

Genomic analysis of head and neck and breast cancers for targeted therapy

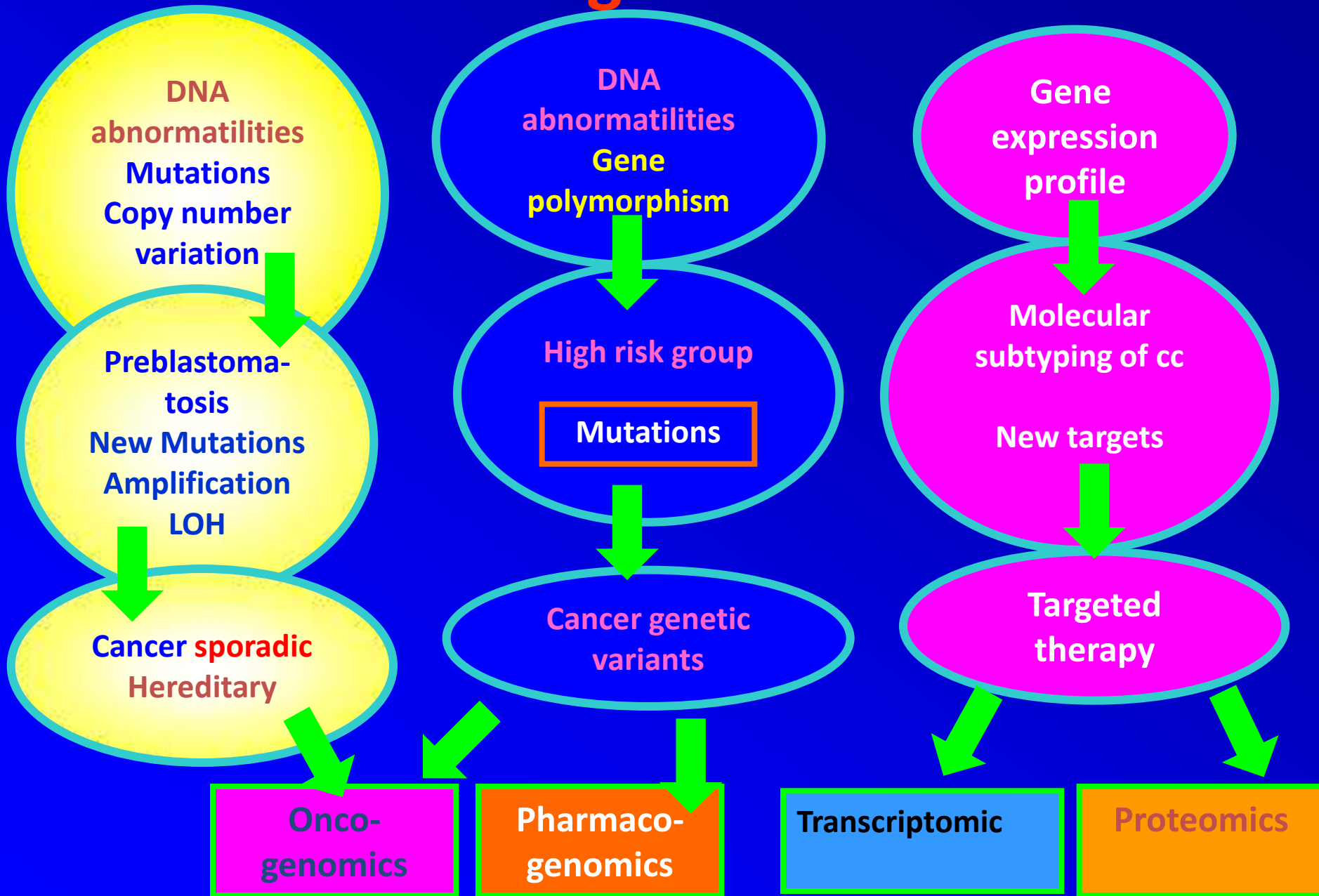
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National Institute of Oncology
Budapest 2011 March

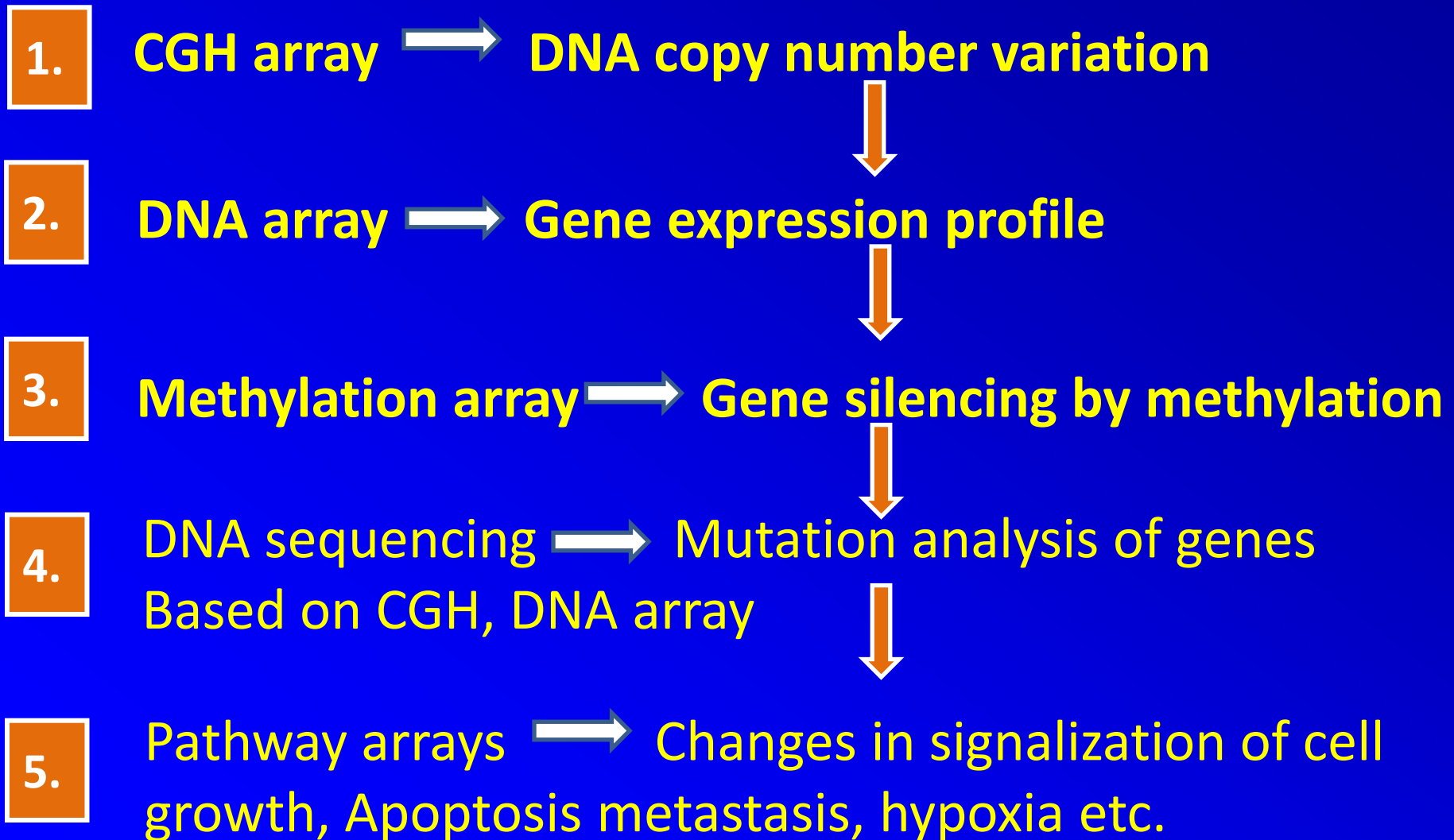
norwegian financial mechanism

norway
grants 

Cancer is a genetic disease



Strategy for integrated genomic analysis of HNSCC and breast cancer



Chromosome aberration in HNSCC by CGH

Aberration Summary for Chromosome 5

Aberration Algorithm: ADM-1 (Threshold: 6.0 , Fuzzy Zero: OFF)

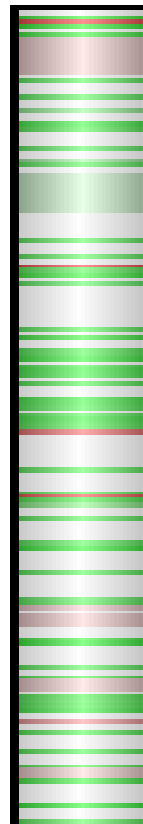
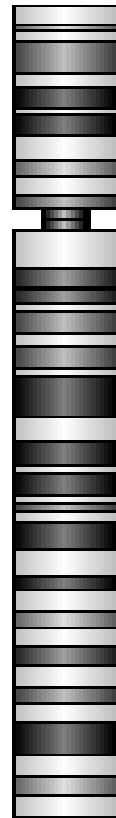
Genome: hg18

Amplification

Deletion

p15.32
p15.2
p14.3
p14.1
p13.2
p12

q12.3
q13.2
q14.1
q14.3
q21.1
q21.3
q22.2
q23.1
q23.3
q31.2
q32
q33.2
q34
q35.2



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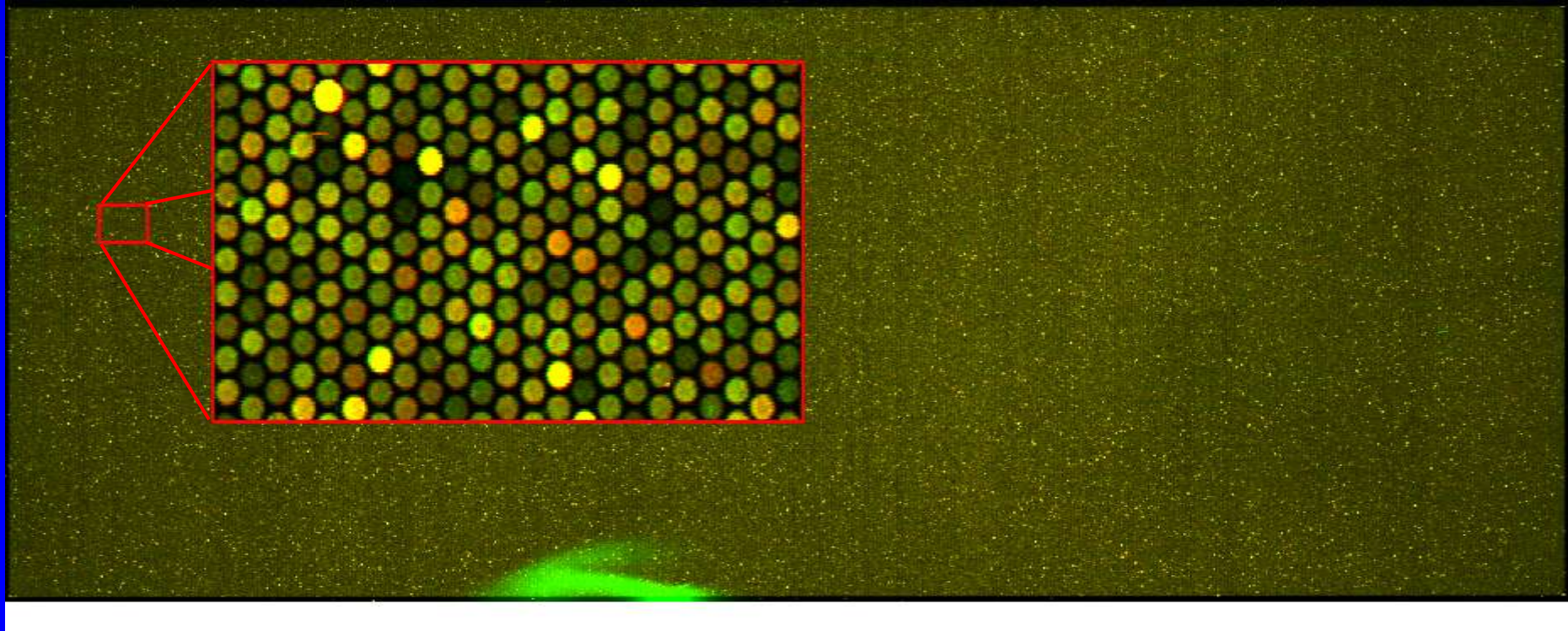
Amplification

Deletion

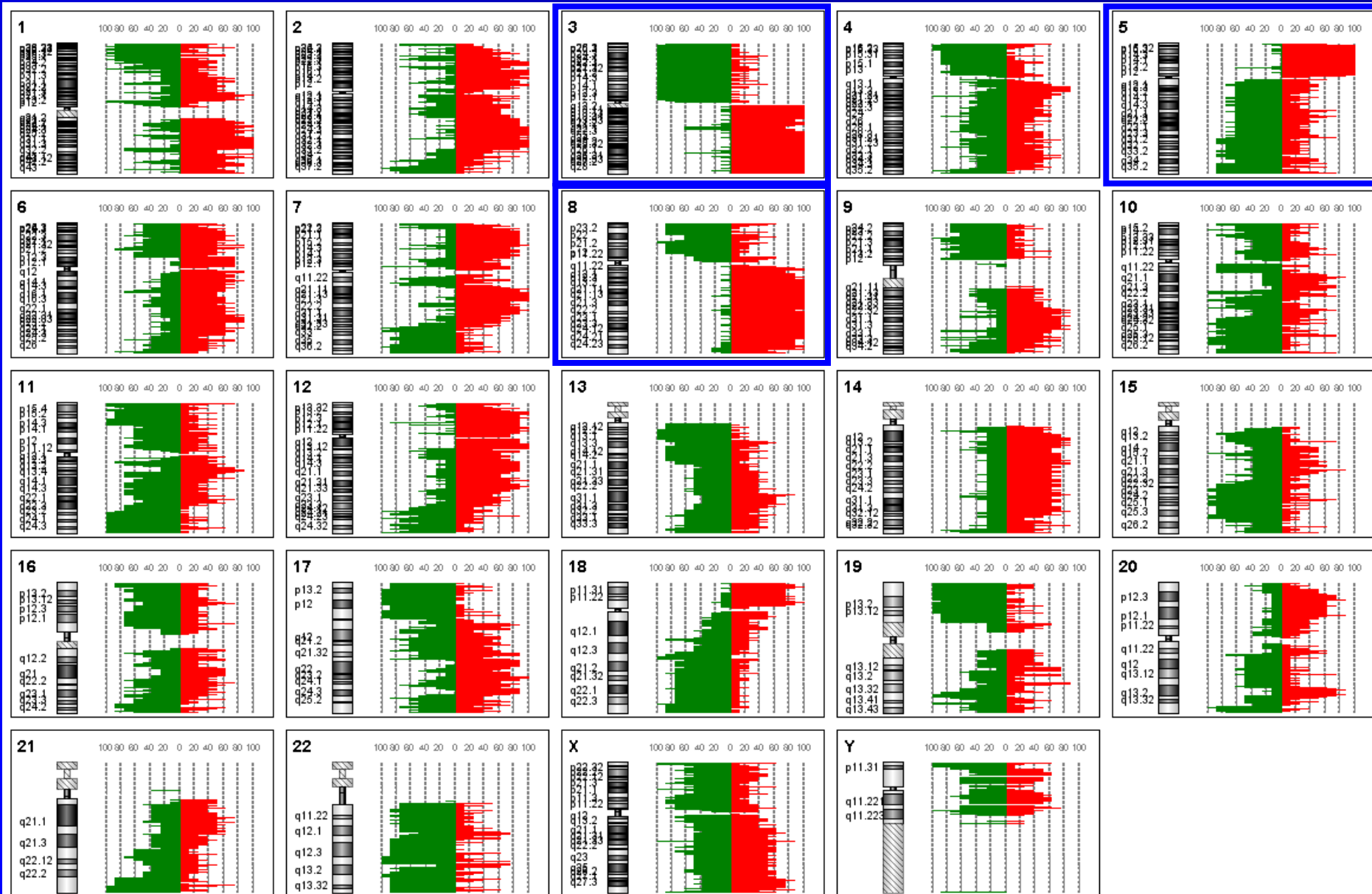
Deleted gene list of 5 chromosome in HNSCC

q33.1	SYNPO	ref Homo sapiens synaptopodin (SYNPO), transcript variant 2, mRNA.	0,3737	0,056320	
			32	366	
q33.1	TCOF1	ref Homo sapiens Treacher Collins-Franceschetti syndrome 1 (TCOF1), transcript variant 3, mRNA.	0,0689	0,056320	
			6	366	
q33.1	TIGD6	ref Homo sapiens tigger transposable element derived 6 (TIGD6), mRNA.	0,1991	0,056320	
			29	366	
			-		
q33.1	TNIP1	ref Homo sapiens TNFAIP3 interacting protein 1 (TNIP1), mRNA.	0,4203	0,056320	
			3	366	
			-		
q33.1	ZNF300	ref Homo sapiens zinc finger protein 300 (ZNF300), mRNA.	0,0529	0,056320	
			3	366	
q33.2	GALNT10	ref Homo sapiens UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (GalNAc-T10) (GALNT10), transcript variant 1, mRNA.	1,9327		1,932720
			2		5
			-		
q33.2	LARPI	ref Homo sapiens La ribonucleoprotein domain family, member 1 (LARPI), transcript variant 1, mRNA.	2,1852		-
			1		2,185213
			-		
q33.3	CCNJL	ref Homo sapiens cyclin J-like (CCNJL), mRNA.	1,9707		1,970737
			4		7
q33.3	CLINT1	ref Homo sapiens clathrin interactor 1 (CLINT1), mRNA.	0,5627	0,713937	
			83	6	

Comparative genomic hybridization (CGH) in head and neck samples



Chromosomal imbalance in HNSCC by CGH array



Subgroups for CGH arrays



Head and neck cancers

1. P53 wt, P53 mut
2. Various anatomical localization: oral cavity, larynx, pharynx



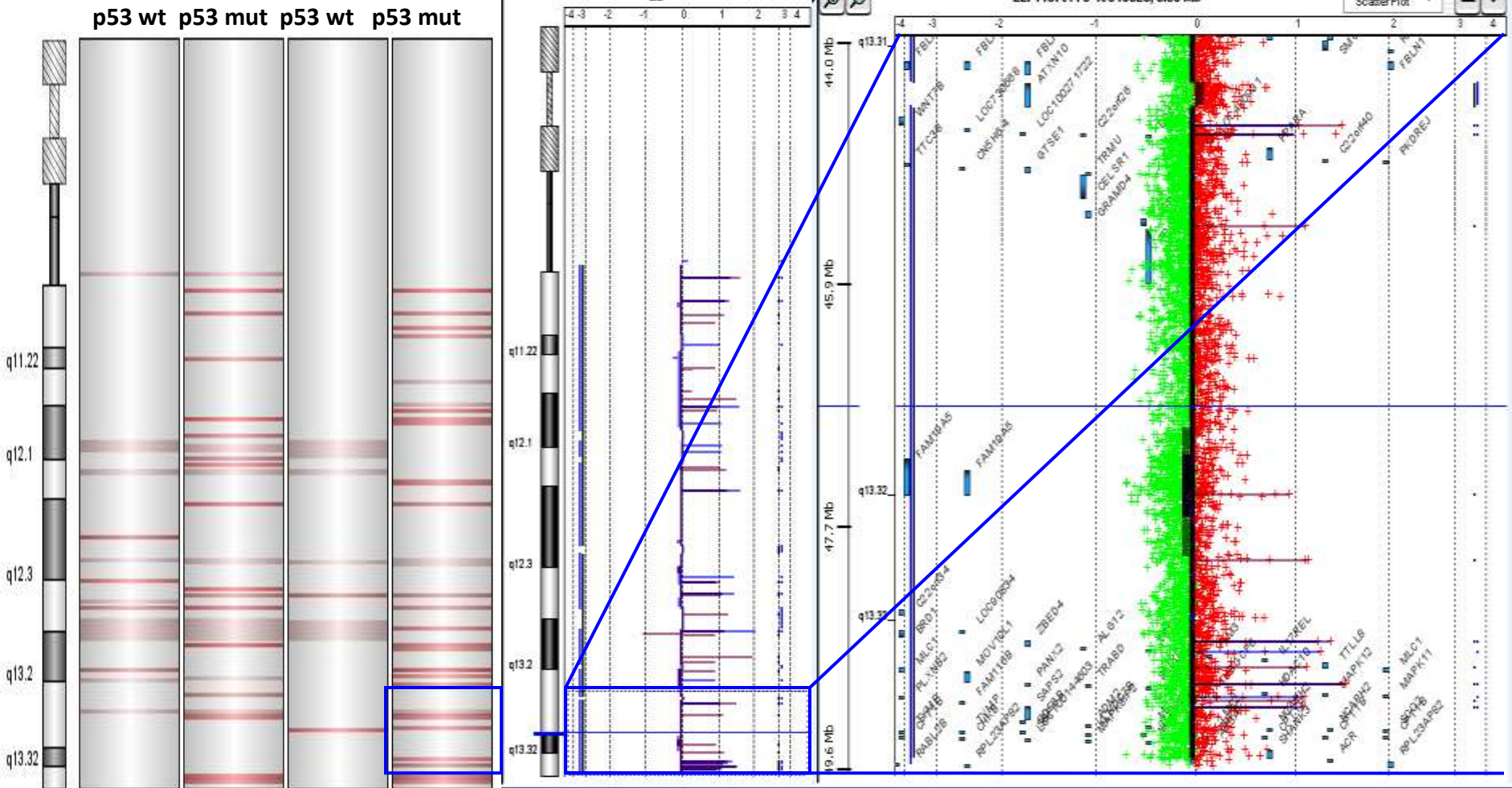
Breast cancers

1. Triple negative
2. Her2 positive

Amplificated genes in chr22q of p53 mutant HNSCC tumors

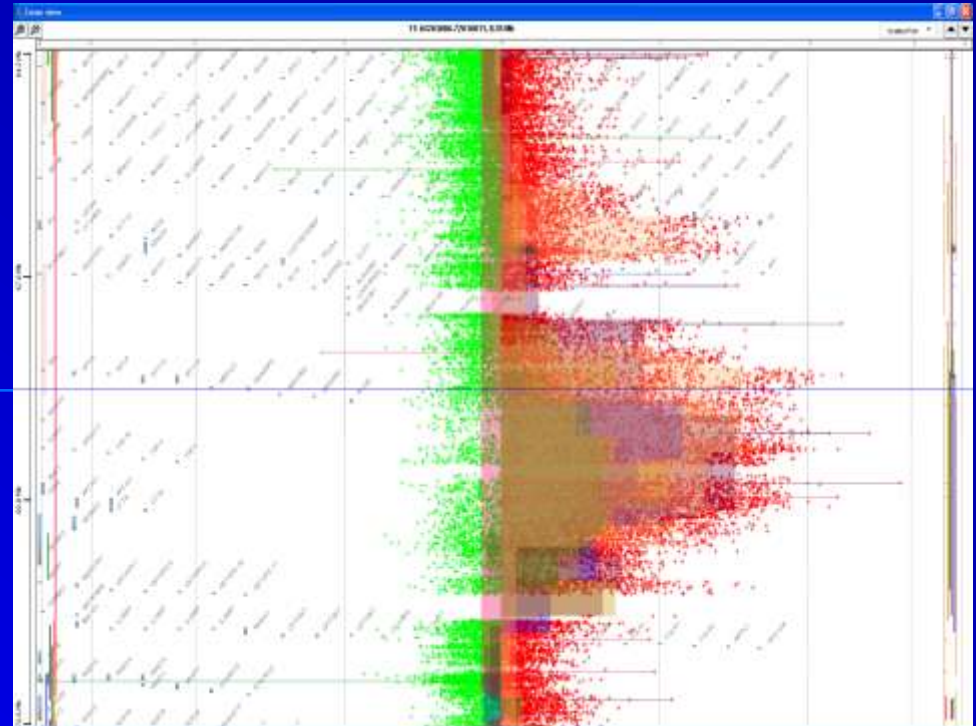
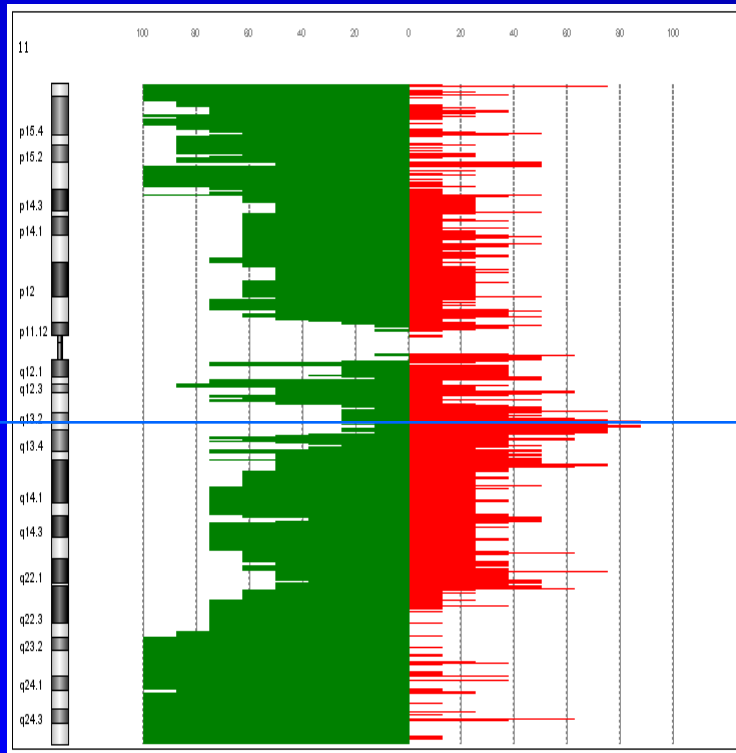
Oral cav. Oropharynx

p53 mut



Amplificated genes: PLXNB2, SELO, PANX2, ZBED4,TTLL8, TRABD, SAPG2
PPARA, ATXN10

Amplificated genes in chr11q13.2-3 of head and neck tumors



chr11 q13.2 - q13.3 68555899 69937613 TPCN2, MYEOV, CCND1, ORAOV1, FGF19, FGF4, FGF3, ANO1, FADD, PPFIA1, CTTN

chr11 q13.2 68601689 68669800 TPCN2

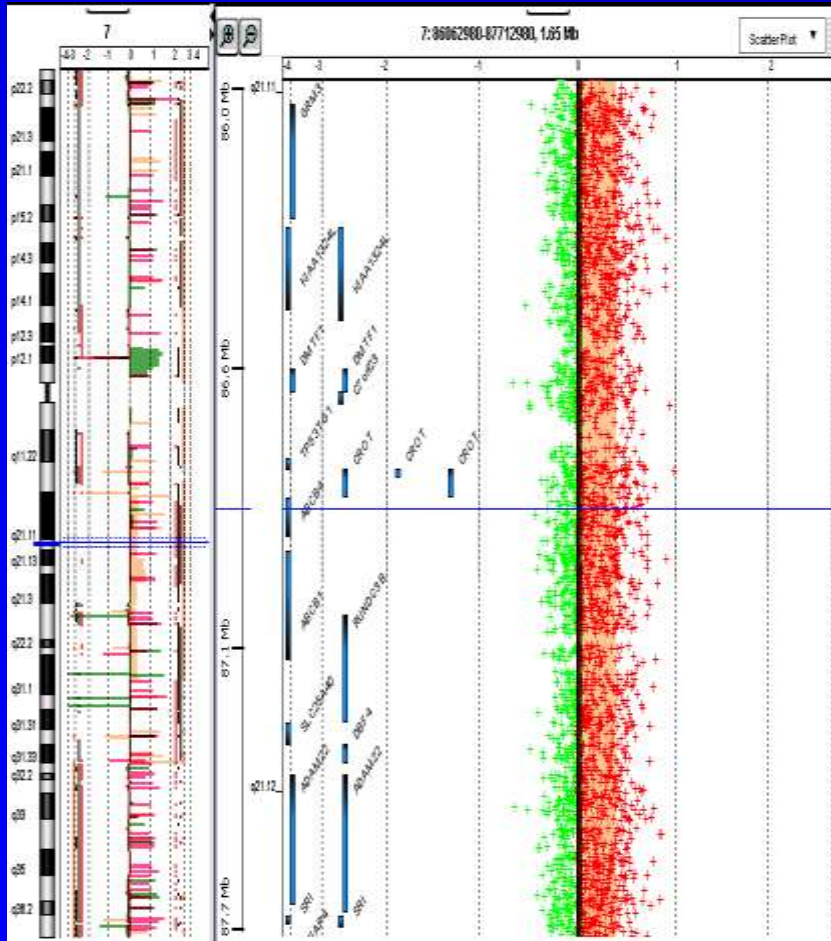
chr11 q13.2 - q13.3 68684682 69508709 MYEOV, CCND1, ORAOV1, FGF19, FGF4, FGF3

chr11 q13.2 68852570 69056107

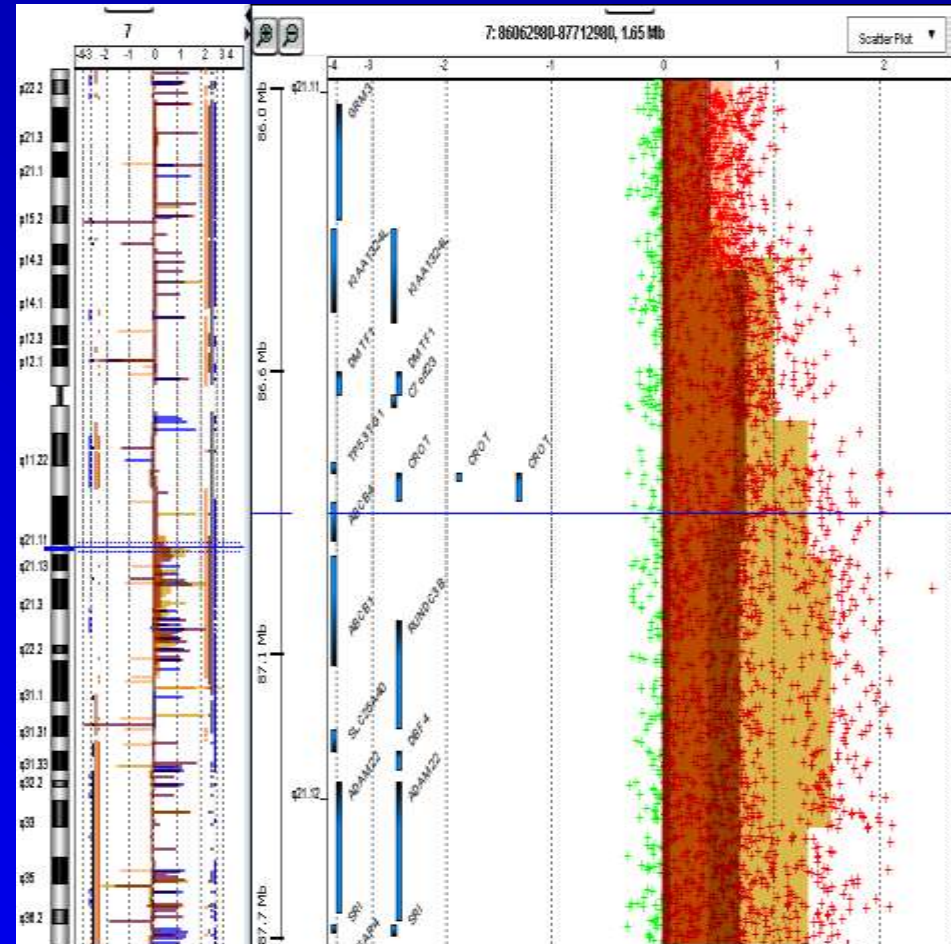
chr11 q13.2 - q13.3 69170348 69222231 CCND1, ORAOV1, FGF19

Amplifications of chr7q21 in p53 mutant head and neck tumors

p53 wt



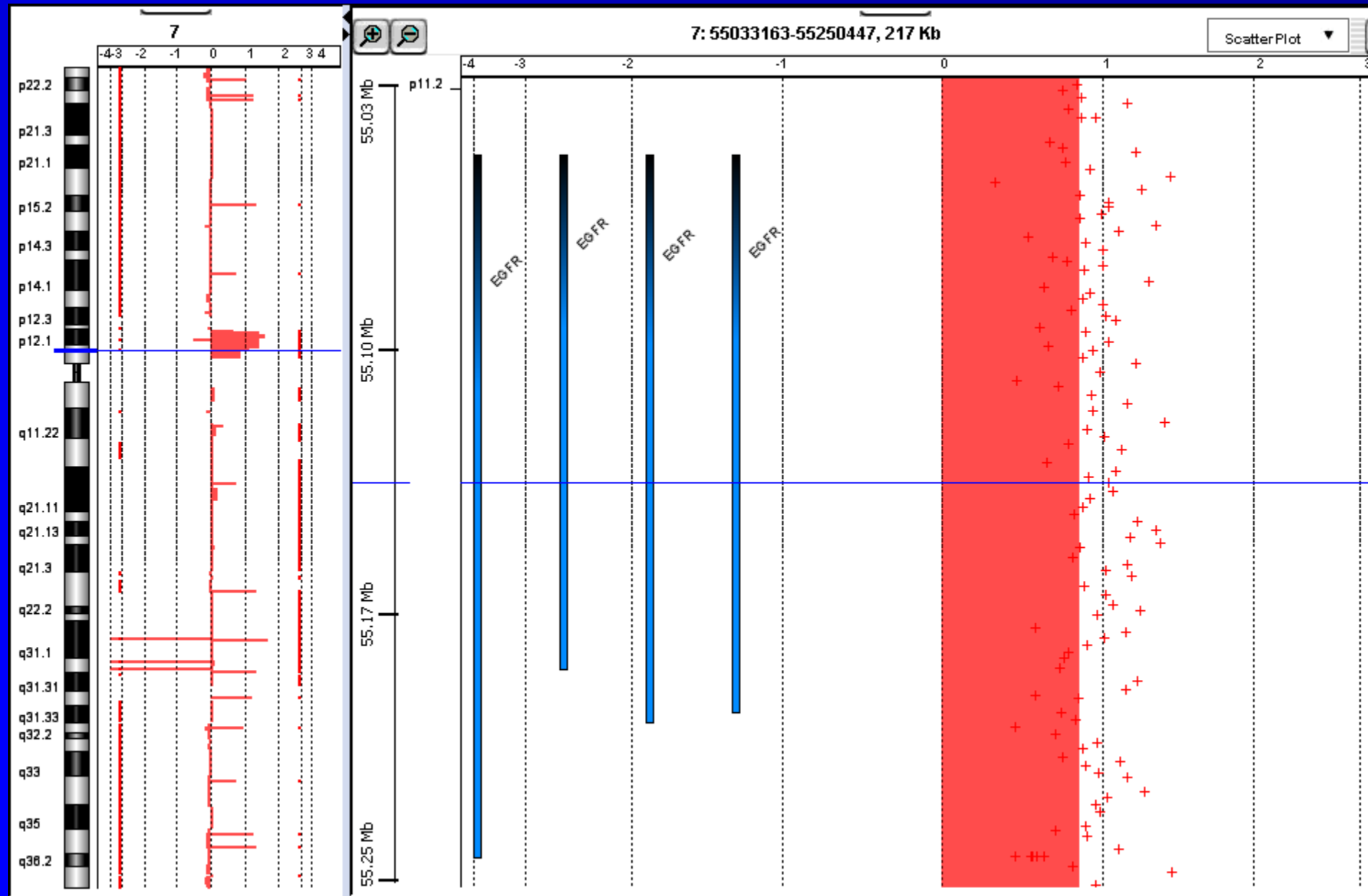
p53 mut



Amplified genes:

ABCB1, ABCB4, ADAM22, CROT, DMTF1, DBF4
TP53TG1, GRM3, RUNDC3B

Amplification of 7p11.2 (EGFR) in HNSCC



CGH array of HNSCC

Amplificated chromosomal regions

3q22, 7p, 14q12, 20 q, 13q21, 11q13

Deleted chromosomal regions

3p21, 9p21, 9p13, 18 q12, 18q21

Significance of CGH array

1.

Genom wide detection of deleted and amplified chromosome regions

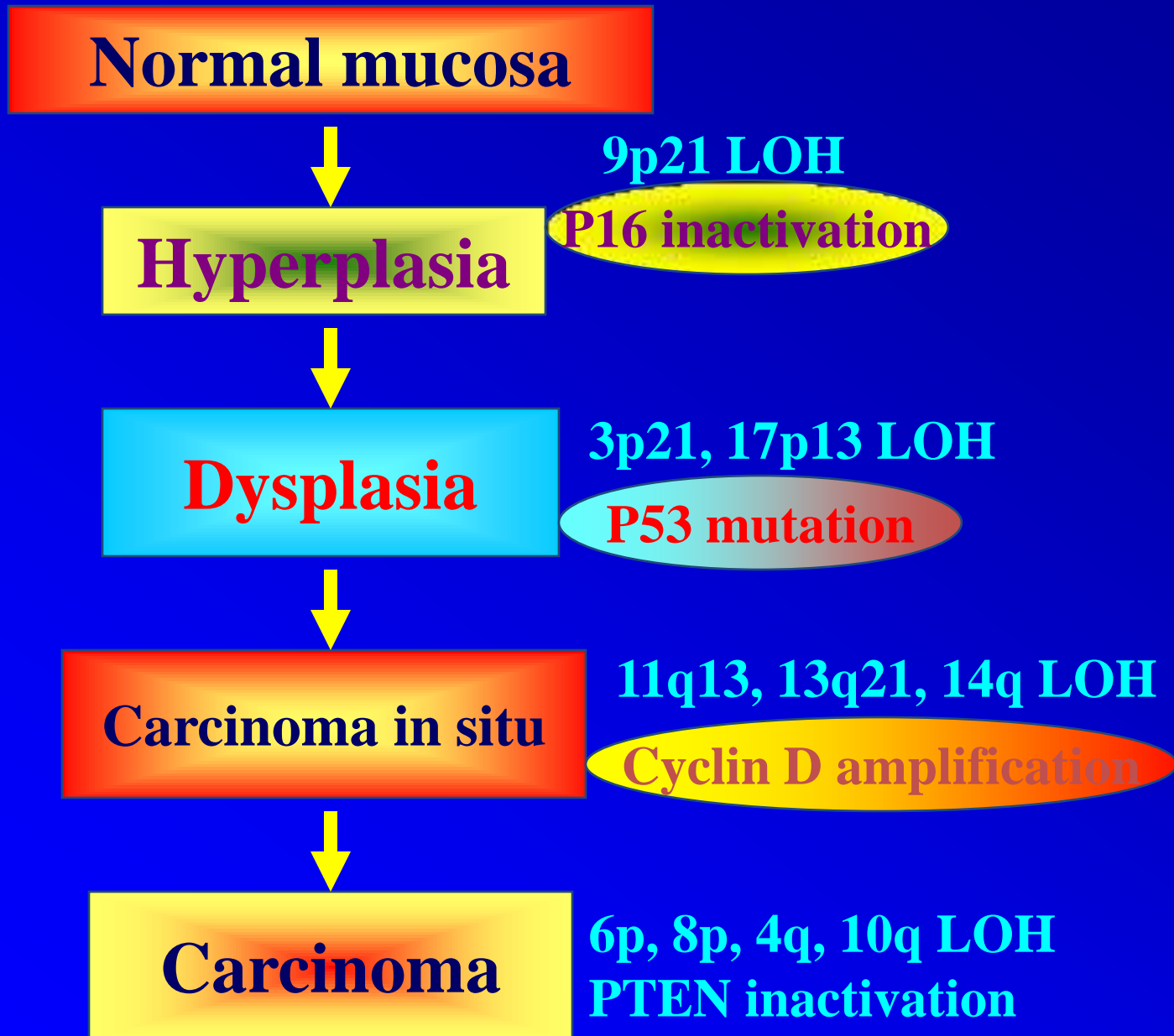
2.

Identification of amplicated and/or deleted genes

3.

Integration of gene expression profile and copy number variations

Development of head and neck cancers



CGH array of breast cancer

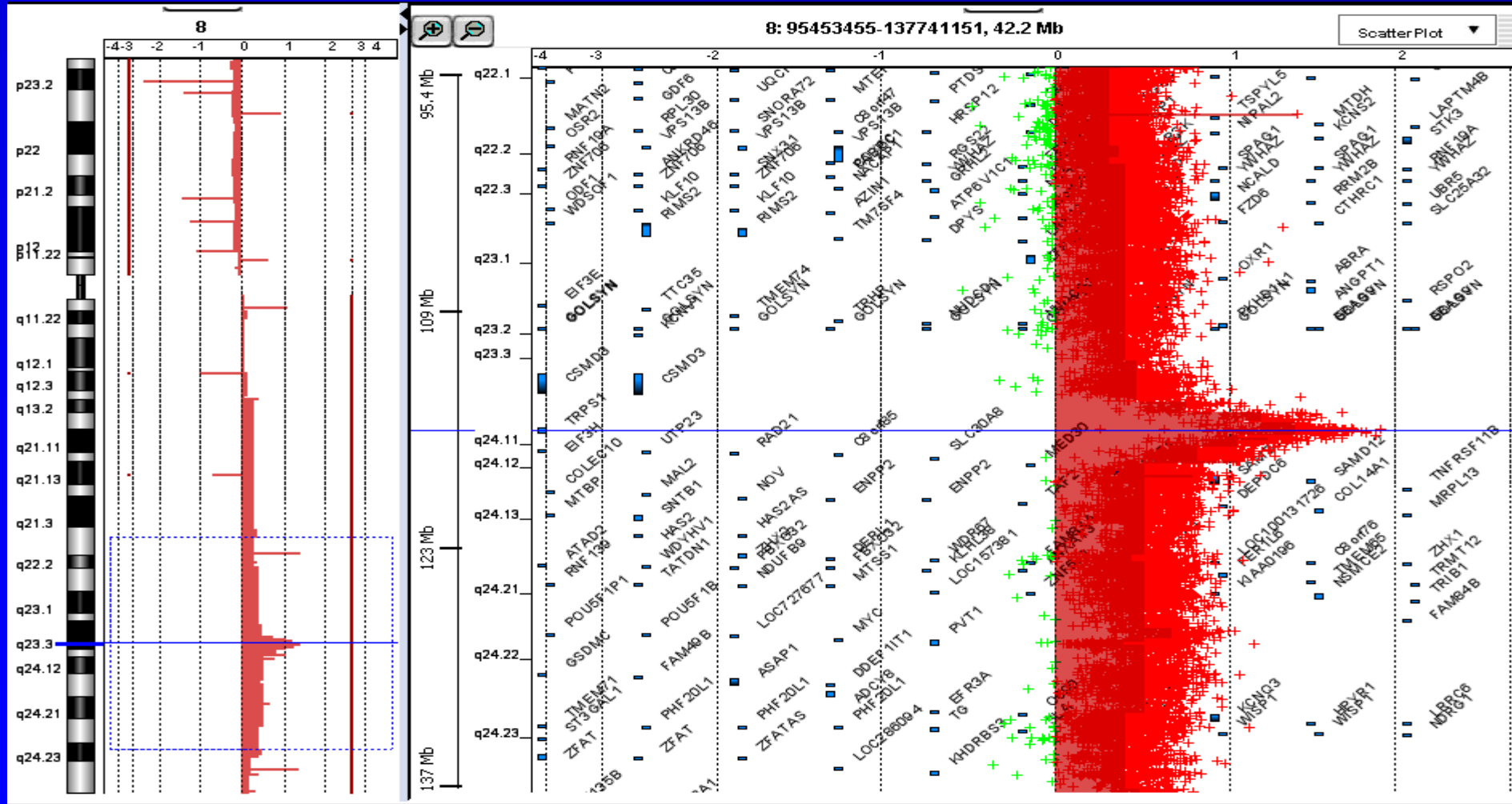


**Triple negative
subgroup**



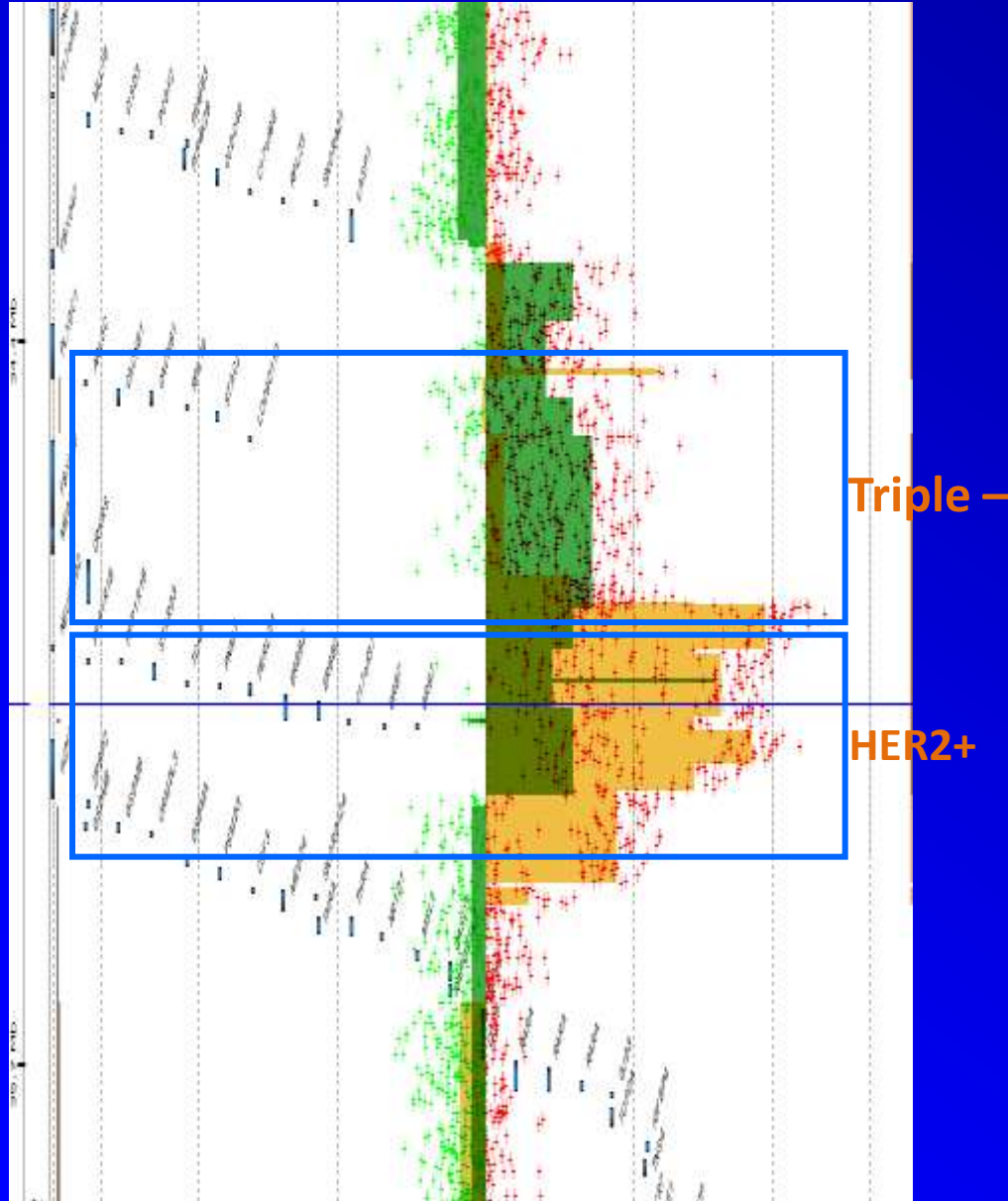
**HER2 positive
subgroup**

Amplifications of 8q22-24 in Triple – breast cancer

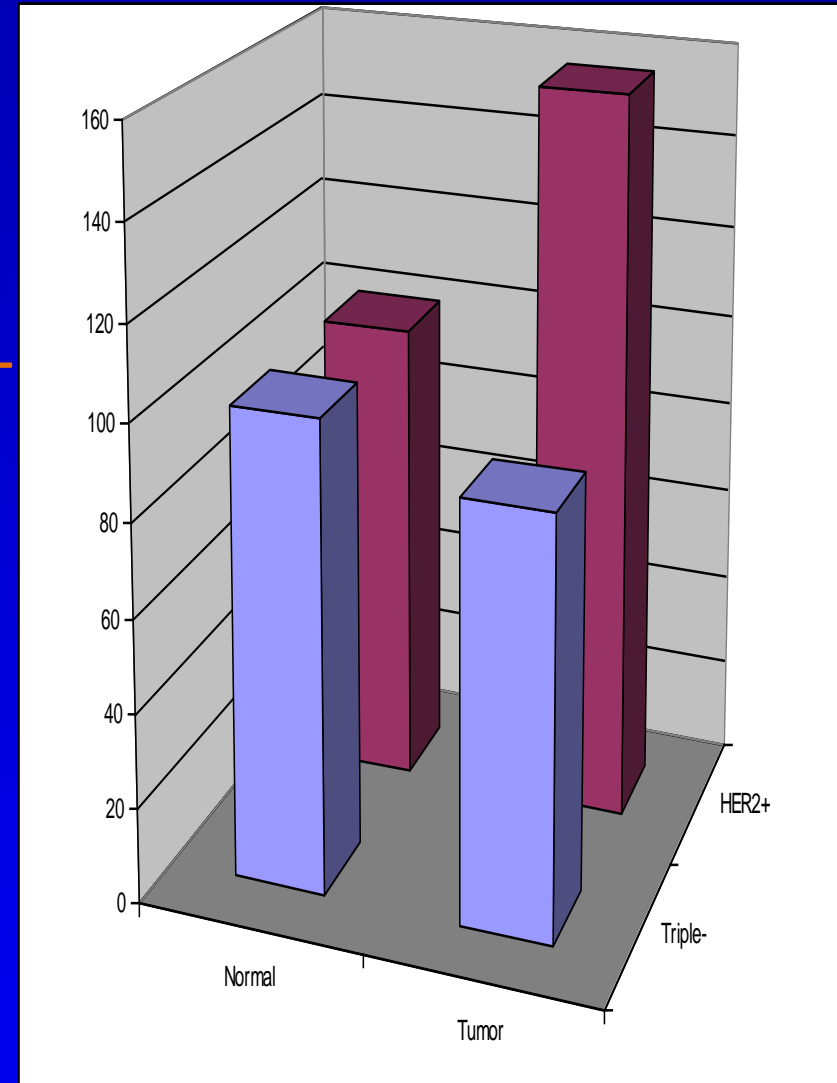


Amplified genes: Cyclin E2, EIF3H, EIF3E, TRPS1, RAD21, SDC2, POP1, MRPL13
 ENPP2, MTSS1, MYC, ASAP1, ST3GAL1, MED30, CSMD3

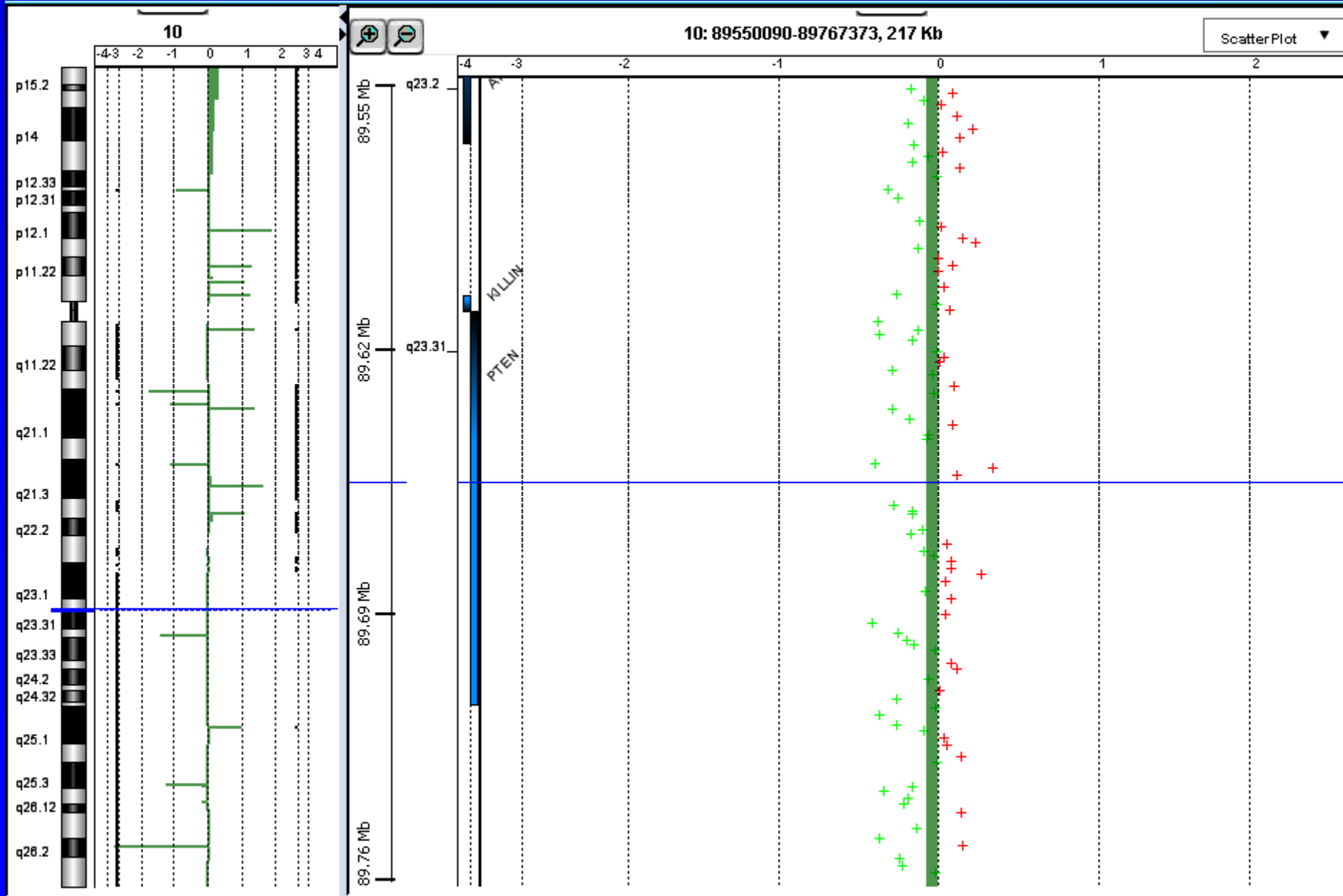
Gene amplifications in 17q chromosome of Triple - és HER2+ breast cancers



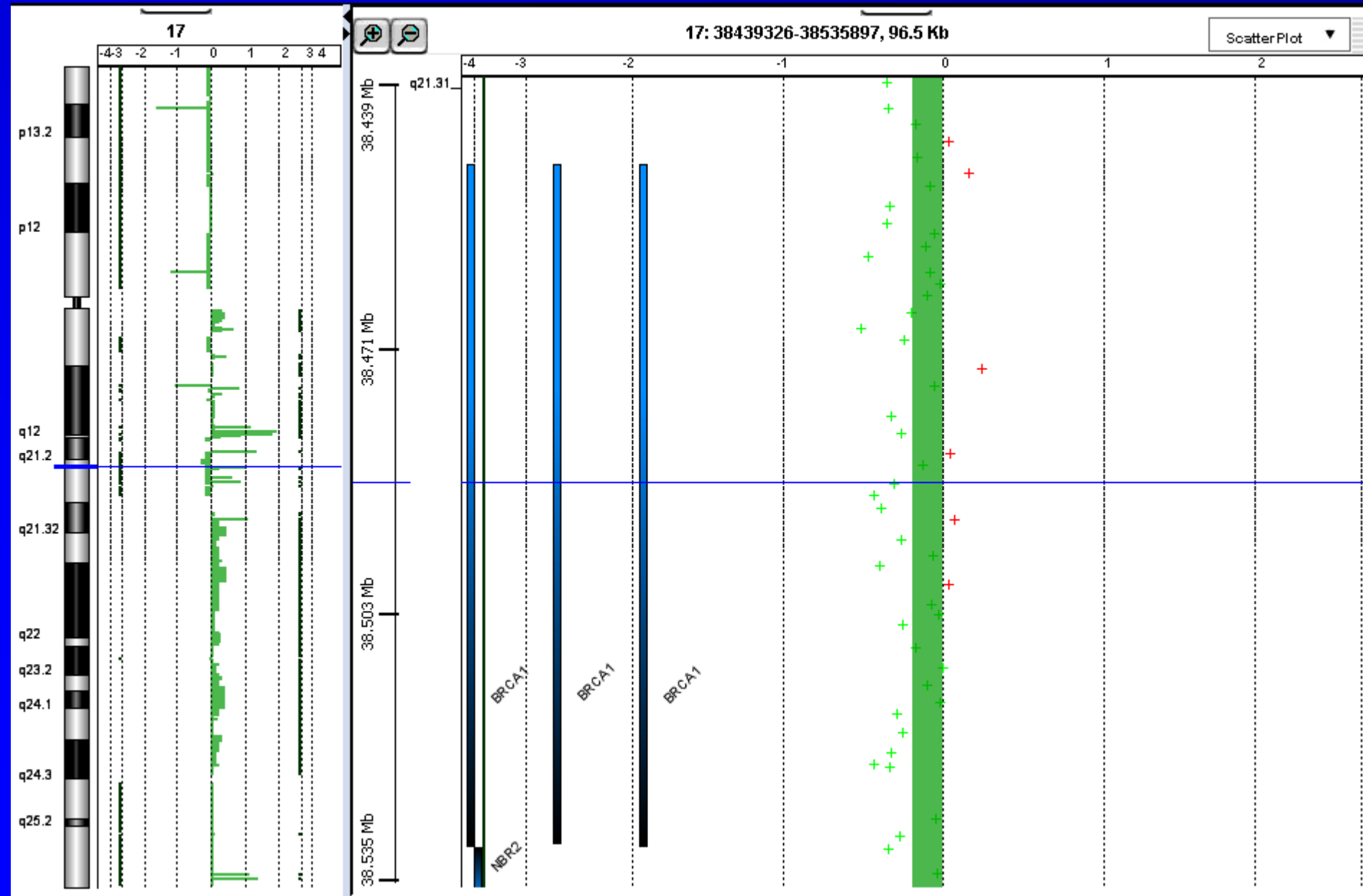
HER2 gene expression



Deletion of 10q23.31 (PTEN) in Triple - breast cancer



Deletion of 17q21.31 (BRCA1) in HER2+ breast cancer



CGH analysis of breast cancer

Amplificated chromosomal regions

13q34, 11q13, 1p12, 4q22, 5p12, 20q
7p15.2, 8p23.3, 9p24, 10p13, 11p13, 17q

Deleted chromosomal regions

1q, 11q22, 12q21, 3p24.1, 22q13.2, 17p, 22q

Significance of CGH analysis in breast cancer

1.

Identification of **BRCA1 negative subgroup** in **HER2 positive breast cancer**

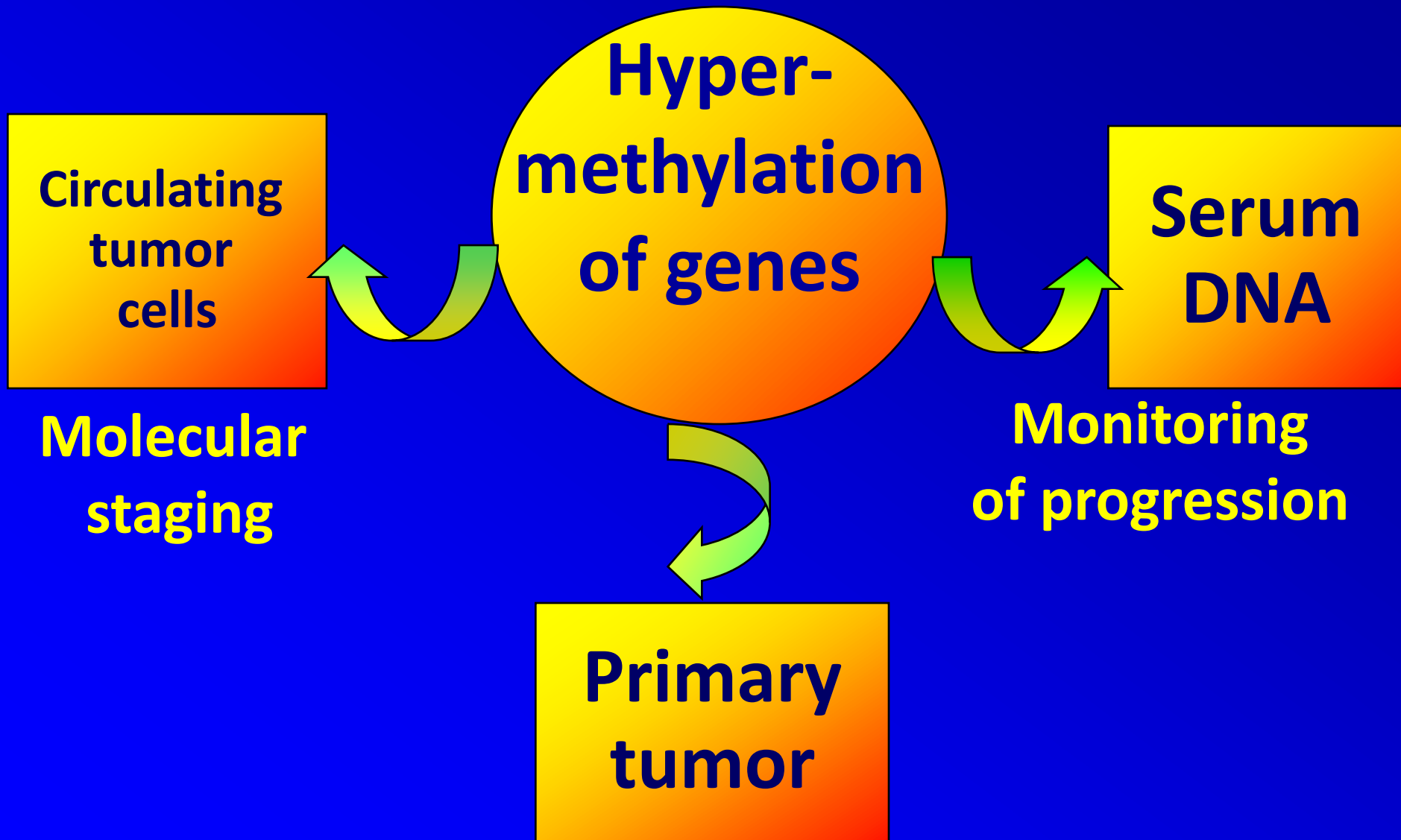
2.

Determination of amplified chromosome regions in Triple negative breast cancer
New therapeutic targets

3.

Identification of amplified metalloprotease signaling in Triple negative Breast cancer

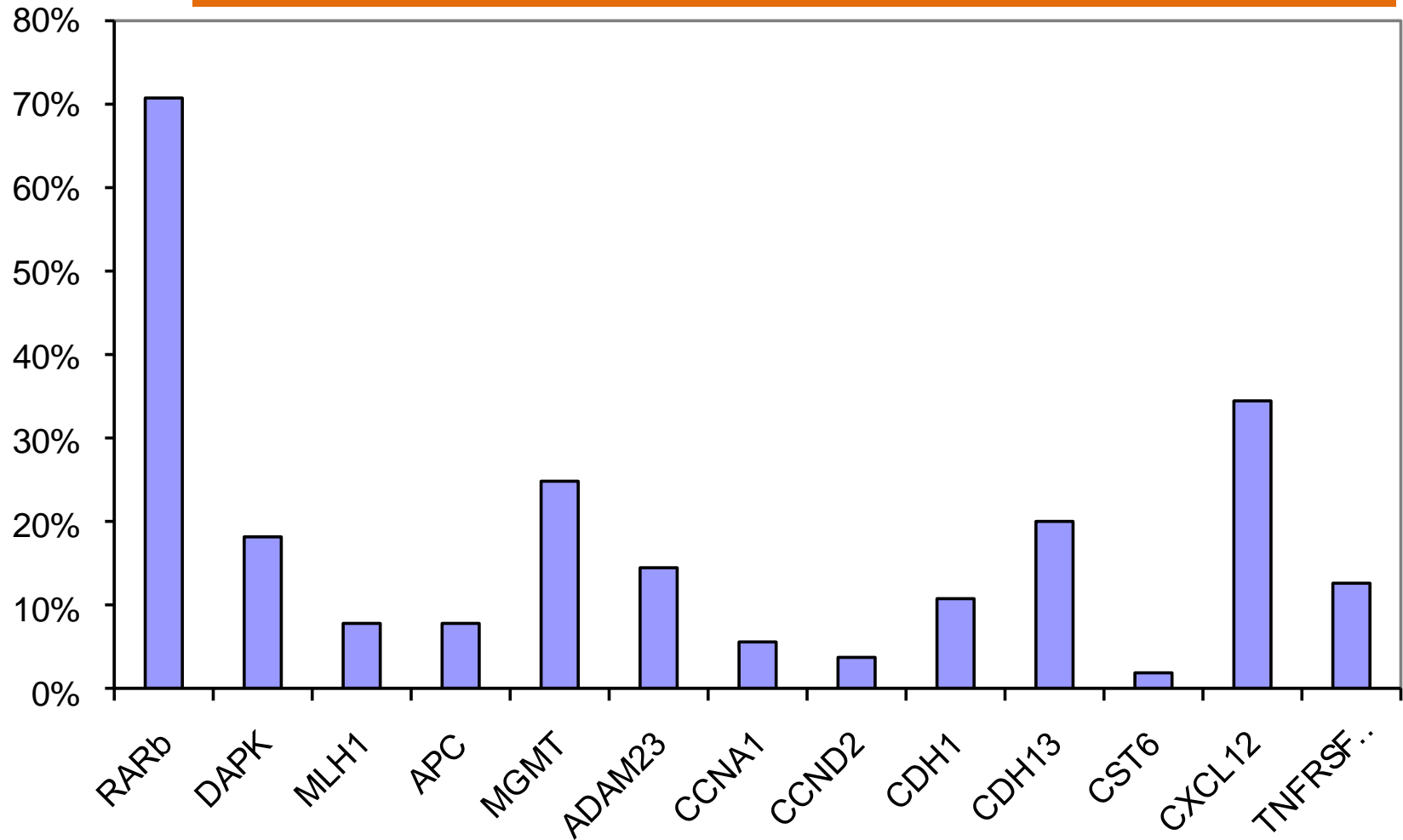
Epigenetic Silencing of cancer genes



Hypermethylated genes specific for head and neck cancer

	Function
ADAM23	Cell adhesion, migration
APC	Cell adhesion
CCNA1	Cell cycle
CCND2	Cell cycle
CDH1	Cell adhesion, invasion
CDH13	Cell adhesion, invasion
CST6	Protease inhibitor, invasion
CXCL12	Activation of lymphocytes
DAPK	Apoptosis
MGMT	DNA-repair
TNFRSF10D	Apoptosis

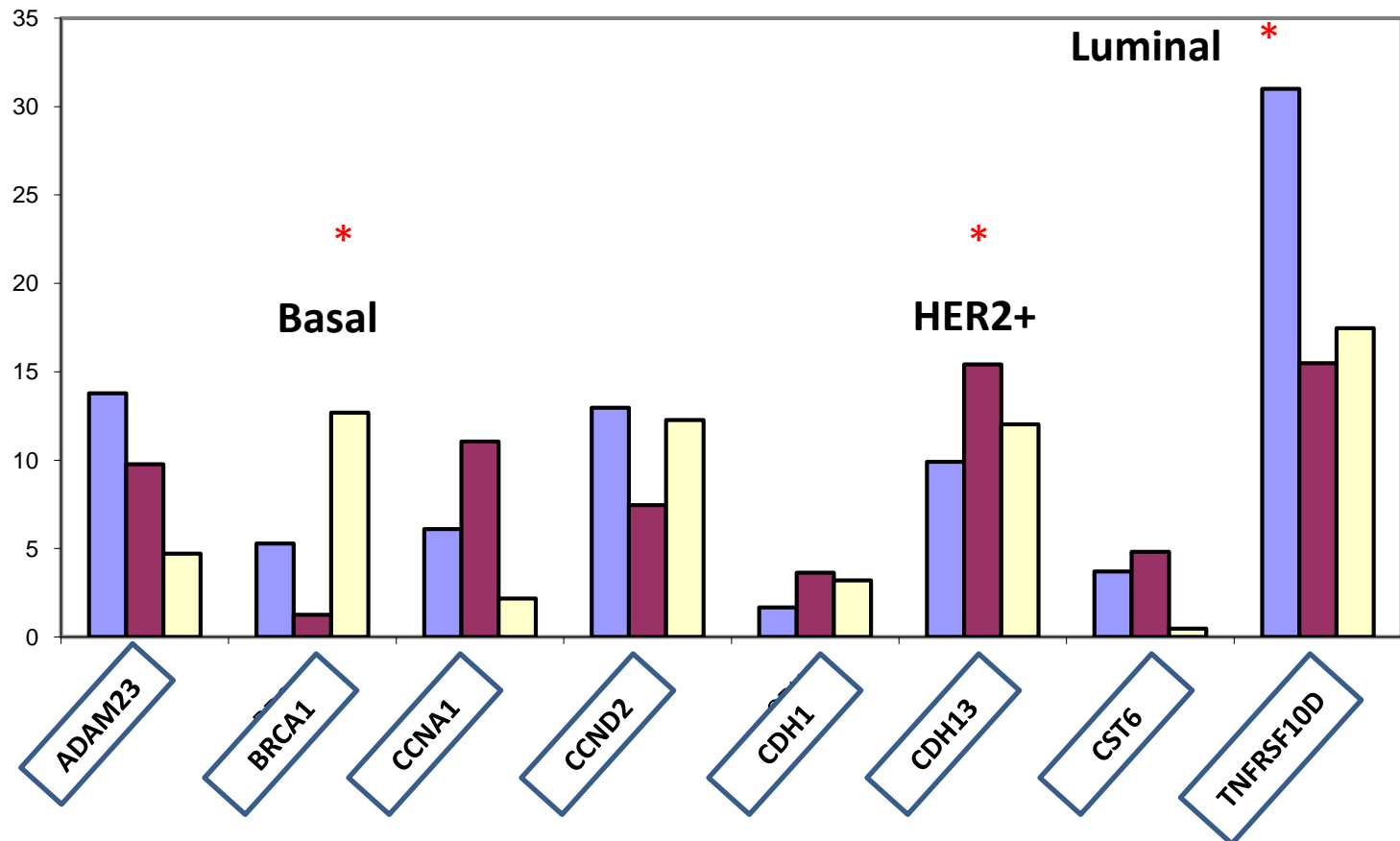
Hypermethylation of various genes in head and neck cancer



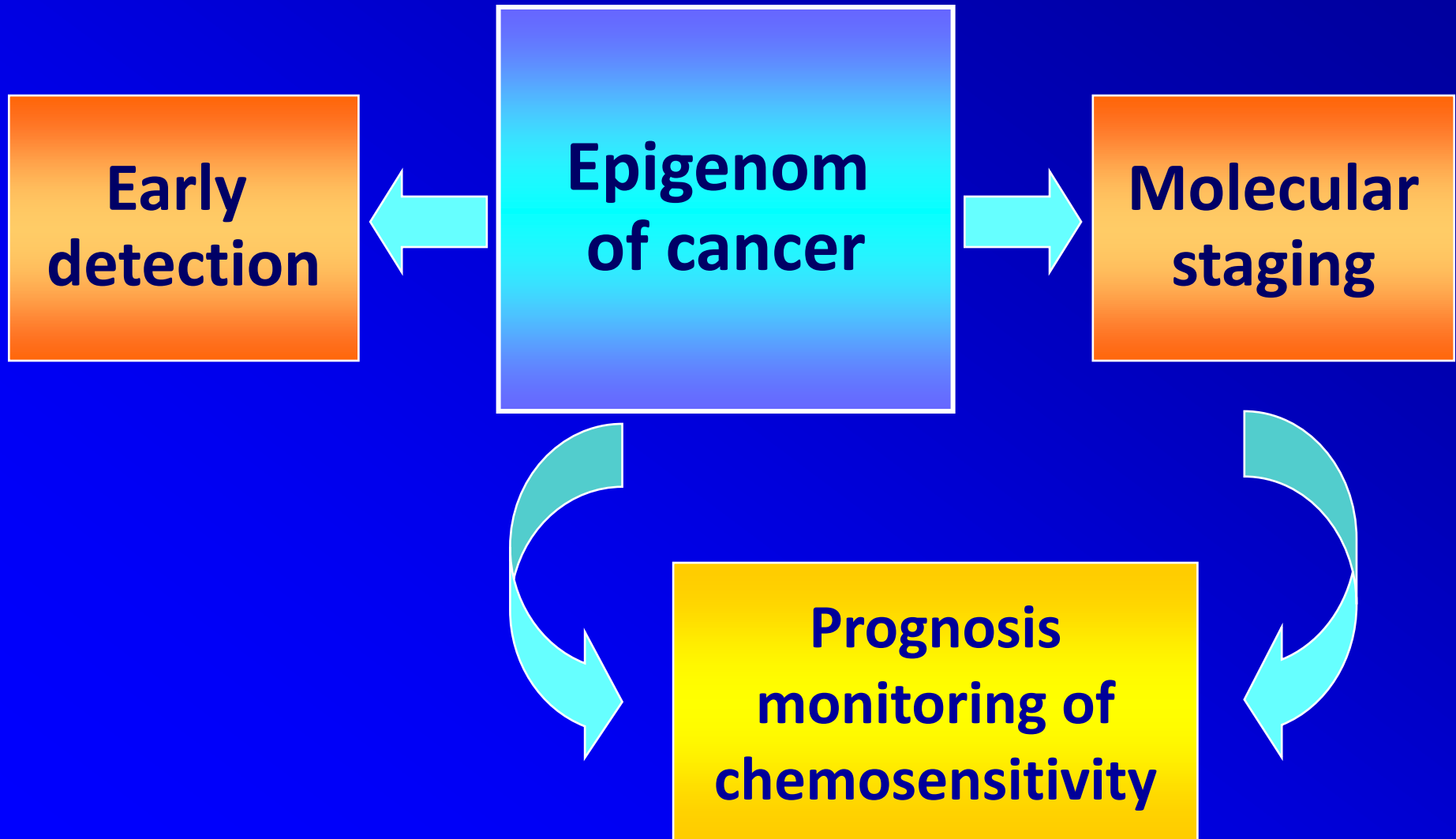
Hypermethylated genes specific for different breast cancer subtypes by methylation array

	Function
Luminal A	
ADAM23	Cell adhesion, migration
CCND2	Cell cycle
TNFRSF10D	Apoptosis
HER2+	
CCNA1	Cell cycle
CDH1	Cell adhesion, invasion
CDH13	Cell adhesion, invasion
CST6	Protease inhibitor, invasion
Basal	
BRCA1	Cell cycle, DNA repair, apoptosis
CCND2	Cell cycle

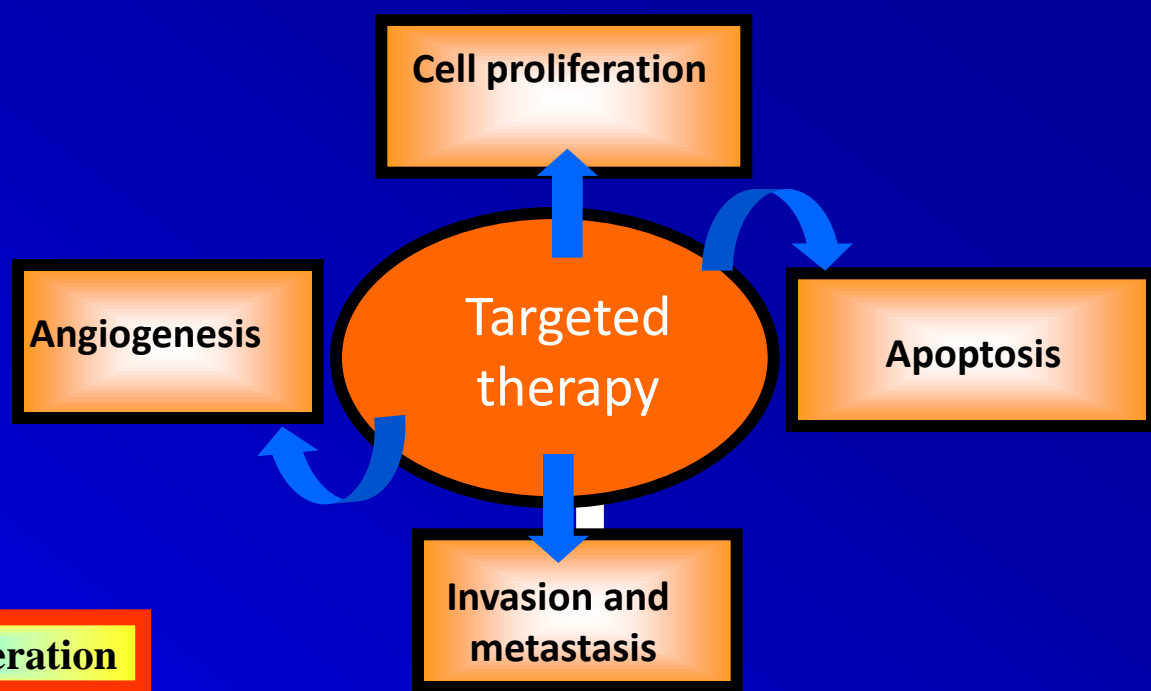
Methylation pattern of breast cancer



Significance of epigenetic signature



Strategy of targeted therapy



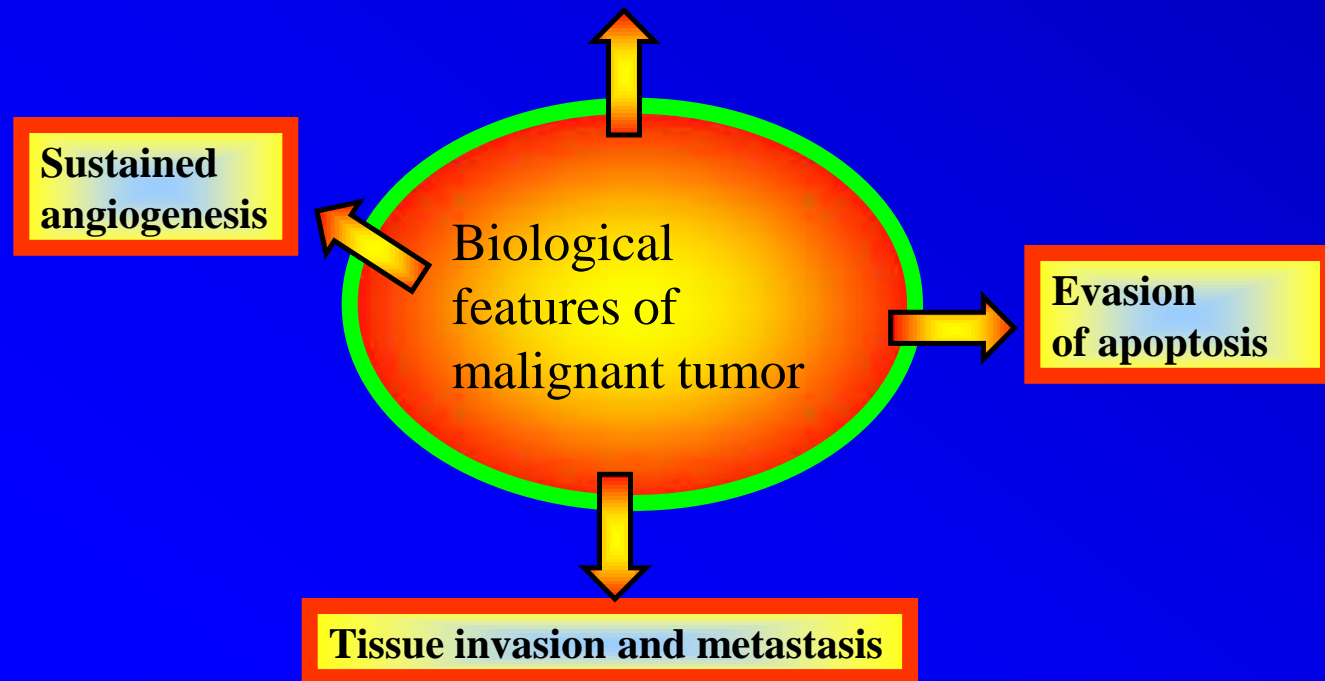
Unlimited cell proliferation

Sustained angiogenesis

Biological features of malignant tumor

Evasion of apoptosis

Tissue invasion and metastasis



Development of targeted therapy

- 1. Identification of gene mutations and genetic variants of cancer**
- 2. Genetic pathway of various cancer**
- 3. Genomic signature of cancers**
- 4. Epigenetic signature of cancers**
- 5. Characterization of cancer type specific signaling pathways**
- 6. Identification of therapeutic targets**
- 7. Development of targeting therapy**

Gene expression pattern of tyrosine kinases



Head and neck cancer

TK expression in various anatomical localizations

Breast cancer

TK expression in luminal, HER2+, triple- breast cancer

**Identification
of new targets**

Cluster analysis of TK Expression in HCNN by DNA chip

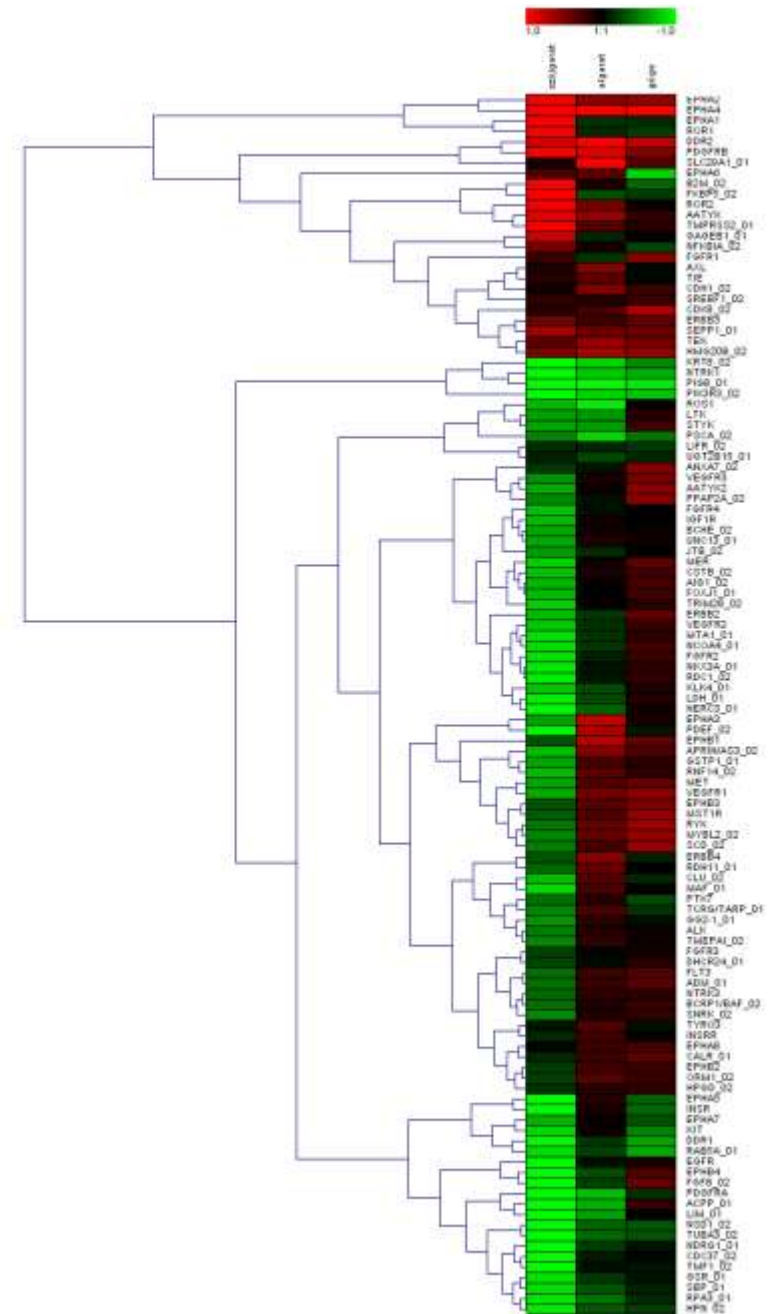
**Tyrosine kinase
over expression**

Oral cavity:

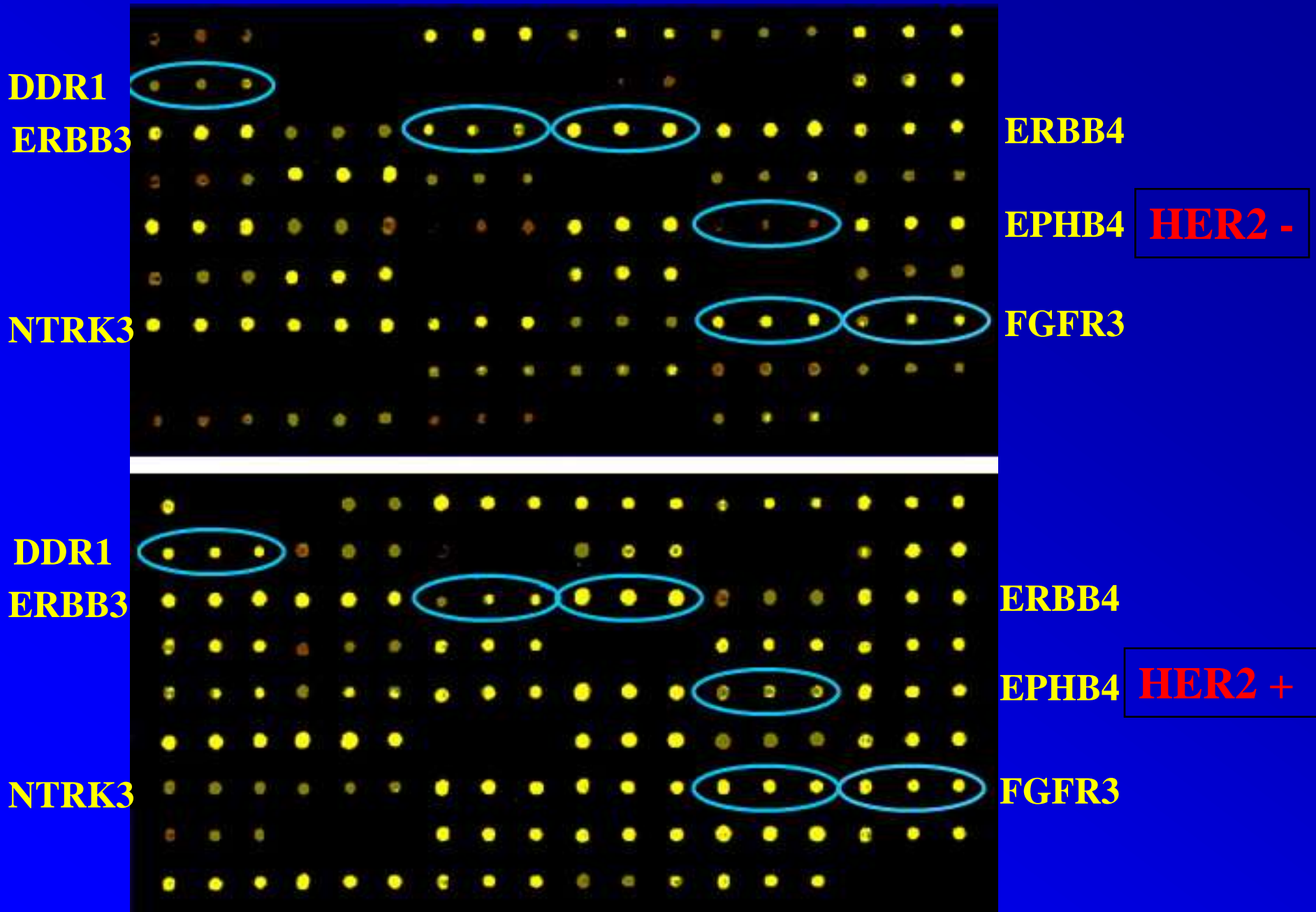
**ERBB2, EPHA1, EPHA2,
ROR1, ROR2, NTRK3,
AATYK2, FGFR4**

Pharynx: ERBB2


**Larynx: ROR1, ROR2,
EPHA1, EPHA2**



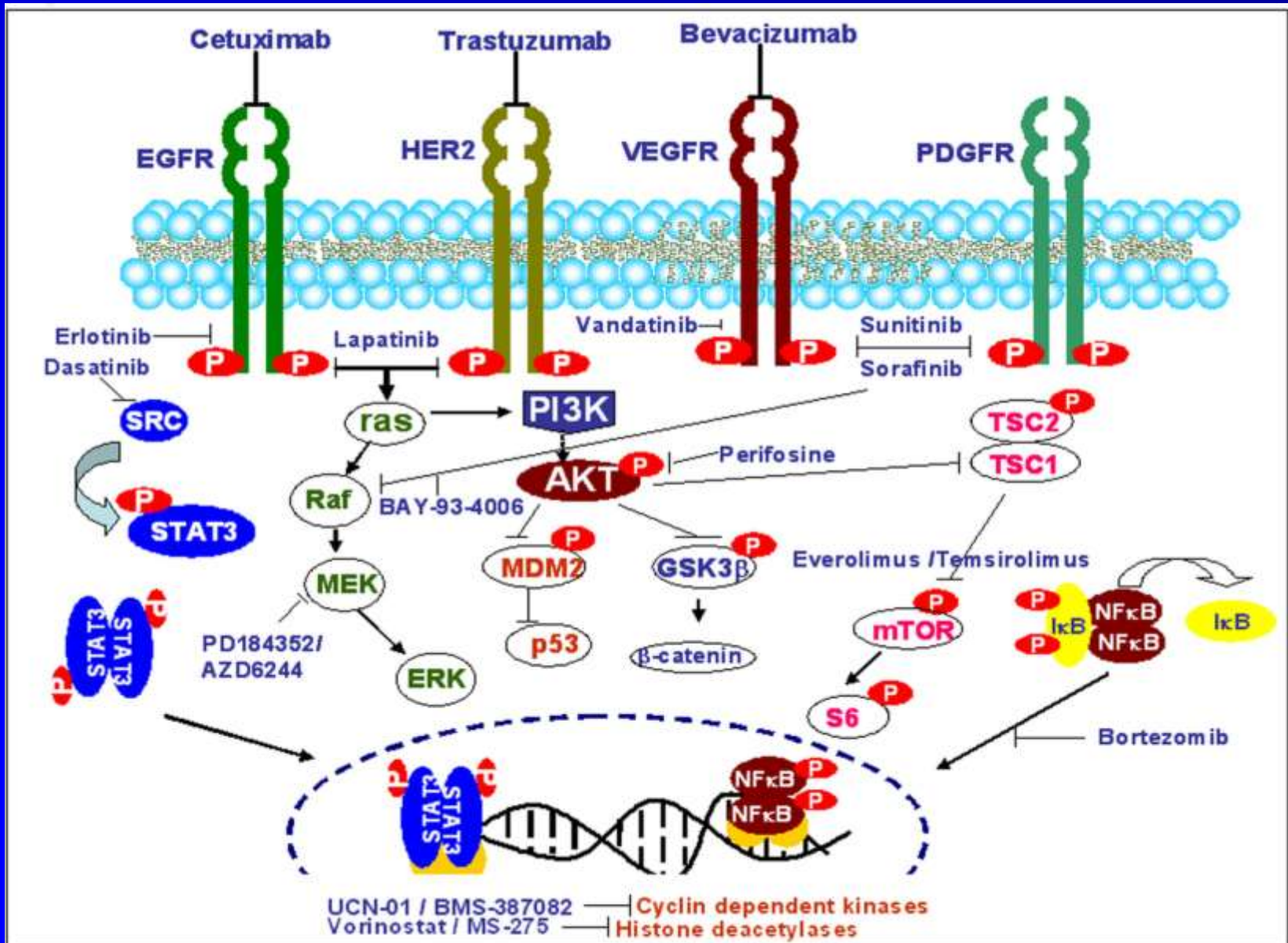
Tyrosine kinase pattern of breast cancer oligo DNS chip



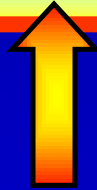
New tyrosine kinases for targeted therapy

1. Breast cancer: **Triple-** ERBB3, NTRK3, AATYK2
HER2+: (EPHA8, EPHB4, TEK, VEGFR1,2 FC, FGFR4)
HER2-: DDR2, INSR, KIT, PDGFR β
2. Head and neck cancer:
Oral cavity:
ERBB2, EPHA1, EPHA2, ROR1, ROR2, NTRK3, AATYK2, FGFR4
Larynx: ERBB2
Pharynx: ROR1, ROR2, EPHA1, EPHA2
3. Colorectal cancer: **Proximal**
ERBB4, EPHA5, INSR, TEK, ROR2, KIT, FGFR3, FGFR4
Distal colon: INSR, KIT, ROR2, TEK, NTRK1 

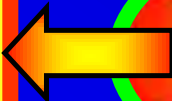
Molecular targets of HNSCC



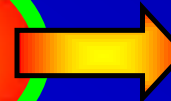
Erbitux



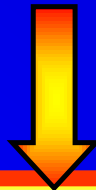
Erlotinib



**TK inhibitors
of HNSCC**

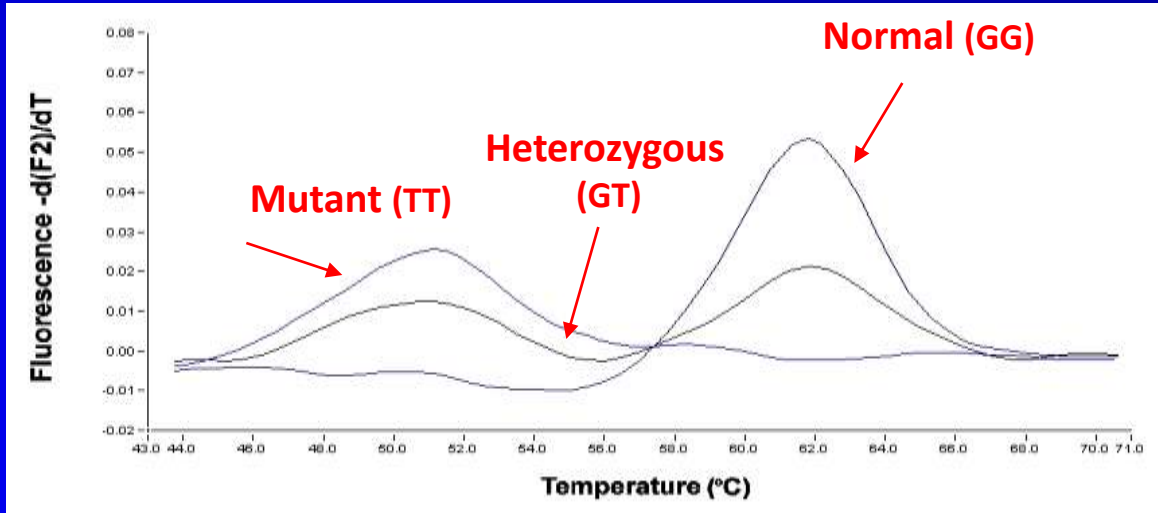


Iressa



Lapatinib

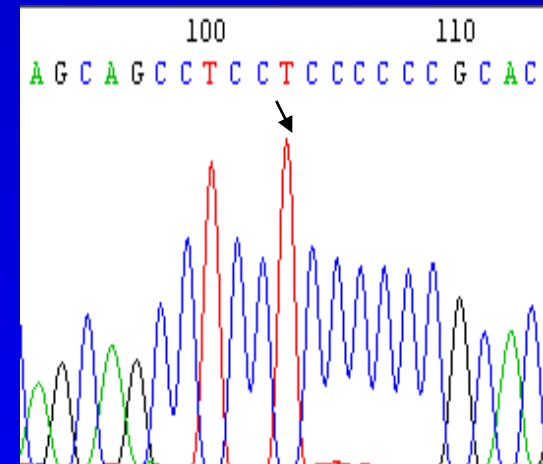
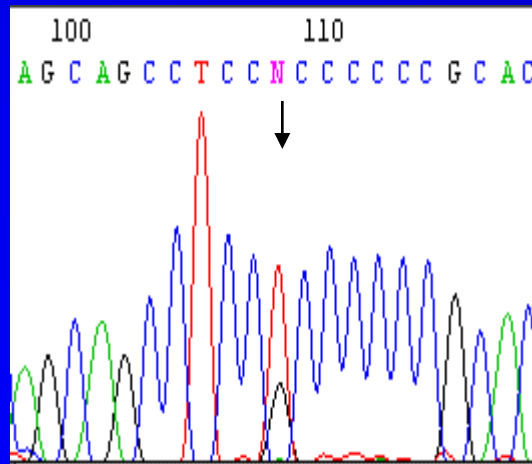
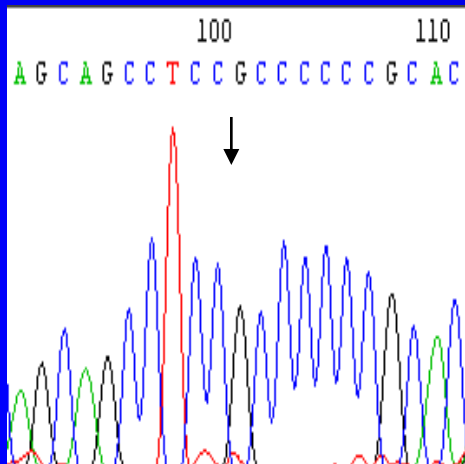
EGFR G-216T polymorphism



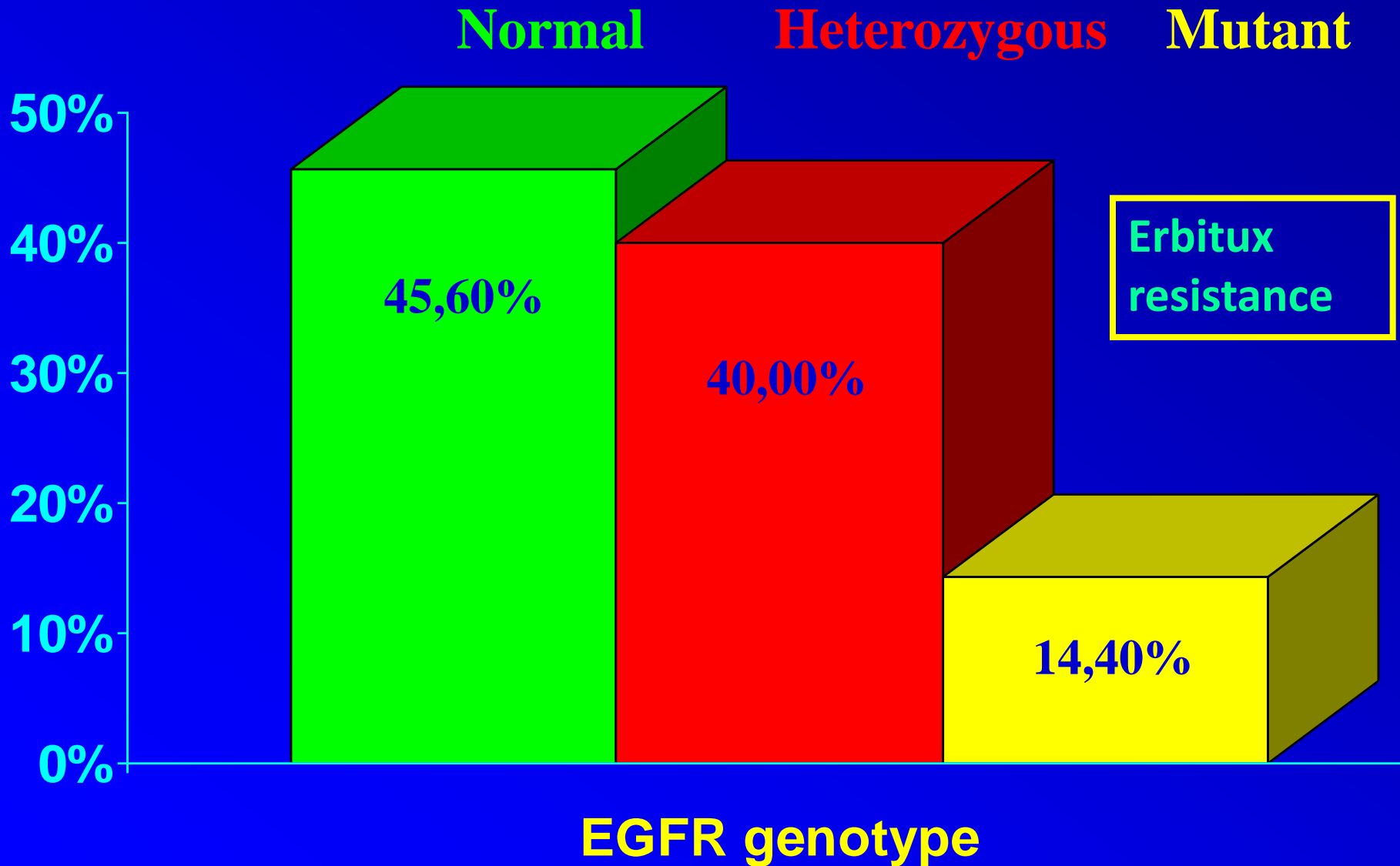
N

H

M



Distribution of EGFR G-216T genotypes



Genomic analysis of head and neck cancer

1. **CGH analysis (40 samples)**

2. **Tyrosine kinase expression by DNA array**

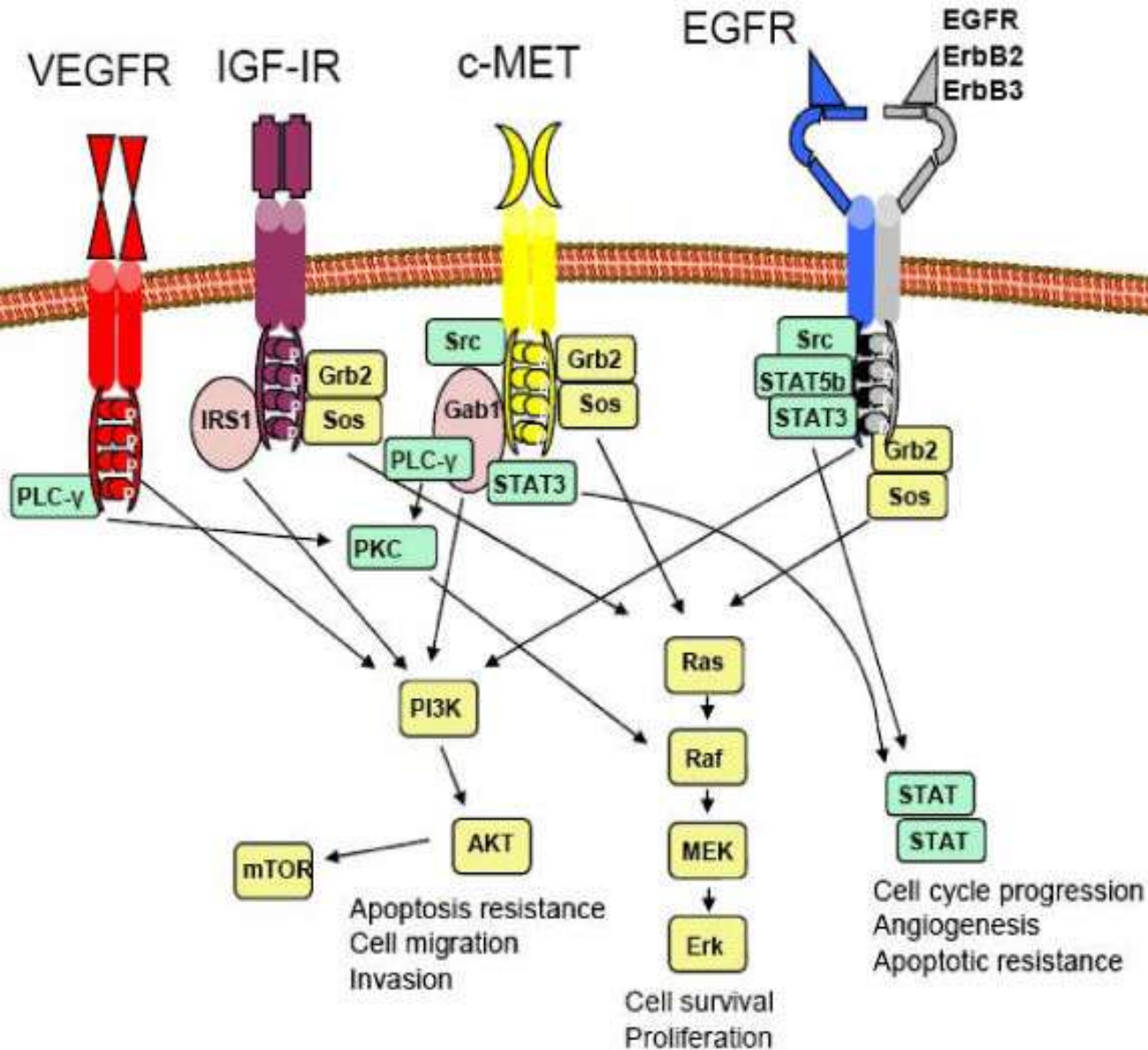
3. **Methylation pattern**

4. **Therapeutic sensitivity of HNSCC**
Cetuximab sensitivity, antiangiogenetic
Drug sensitivity, cytotoxic drug sensitivity

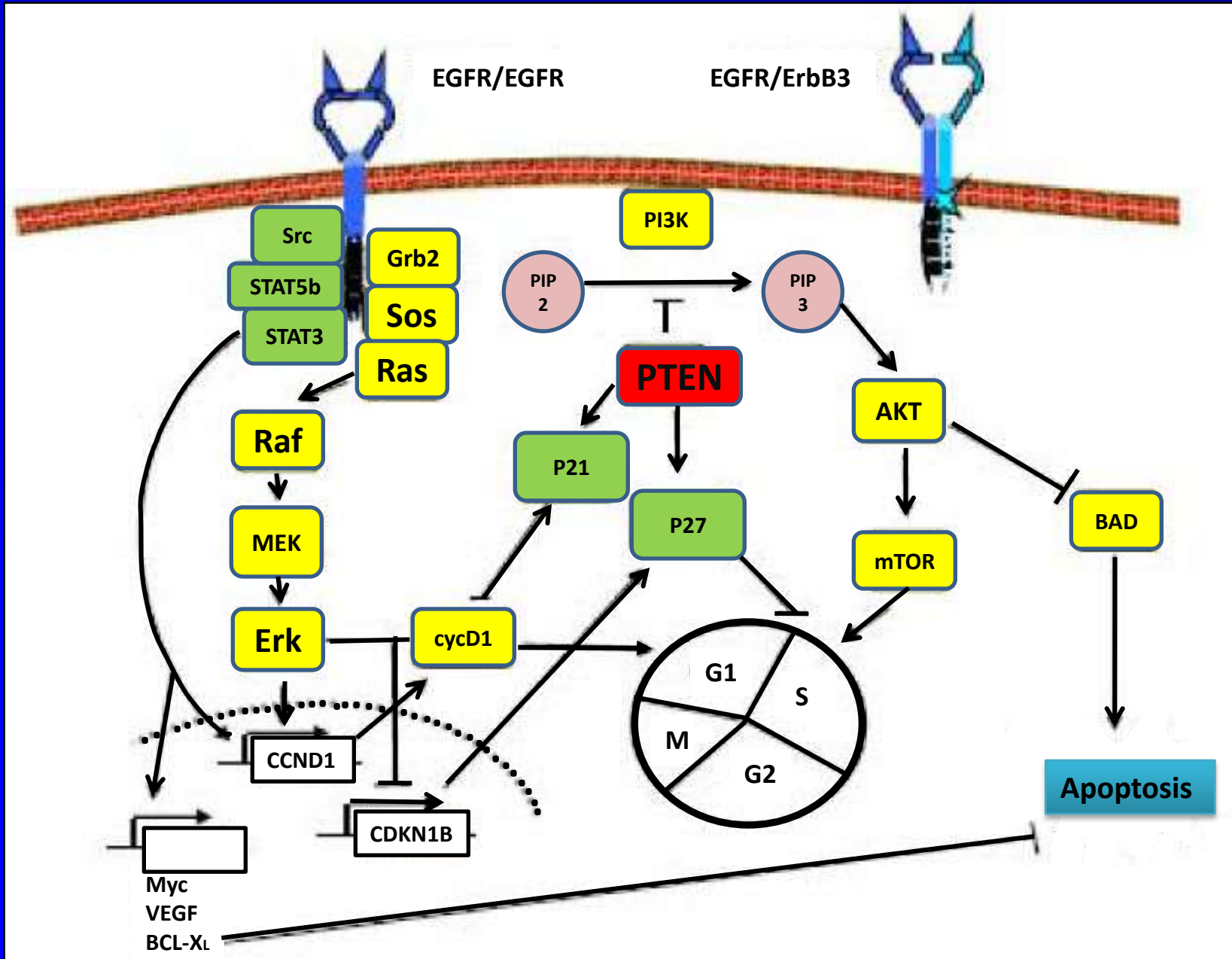
Resistance to TK inhibitors

1. Mutation of targets -
» cKIT, PI3K, EGFR
2. Activation of alternative signaling pathway -
» Ras, Raf, PI3K, Akt, mTOR
3. Crosstalk of receptors -
» EGFR, HER2, Erb3
4. Inactivation of growth inhibitors PTEN -
» PTEN, LOH, Mutation, Hypermethylation

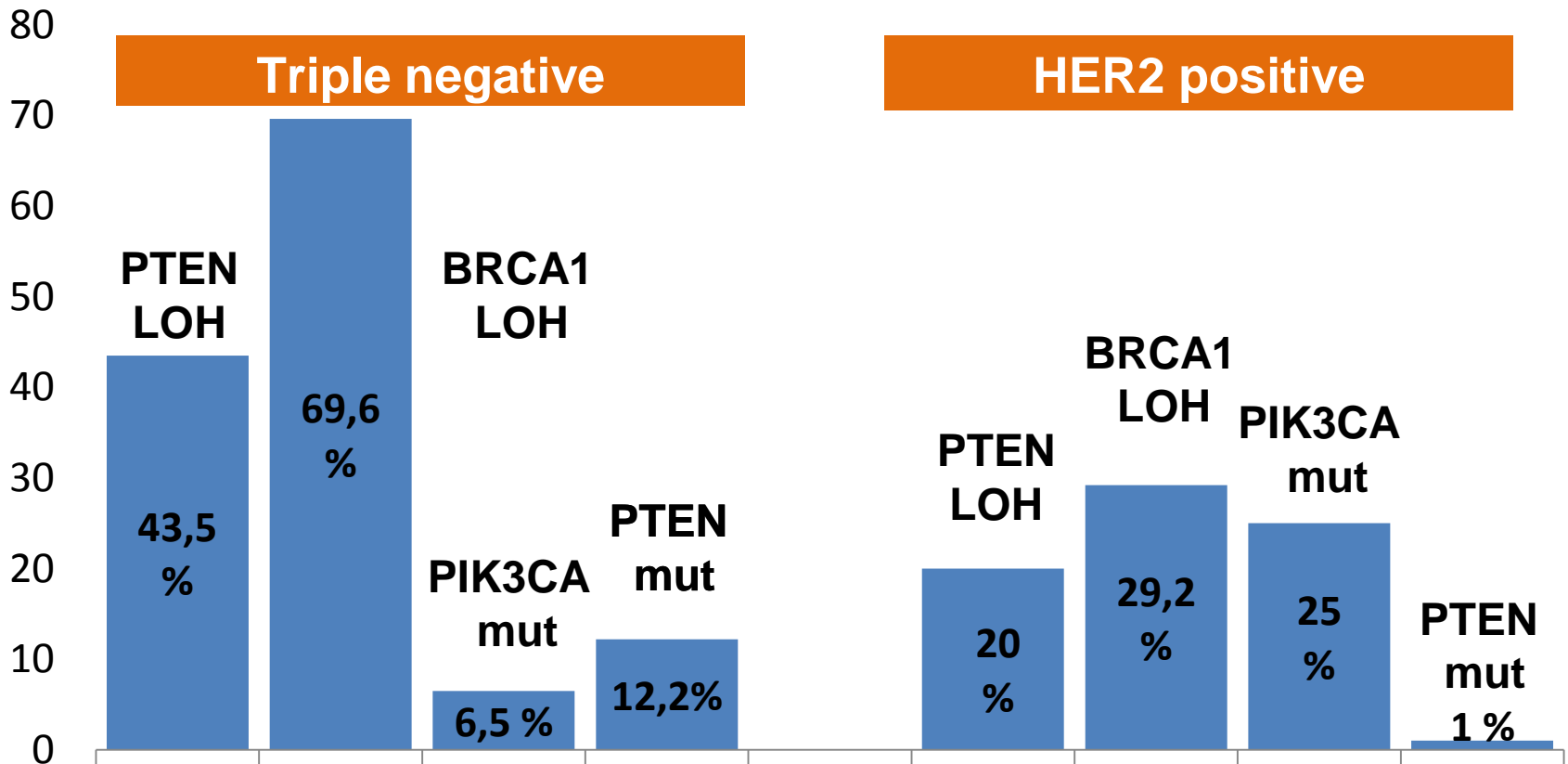
Resistance mechanisms originating from parallel growth factor receptors



Resistance mechanisms originating from EGFR downstream effectors



Genomic feature of triple negative breast cancer



Systems Biological approaches for targeted cancer therapy

Network and functional analysis

- 1. Targeting signal transduction pathways**
- 2. Targeting angiogenesis**
- 3. Targeting anti-apoptotic proteins**
- 4. Targeting metastasis cascade**
- 5. Targeting cancer stem cells**
- 6. Targeting epigenetic changes**

PCR array of cancer

Angiogenesis

Apoptosis

Brain Cancer

Breast Cancer

Cancer

Cancer Drug Targets

Cancer miRNA Genes

Cancer

PathwayFinder

Cell Cycle

Cell Motility

Colon Cancer

DNA Damage

Signaling Pathway

DNA Repair

Epithelial to

Mesenchymal

Transition (EMT)

Leukemia &

Lymphoma

Liver Cancer

Lung Cancer

Melanoma

mTOR Signaling

Prostate Cancer

Serum

Telomeres &

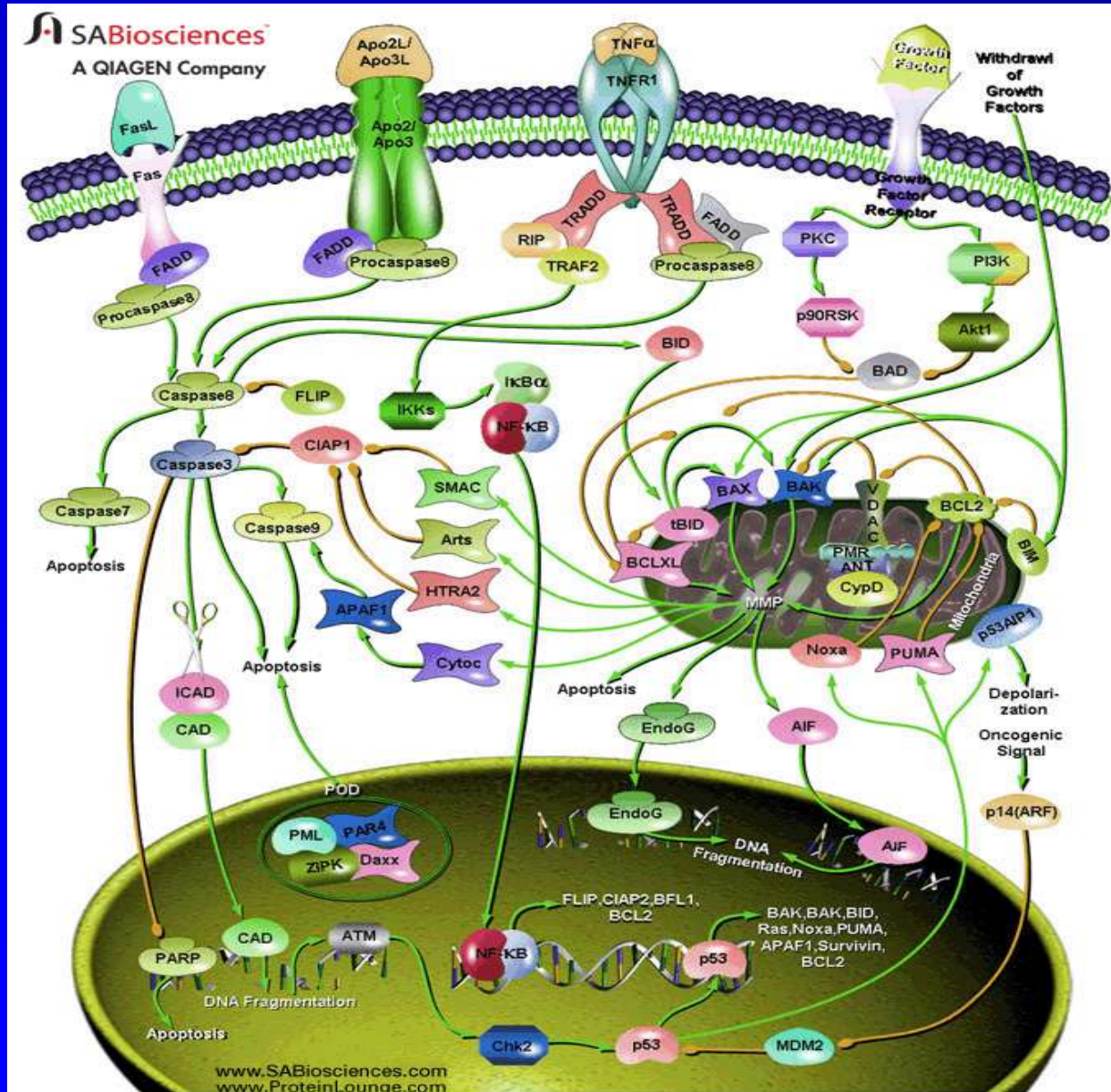
Telomerase

Tumor Metastasis

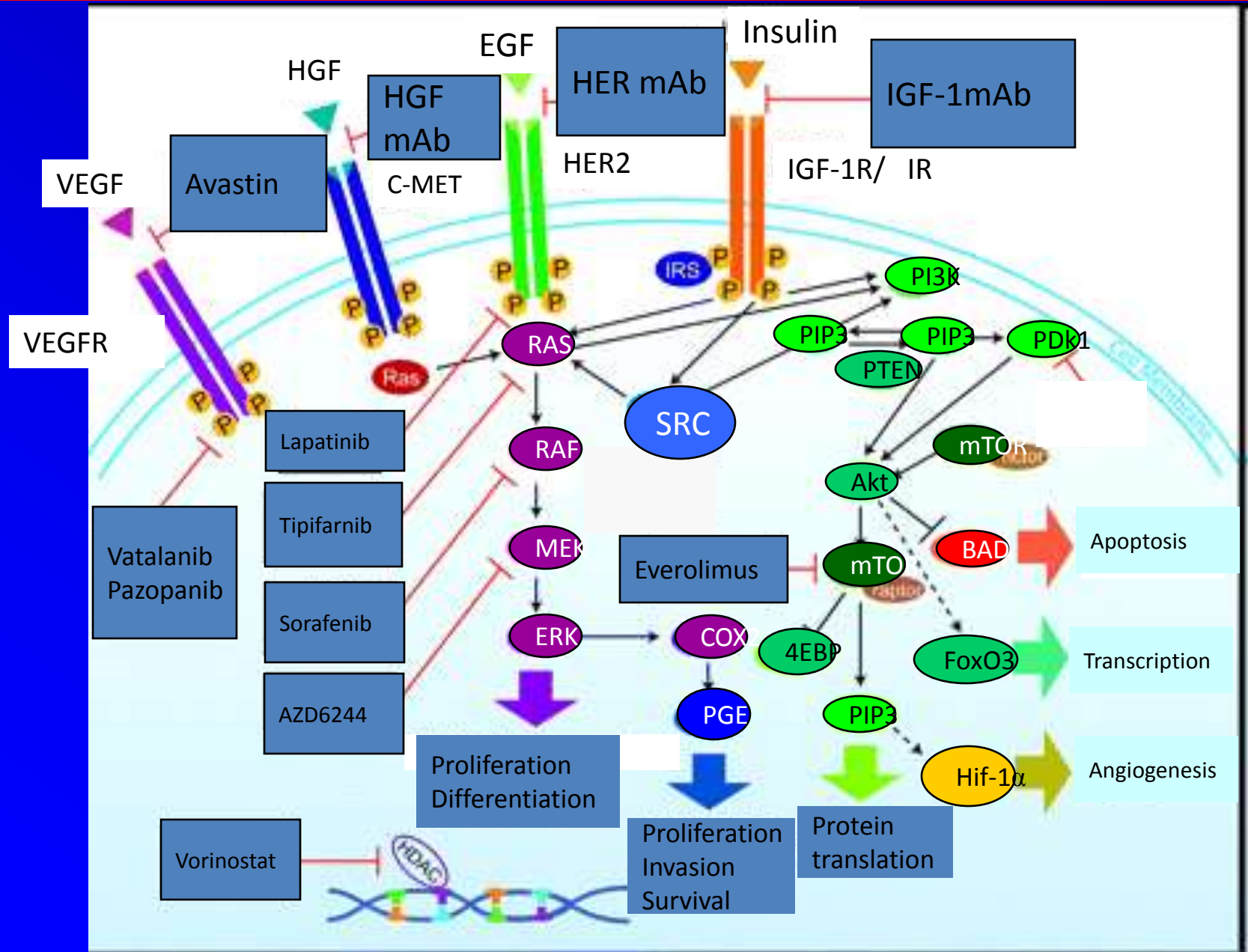
Tumor Suppressor

Genes

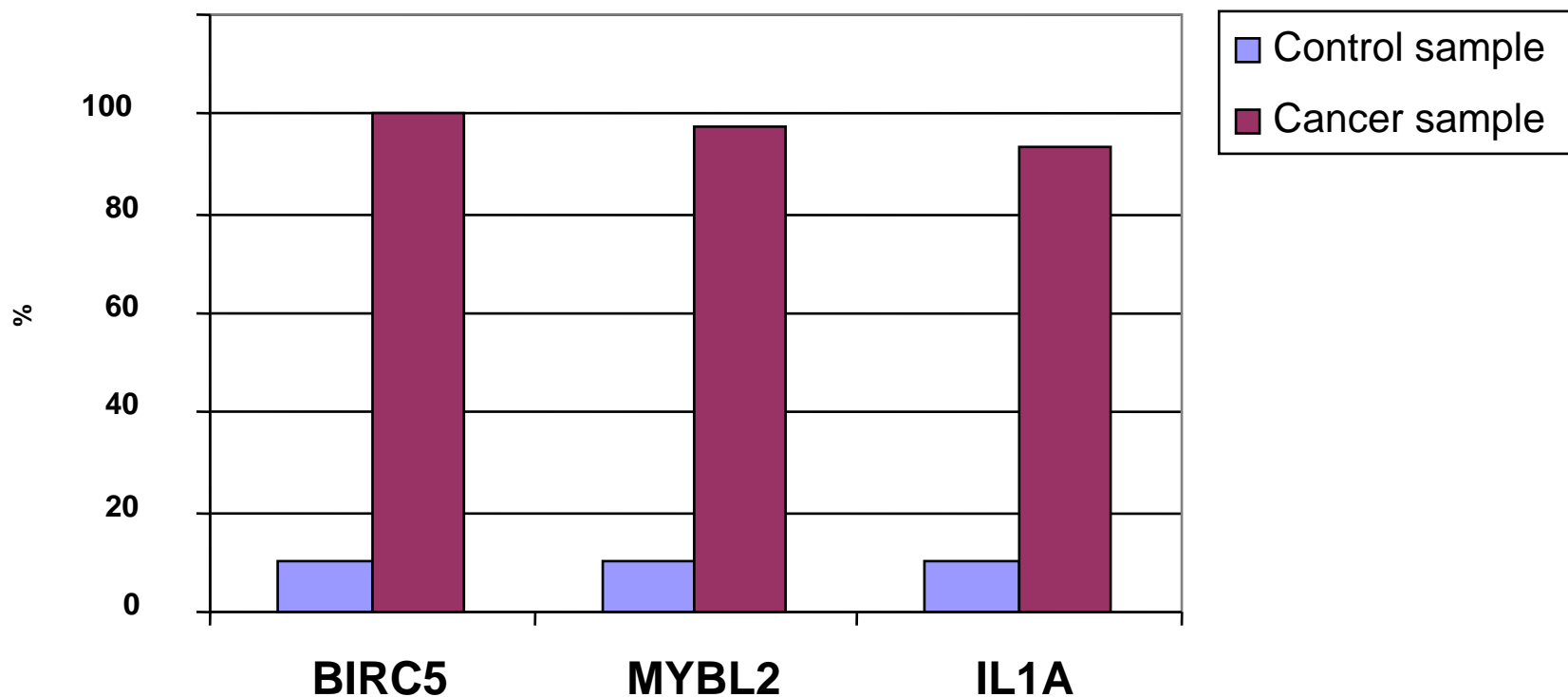
Cellular Apoptosis Pathway



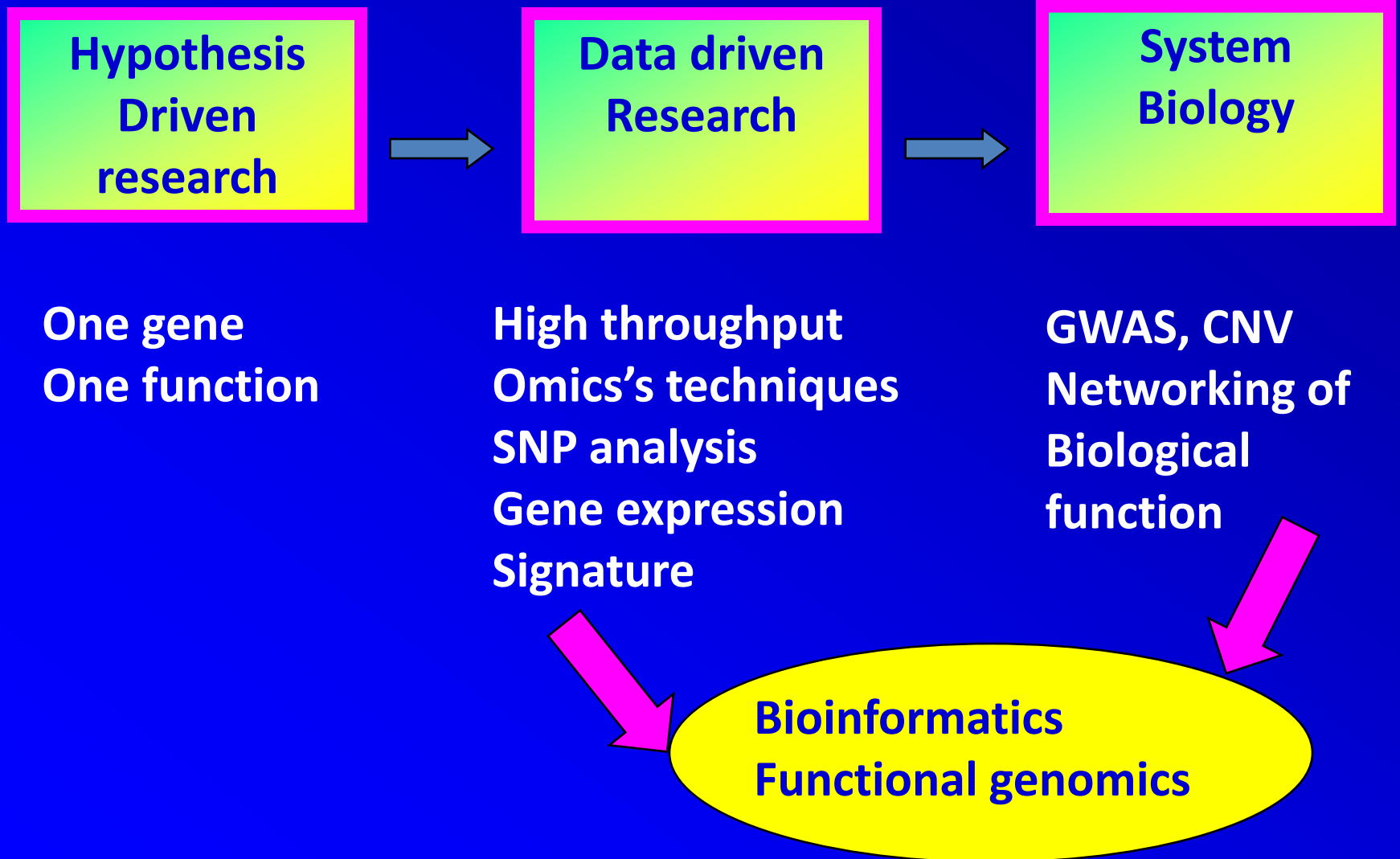
Signaling pathways in colorectal cancer



Over expression of genes in HIF α pathway HNSCC



Evolution of Cancer Research Strategy



Systems Biological approaches for personalized cancer therapy

1. Genome wide associations Studies (GWAS)
2. DNA copy number aberration (CNA)
3. DNA mutation detection
4. Epigenetic profiling
5. Gene expression profiling
6. Network and functional analysis

Significance of genomic analysis of HNSCC and breast cancer

1. Genetic markers of tumor progression
2. Molecular subtyping of tumors
3. Identification of new therapeutic target
4. Determination of resistance factors
5. Development of individualized therapeutic protocols