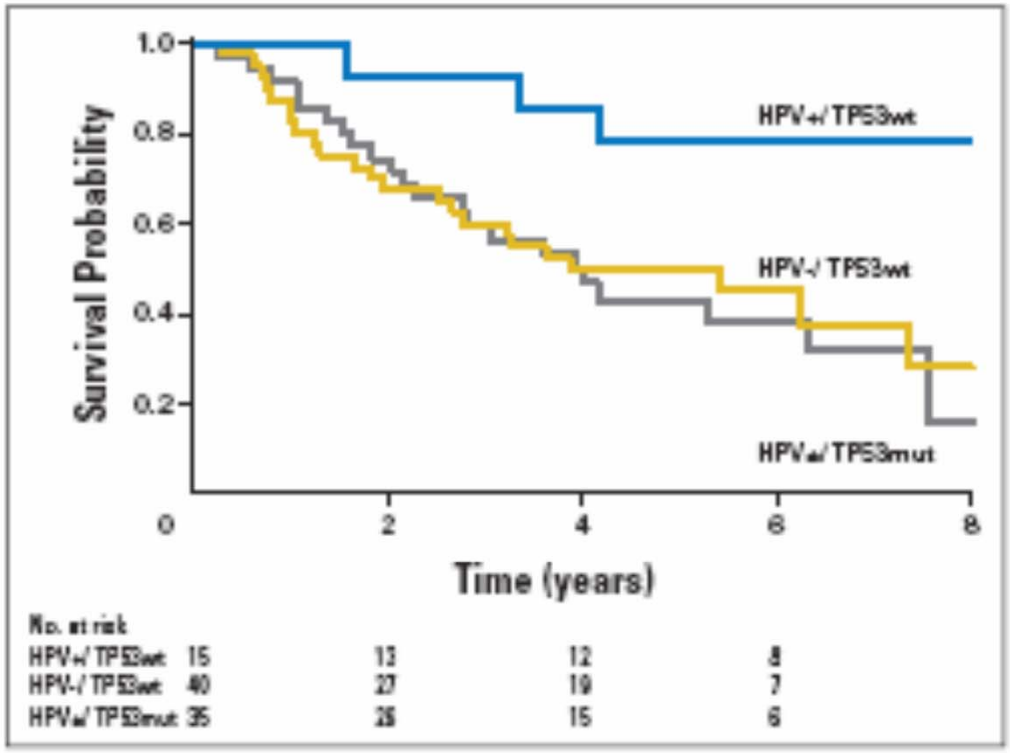


Fig 1. Overall survival according to human papillomavirus (HPV)/TP53 status. mut, mutated; wt, wild type.

90 surgical pts
64% post op RT

HPV 16 – 18 by PCR
E2 and E6
TP53 gene sequence
p16INK4a gene
p16 immunohistochemistry

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ORIGINAL REPORT

Molecular Classification Identifies a Subset of Human Papillomavirus–Associated Oropharyngeal Cancers With Favorable Prognosis

Paul M. Weinberger, Ziwei Yu, Bruce G. Haffty, Diane Kowalski, Malini Harigopal, Janet Brandsma, Clarence Sasaki, John Joe, Robert L. Camp, David L. Rimm, and Amanda Psyrri

Conclusion

Using this system for classification, we define the molecular profile of HPV+ OSCC with favorable prognosis, namely HPV+/p16 high (class III). This study defines a novel classification scheme that may have value for patient stratification for clinical trials testing HPV-targeted therapies.

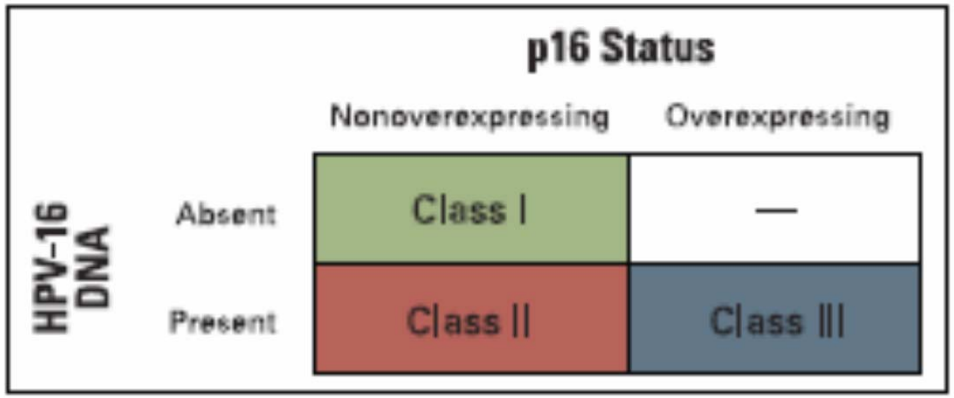
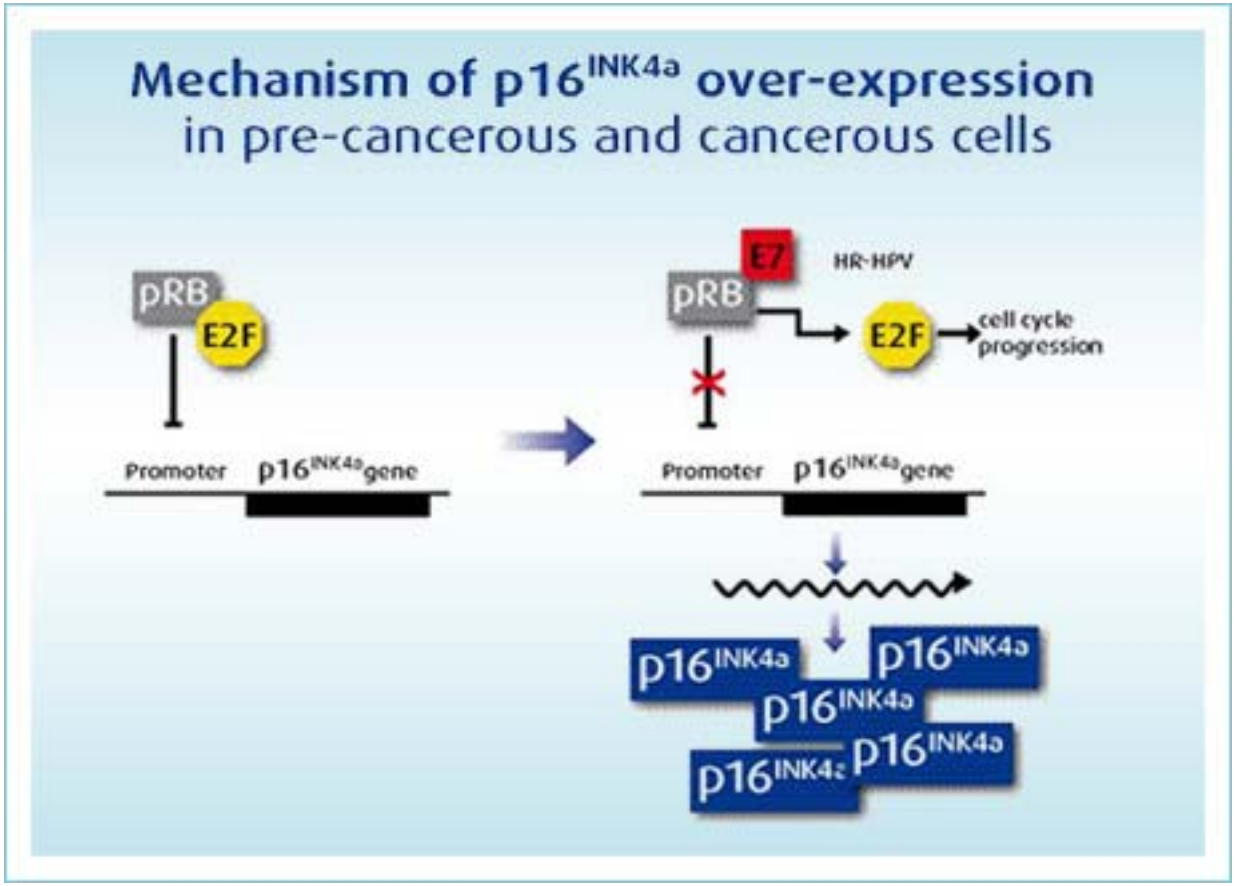


Table 2. Antibodies Used for Immunohistochemistry and Immunofluorescence

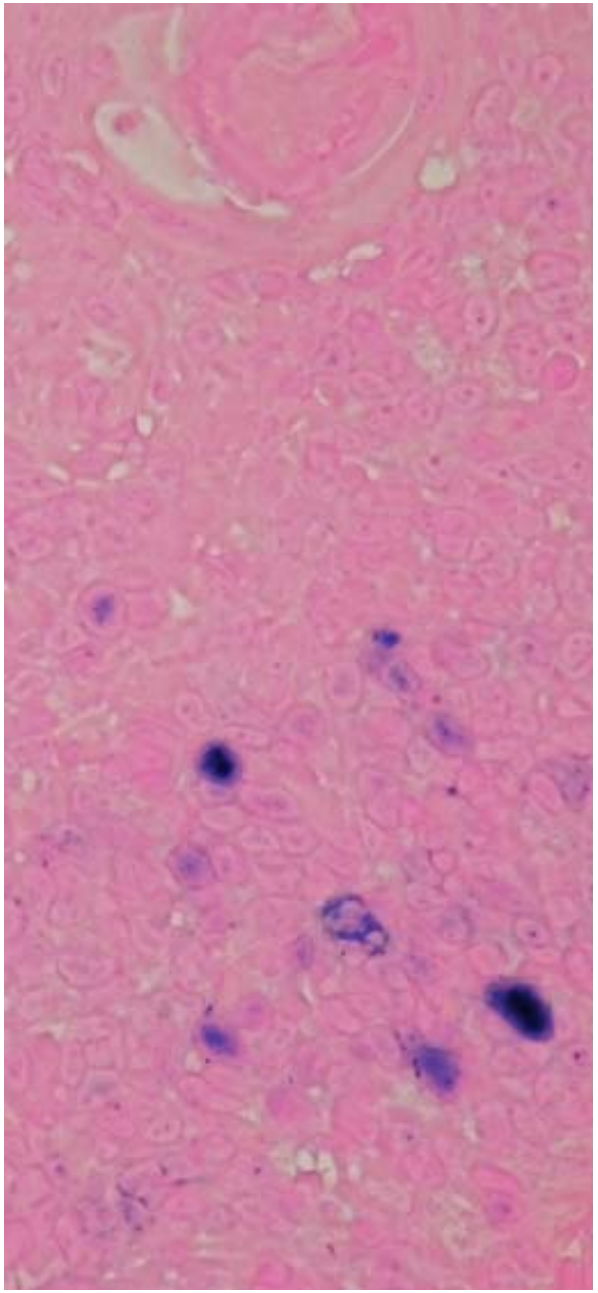
Antibody Target	Species	Type	Dilution	Company	Identifier
Rb	Mouse	Monoclonal	1:50	NeoMarkers*	Clone 1F8
p53	Mouse	Monoclonal	1:100	DAKO†	Clone DO7
p16	Mouse	Monoclonal	1:25	DAKO	k5334

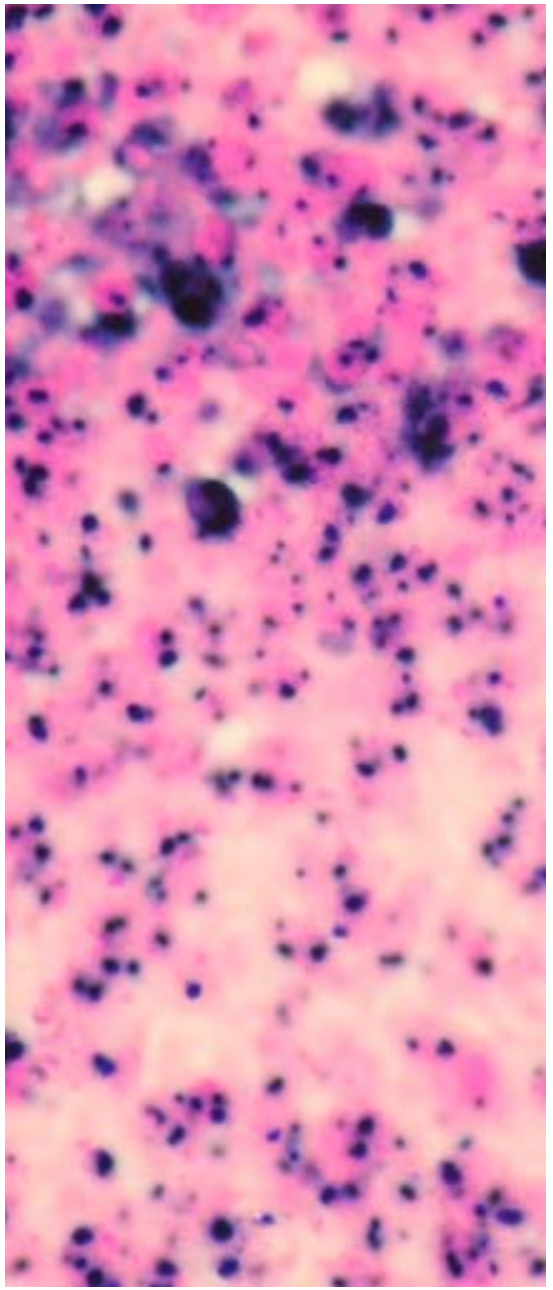
Abbreviation: Rb, retinoblastoma.
*Fremont, CA.
†Carpinteria, CA.

In

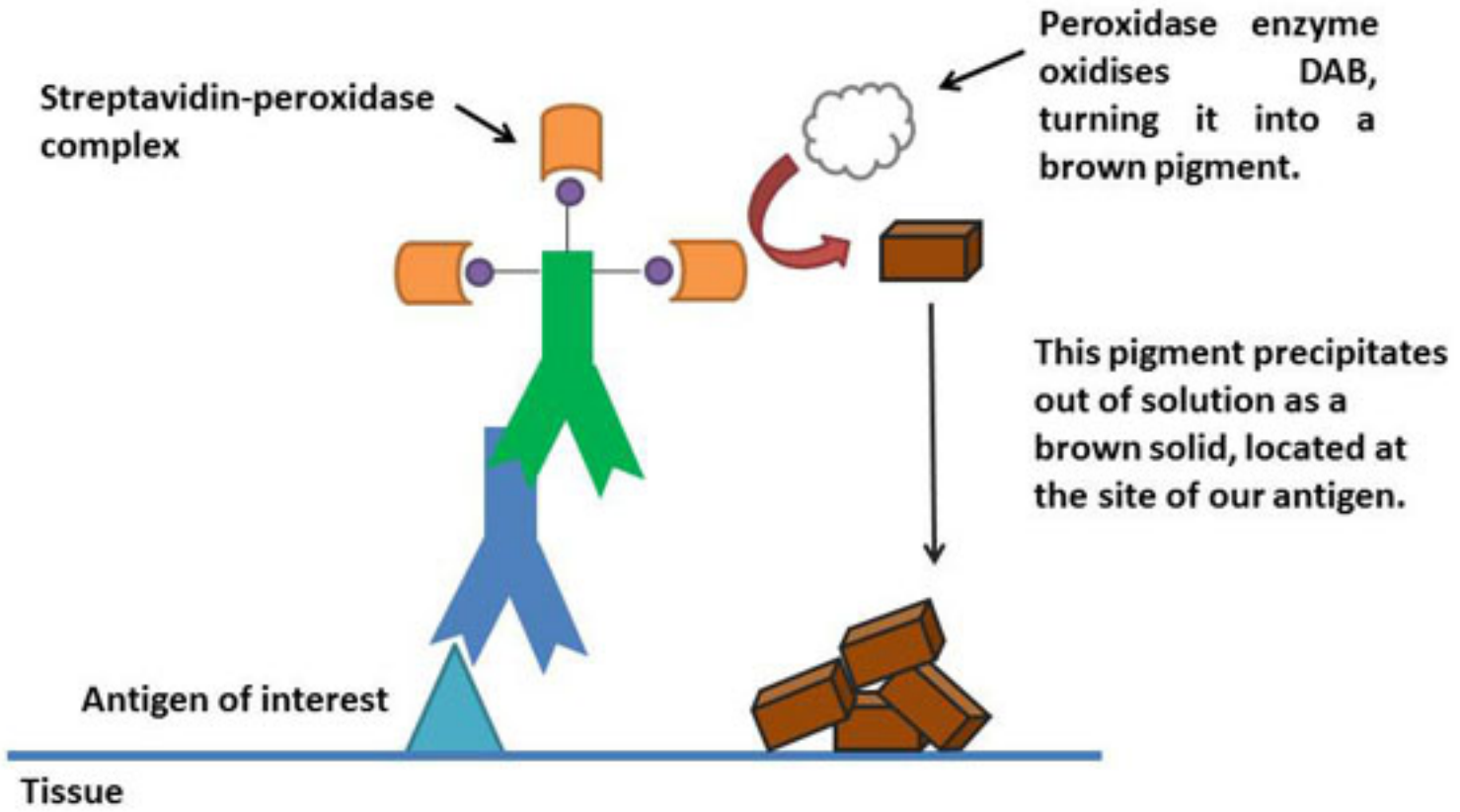

Mechanism of p16INK4a over-expression
in pre-cancerous and cancerous cells
pRB
E2F
Promoter
p16INK4a gene
E7
HR-HPV
pRB
E2F
cell cycle progression
Promoter
p16INK4a gene
p16INK4a
p16INK4a
p16INK4a
p16INK4a
p16INK4a

HPV in situ

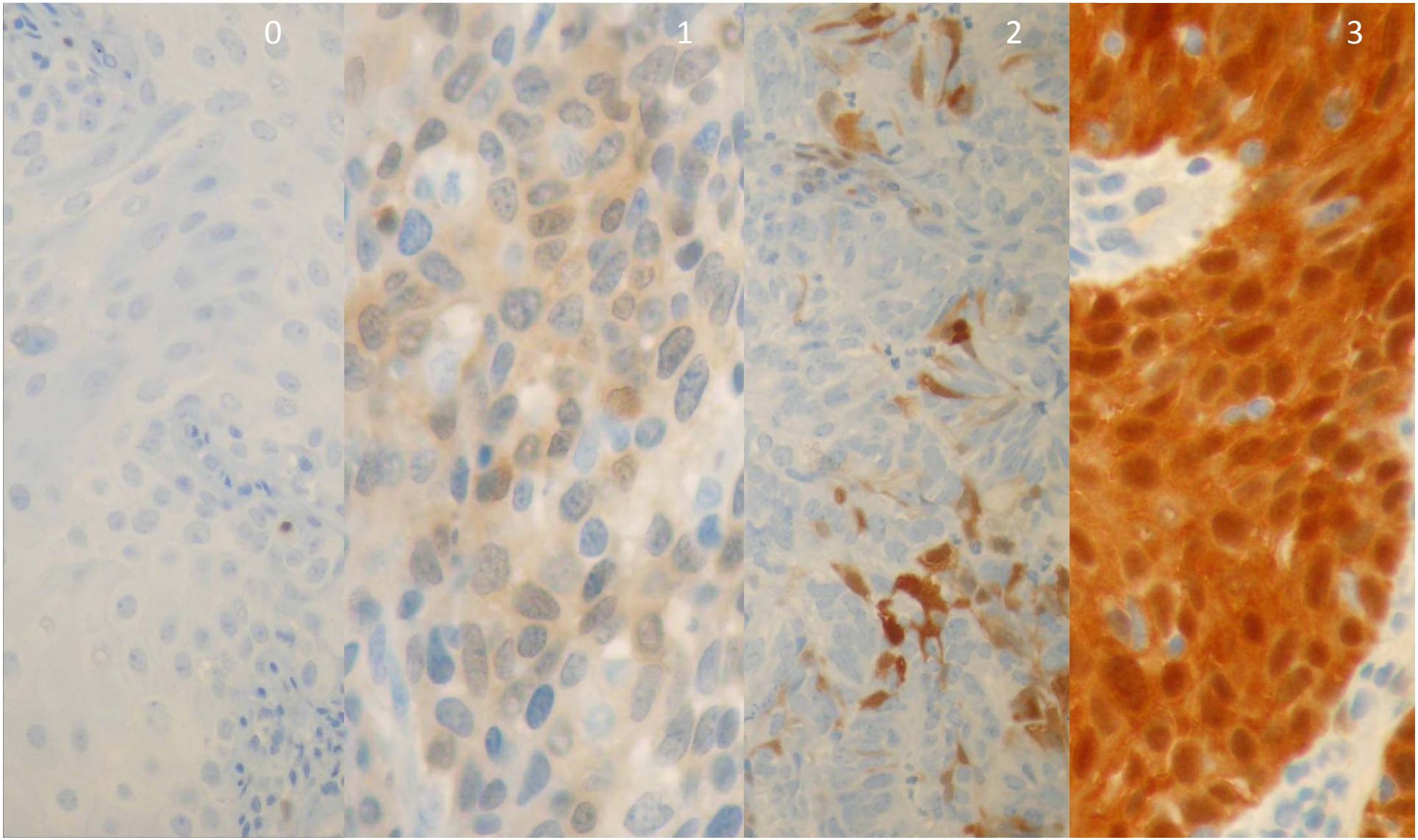


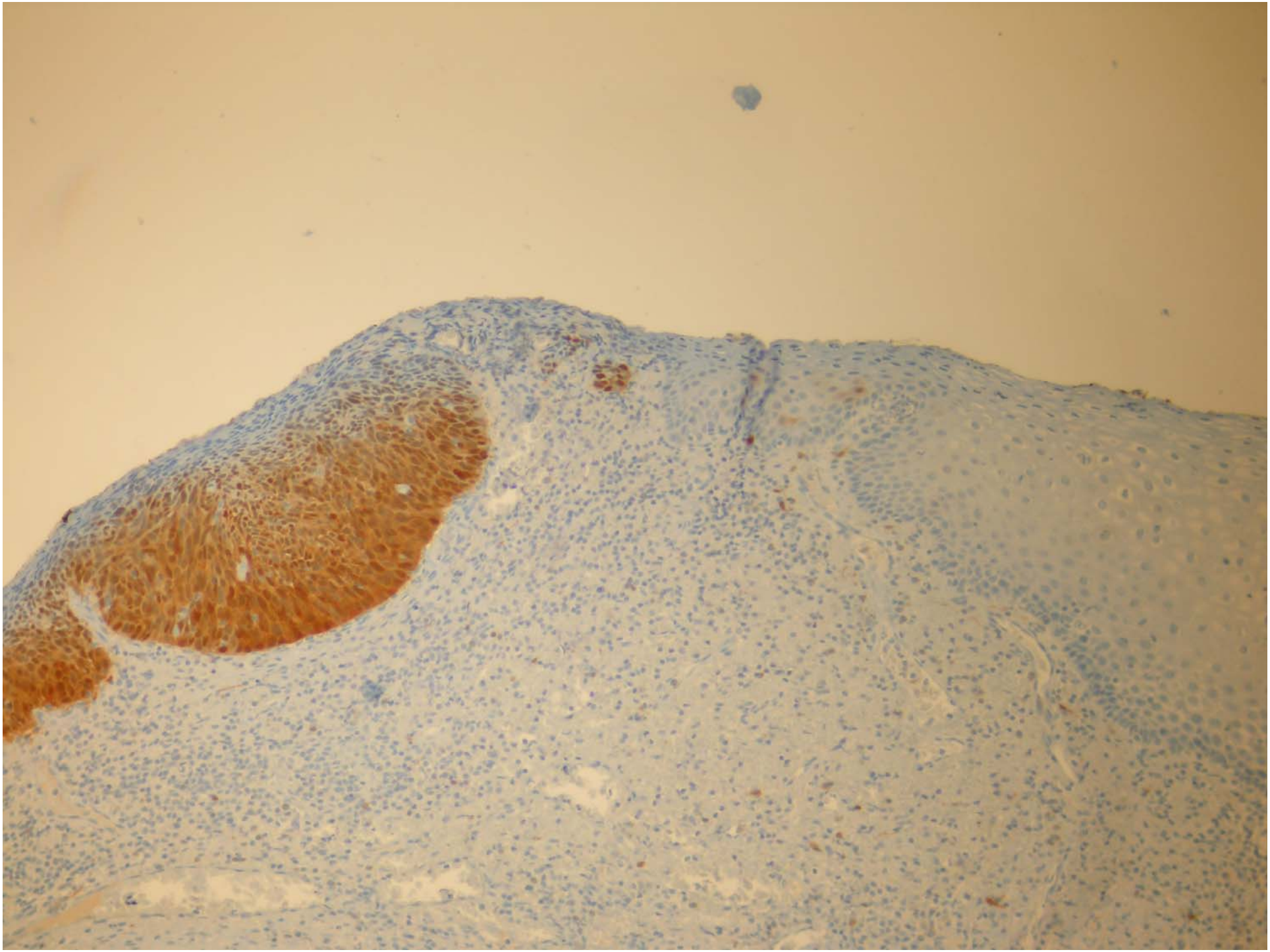


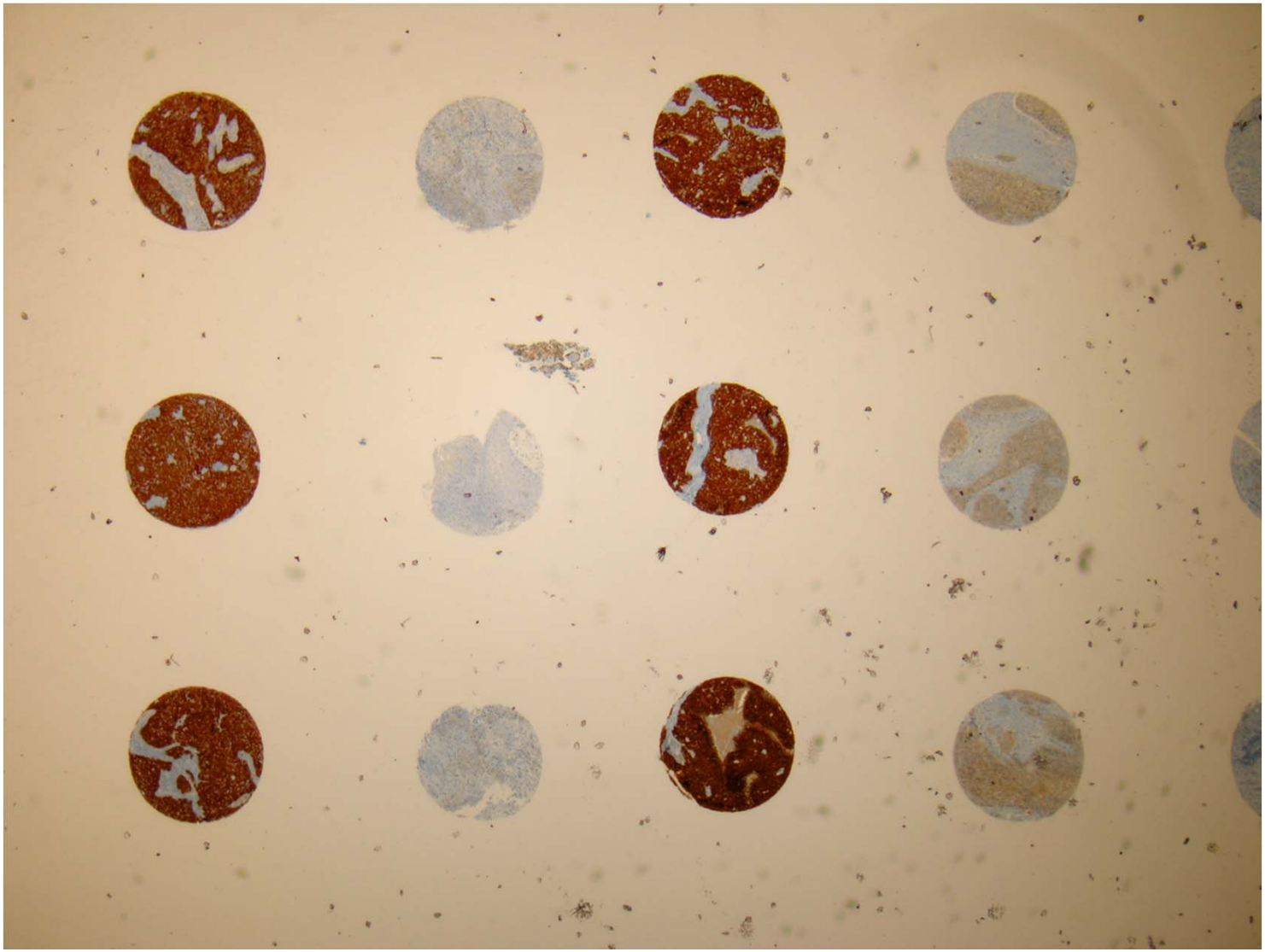
IMMUNOHISTOCHEMISTRY



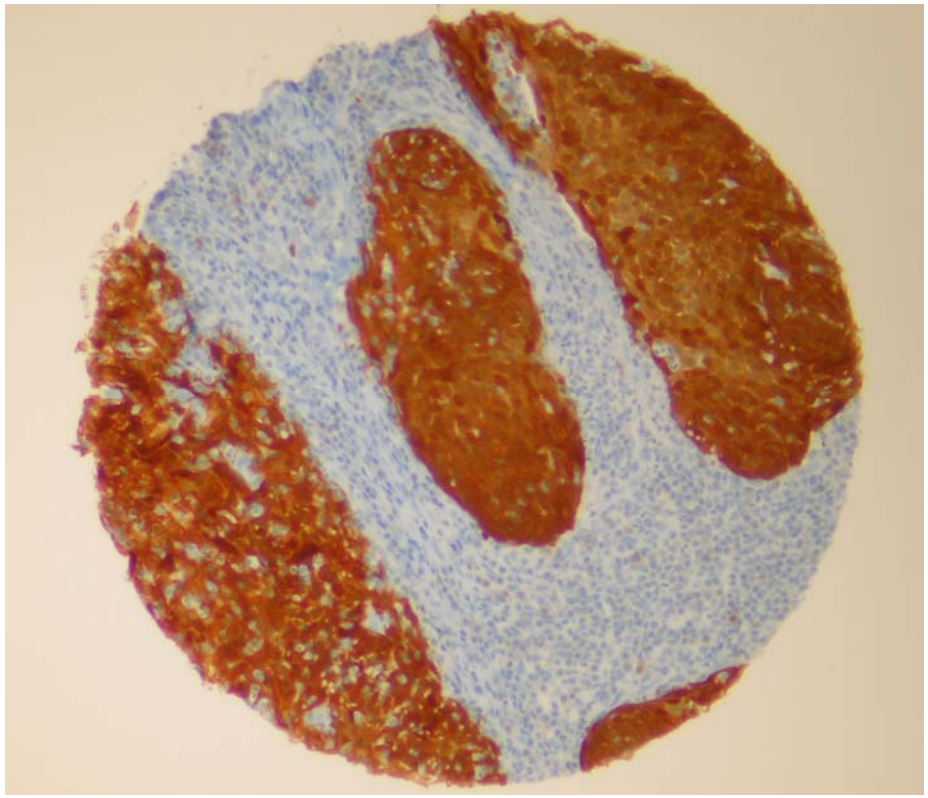
P16 IHC Invasive Cervical Carcinoma

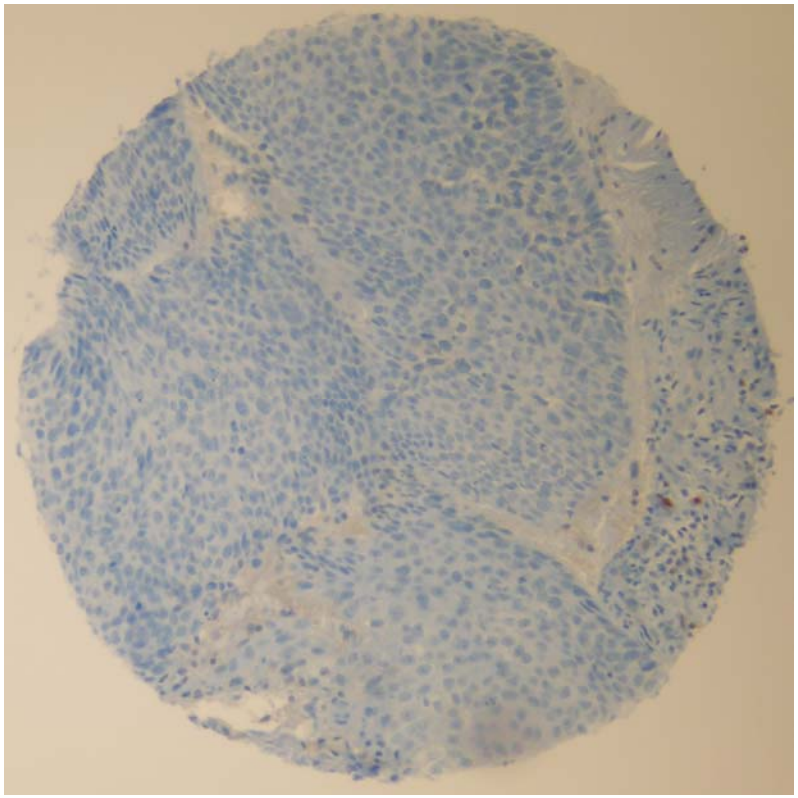


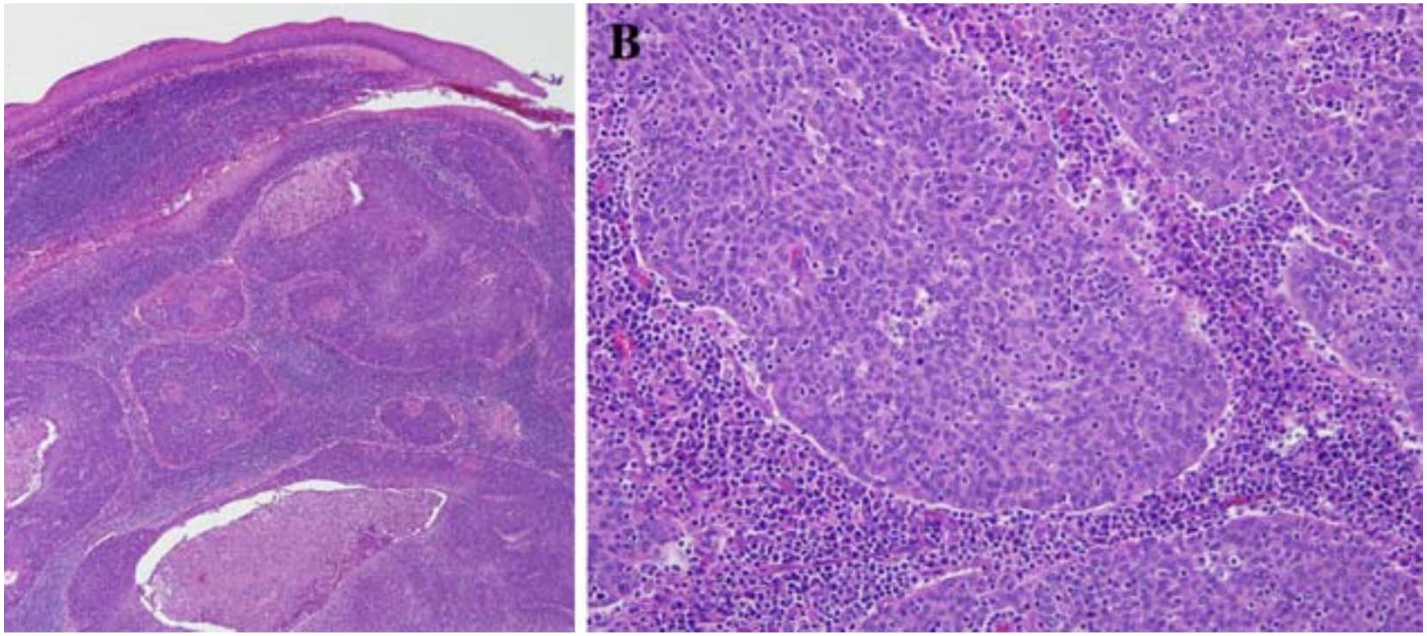




P16 Head and Neck






B

TBCC patient cohort

P16 status	P16 positive N=29	P16 negative N=26
Median Age	52	62
Male: Female	24 : 5	21 : 5
Primary Site		
Oral Cavity	0	1
Oropharynx	21	7
Hypopharynx	3	6
Larynx	1	12
Unknown	4	0
Stage II	1	2
III	1	9
IV	23	15
X	4	0
Smoking Status		
Never	11	2
Prior	7	9
Current	11	15
% CR	28 (97%)	20 (77%)

TBCC patient cohort

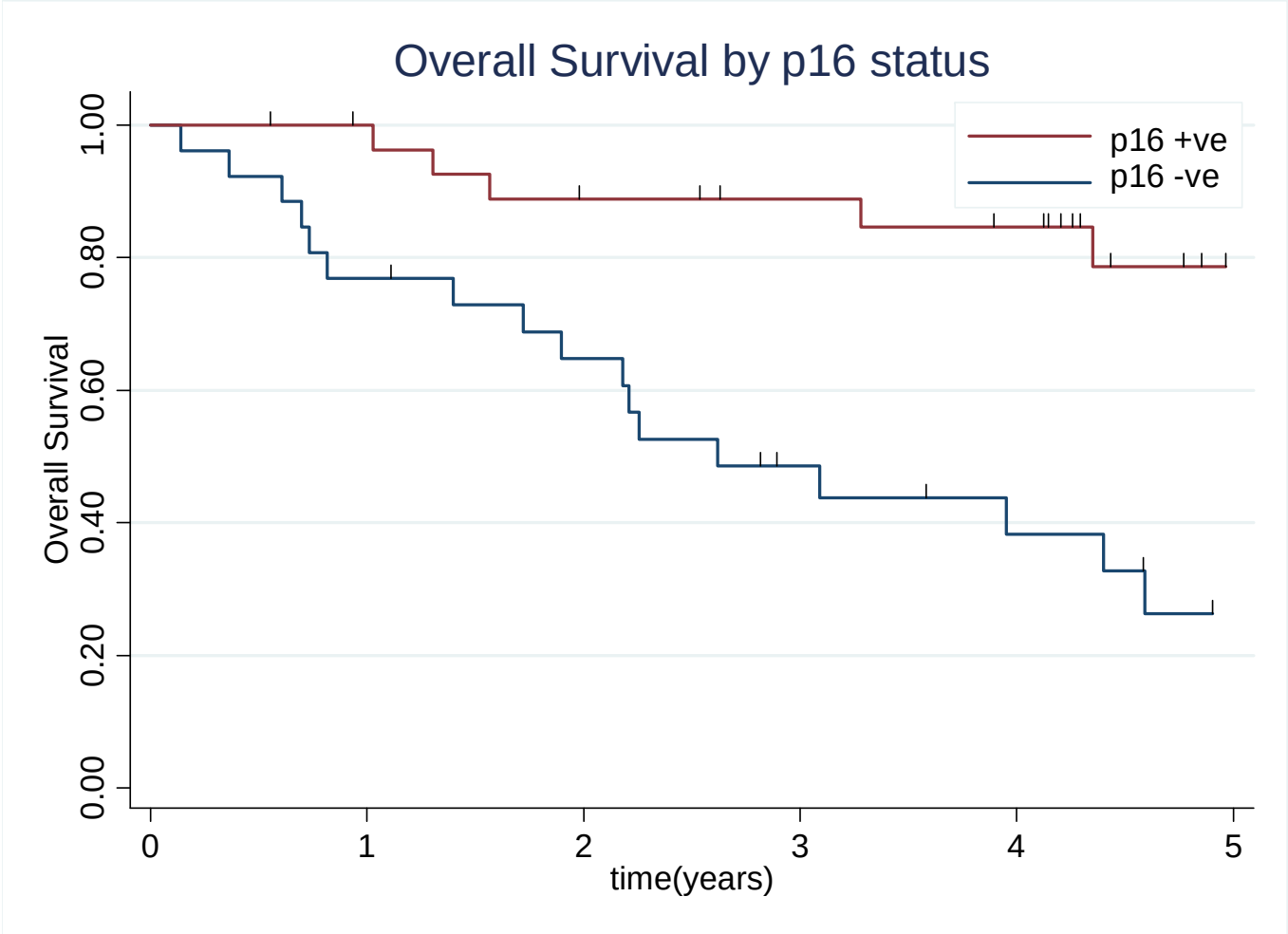
p16 status	P16+ve N=29		P16-ve N=26		p-value
Mean Age (±SD)	52 (± 8)		62 (± 8)		<0.0001
Stage IV at diagnosis	24	83%	15	58%	0.04
Never smokers	11	38%	2	8%	0.008
Oropharyngeal Primary	21	72%	7	27%	<0.0001

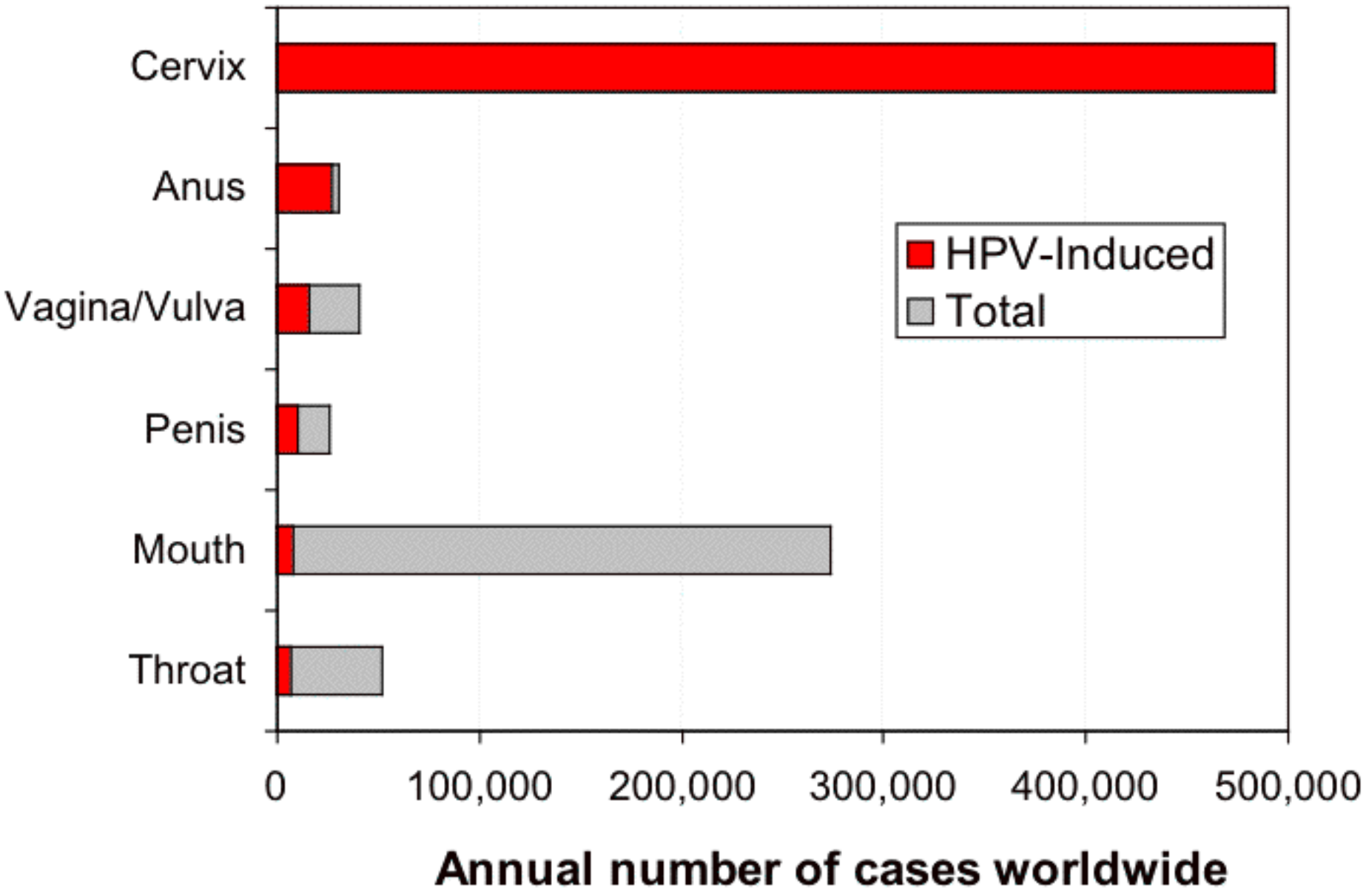
TBCC cohort: results

p16 status	P16+ve N=29	P16-ve N=26	p-value
Overall survival*	79%	26%	<0.0001
Disease-specific survival*	91%	34%	<0.0001
Locoregional recurrence	11%	60%	<0.0001

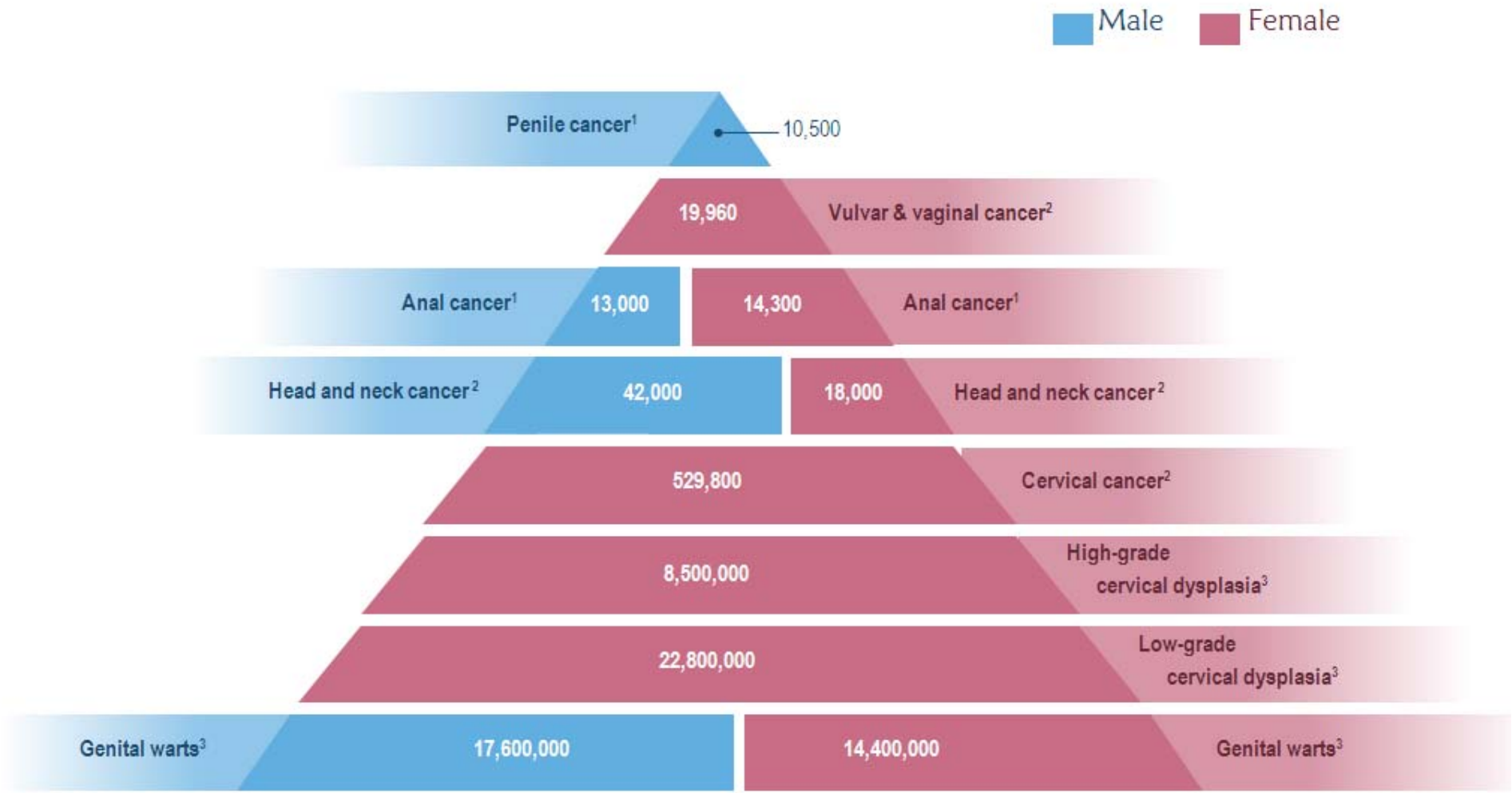
*5-year survival rate; median follow-up 38 months (range 3 - 85months)

Overall Survival by p16 status




Cervix
Anus
Vagina/Vulva
Penis
Mouth
Throat
HPV-Induced
Total
0
100,000
200,000
300,000
400,000
500,000
Annual number of cases worldwide

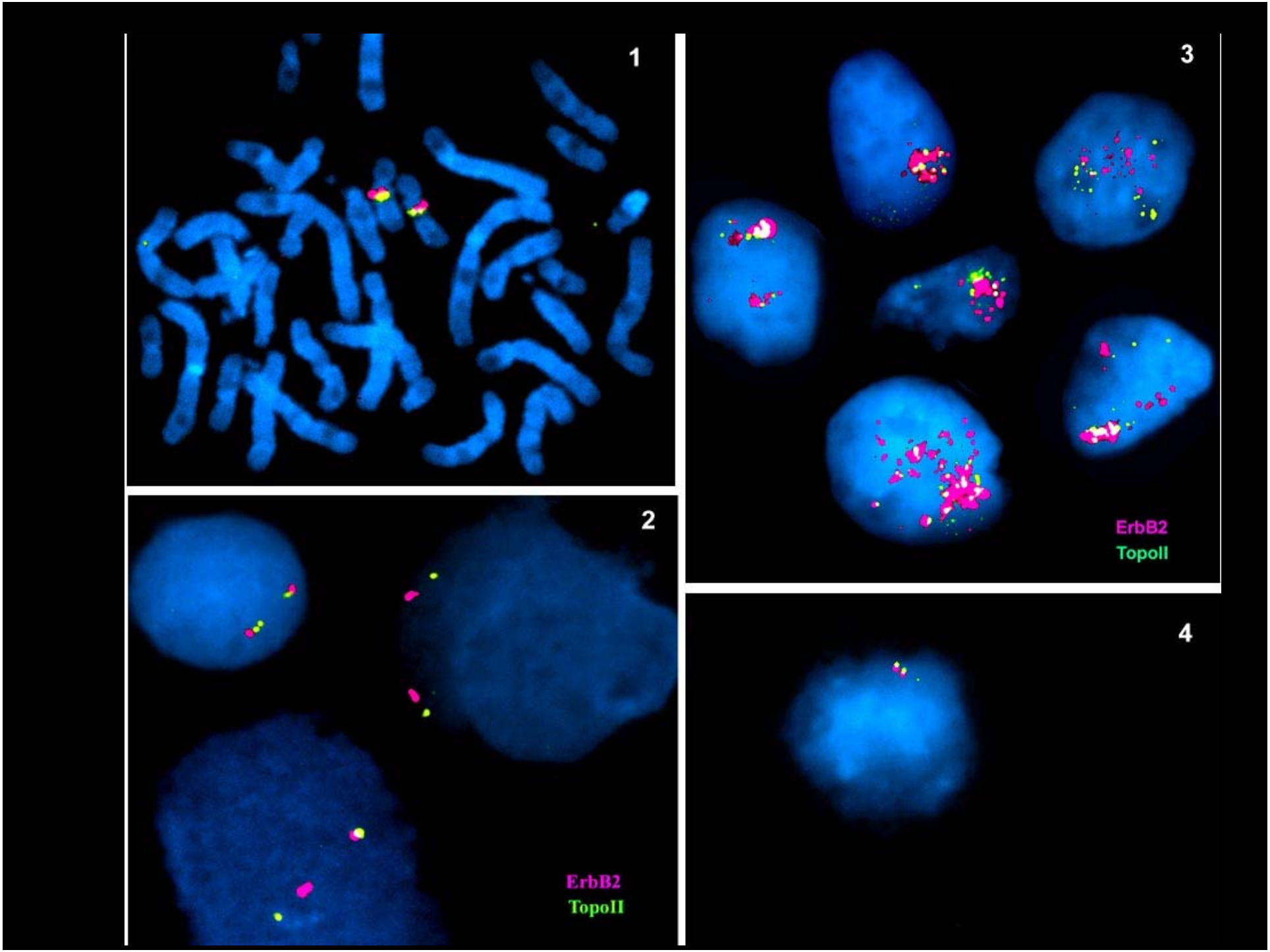
Estimated annual new HPV-related disease cases in males and females globally

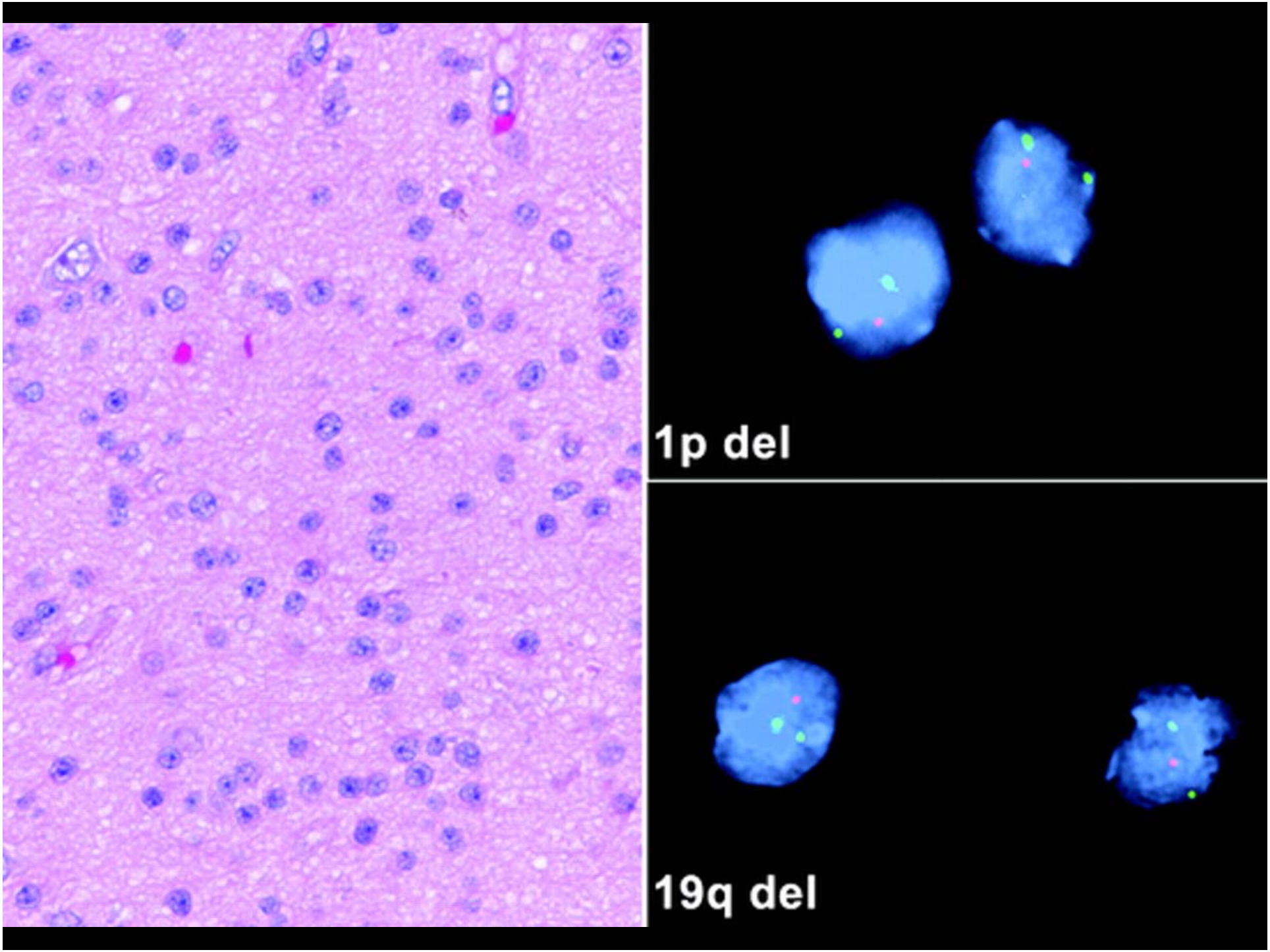


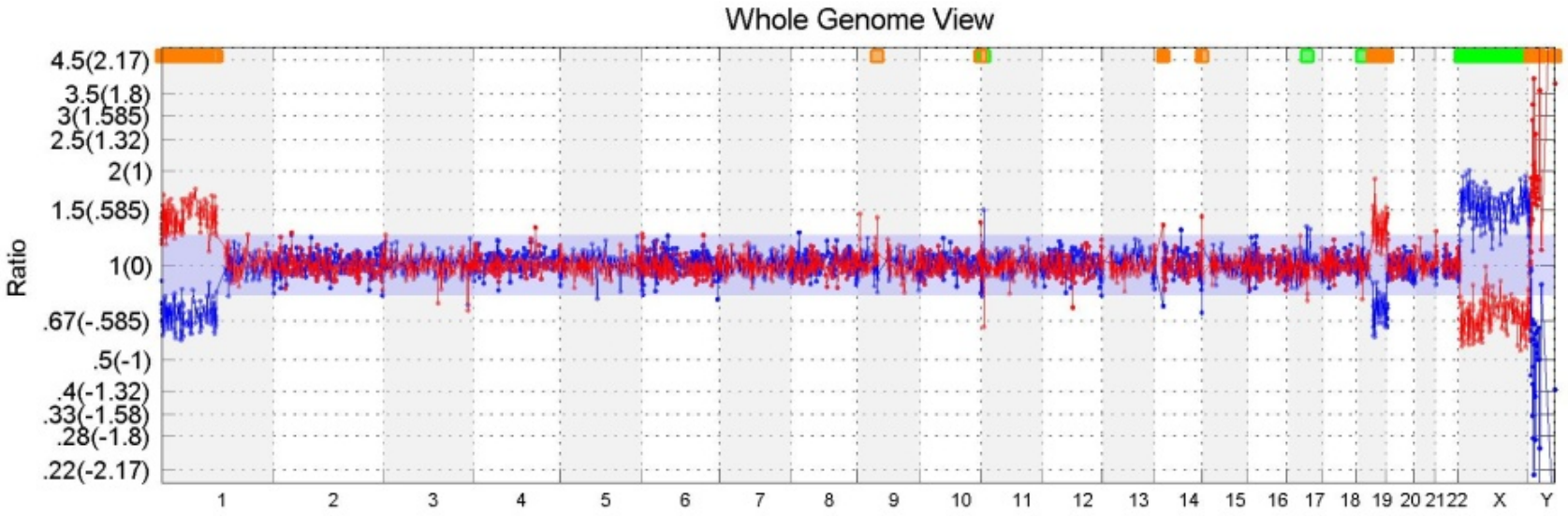
Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. *Vaccine*. 2006;24(Suppl 3):S3/11–S3/25.

Glioma- Use of FISH for Prognosis

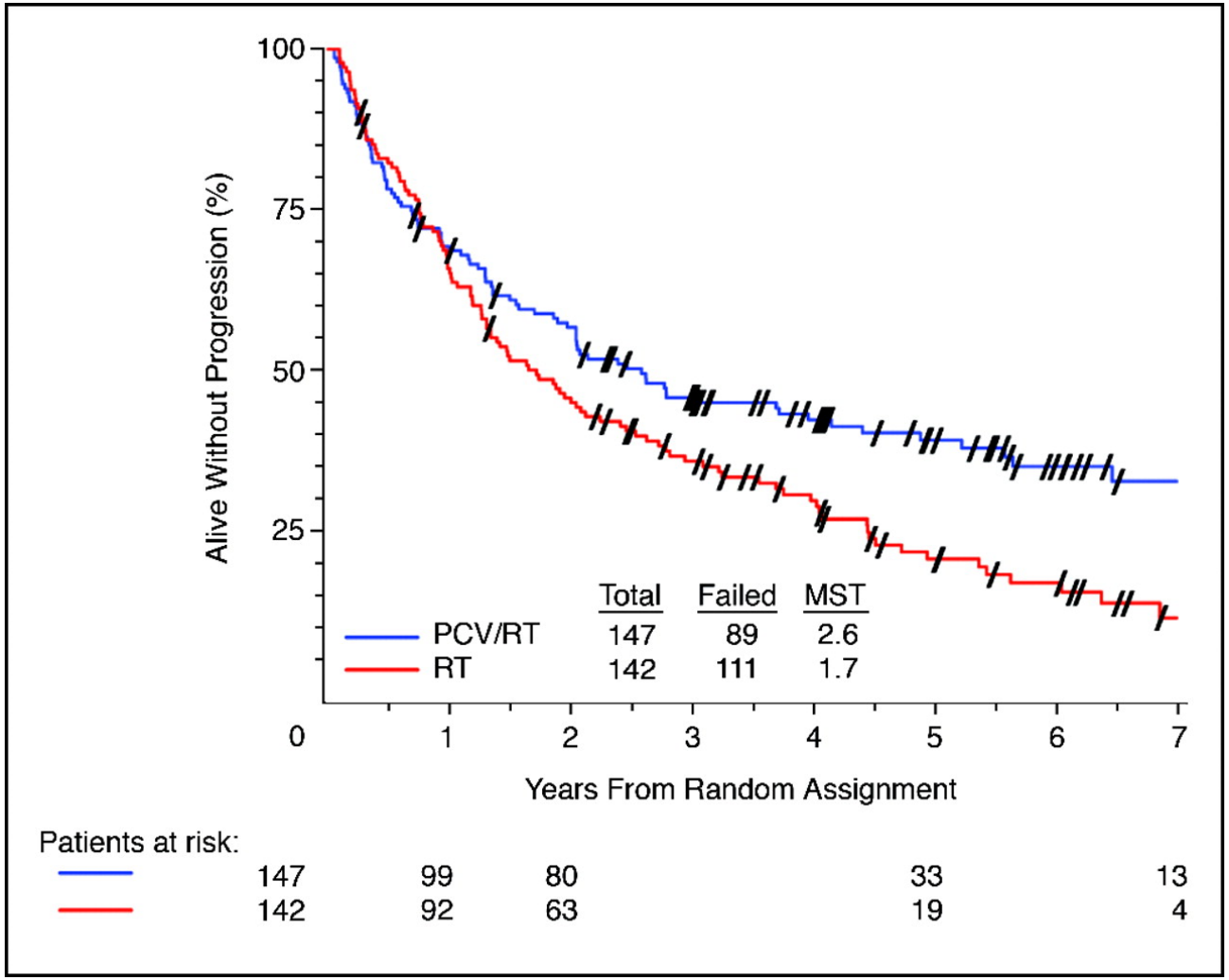
- Insert diagram of FISH Procedure


1
2
ErbB2
TopoII
3
ErbB2
TopoII
4


1p del
19q del

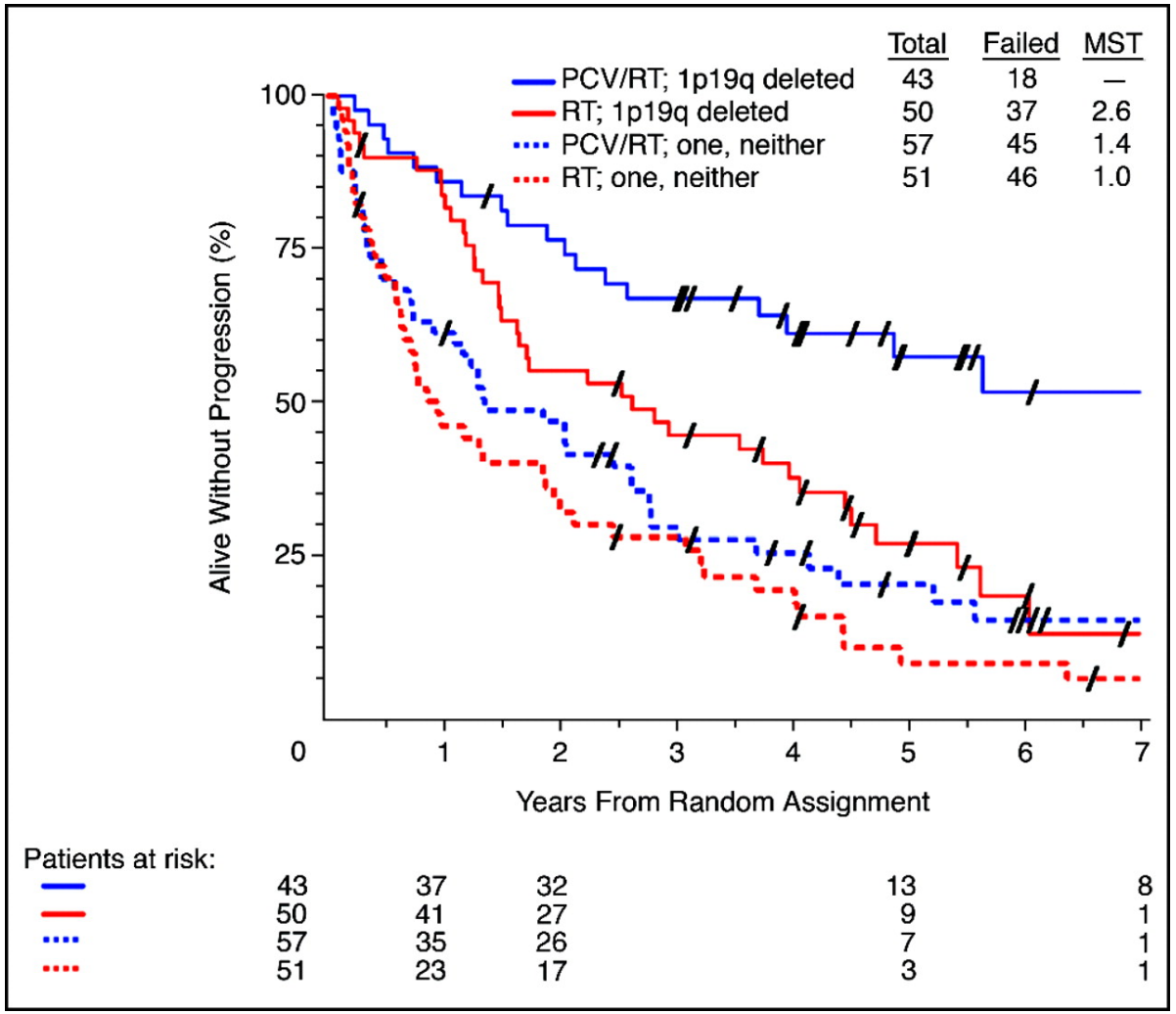

Whole Genome View
Ratio
4.5(2.17)
3.5(1.8)
3(1.585)
2.5(1.32)
2(1)
1.5(.585)
1(0)
.67(-.585)
.5(-1)
.4(-1.32)
.33(-1.58)
.28(-1.8)
.22(-2.17)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y

Kaplan-Meier estimates of progression-free survival by treatment group.



Cairncross G et al. JCO 2006;24:2707-2714

Kaplan-Meier estimates of progression-free survival by treatment and genotype.



Cairncross G et al. JCO 2006;24:2707-2714

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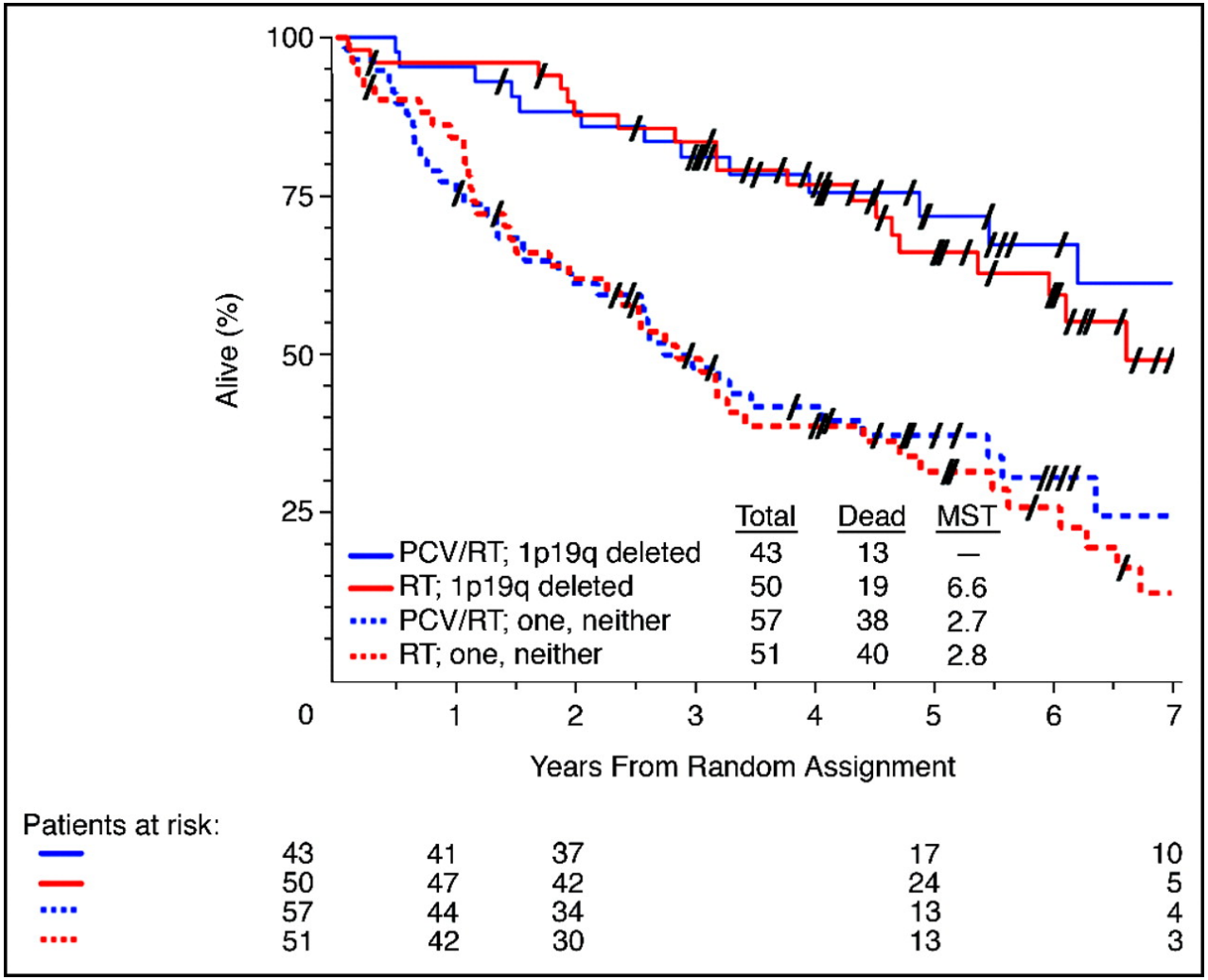
Kaplan-Meier estimates of overall survival by treatment and genotype.



Cairncross G et al. JCO 2006;24:2707-2714

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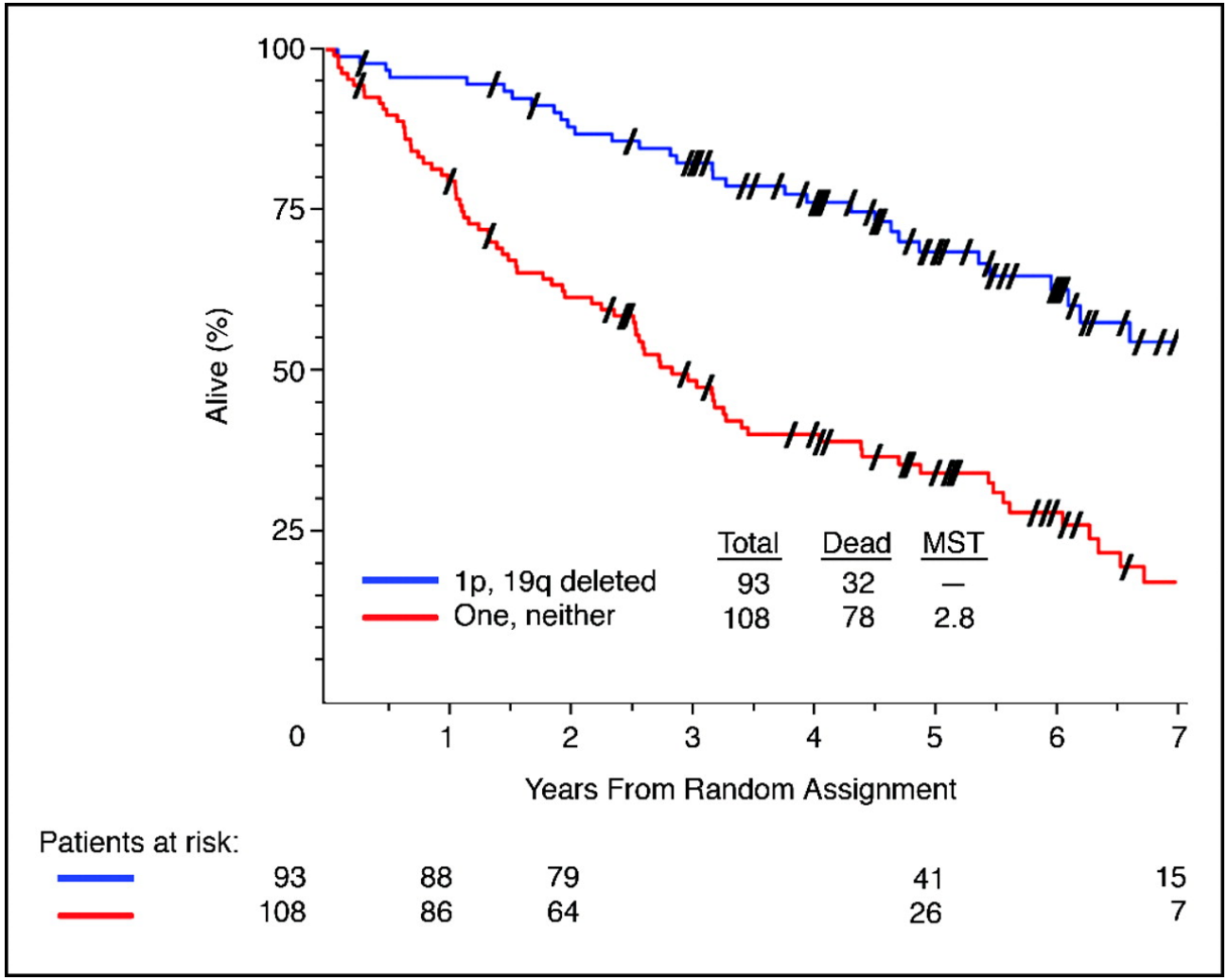
Kaplan-Meier estimates of overall survival by 1p and 19q deletion.



Cairncross G et al. JCO 2006;24:2707-2714

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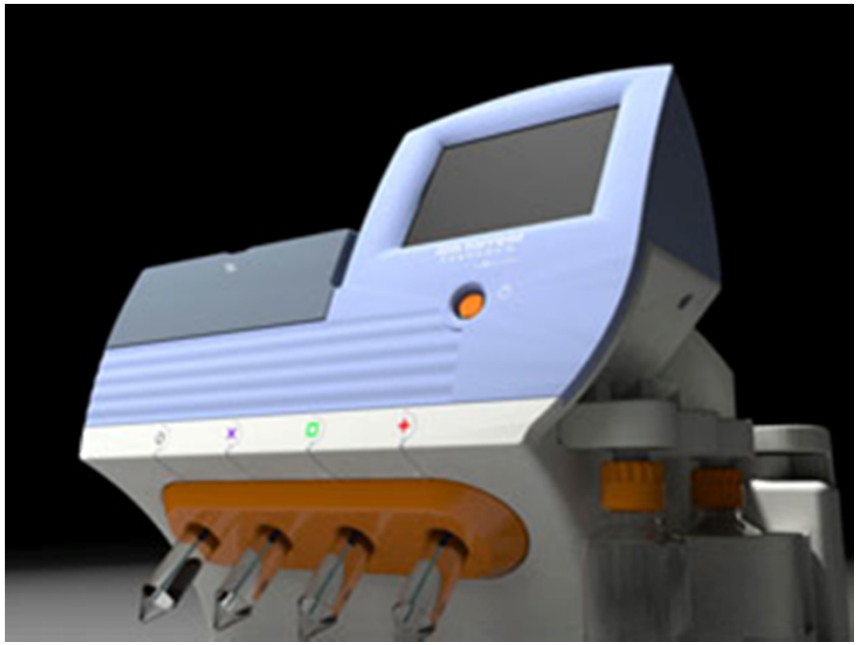
Next Generation Sequencing

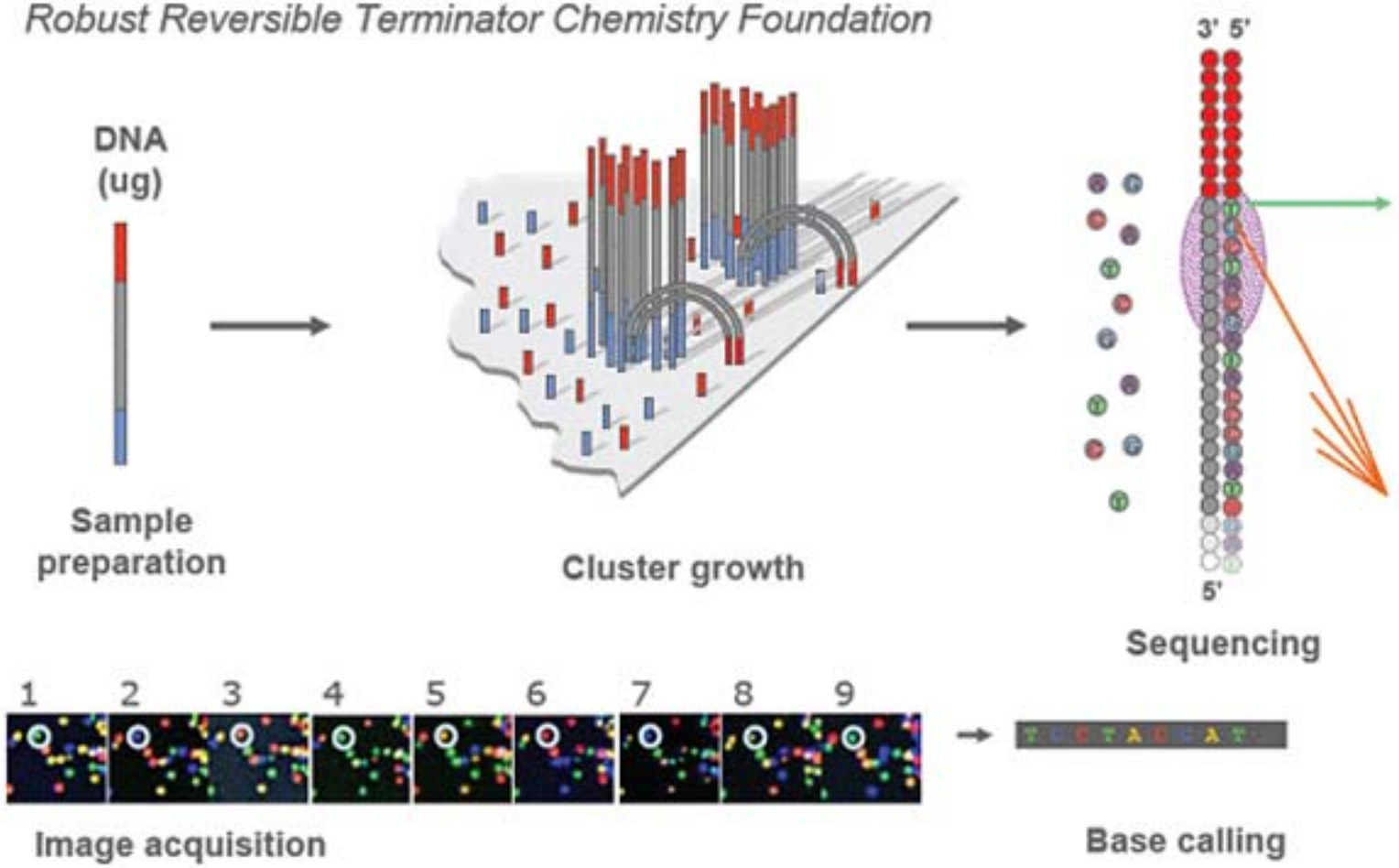


Ion Torrent

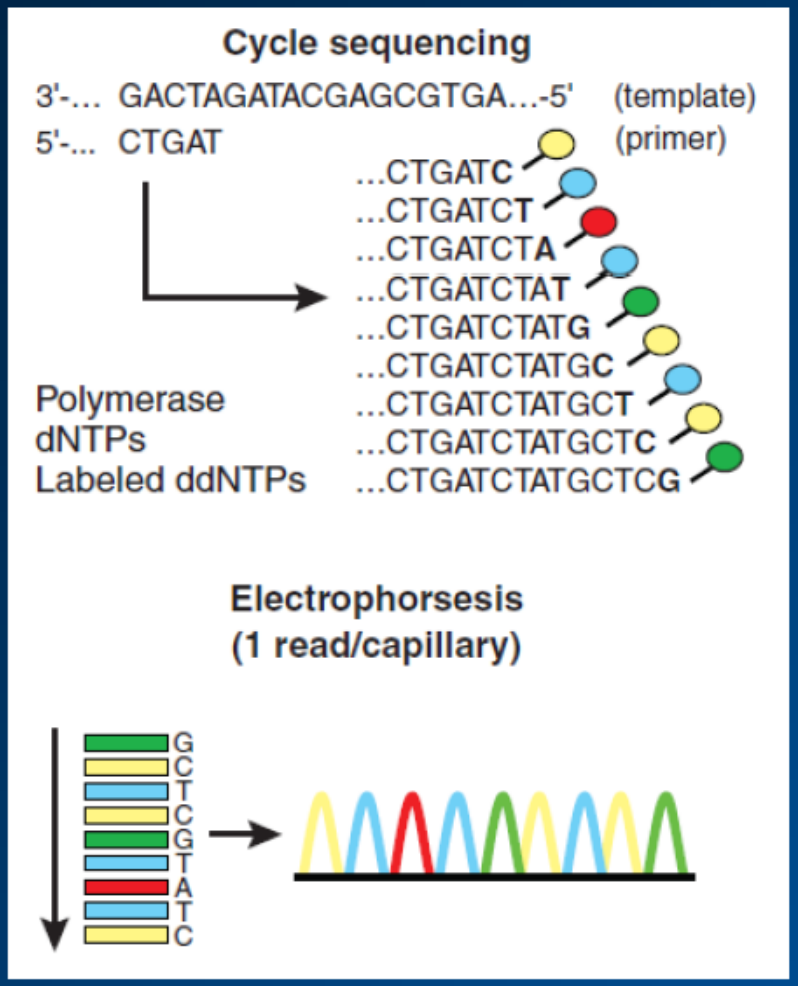
MiSEQ

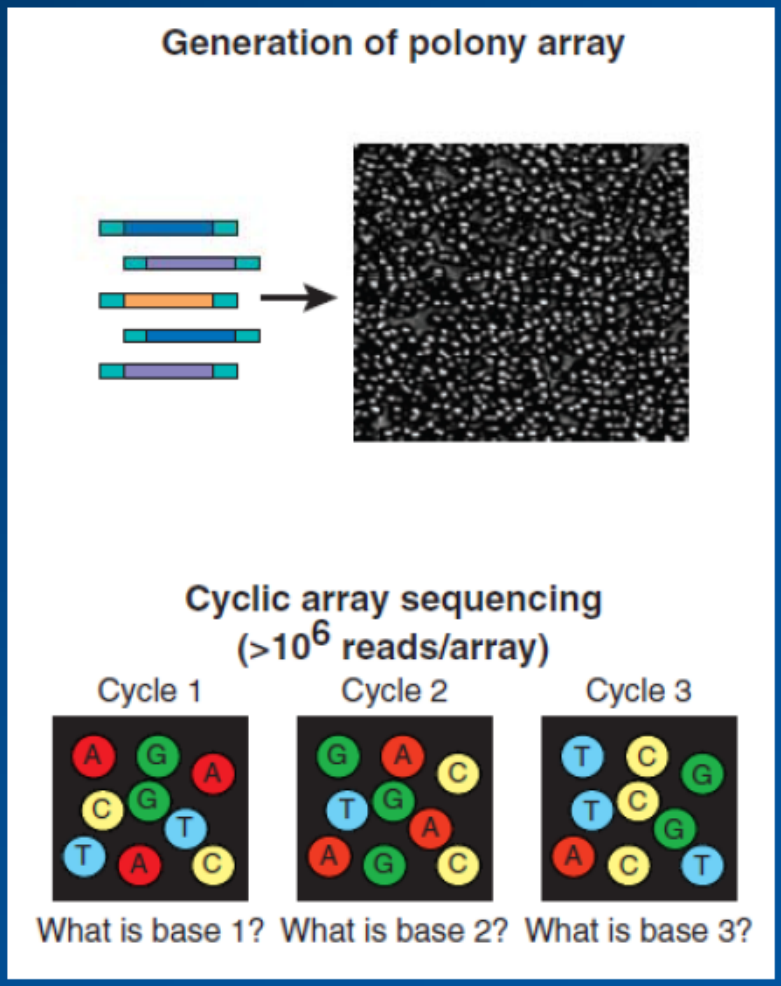
Illumina Sequencing Technology

Robust Reversible Terminator Chemistry Foundation



Sanger vs Next Generation Sequencing





Illumina Genome Analyzer

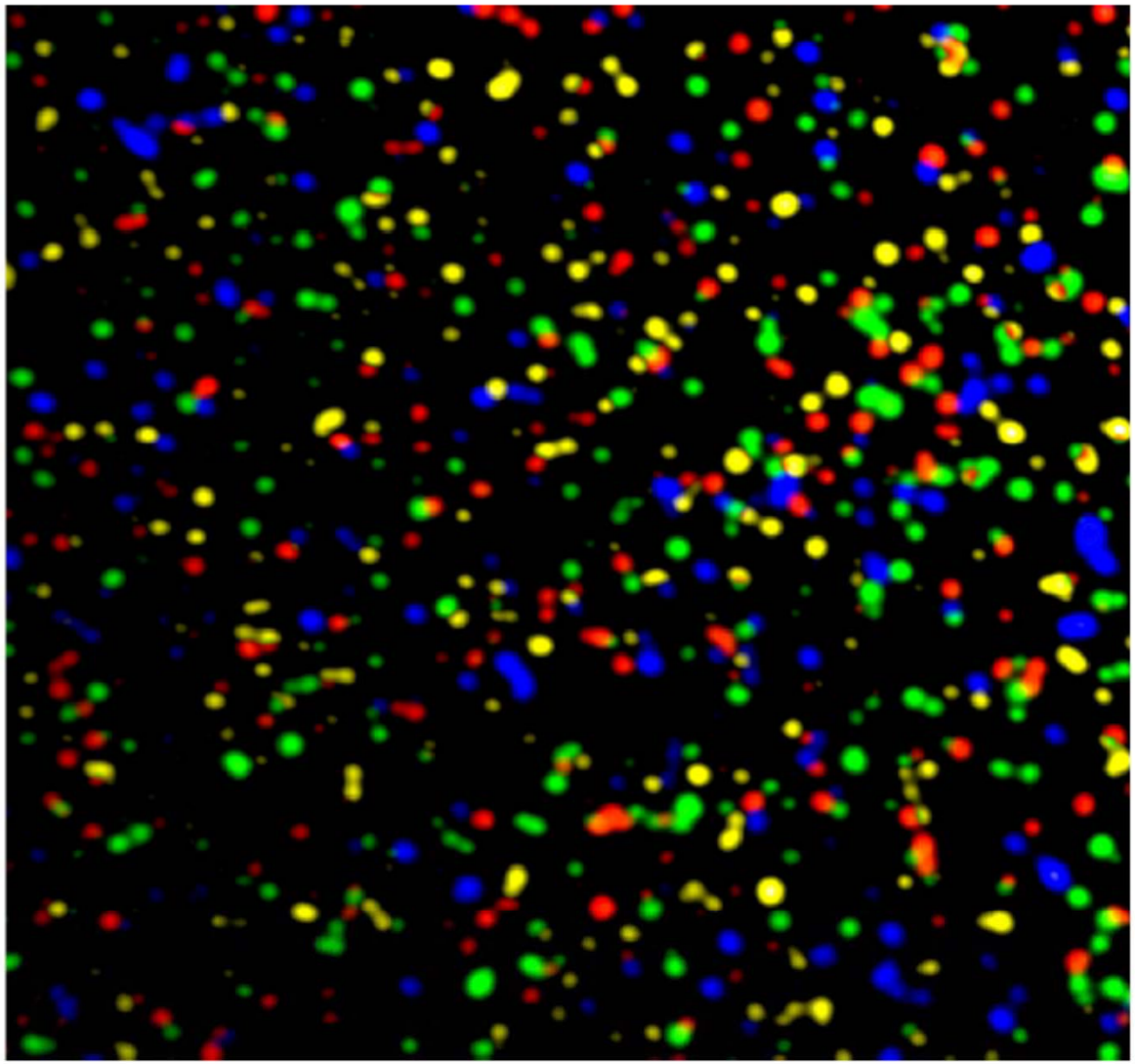
Compiled Image

A

T

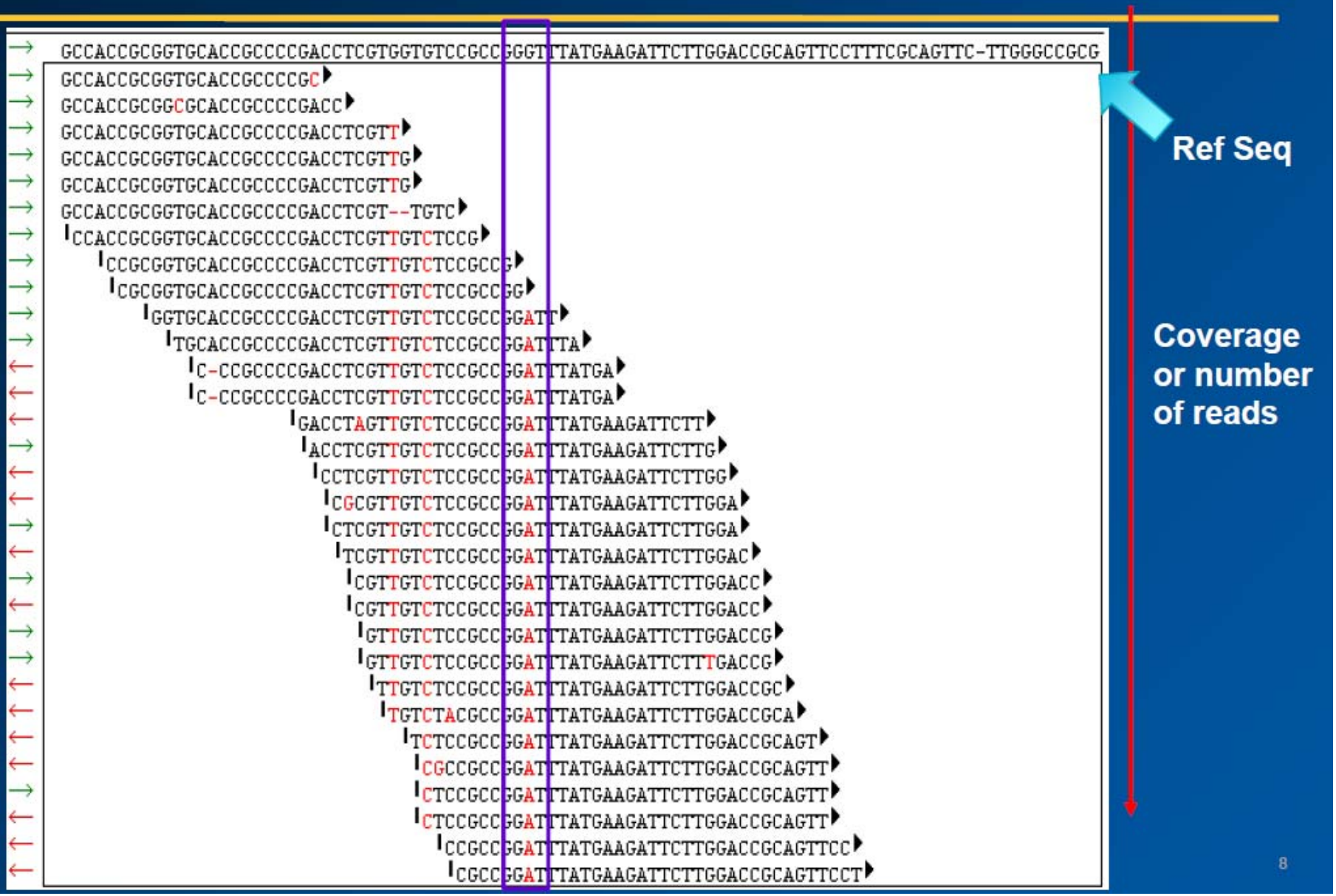
C

G



Flow Cell Clusters

Qualitative and Quantitative Information



Next Generation Sequencing: Steps in Workflow

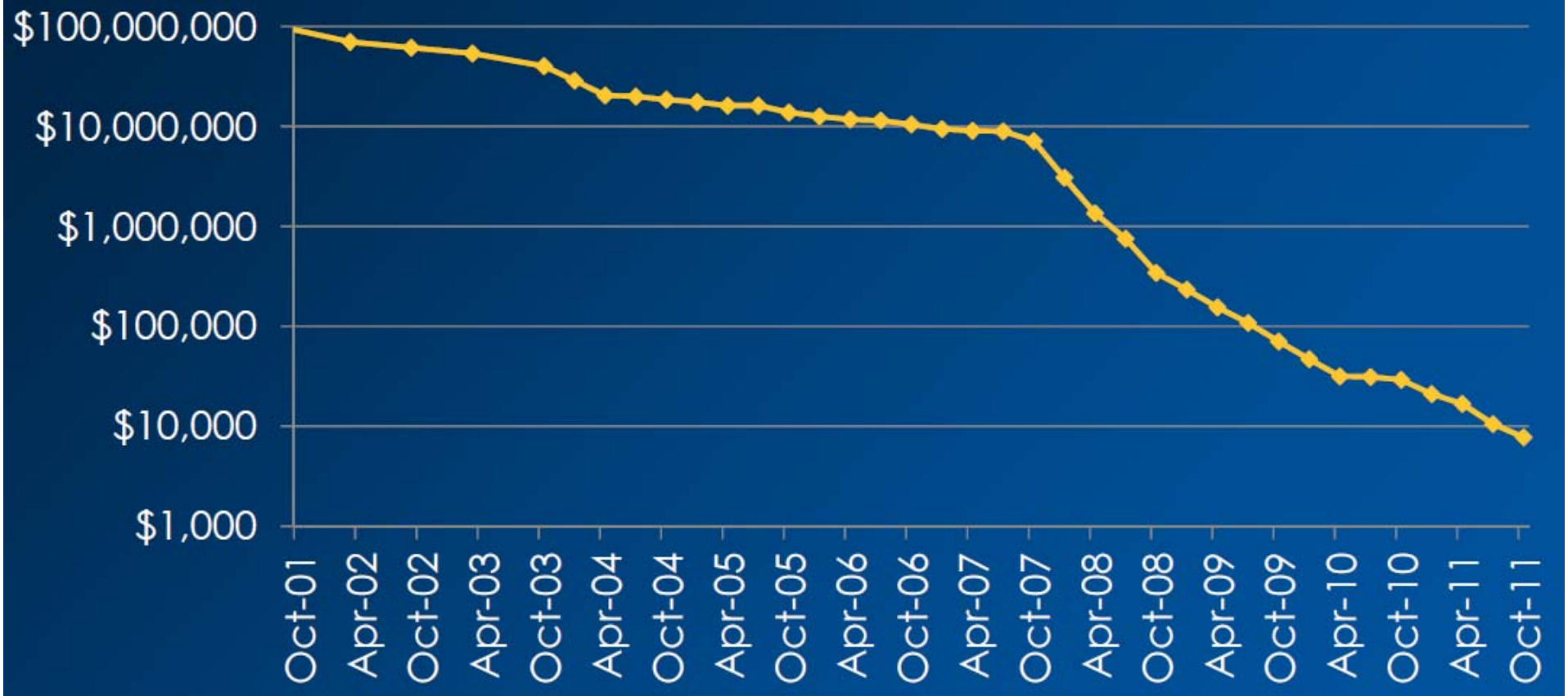
Analytical Wet Bench Process: sample handling, library preparation, sequence generation

Bioinformatics process: Alignment, variant calling, and variant annotation

Clinical Interpretation

The Cost of Genome Sequencing is Decreasing Rapidly and Driving Clinical Adoption of Genomic Analysis

Cost per Genome Data Generation, Sep 2001 – Oct 2011



Cost for genome sequence data generation today is <$3,000

Source: National Human Genome Research Institute

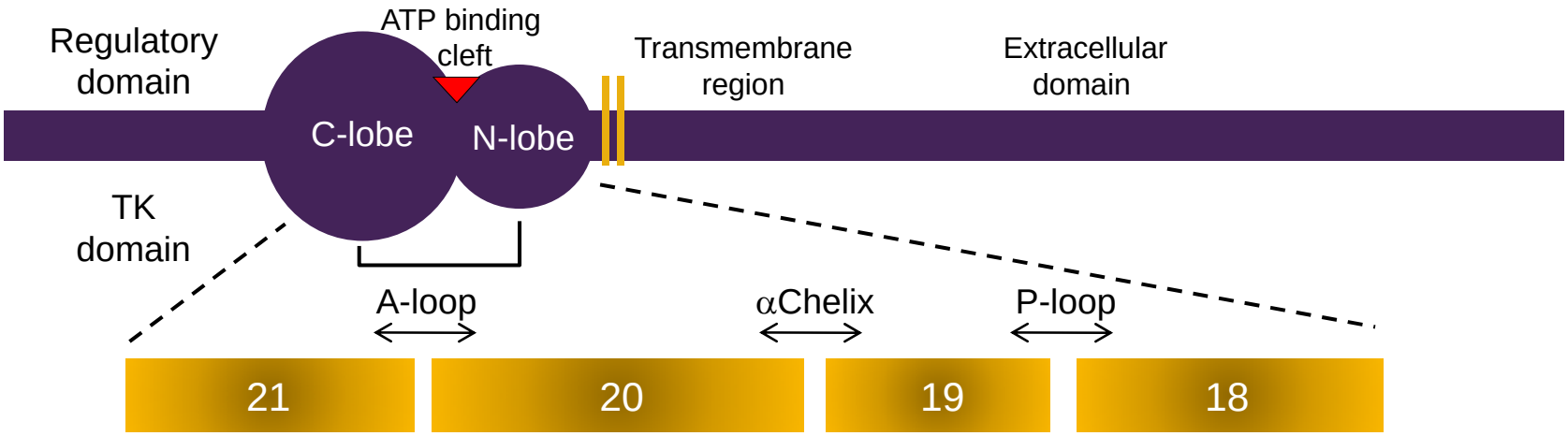
New Clinical Molecular Testing

EGFR as an Example

The significance of EGFR activating mutations

- The EGFR is a transmembrane receptor
- Somatic mutations in the kinase domain increase activity of the EGFR
  - EGFR mutations are 10-15% of cases in North America and Western Europe, but 30%-40% in East Asian
  - Mutations are associated with adenocarcinoma and bronchoalveolar histology
  - Mutations are also observed more frequently in women and non-smokers with NSCLC

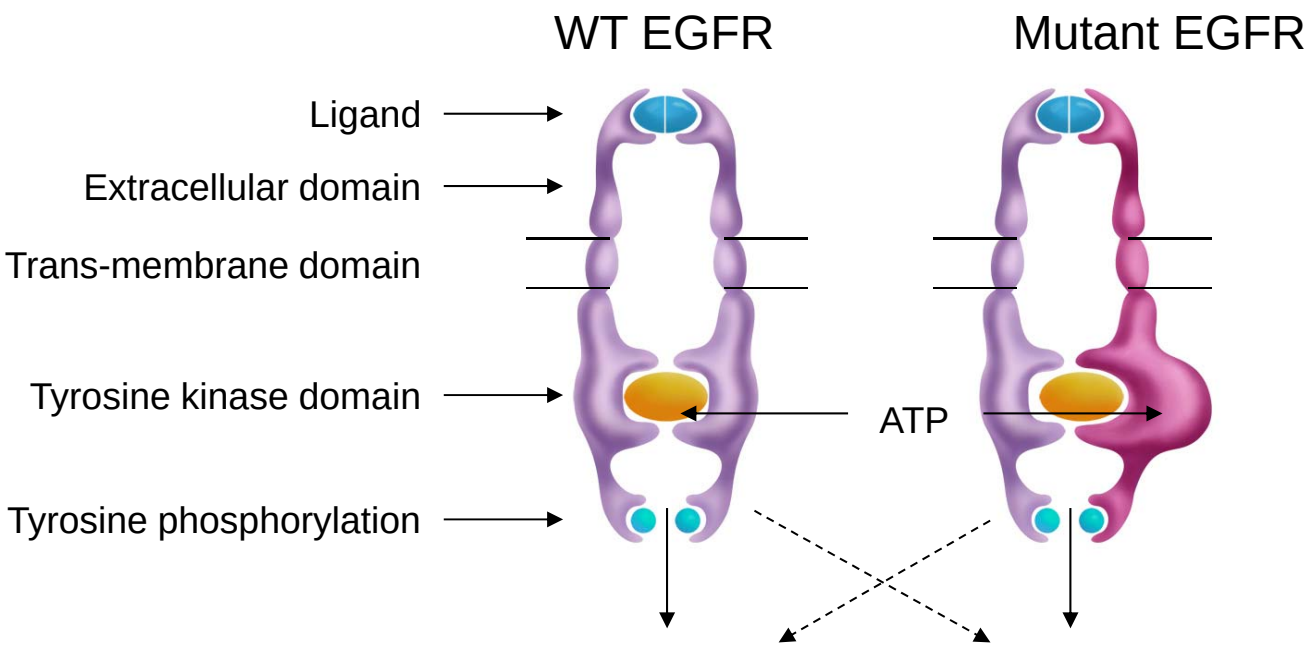
The distribution of activating mutations among EGFR mutation positive patients is similar in Asian and Non Asian studies



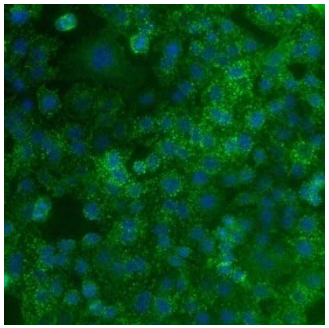
Distribution of mutation types (% of mutations)		
Literature review	**Asian studies**	**Non-Asian studies**
Most prevalent mutation types	**Literature (n=1523)**	**Literature (n=583)**
Exon 19 deletion	**51%**	**58%**
Exon 21 point mutation L858R	**42%**	**32%**
Exon 20	**2%**	**6%**
Exon 18 G719A/C	**3%**	**2%**
Exon 21 L861Q	**1%**	**1%**

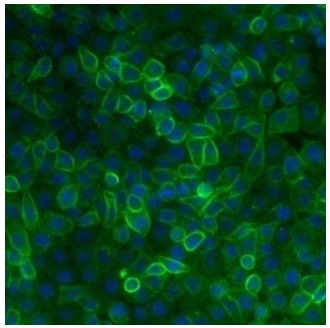
Some patients had more than one mutation type

Mutation status causes conformational change and increased activation



EGFR internalisation
Degradation/recycling





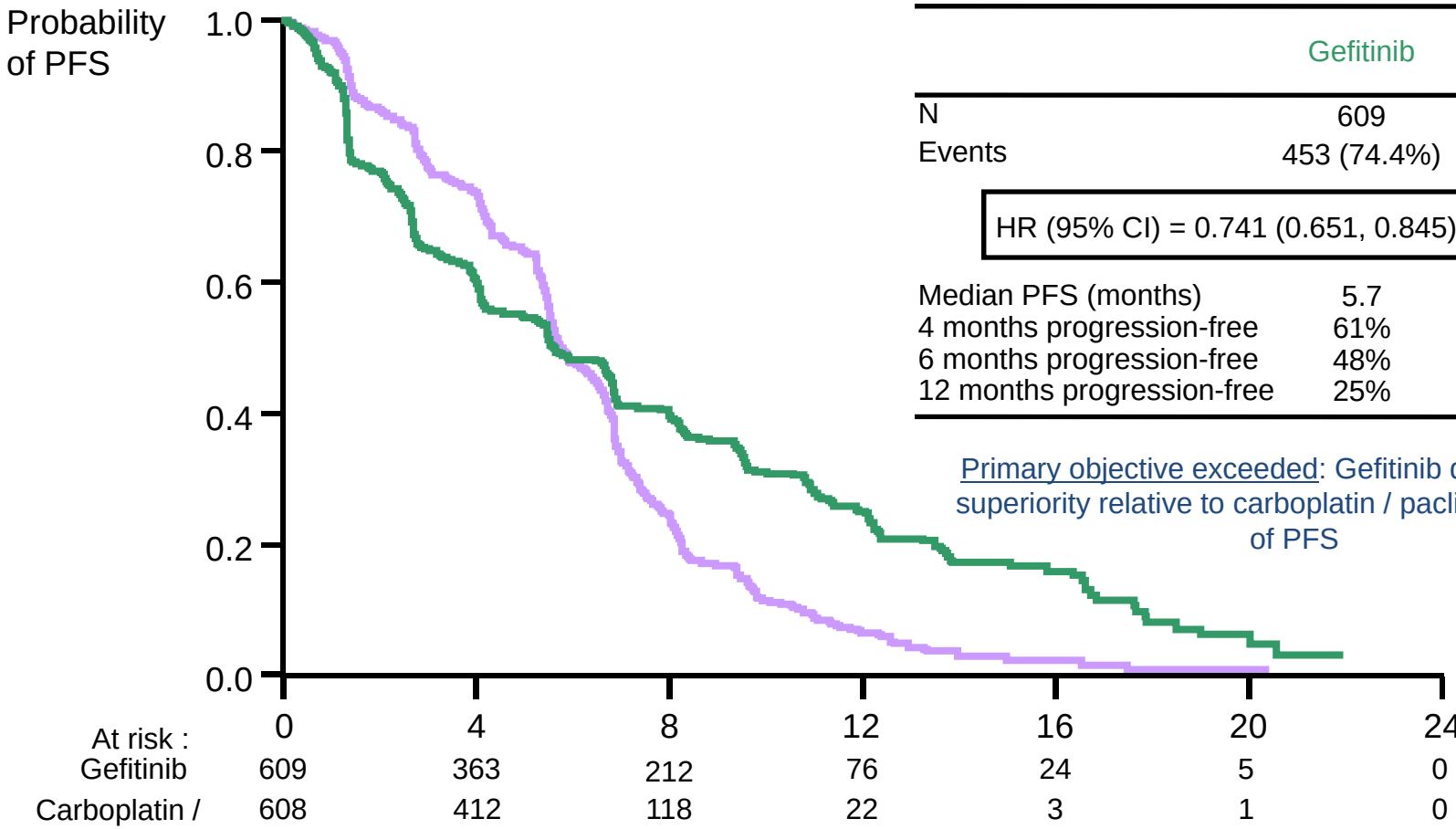
EGFR signals longer
at the cell membrane

Arteaga 2006, Gadzar et al 2004, Hendricks et al 2006, Sordella et al 2004

The IPASS Trial

The Phase III IRESSA Pan-Asia Study (IPASS) compared the efficacy, safety and tolerability of IRESSA vs. carboplatin/paclitaxel in clinically selected chemonaïve patients in Asia with advanced non-small-cell lung cancer (NSCLC).

IPASS: Superior PFS and ORR with gefitinib vs doublet chemotherapy; PFS effect not constant over time



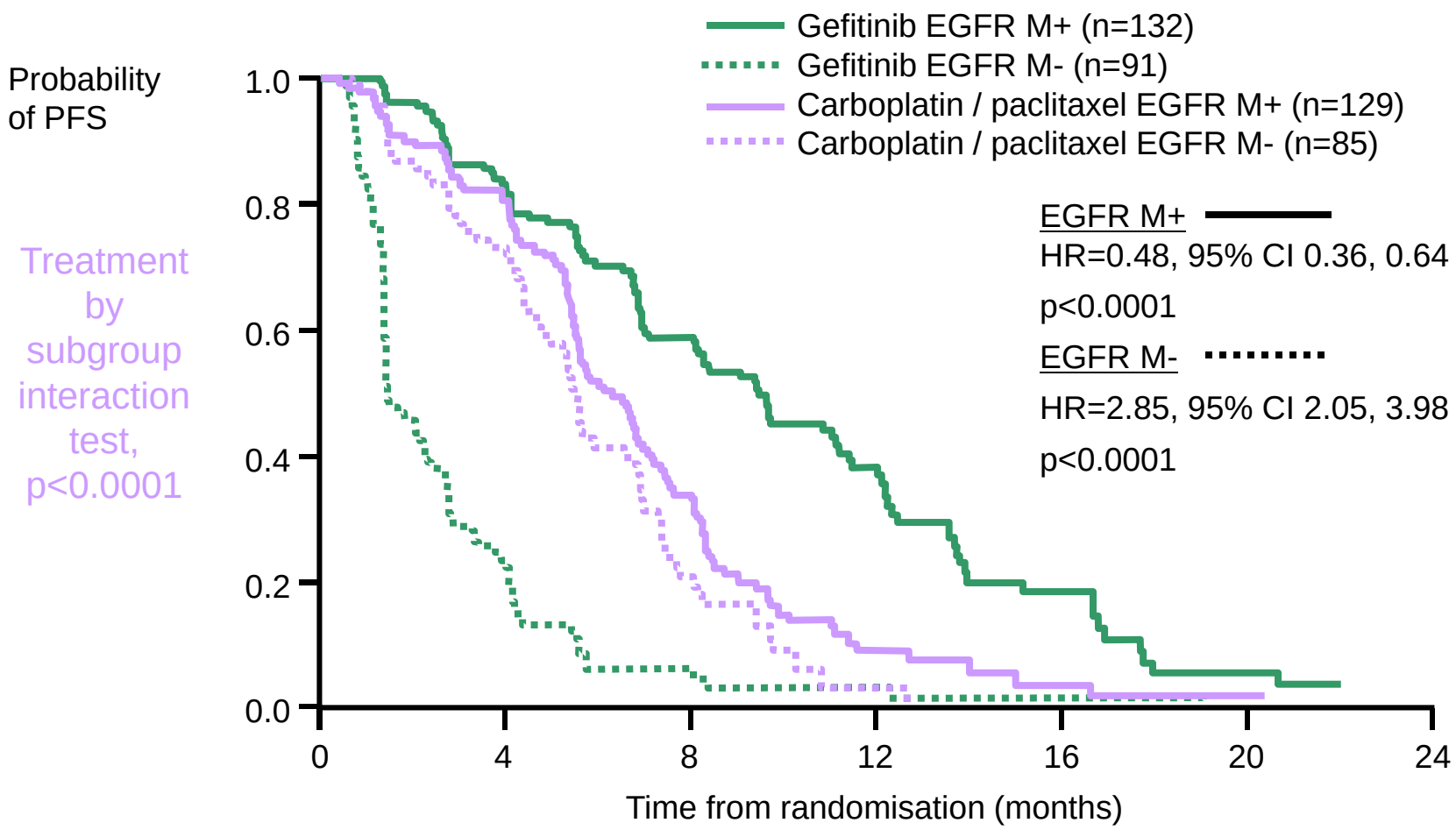
	Gefitinib	Carboplatin / paclitaxel
N	609	608
Events	453 (74.4%)	497 (81.7%)
HR (95% CI) = 0.741 (0.651, 0.845) p<0.0001		
Median PFS (months)	5.7	5.8
4 months progression-free	61%	74%
6 months progression-free	48%	48%
12 months progression-free	25%	7%

Primary objective exceeded: Gefitinib demonstrated superiority relative to carboplatin / paclitaxel in terms of PFS

Objective response rate 43% vs 32% p=0.0001

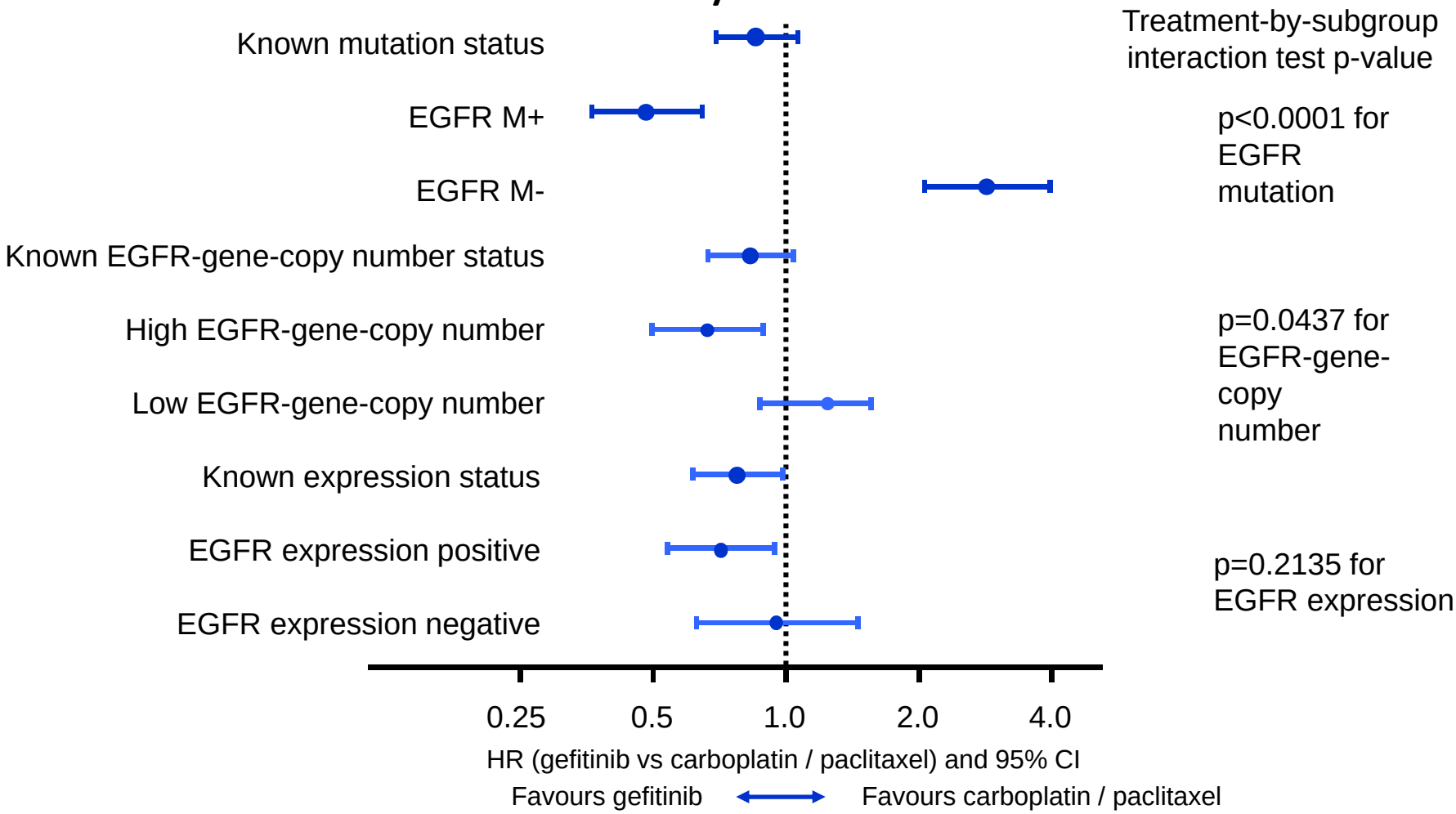
Primary Cox analysis and logistic regression with covariates; ITT population
HR <1 implies a lower risk of progression on gefitinib

IPASS: EGFR mutation is a strong predictor for differential PFS benefit between gefitinib and doublet chemotherapy



M+, mutation positive; M-, mutation negative

IPASS: PFS by biomarkers

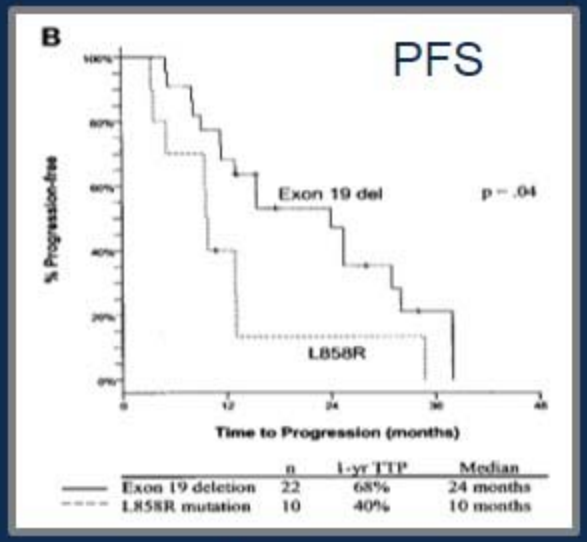


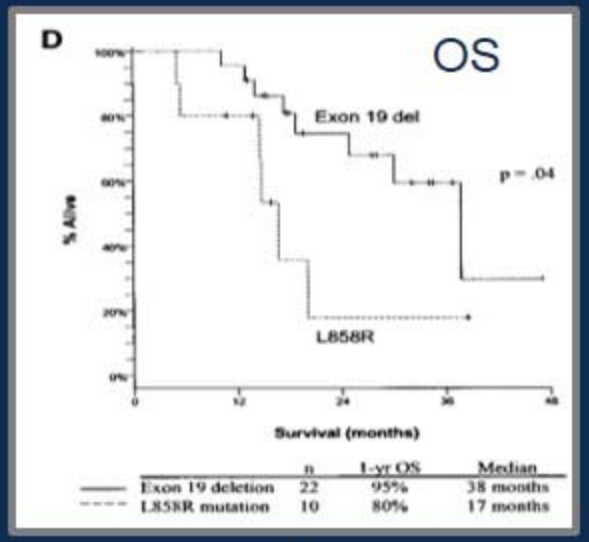
ITT population; Cox analysis with covariates;
HR <1 implies a lower risk of progression on gefitinib

Fukuoka et al 2009

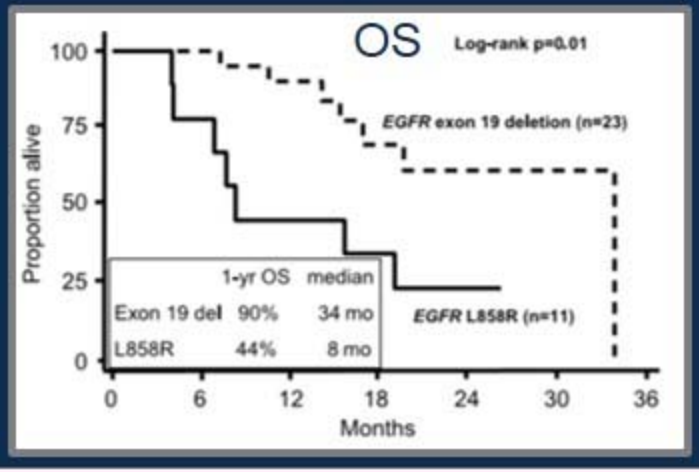
Differences in Efficacy of Gefitinib/ Erlotinib: Exon 19 Del vs. L858R

Jackman,
Clin Cancer Res,
2006





Riely,
Clin Cancer Res,
2006



Presented by: H. Jack West

PRESENTED AT:

ASCO 50th ANNUAL MEETING SCIENCE & SOCIETY

Clinical factors that independently predict EGFR mutation status in Caucasian patients

Analysis in Caucasian patients
(INTEREST, INVITE, ISEL, INTACTs and IDEALs combined [n=786])

- **Smoking status** p<0.0001
  - Odds of mutation 6.5 times higher in never-smokers than ever-smokers
- **Histology** p<0.0001
  - Odds of mutation 4.4 times higher in adeno than non-adeno
- **Gender** p=0.0397
  - Odds of mutation 1.7 times higher in females than males

Other factors tested: Age (<65yrs, ≥ 65yrs), WHO PS (0-1, ≥2)
Overall EGFR mutation positive rate 9.5%

Summary

- EGFR mutation status is the most robust predictive biomarker of clinical benefit in NSCLC
- EGFR mutation status is predictive irrespective of ethnicity
  - Incidence rates may differ, but response rates do not
- Clinical characteristics can not be used to determine EGFR mutation status but may be helpful in determining who to test

Types of Biomarkers

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

Level of Evidence

- Level 1
  - prospective, high power, specifically addressing utility of marker in question
  - meta analysis of several small studies
- Level 2
  - Clinical Trial companion study in which marker is also evaluated
- Level 3
  - performed on assembly of cases taken for other reasons

Integral Markers in Trials

- Marker is used to make a CLINICAL DECISION
  - Assignment to a specific treatment
    - Ie Kinase mutation
  - Witholding treatment
    - Ie Low risk prognostically

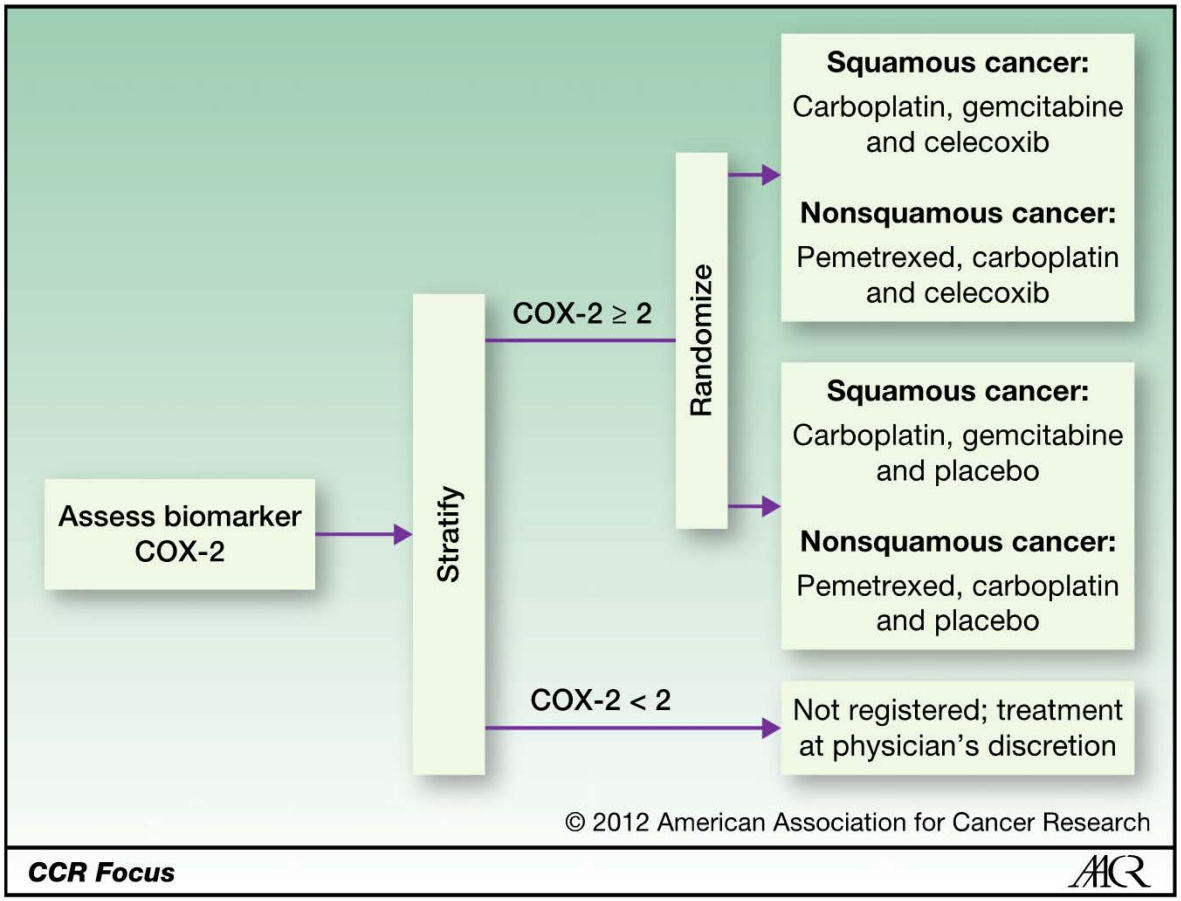
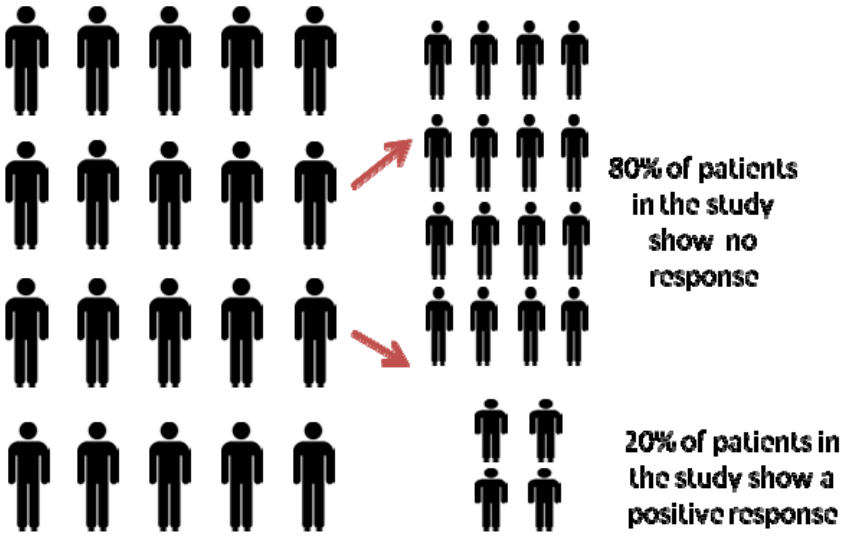


Figure 1. CALGB-30801. Randomized phase III trial for stage IIIB or IV NSCLC (adenocarcinoma, large cell, squamous, or mixture). The trial will allow evaluation of the cut point for the COX-2 assay.

Typical Drug Development Example

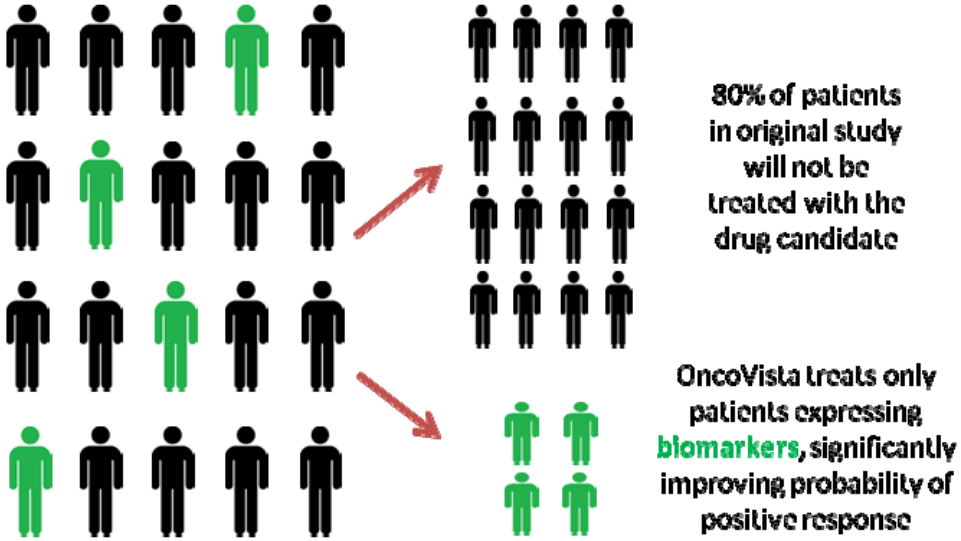
A pharmaceutical company performs trials on patients who have identical cancer diagnosis with a drug candidate



Efficacy below minimum threshold, drug is abandoned

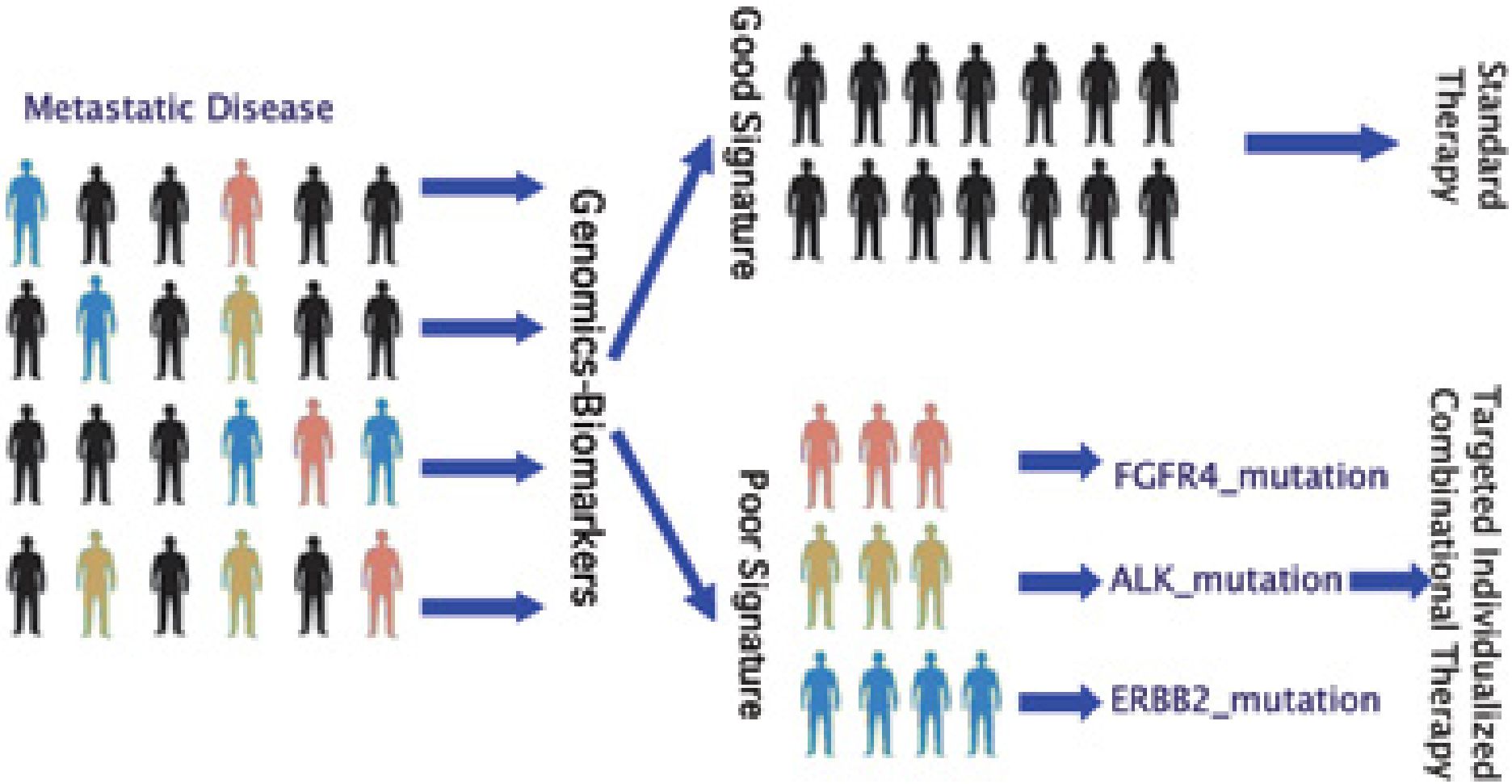
Targeted Drug Development Example

OncoVista licenses a drug candidate & identifies patients expressing biomarkers correlated with drug efficacy



Efficacy exceeds minimum threshold, OncoVista has a viable drug candidate

Personalized medicine–The Goal



FDA, CLIA, and Assays

- FDA Approved
- FDA Cleared

CENTERS FOR MEDICARE & MEDICAID SERVICES
CLINICAL LABORATORY IMPROVEMENT AMENDMENTS
CERTIFICATE OF REGISTRATION

LABORATORY NAME AND ADDRESS
AP LABORATORY OF FLORIDA
13365 OVERSEAS HWY STE 104
MARATHON, FL 33050

CLIA ID NUMBER
10D1097203

EFFECTIVE DATE
03/20/2009

LABORATORY DIRECTOR
SEAN M KAUFMAN MD

EXPIRATION DATE
03/19/2011

Pursuant to Section 353 of the Public Health Services Act (42 U.S.C. 263a) as revised by the Clinical Laboratory Improvement Amendments (CLIA), the above named laboratory located at the address shown hereon (and other approved locations) may accept human specimens for the purposes of performing laboratory examinations or procedures.
This certificate shall be valid until the expiration date above, but is subject to revocation, suspension, limitation, or other sanctions for violation of the Act or the regulations promulgated thereunder.

CMS

Judith A. Yost, Director
Division of Laboratory Services
Survey and Certification Group
Center for Medicaid and State Operations

493 certs1_032809

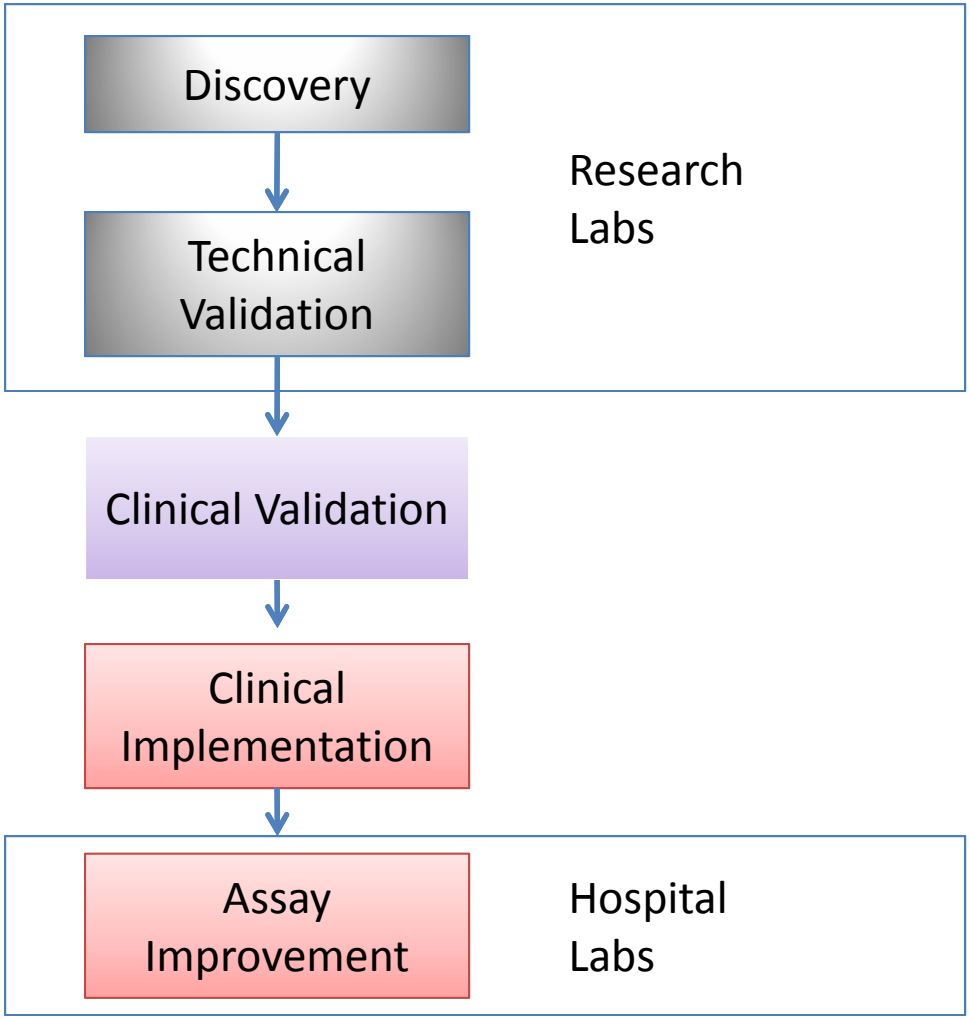
- If this is a Certificate of Registration, it represents only the enrollment of the laboratory in the CLIA program and does not indicate a Federal certification of compliance with other CLIA requirements. The laboratory is permitted to begin testing upon receipt of this certificate, but is not determined to be in compliance until a survey is successfully completed.
- If this is a Certificate for Provider-Performed Microscopy Procedures, it certifies the laboratory to perform only those laboratory procedures that have been specified as provider-performed microscopy procedures and, if applicable, examinations or procedures that have been approved as waived tests by the Department of Health and Human Services.
- If this is a Certificate of Waiver, it certifies the laboratory to perform only examinations or procedures that have been approved as waived tests by the Department of Health and Human Services.

CMS

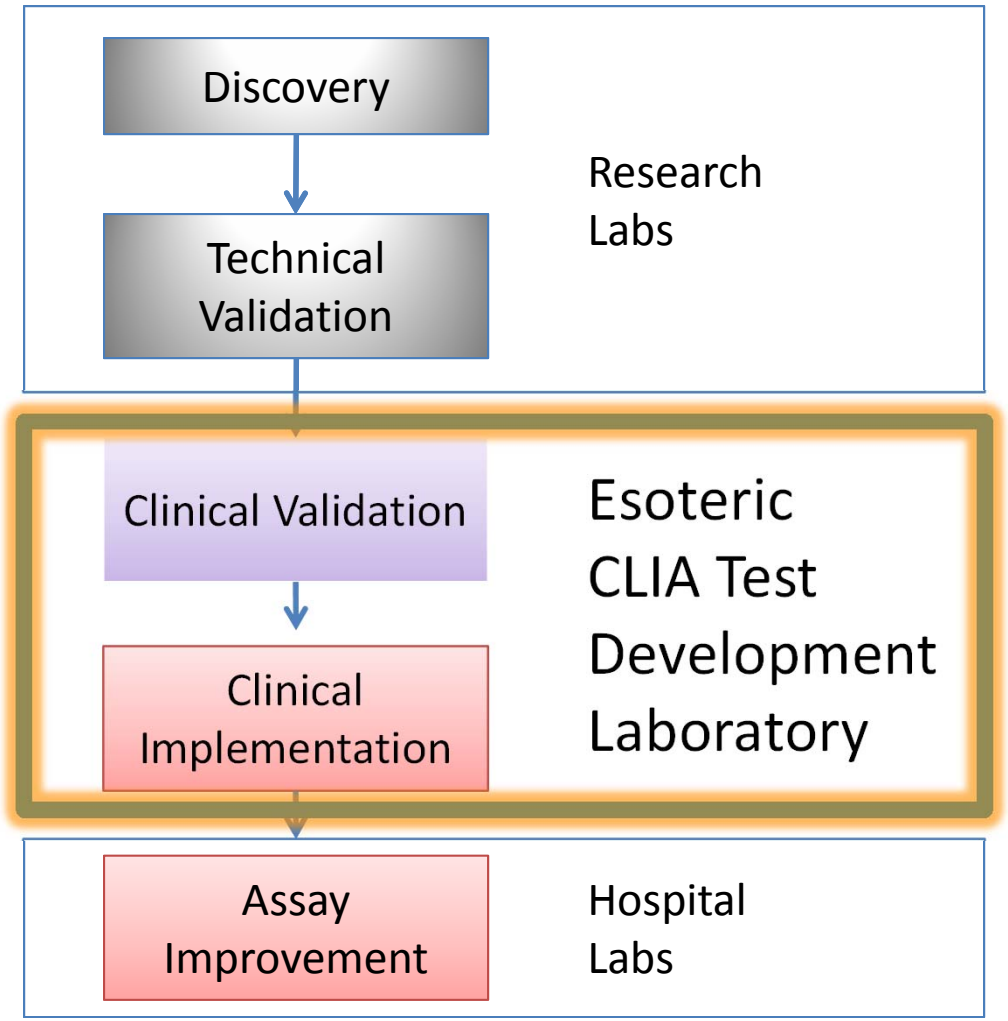
FOR MORE INFORMATION ABOUT CLIA, VISIT OUR WEBSITE AT WWW.CMS.HHS.GOV/CLIA
OR CONTACT YOUR LOCAL STATE AGENCY. PLEASE SEE THE REVERSE FOR
YOUR STATE AGENCY'S ADDRESS AND PHONE NUMBER.
PLEASE CONTACT YOUR STATE AGENCY FOR ANY CHANGES TO YOUR CURRENT CERTIFICATE.

- LDTs (Laboratory Developed Assays)

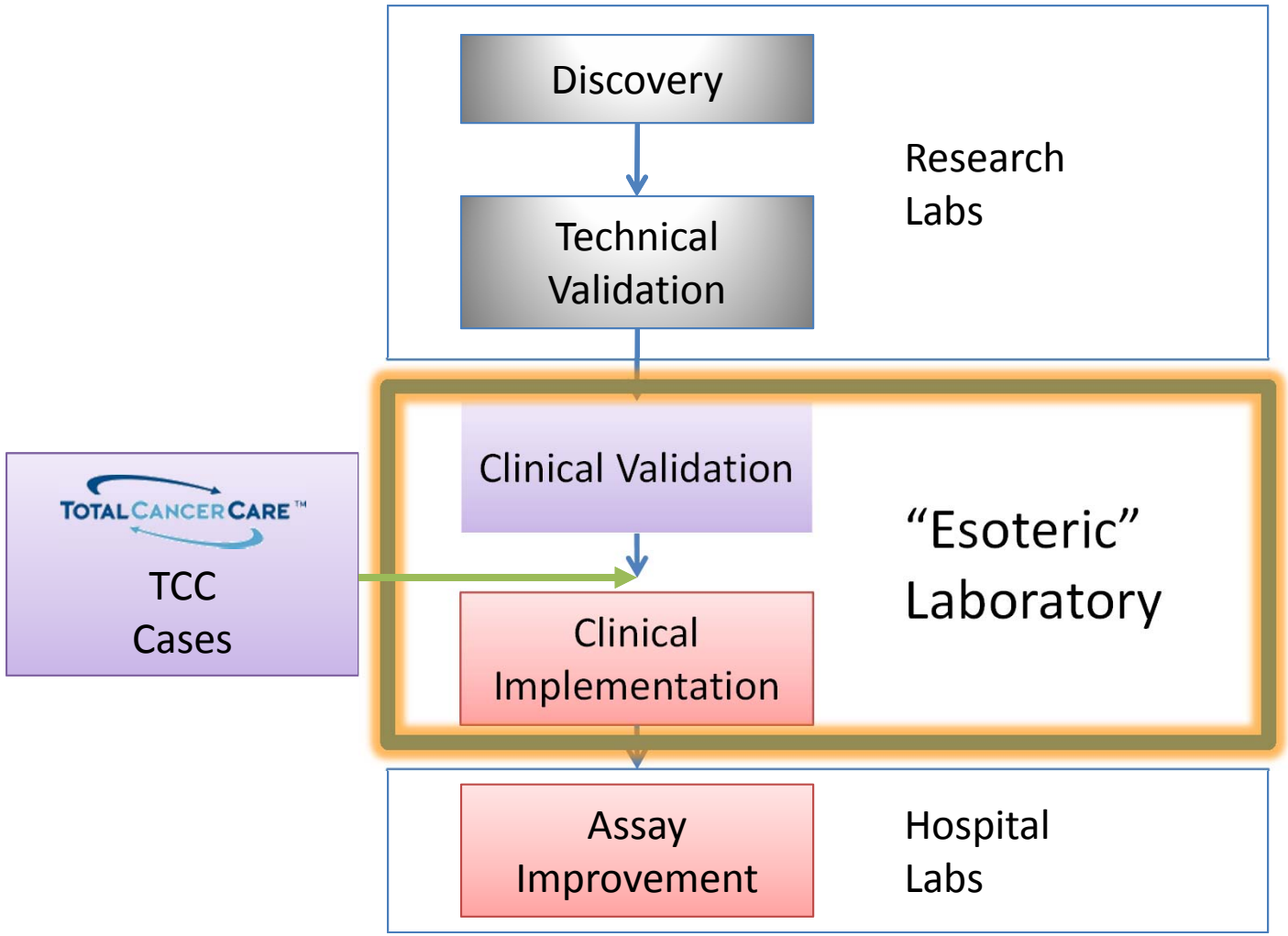
Development of a Clinical Assay



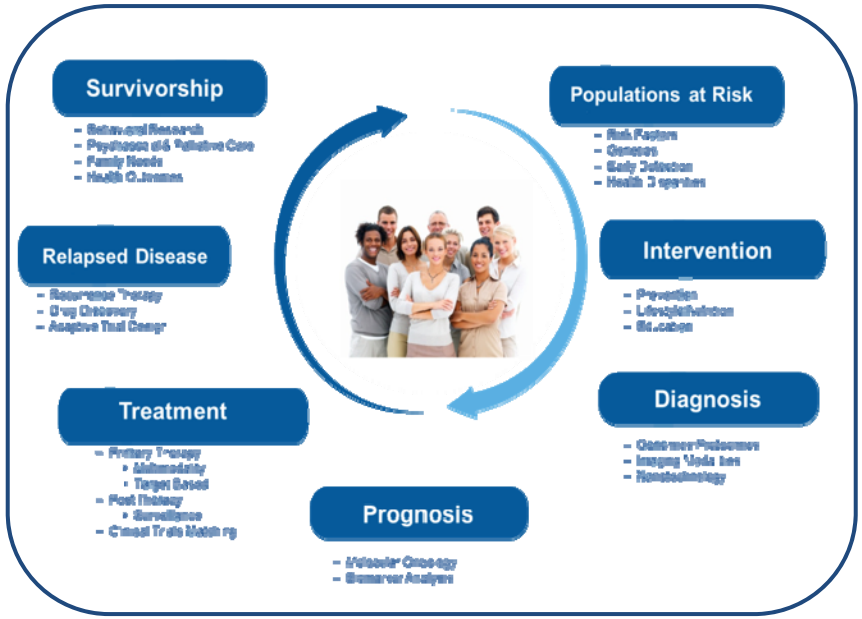
Product Cycle of Biomarker



Product Cycle of Biomarker



Vision of Total Cancer Care



- Identify the needs of the patient & their families
- Develop an evidence-based approach to meet those needs
- Develop markers to predict need so they can be prevented

The TCC Protocol

The Total Cancer Care™ Protocol

- May we follow you throughout your lifetime?
- May we study your tumor using molecular technology?
- May we re-contact you?

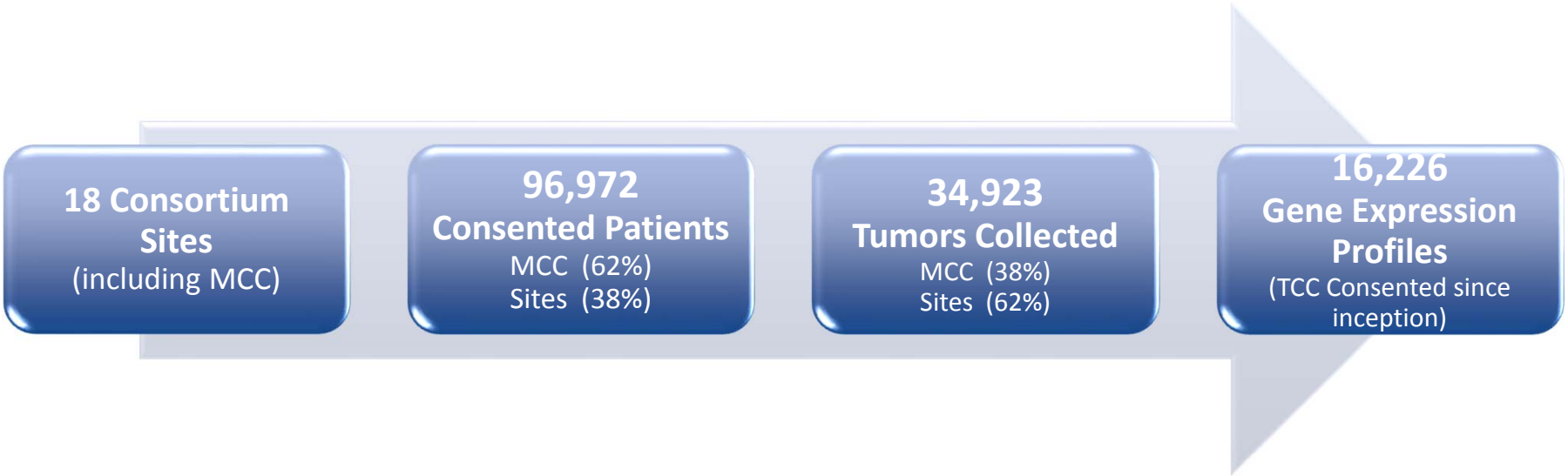
The Total Cancer Care Protocol represents Moffitt's unique approach to Personalized Medicine, and is the foundation upon which M2Gen is built.

The TCC Consortium

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

- Hartford Hospital, Hartford, CT
- St. Joseph's Candler Health System, Savannah, GA
- Greenville Hospital System, Greenville, SC
- Moffitt Cancer Center, Tampa, FL (Coordinating Site)
- Baptist Health South Florida, Miami, FL
- Martin Memorial Medical Center, Stuart, FL
- Morton Plant Mease Health Care, Clearwater, FL
- Sarasota Memorial Health Care, Sarasota, FL
- Watson Clinic Center for Research, Lakeland, FL
- Norton Healthcare, Louisville, KY
- Lehigh Valley Health Network, Allentown, PA
- Billings Clinic, Billings, MT

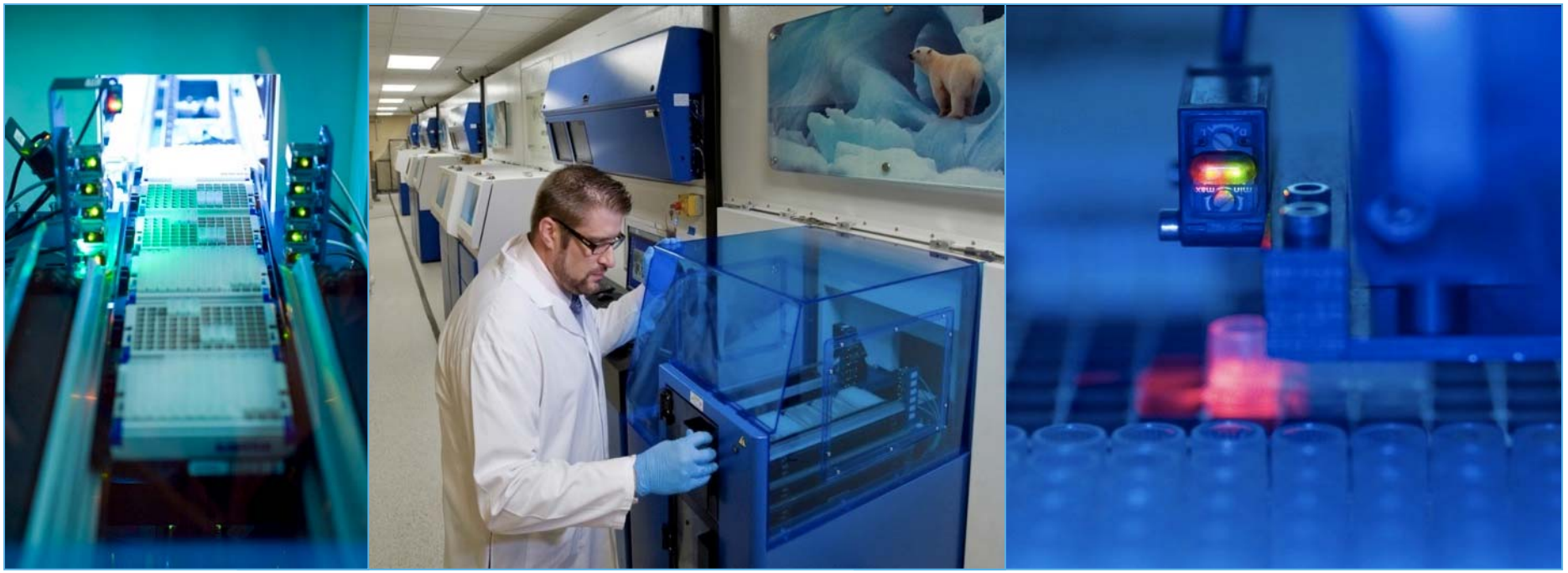
Total Cancer Care™ to Date



As of May 29, 2013

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

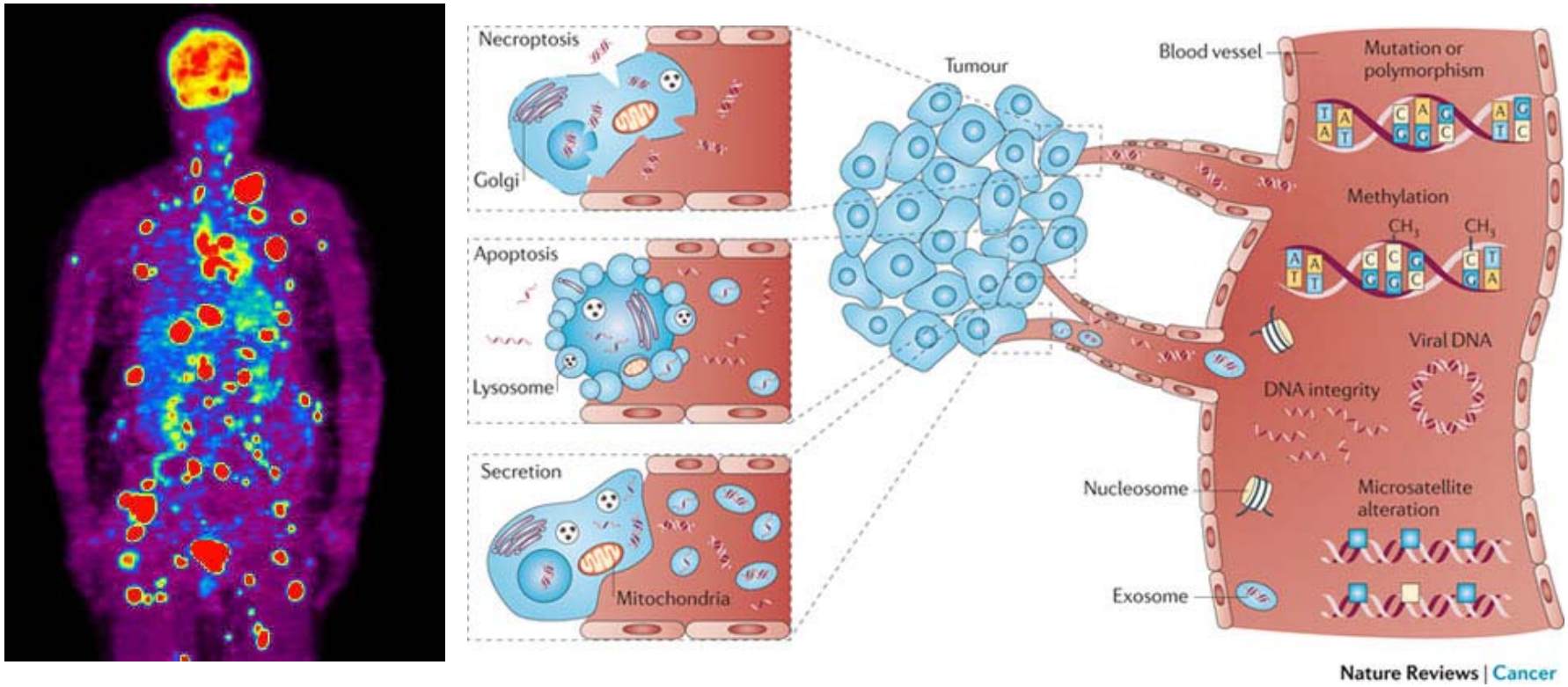
Nexus Biostore



- **Four unit capacity** of 2.4 Million samples
- **Stores** samples in a -80°C environment
- **Handles** samples in a -20°C environment
- **Retrieves** samples using NEXUS proprietary **'Cool Transition'** technology
- **Flexibility** to accommodate a wide variety of samples, vessels and labware
- **Automated** 24/7 monitoring system in place
- **Automated Inventory** functionality provides real-time inventory tracking of stored biospecimens

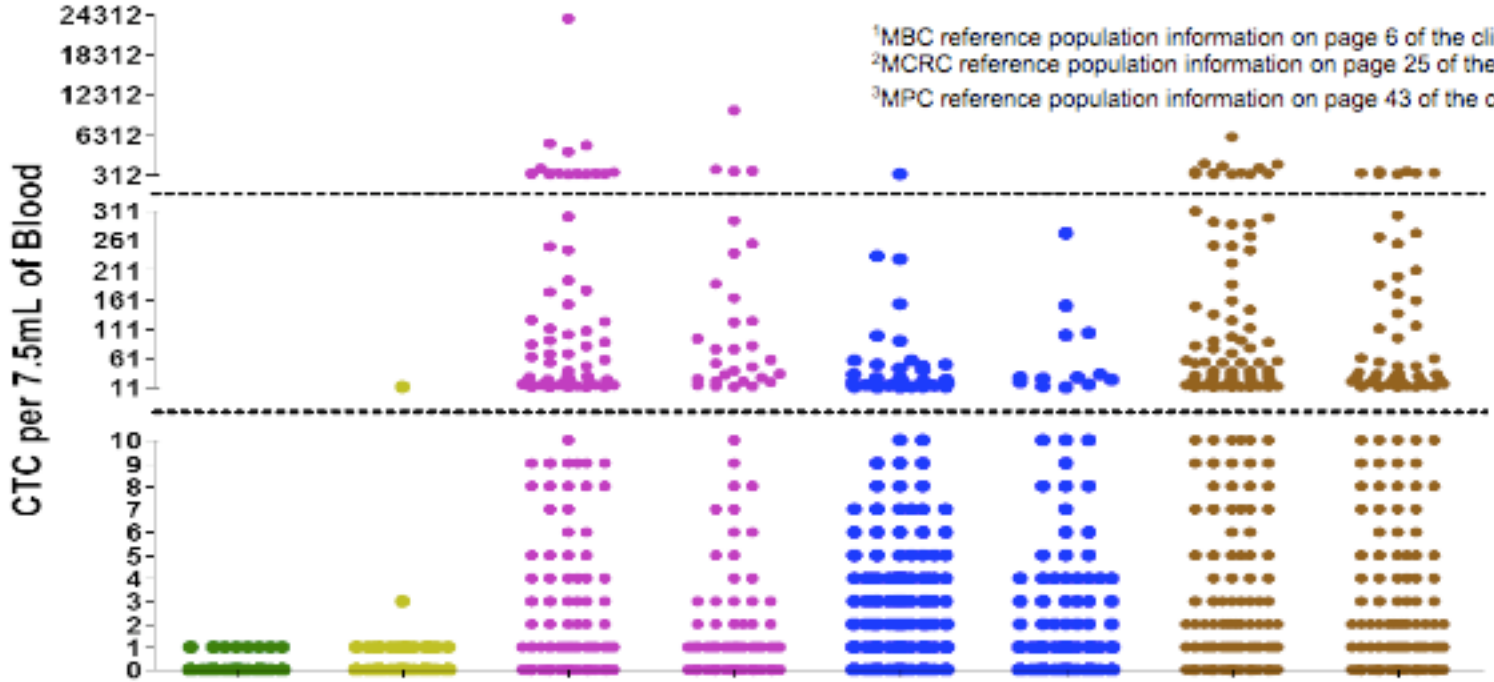
CIRCULATING TUMOR CELLS

METASTASIS


Necroptosis
Golgi
Apoptosis
Lysosome
Secretion
Mitochondria
Tumour
Blood vessel
Mutation or polymorphism
Methylation
CH3
CH3
Viral DNA
DNA integrity
Nucleosome
Microsatellite alteration
Exosome
Nature Reviews | Cancer

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.

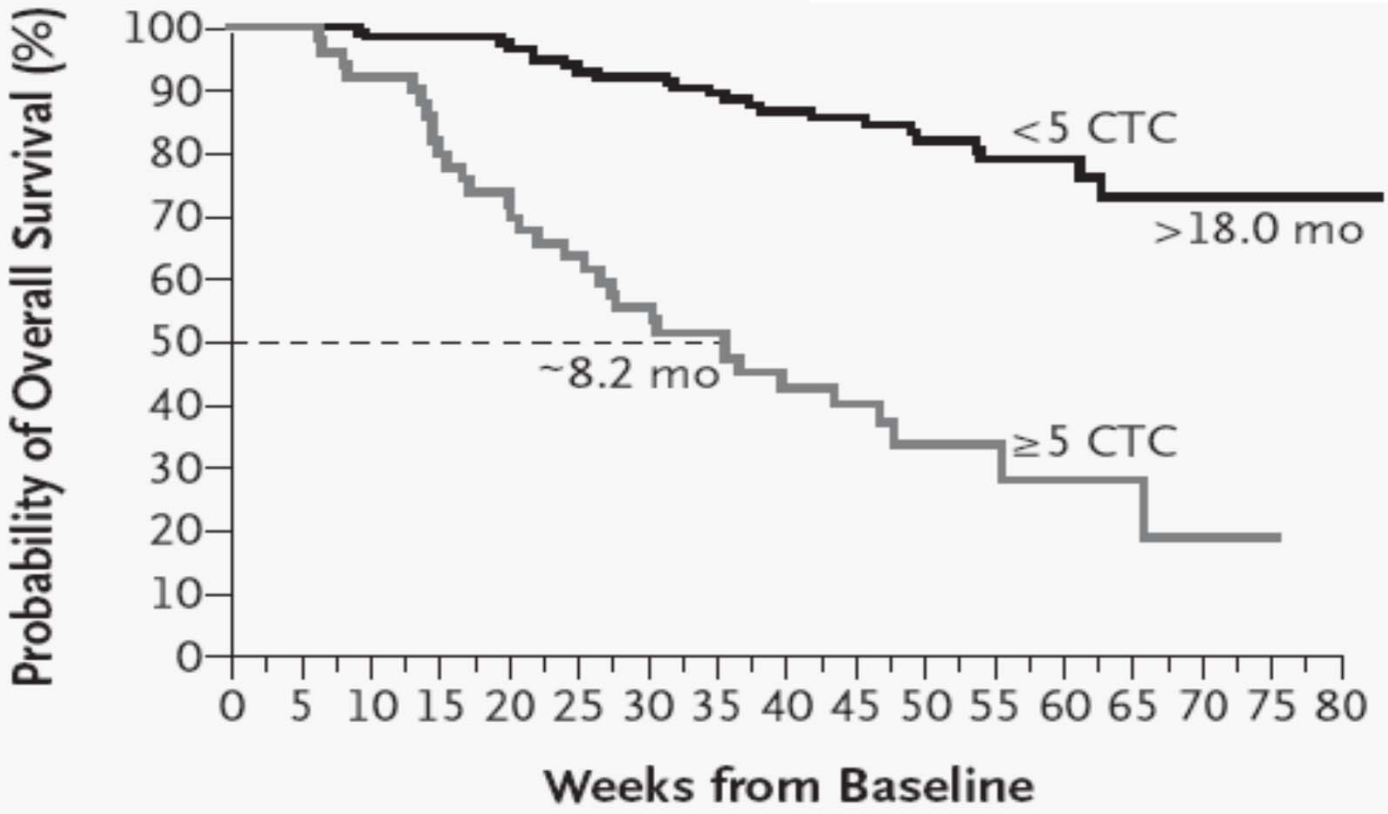


# of Cases	Healthy Controls	Benign Diseases	MBC Patients[1] Baseline	MBC Patients[1] 3-5 Weeks	MCRC Patients[2] Baseline	MCRC Patients[2] 3-5 Weeks	MPC Patients[3] Baseline	MPC Patients[3] 2-5 Weeks
	296	266	177	132	413	334	219	203
N (%) ≥1 CTC	10 (3%)	19 (7%)	125 (71%)	73 (55%)	196 (47%)	89 (27%)	170 (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%)	108 (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%)

Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer

Massimo Cristofanilli, M.D., G. Thomas Budd, M.D., Matthew J. Ellis, M.B., Ph.D., Alison Stopeck, M.D., Jeri Matera, B.S., R.Ph., M. Craig Miller, B.S., James M. Reuben, Ph.D., Gerald V. Doyle, D.D.S., W. Jeffrey Allard, Ph.D., Leon W.M.M. Terstappen, M.D., Ph.D., and Daniel F. Hayes, M.D.

F Full Set of Data



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

N Engl J Med 2004;351:781-91.

CellSearch™ System

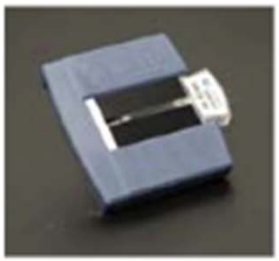
CellSave Tube

CellSearch™

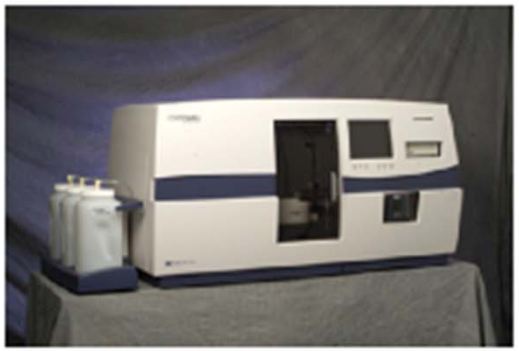
Circulating Tumor Cell Kit

Circulating Tumor Cell Control Kit

MagNest®

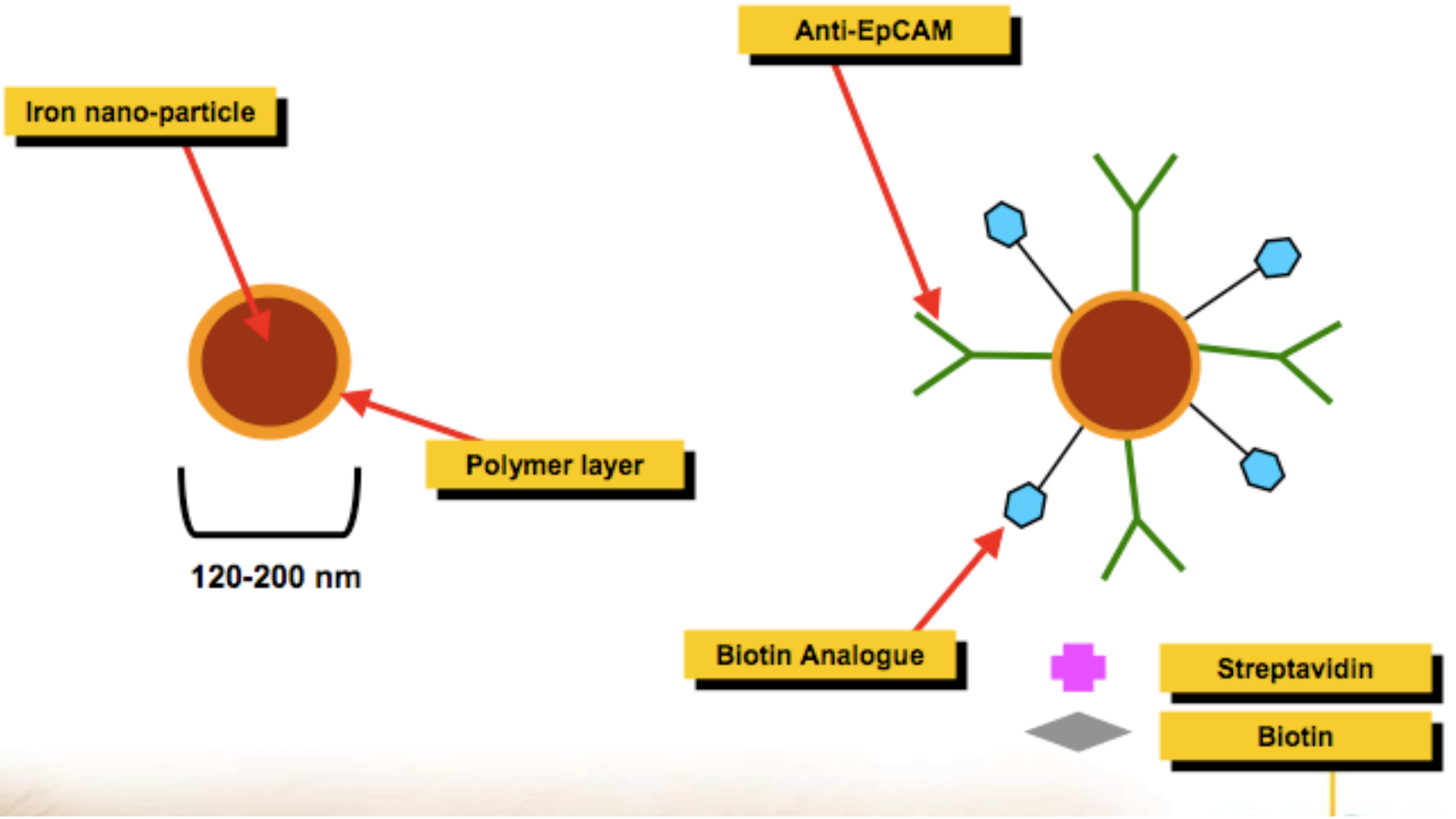


CellTracks® AutoPrep® System



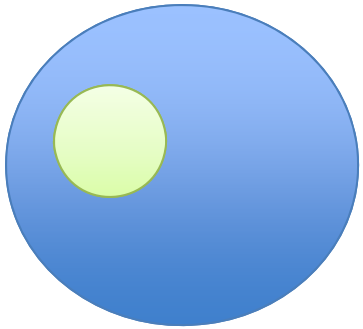
CellTracks® Analyzer II

Anatomy of Ferrofluid

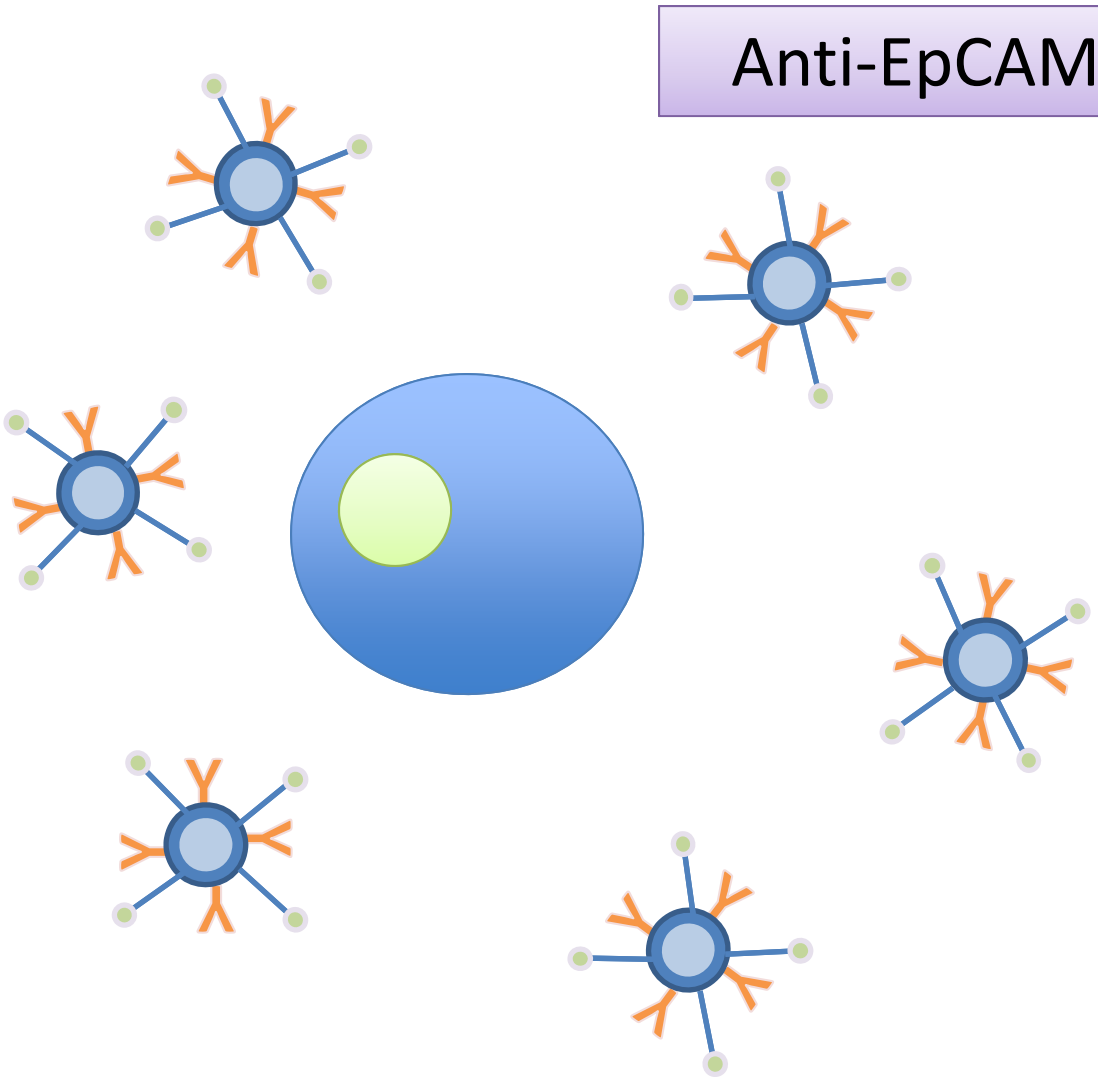


Automated Optimization of Ferrofluid Binding Activity

Circulating Tumor Cell

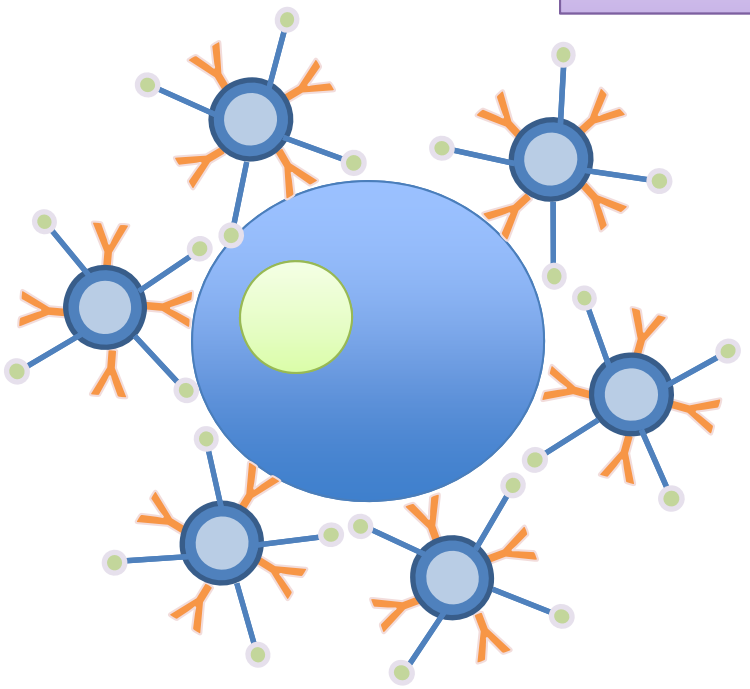


Automated Optimization of Ferrofluid Binding Activity

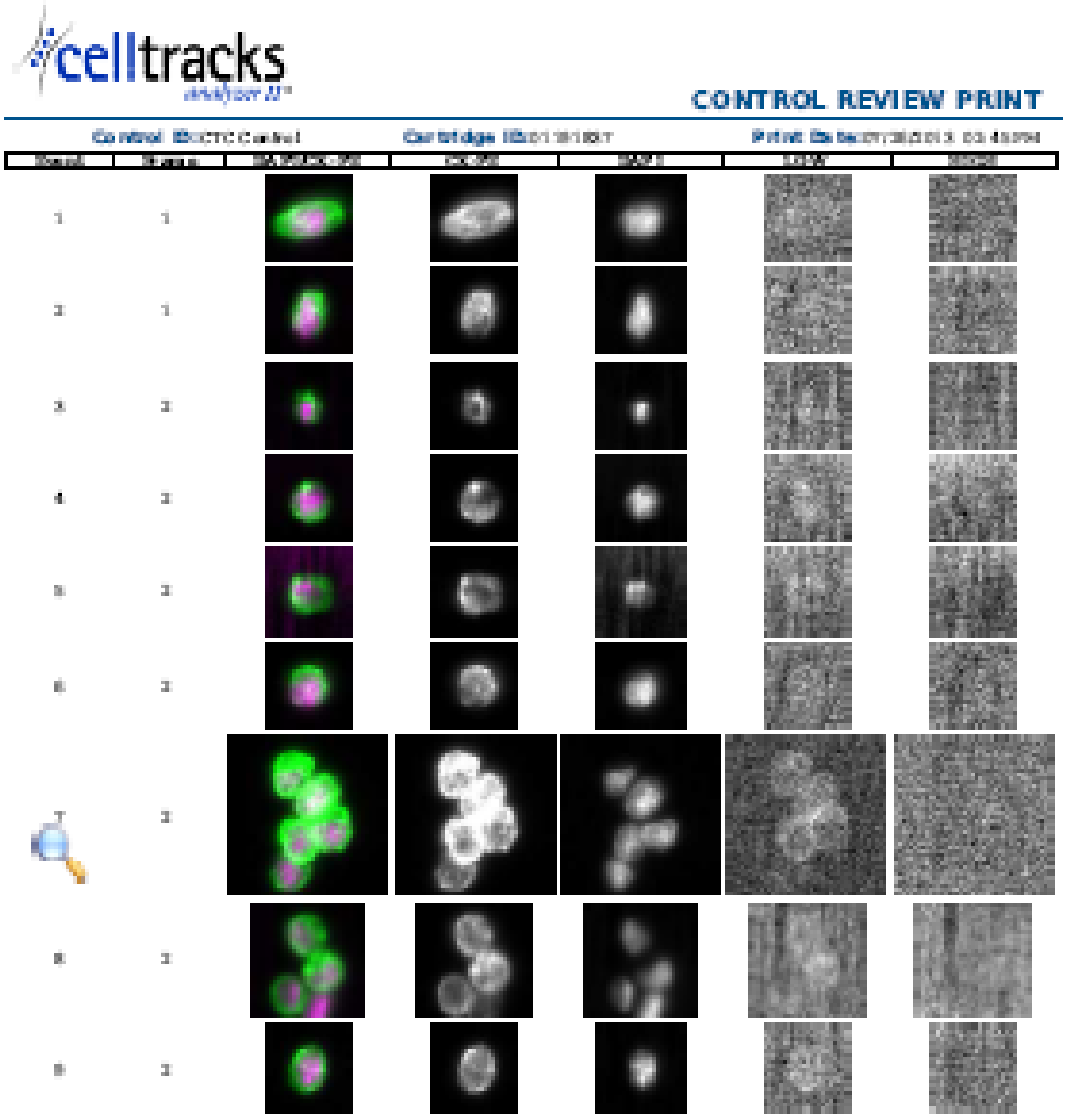


Automated Optimization of Ferrofluid Binding Activity

Anti-EpCAM-Ferrofluid binds to CTCs



Control HCT-116 cells

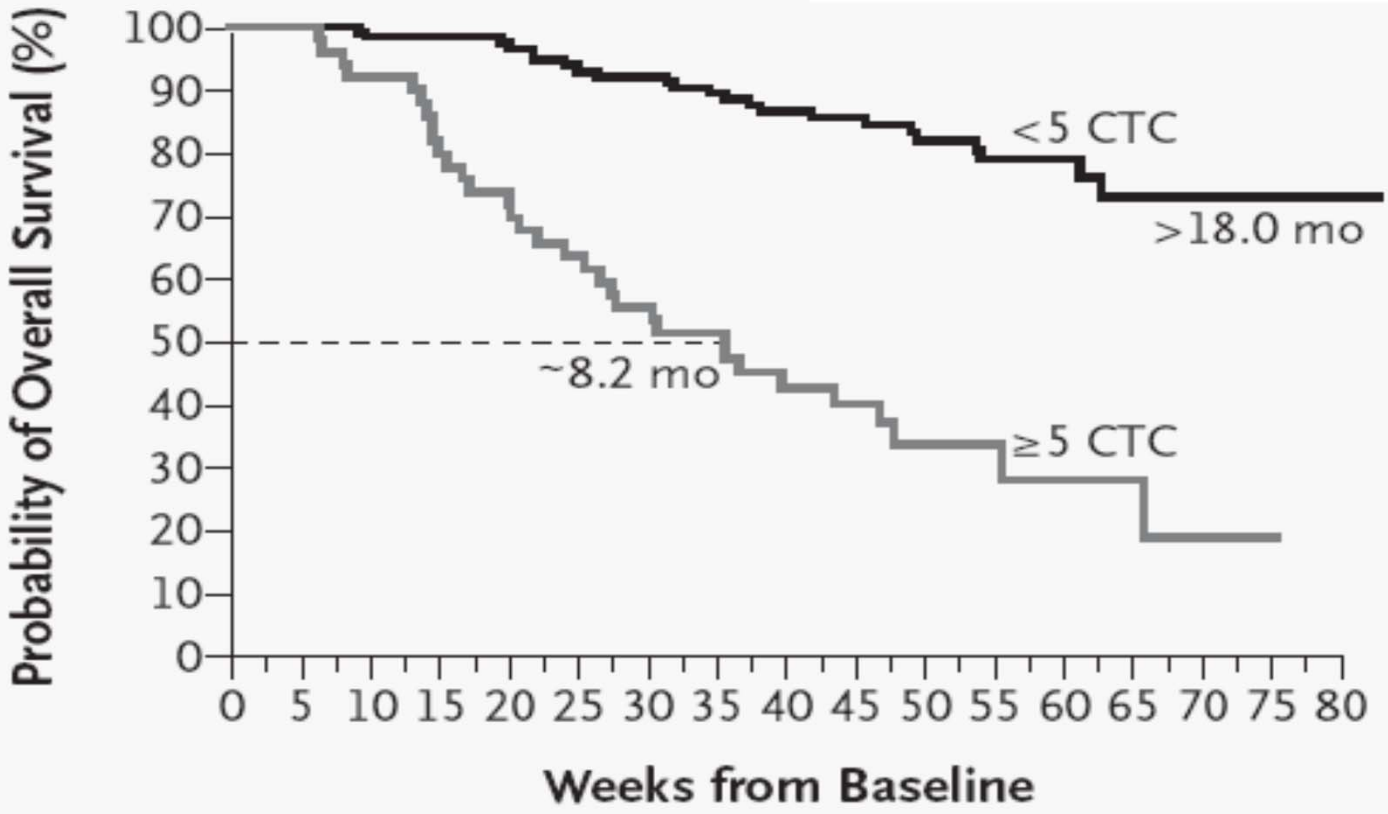


Cultured HCT-116 EpCaM+ cells were run on the Veridex/Janssen Celltracks CTC system and evaluated for CK and DAPI expression.

Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer

Massimo Cristofanilli, M.D., G. Thomas Budd, M.D., Matthew J. Ellis, M.B., Ph.D., Alison Stopeck, M.D., Jeri Matera, B.S., R.Ph., M. Craig Miller, B.S., James M. Reuben, Ph.D., Gerald V. Doyle, D.D.S., W. Jeffrey Allard, Ph.D., Leon W.M.M. Terstappen, M.D., Ph.D., and Daniel F. Hayes, M.D.

F Full Set of Data



No. at Risk

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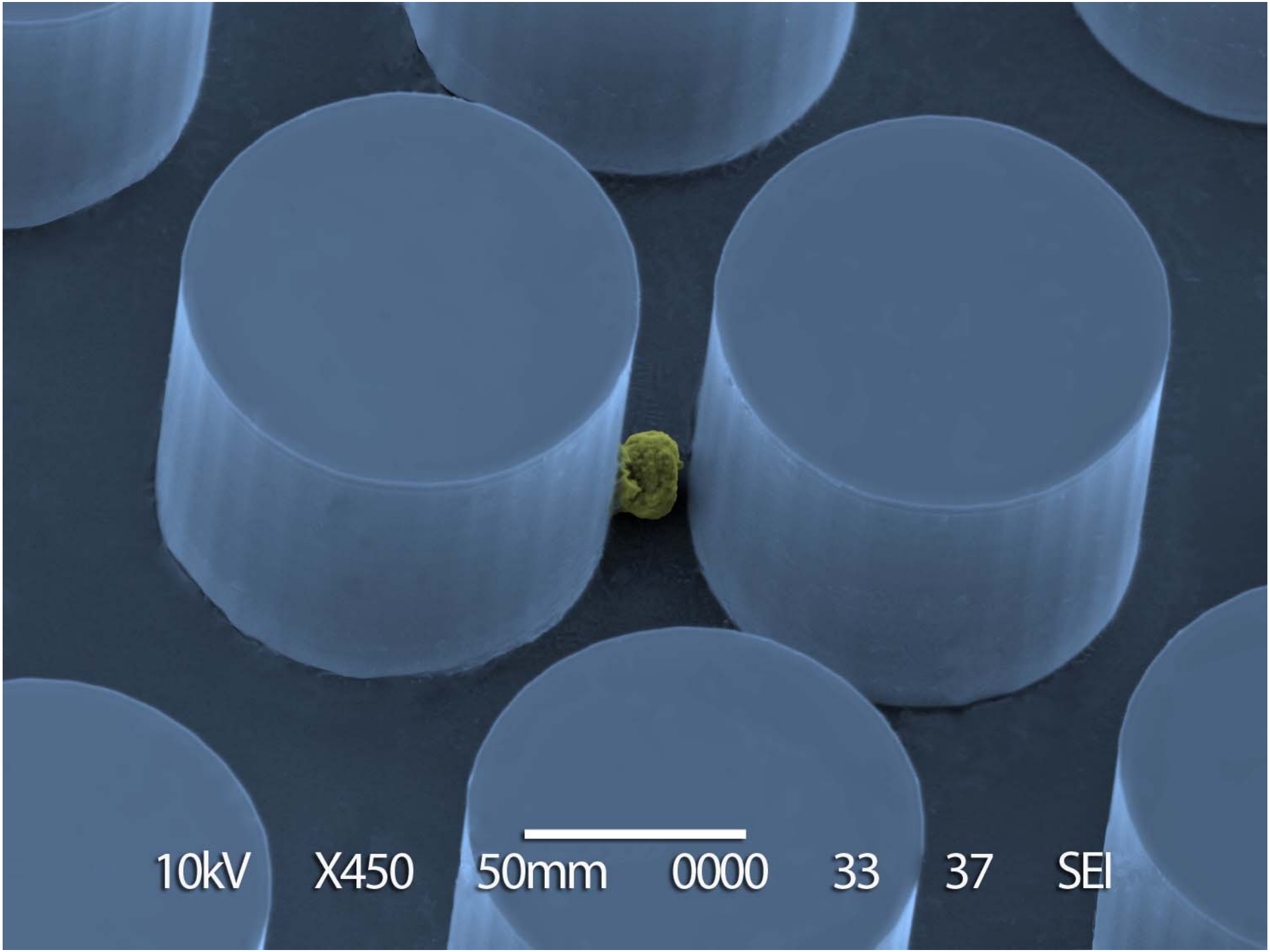
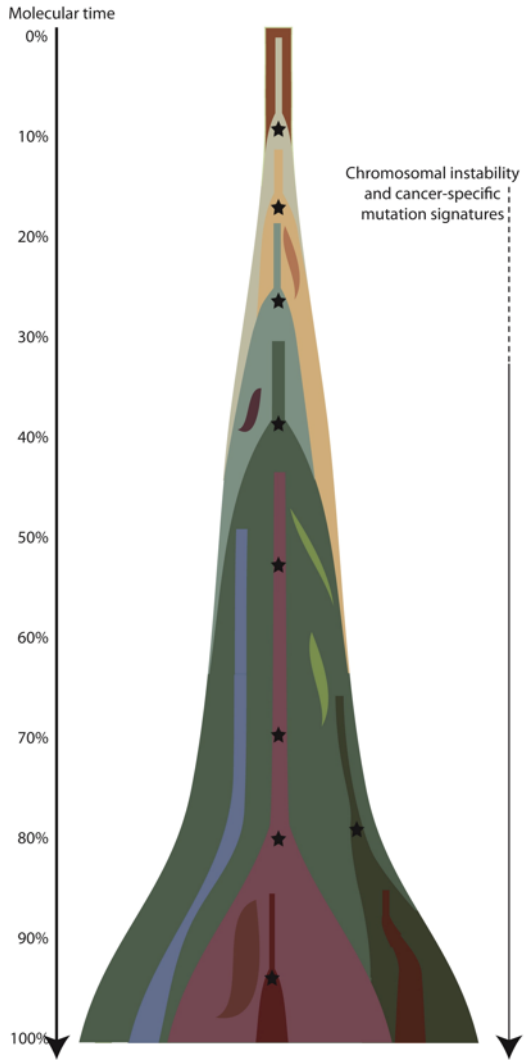
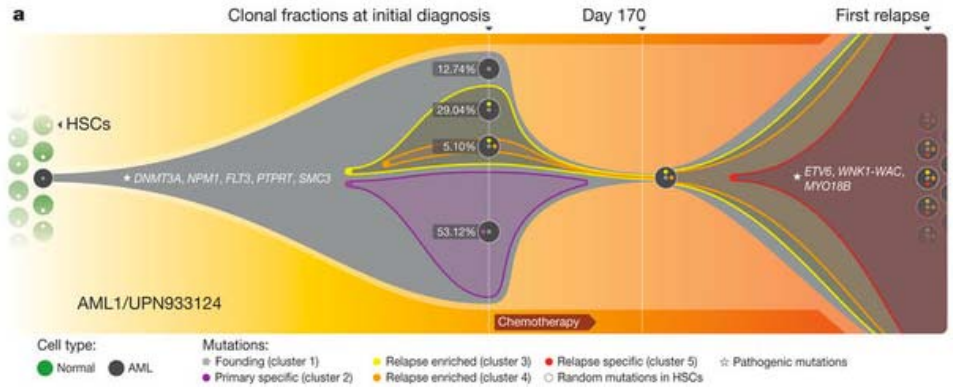
≥5 CTC	49	49	45	39	35	31	27	24	18	14	9	6	3	3	2	1	0

N Engl J Med 2004;351:781-91.


10kV X450 50mm 0000 33 37 SEI

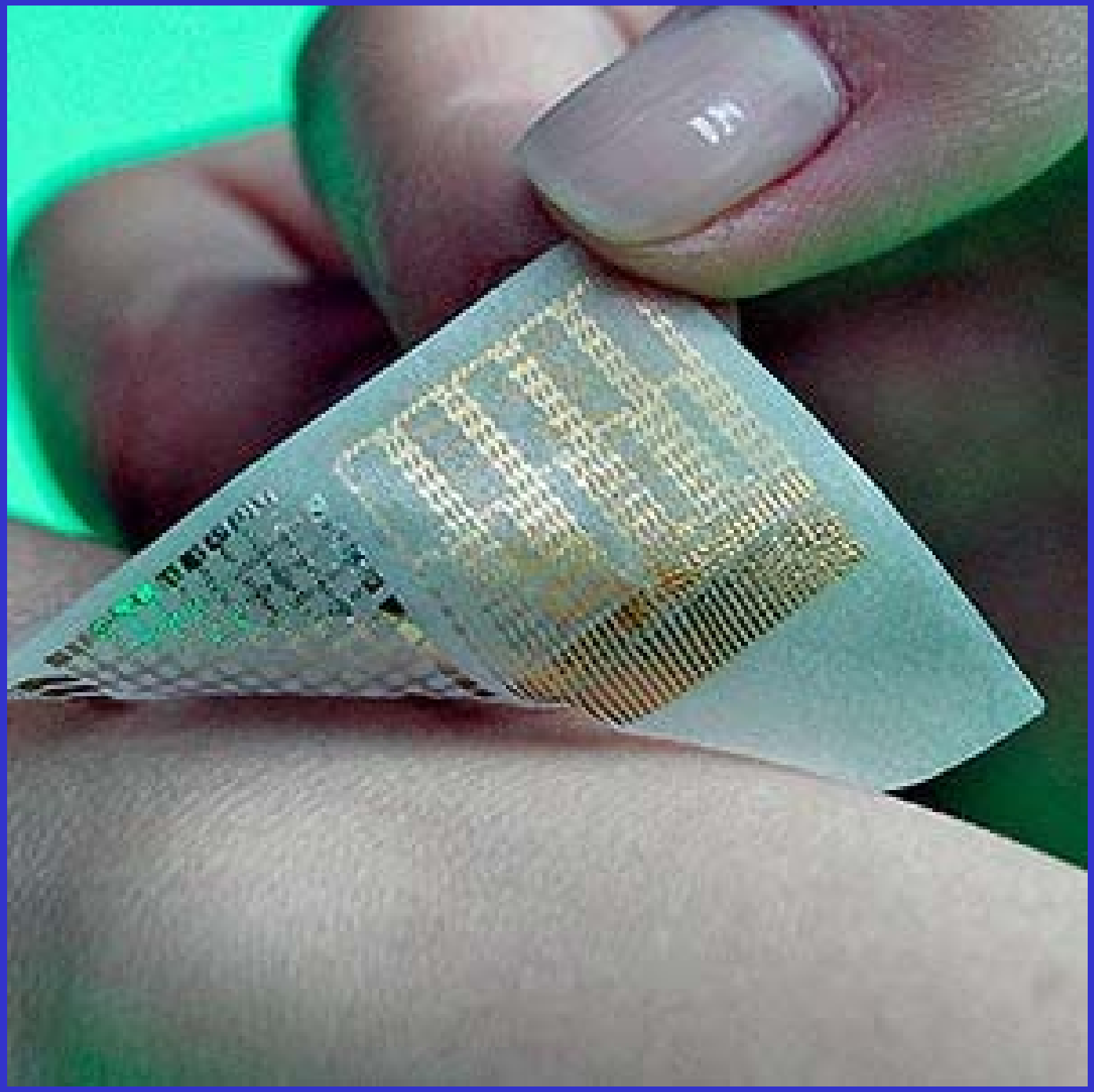
Heterogeneity and tumor evolution





Ding et al, Nature 2012

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



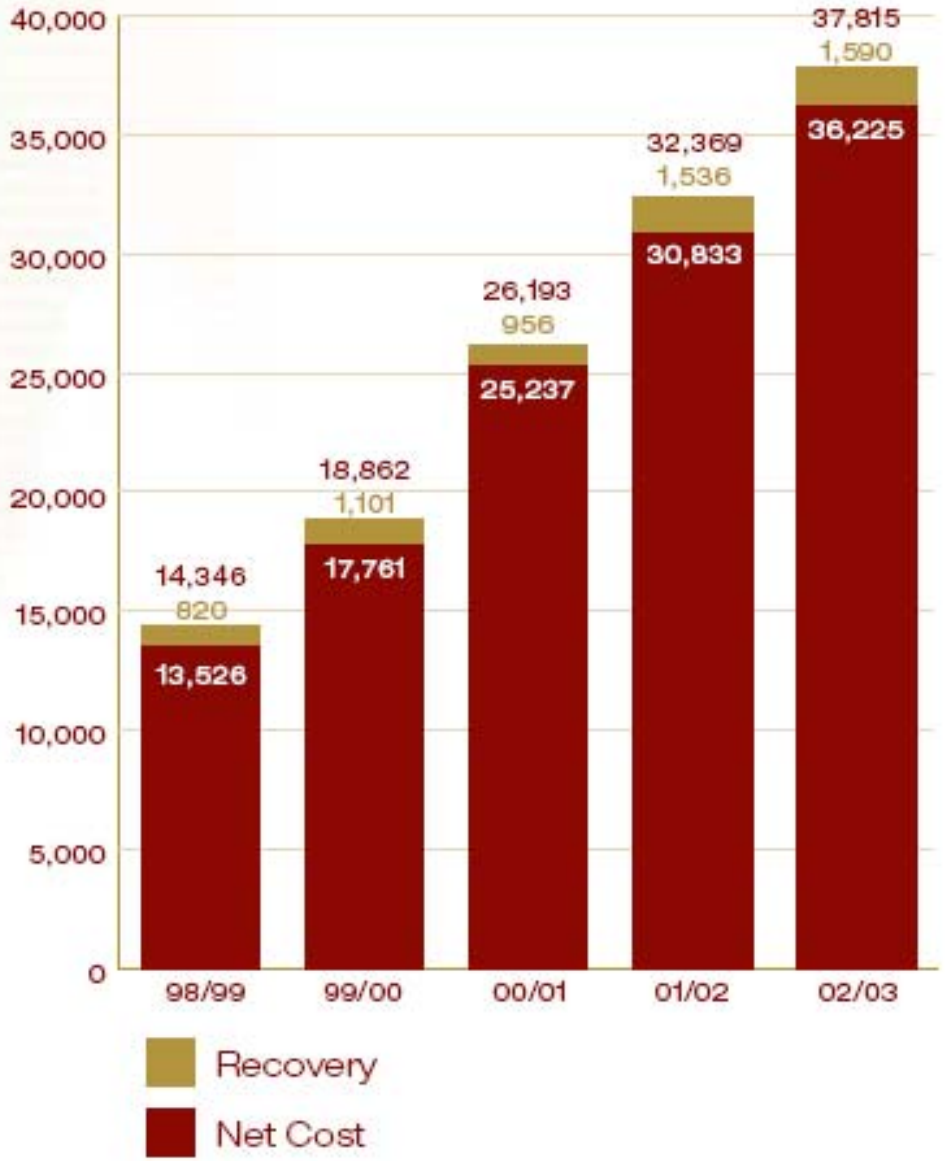
CTCs

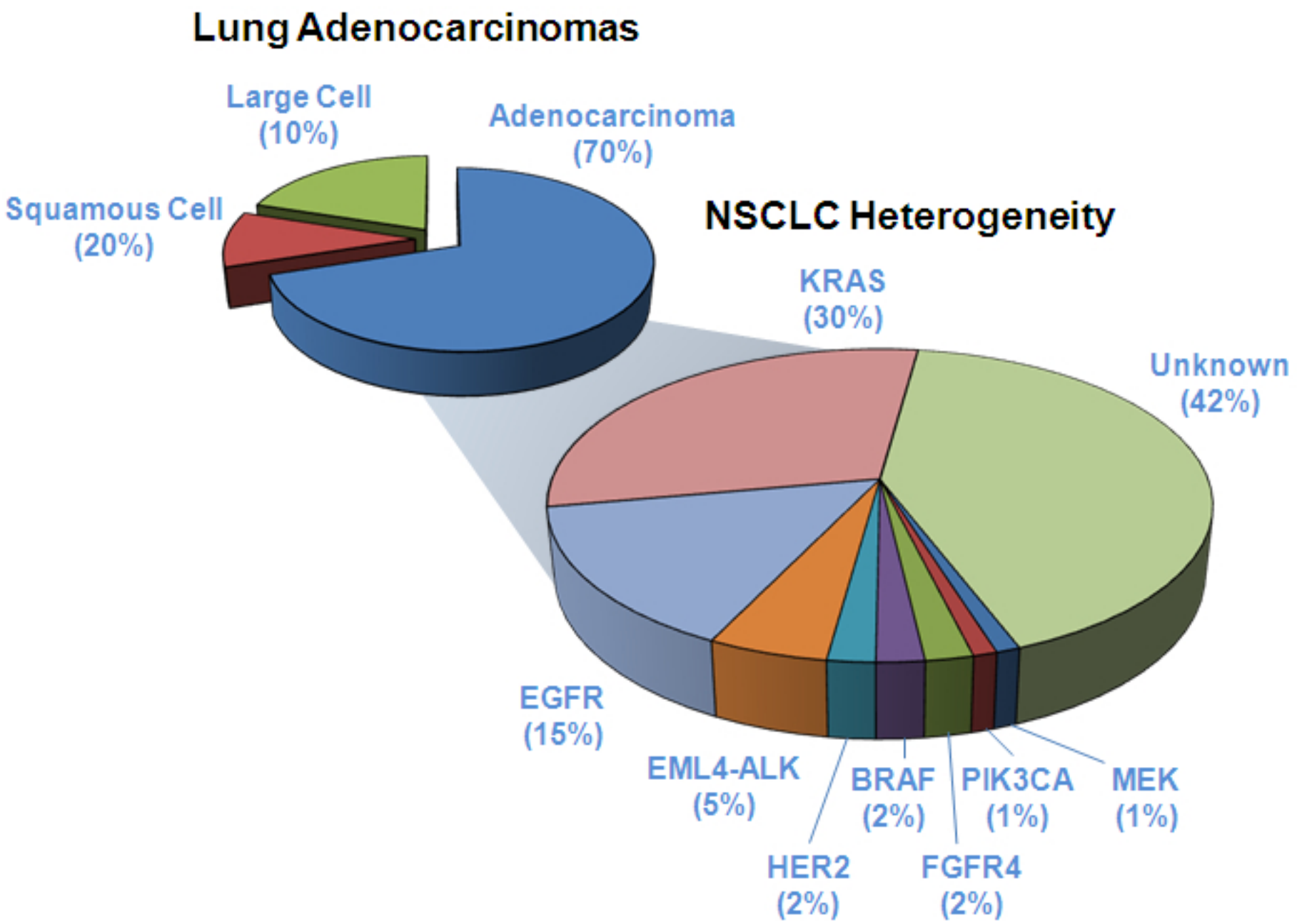
- FDA approved for Breast, Colon, Prostate
- Must be analyzed within 48 hrs
- Monitor response to therapy / early recurrence in metastatic setting
- Opportunity to use open channel- other antibody, FISH

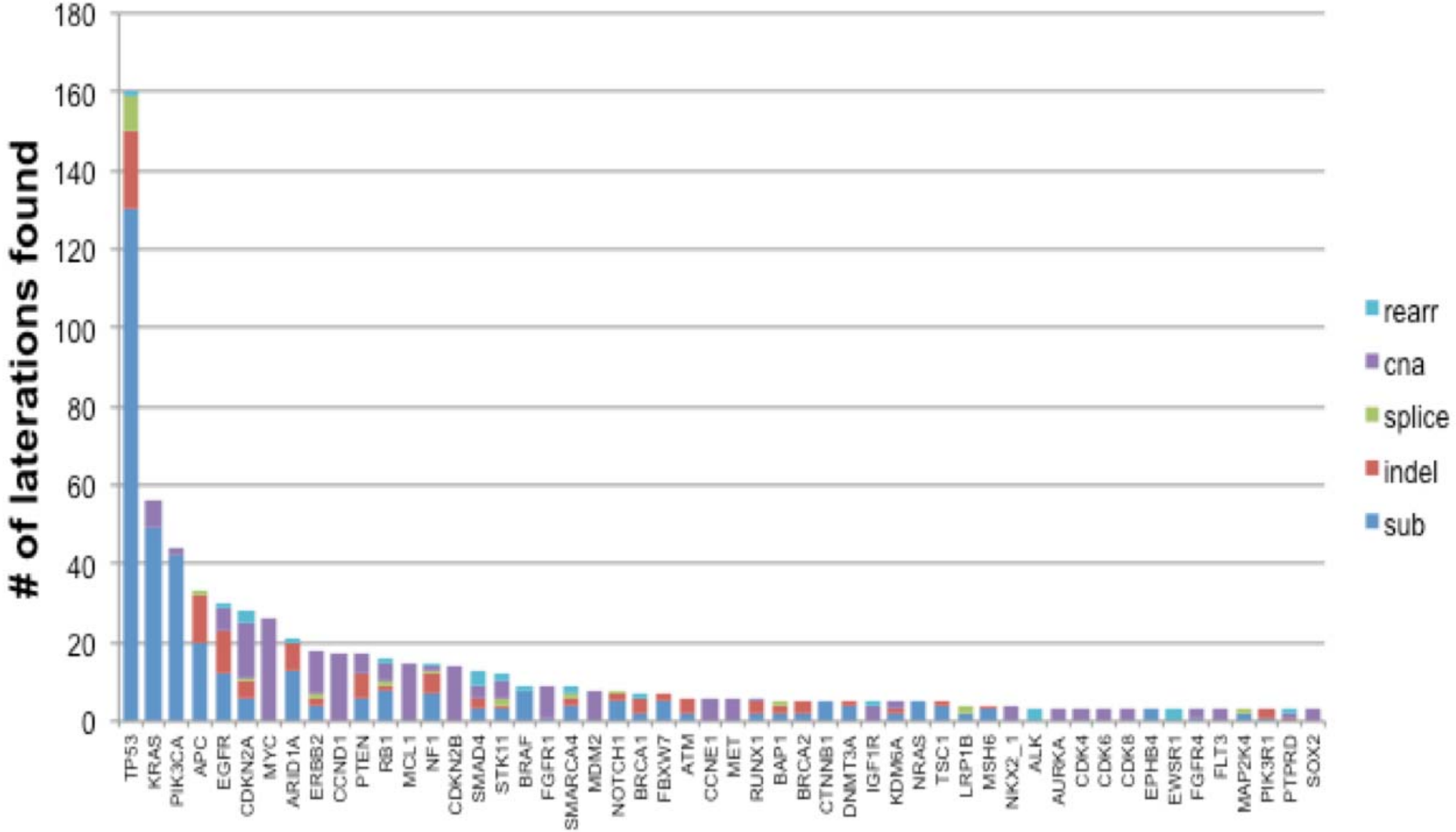
- May be complementary to cell free serum circulating DNA studies

- Why do we need better biomarkers?

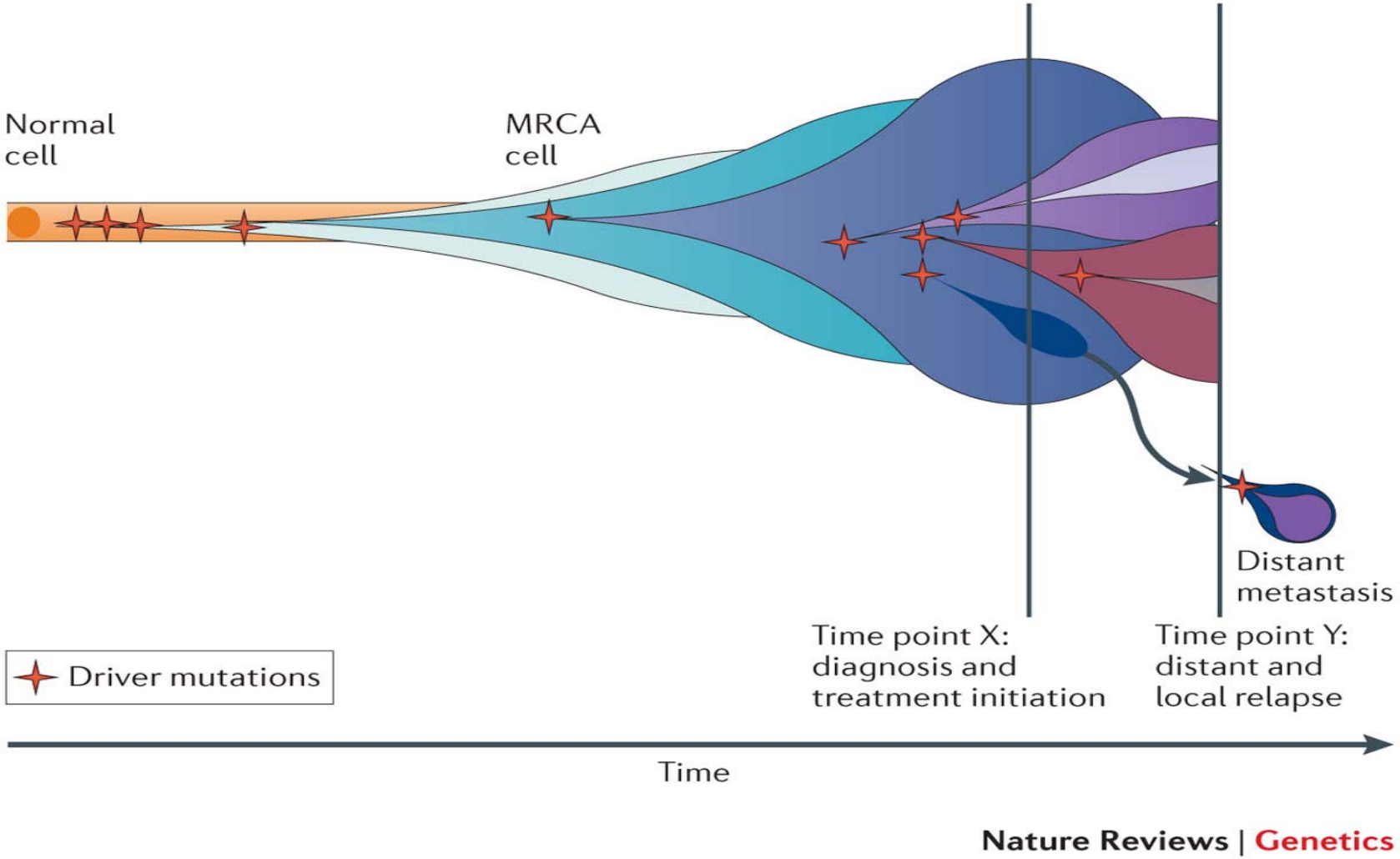
Drug Costs

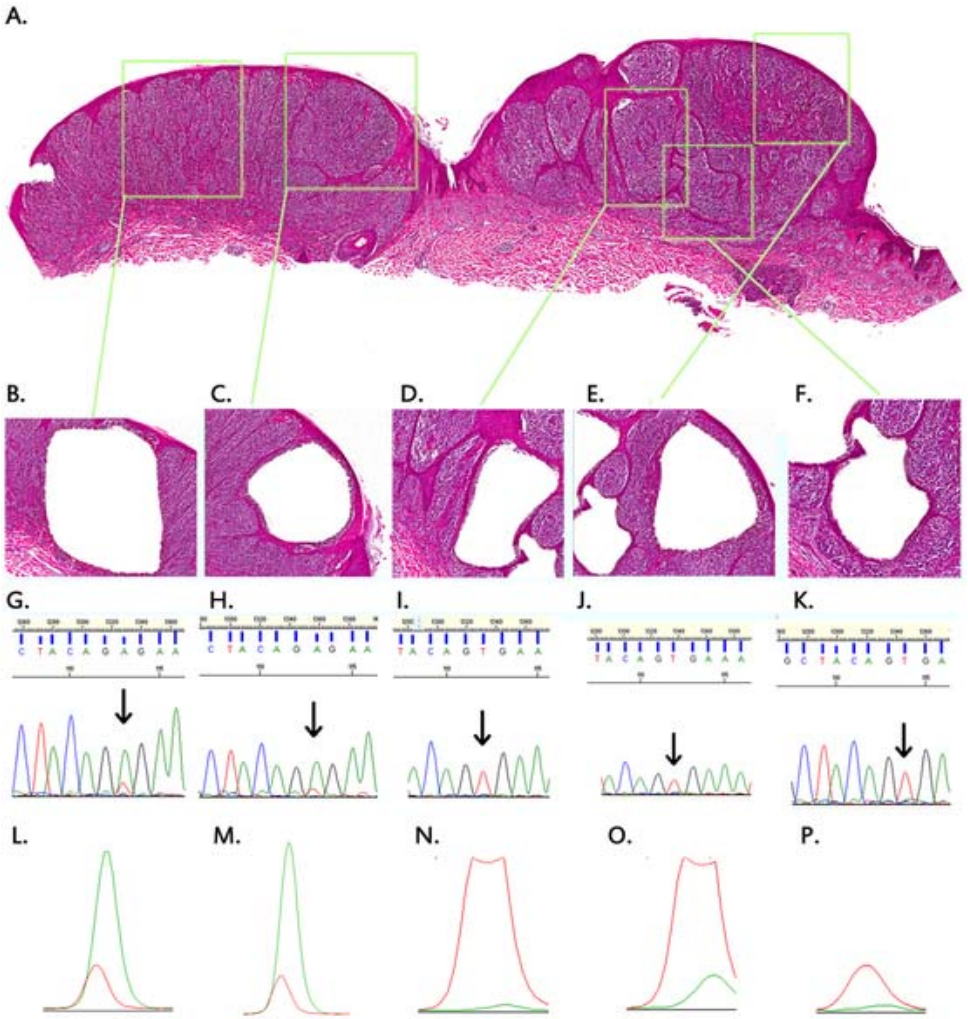

40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
14,346
820
13,526
18,862
1,101
17,761
26,193
956
25,237
32,369
1,536
30,833
37,815
1,590
36,225
98/99
99/00
00/01
01/02
02/03
Recovery
Net Cost


Lung Adenocarcinomas
Large Cell
(10%)
Adenocarcinoma
(70%)
Squamous Cell
(20%)
NSCLC Heterogeneity
KRAS
(30%)
Unknown
(42%)
EGFR
(15%)
EML4-ALK
(5%)
HER2
(2%)
BRAF
(2%)
FGFR4
(2%)
PIK3CA
(1%)
MEK
(1%)


# of laterations found
180
160
140
120
100
80
60
40
20
0
TP53
KRAS
PIK3CA
APC
EGFR
CDKN2A
MYC
ARID1A
ERBB2
CCND1
PTEN
RB1
MCL1
NF1
CDKN2B
SMAD4
STK11
BRAF
FGFR1
SMARCA4
MDM2
NOTCH1
BRCA1
FBXW7
ATM
CCNE1
MET
RUNX1
BAP1
BRCA2
CTNNB1
DNMT3A
IGF1R
KDM6A
NRAS
TSC1
LRP1B
MSH6
NKX2_1
ALK
AURKA
CDK4
CDK6
CDK8
EPHB4
EWSR1
FGFR4
FLT3
MAP2K4
PIK3R1
PTPRD
SOX2
rearr
cna
splice
indel
sub

Cancer Biology and Metastasis





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

The Future

Better trials based on molecular selection

Adaptive designs

Better monitoring

The right treatment for the right patient at the right time!