Precision Medicine Based on Genomics in Breast Cancer

Kathleen Burke, PhD
Bioinformatics Postdoctoral Fellow
Laboratory of Dr. Jorge Reis-Filho

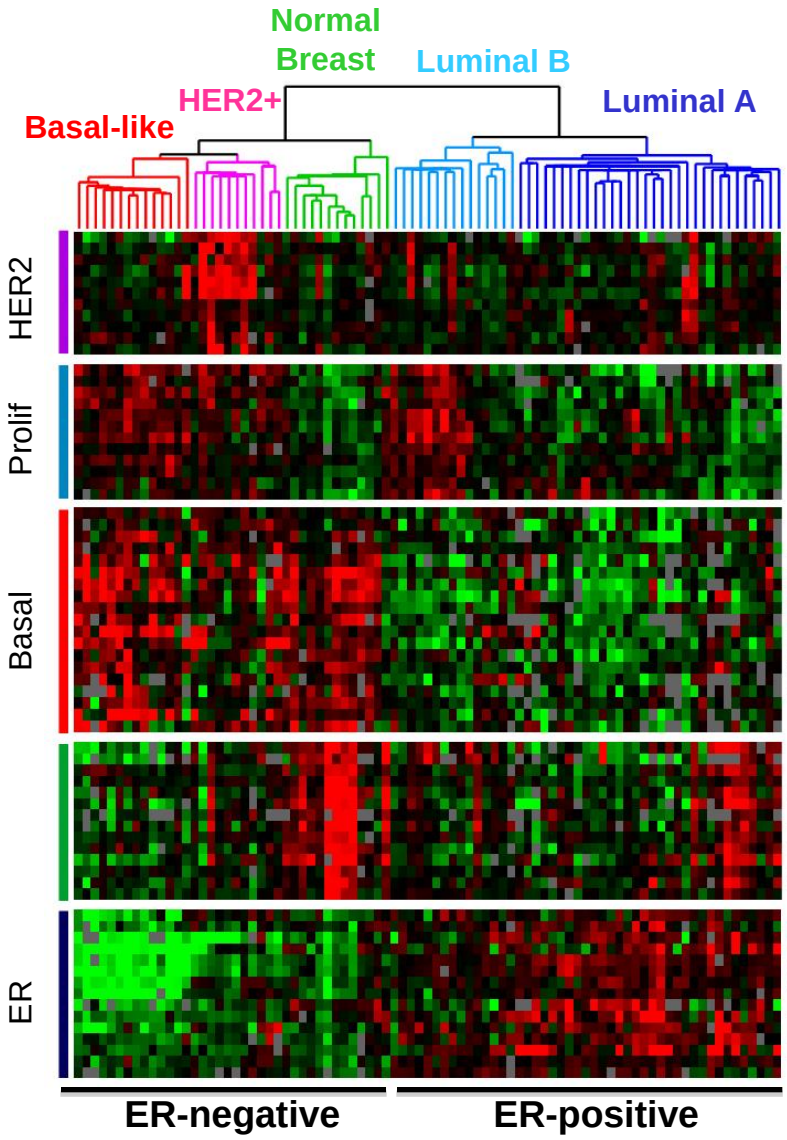
Memorial Sloan Kettering
Cancer Center™

Summary

- Current genomics tools
- Precision medicine
- Massively parallel sequencing
- Delivery of precision medicine

Current genomics tools

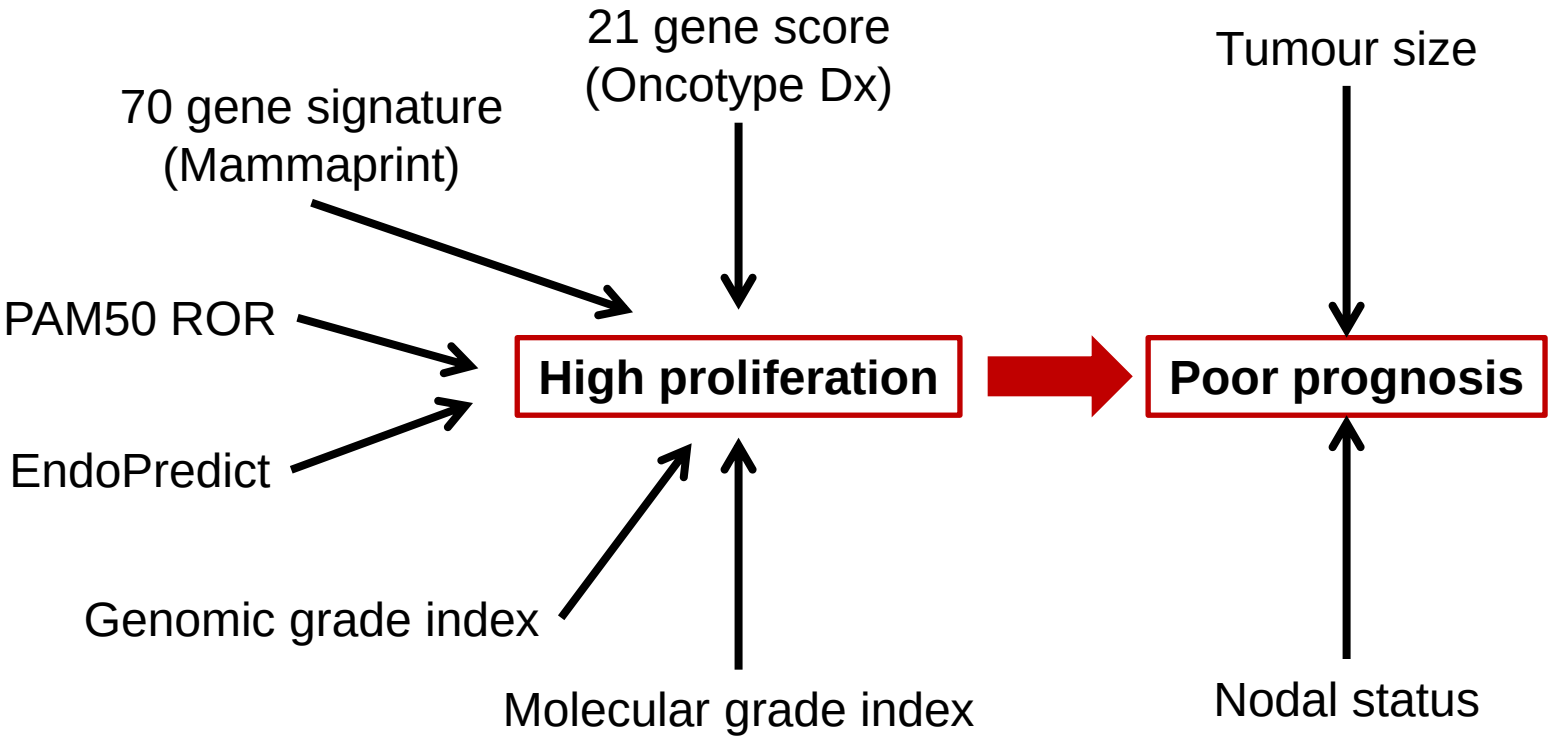
Molecular subtypes of breast cancer



- **Additional molecular subtypes**
  - Claudin-low
    - approx 60-70% TN phenotypes
  - Molecular subtypes of TNBC
    - Basal-like I, Basal-like II, Mesenchymal, Mesenchymal stem-like, Immunomodulatory, and Luminal androgen receptor (molecular apocrine)
  - METABRIC subtypes
    - 10 subtypes

Perou et al, Nature 2000; Sorlie et al, PNAS 2001; Hu et al, BMC Genomics 2006; Parker et al. JCO 2009

First generation prognostic signatures



First generation prognostic signatures are associated with chemotherapy response

Recurrence Score	≤18	>18 and <31	≥31
Prognosis	Good	Intermediate	Poor
Endo benefit	High	Undetermined	Low
Chemo benefit	Negligible	Undetermined	High

Fan et al. NEJM 2006; Sotiriou et al. JNCI 2006; Reis-Filho & Puztai. Lancet 2011

15 years of microarray analysis

- ER+ and ER- negative tumours
  - Fundamentally different diseases
- The outcome of ER-positive cancers can be predicted by proliferation-related genes
- The prognosis of ER-negative breast cancers is determined by immune response-related genes
- Microarrays did not result in ways to define the best therapy for individual patients

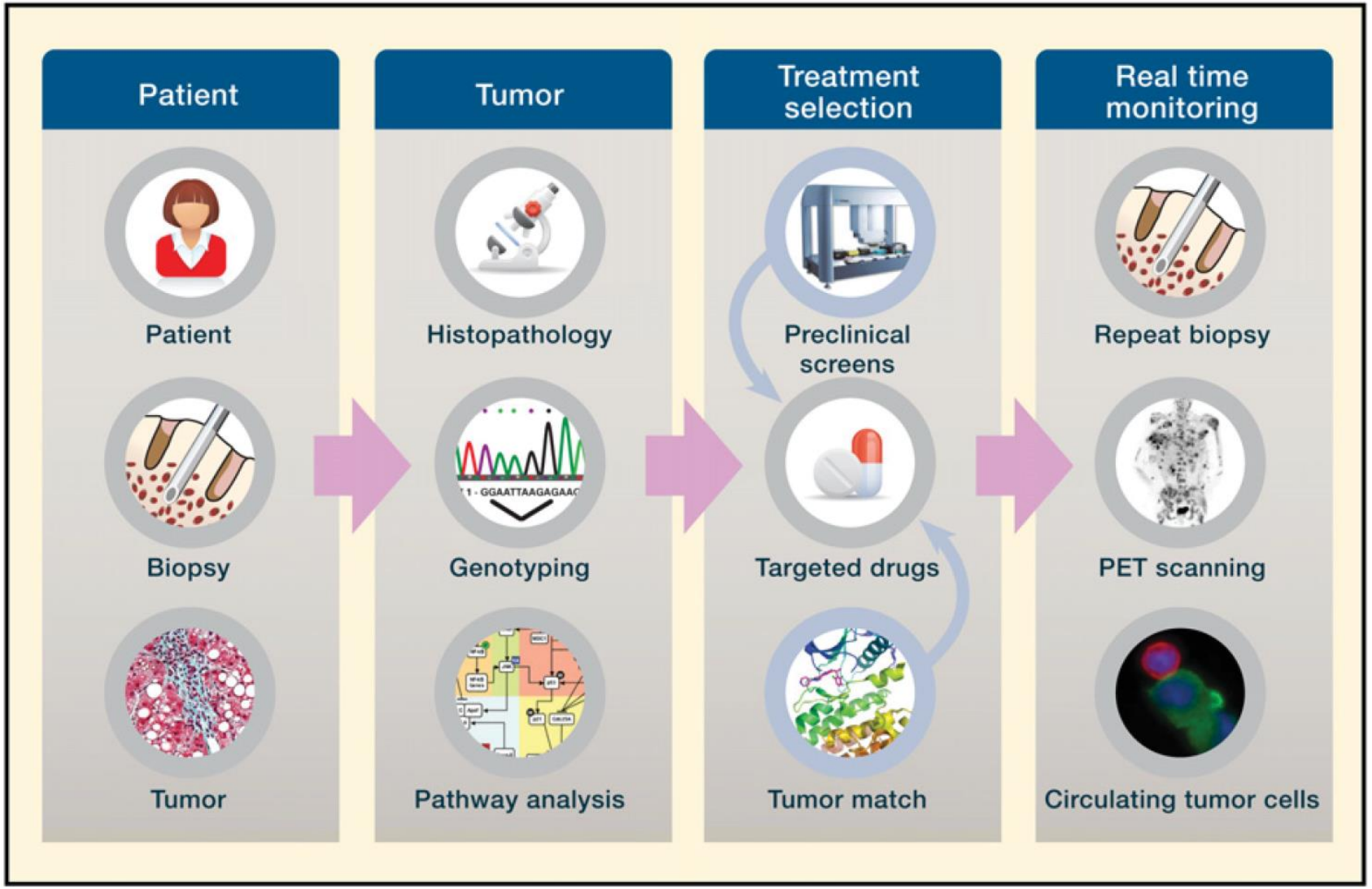
Precision Medicine

The use of genomic, epigenomic, exposure, and other data to define individual patterns of disease, potentially leading to better individual treatment.

National Academy of Sciences (NAS), 2011

Breast Cancer Patient Management

“Precision medicine”-based breast cancer patient therapy



Haber DA, Gray NS, Baselga J. Cell 2011

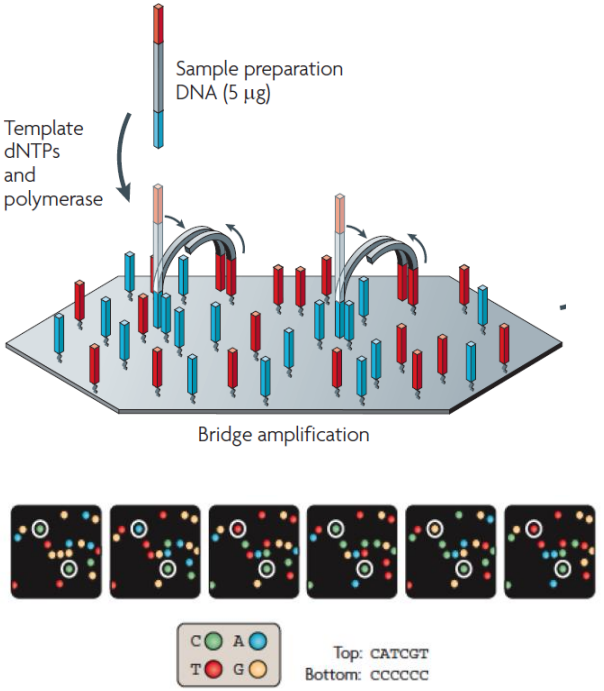
Precision medicine is now possible

Development of targeted treatments

- Small molecule inhibitors
- Monoclonal antibodies

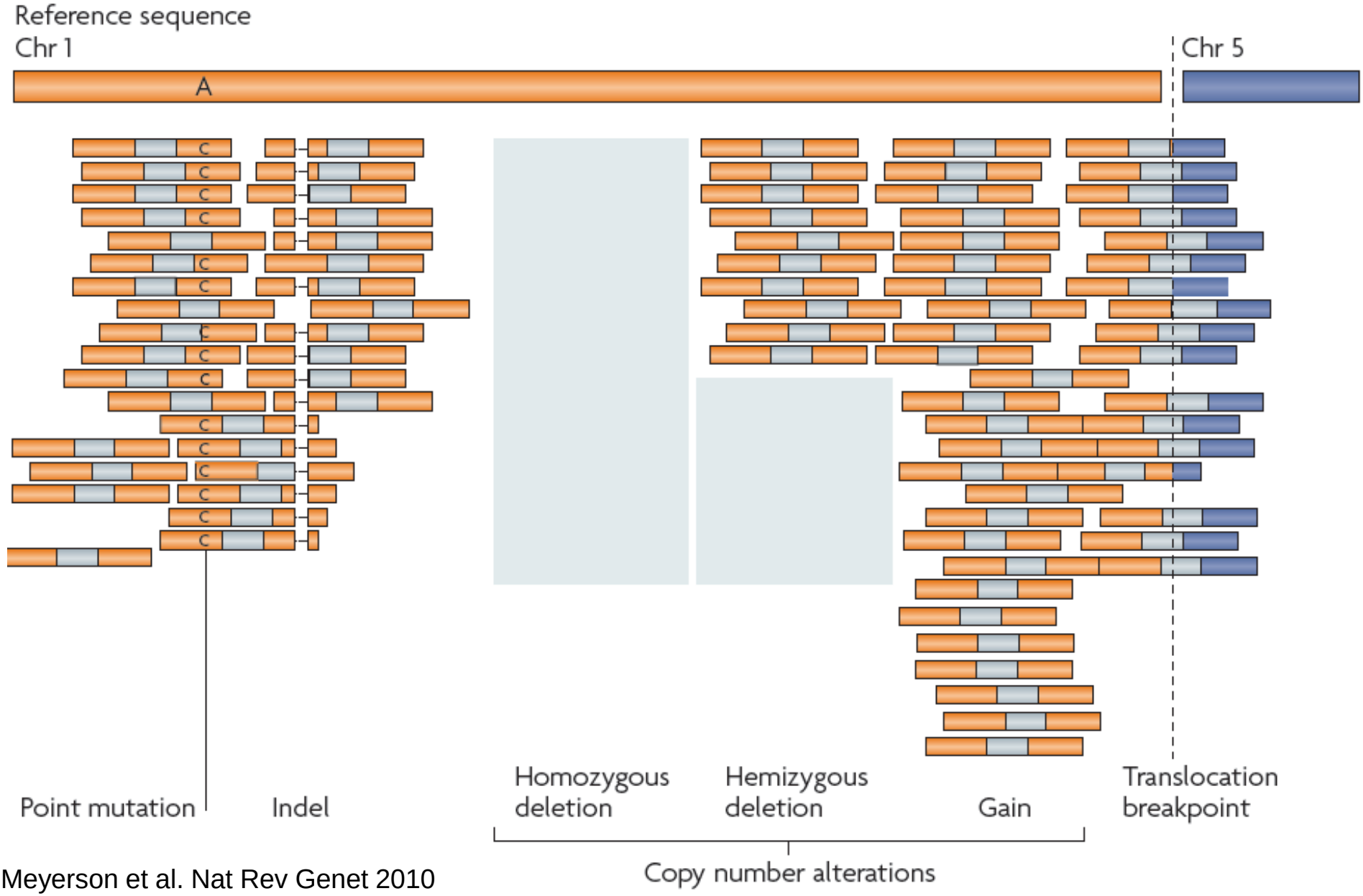
Massively Parallel Sequencing (NGS)

- Tumour genomes



Metzker et al. Nat Rev Genet 2010

Genetic changes identified by NGS



Meyerson et al. Nat Rev Genet 2010

Oncogene ‘addiction’ as the basis for predictive markers

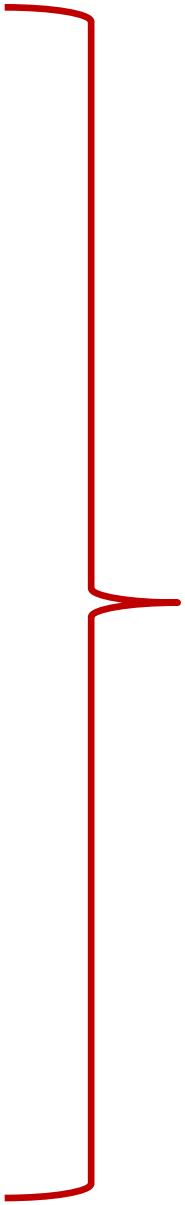
Oncogene addiction:

“…cancer cells are often "addicted to" (that is, physiologically dependent on) the continued activity of specific activated or overexpressed oncogenes for maintenance of their malignant phenotype.”

I. Bernard Weinstein

Oncogene ‘addiction’

- *HER2* amplification
  Breast and gastric cancer
- *KIT* mutation
  Gastrointestinal stromal tumours
- *BCR-ABL* fusion
  Chronic myeloid leukaemias
- *EGFR* mutations and/ or amplification
  NSCLC
- *EML4-ALK* fusion
  NSCLC
- *BRAF* mutation (V600E)
  Melanoma

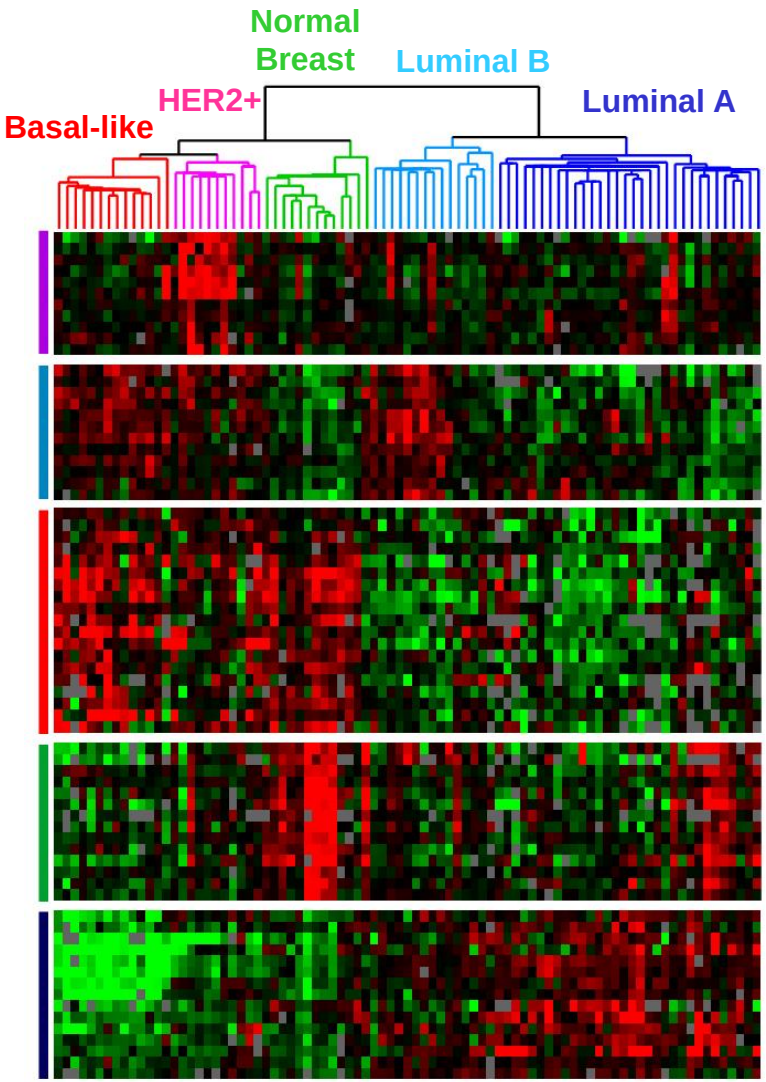


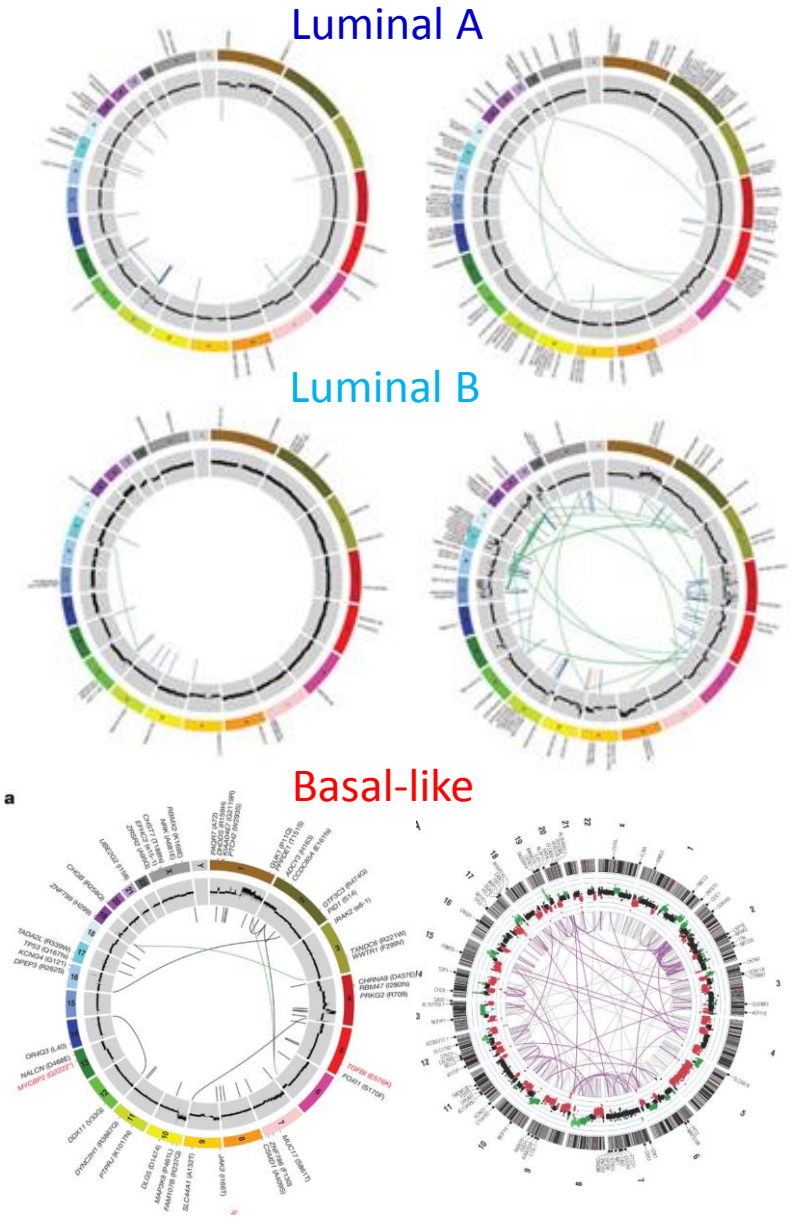
Activated through genetic hits

Inhibition is selectively lethal

Breast cancer massively parallel sequencing analysis

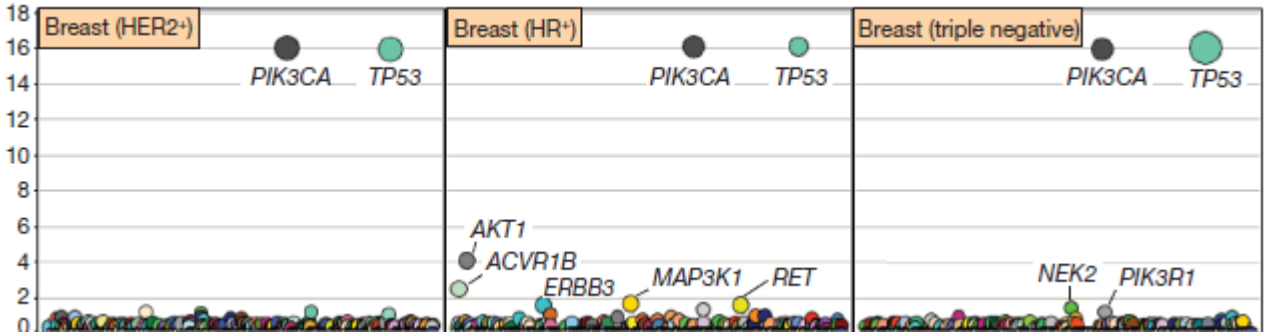
Inter-tumour genetic heterogeneity

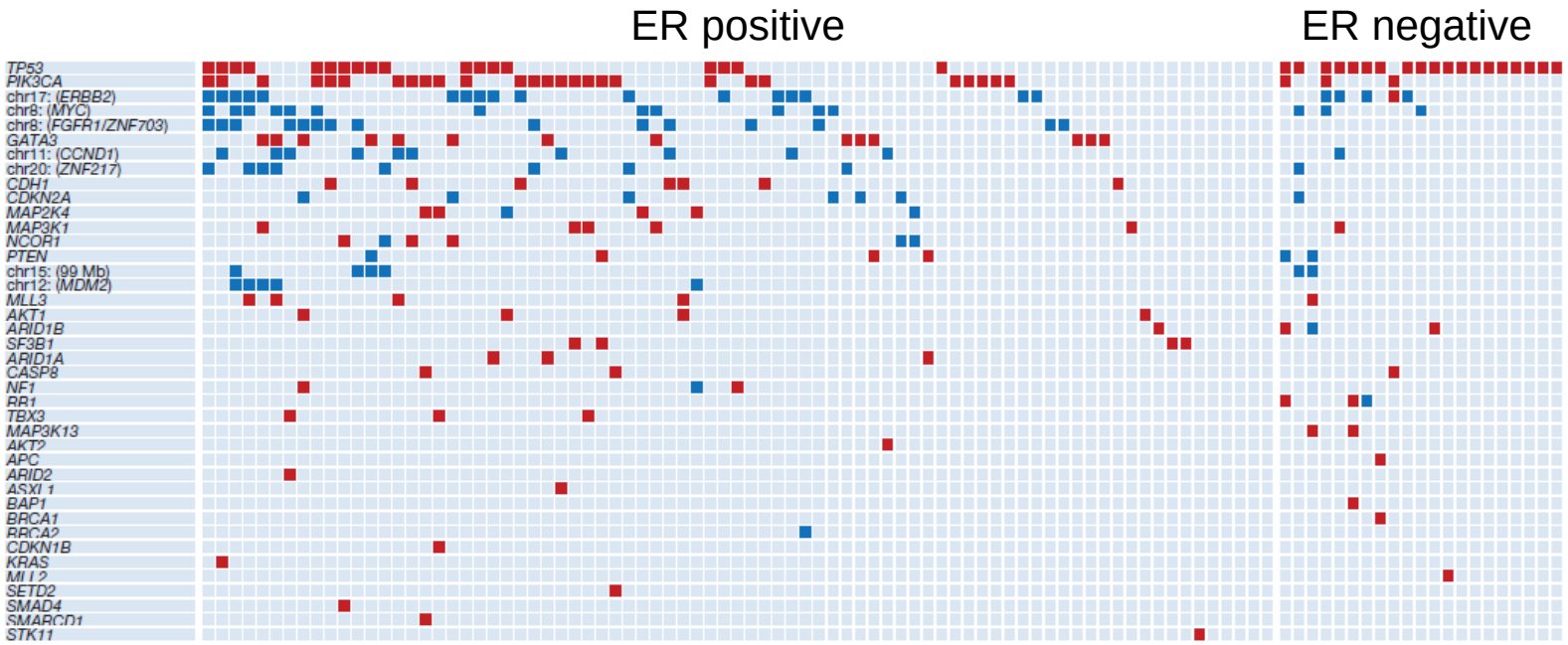




Shah et al. Nature 2009; Ding et al. Nature 2010; Natrajan et al. J Pathol 2012; Ellis et al. Nature 2012

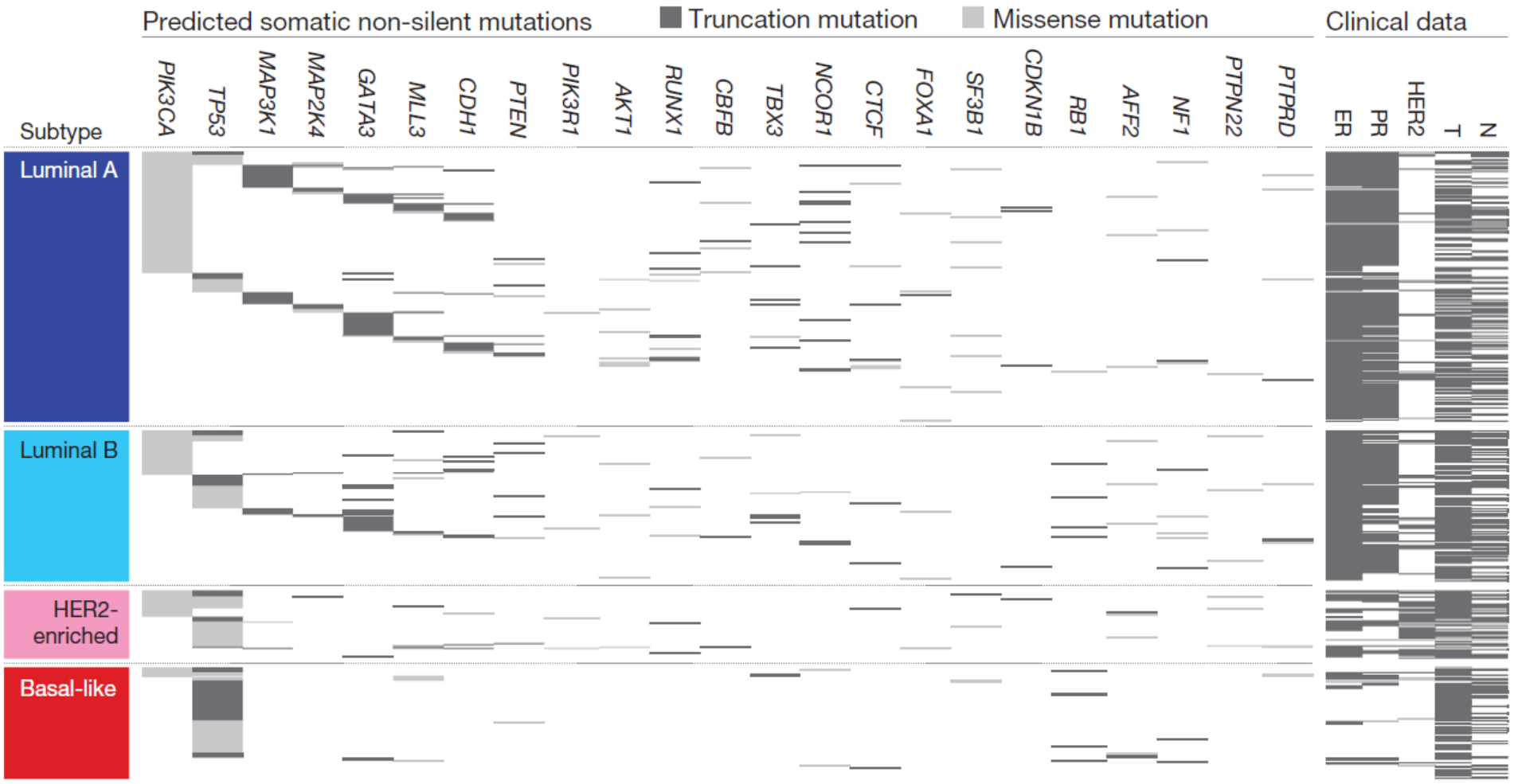
Few highly recurrent mutations in breast cancer





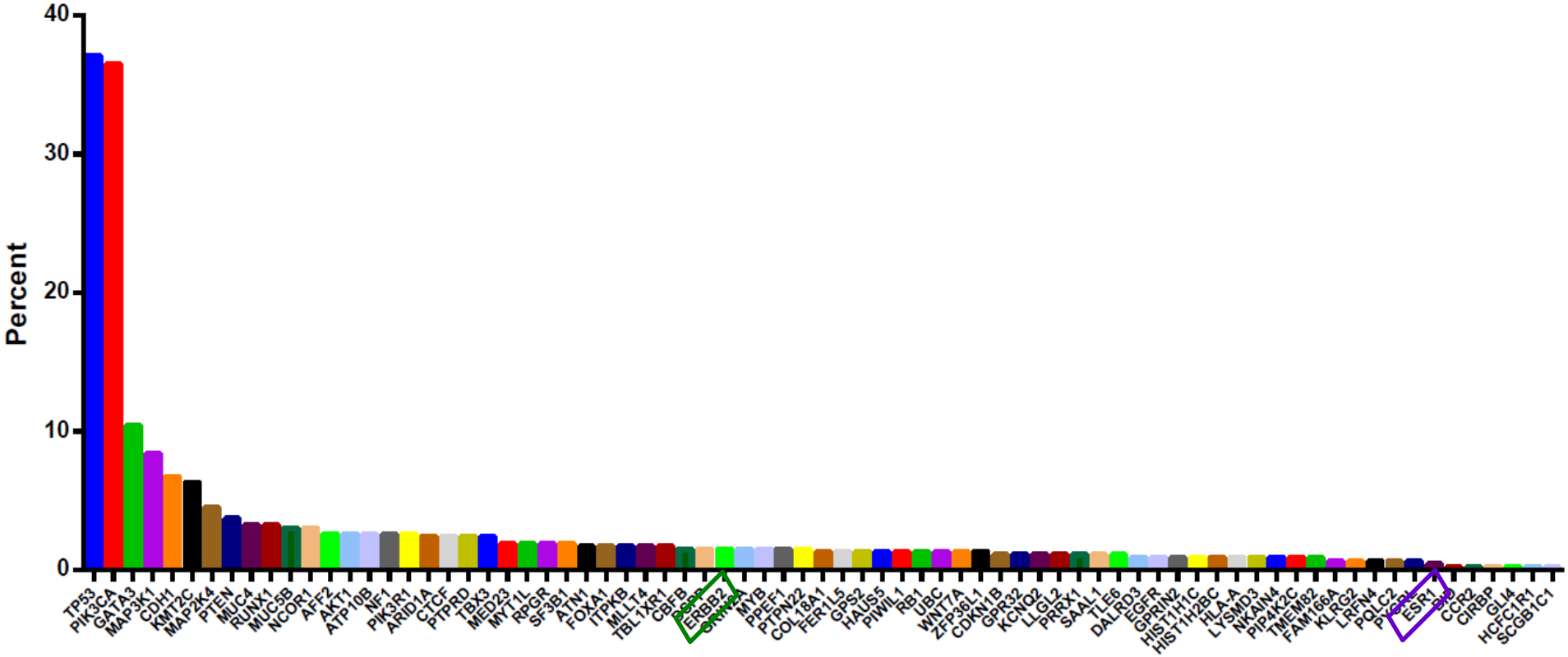
Kan et al, Nature 2010; Stephens et al. Nature 2012

Distinct subtypes have different repertoires of mutations, but no highly recurrently mutated gene is subtype specific



Nature 2012; Stephens et al, Nature 2012; Shah et al. Nature 2012; Ellis et al. Nature 2012; Banerji et al. Nature 2012

Few highly recurrently mutated driver genes…

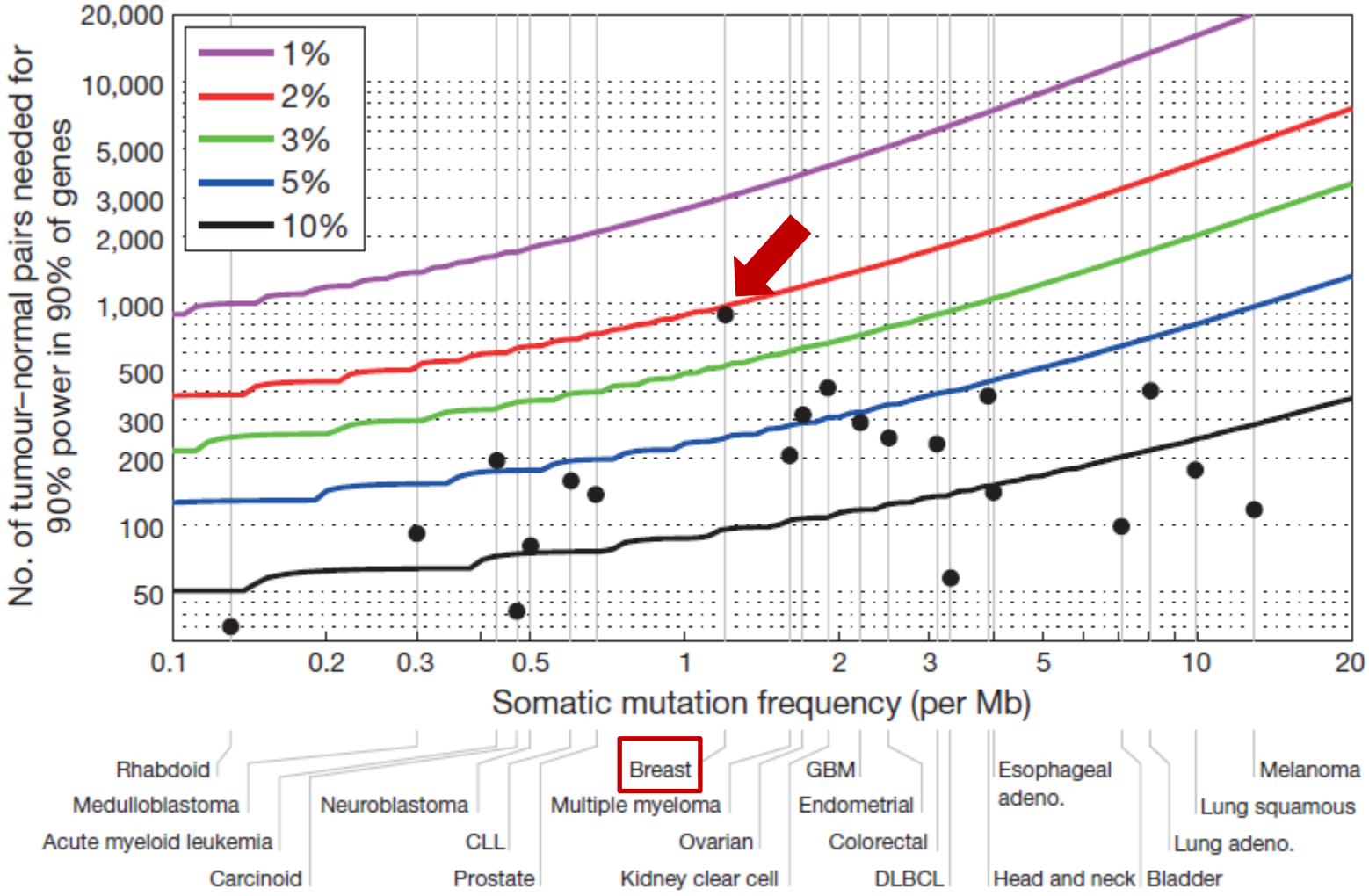


HER2 mutations
1.5% of breast cancers

ESR1 mutations
0.6% of luminal cancers

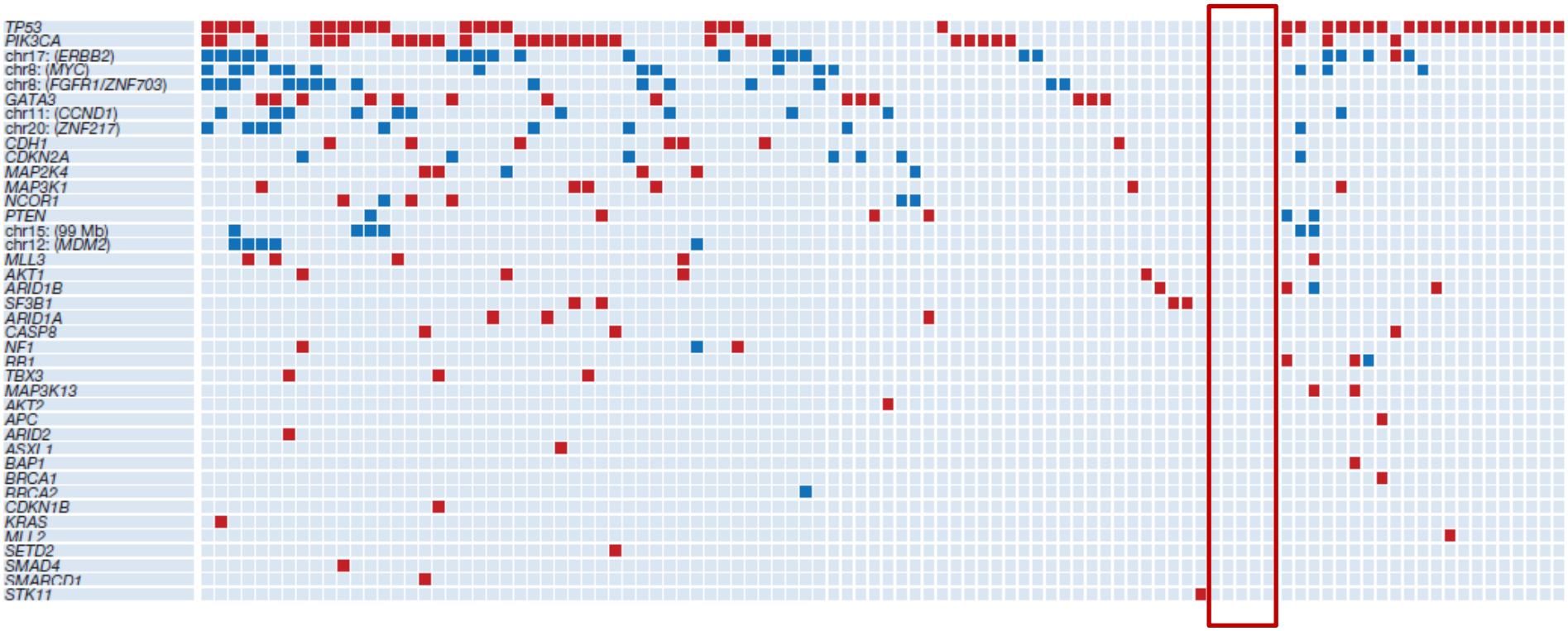
cbioportal.org; TCGA Breast (provisional); n=962

Have we found all drivers in breast cancers?



Lawrence et al. Nature 2014

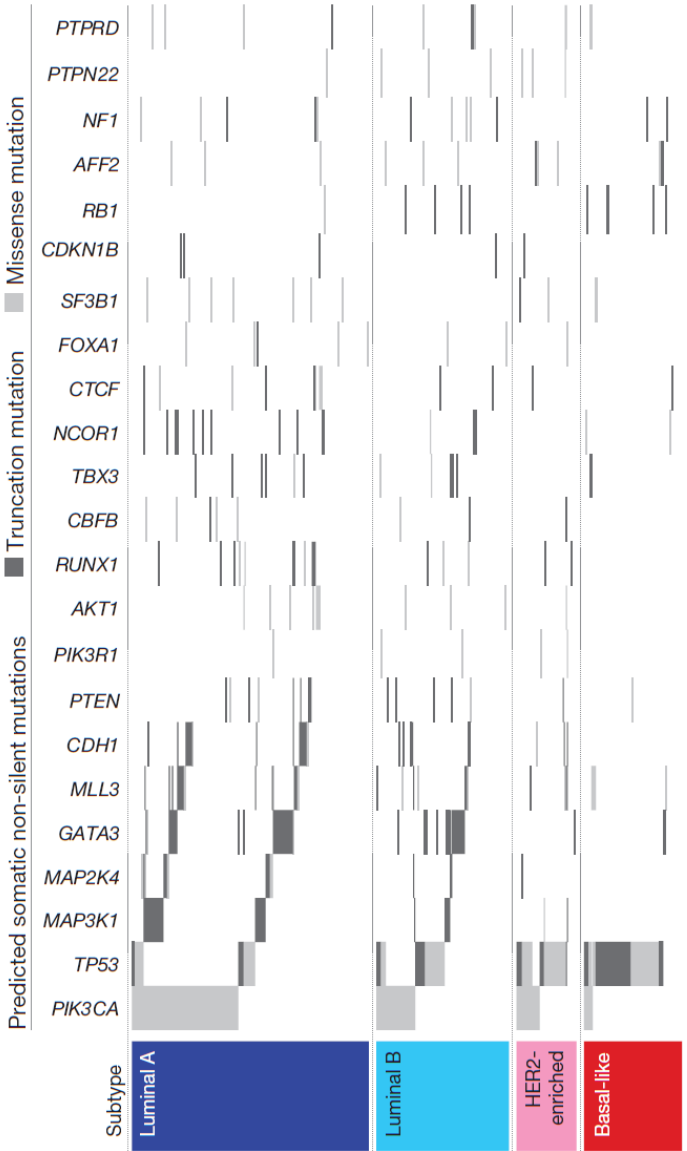
Exome analysis of 101 breast cancers



No driver genetic aberrations in a subset of breast cancers

Stephens et al. Nature 2012

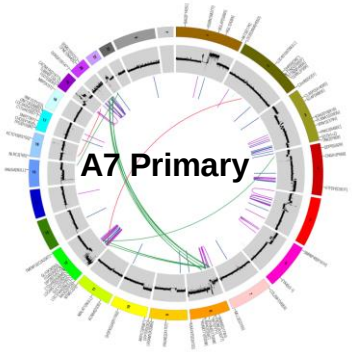
Methods to identify significantly mutated genes in breast cancer focus on highly recurrently mutated genes

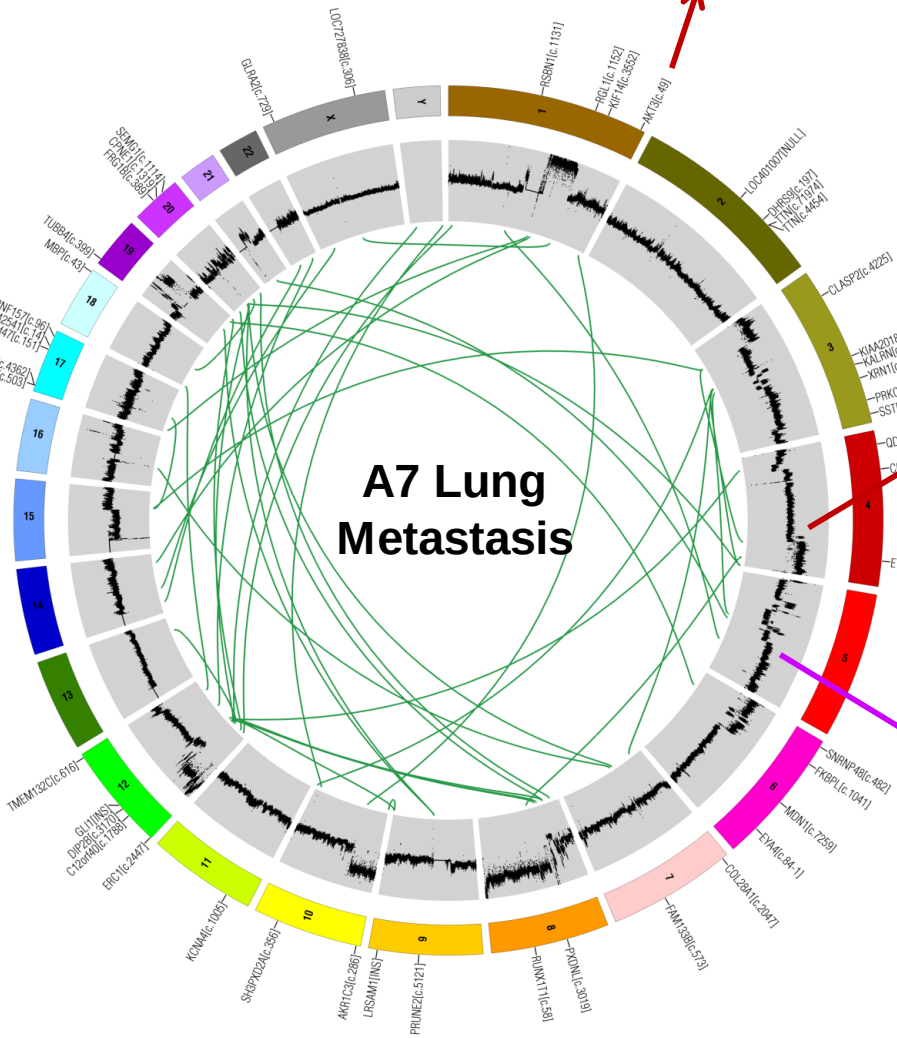


- Rare driver genes can be missed
  - *ESR1* mutations
    - 0.6% of luminal tumours
  - *HER2* mutations
    - Approx 1.5% of breast cancers

TCGA. Nature 2012

And even when we believe we know the drivers...





AKT3 E17K activating mutation

AKT inhibitors

TP53 mutation

Chemotherapy sensitivity Gluck et al., BCRT 2012 (PMID:21373875)

INPP4B deletion, FBXW7 fusion

PIK3CA inhibitors, mTOR inhibitors, PIK3CA/mTOR inhibitors

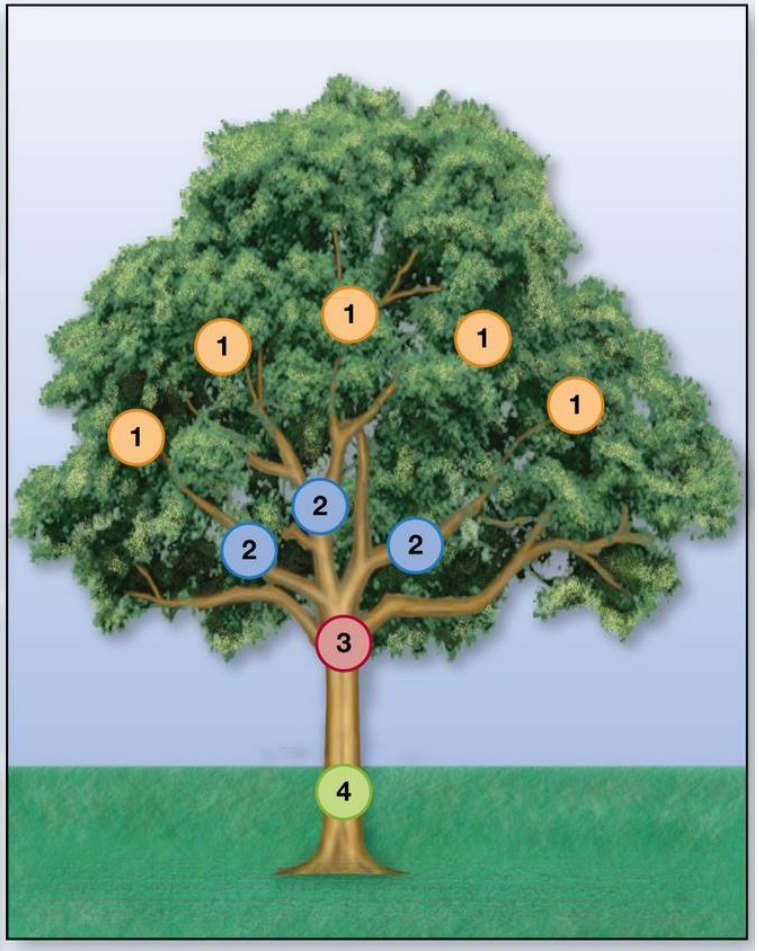
RAD17 + RAD50 loss

Chemotherapy + PARPi sensitivity, Weigman et al., BCRT 2012 (PMID:22048815)

How do we prioritise them?

Courtesy Chuck Perou

Intra-tumour genetic heterogeneity



1 Tracking heterogeneity/ bottlenecks

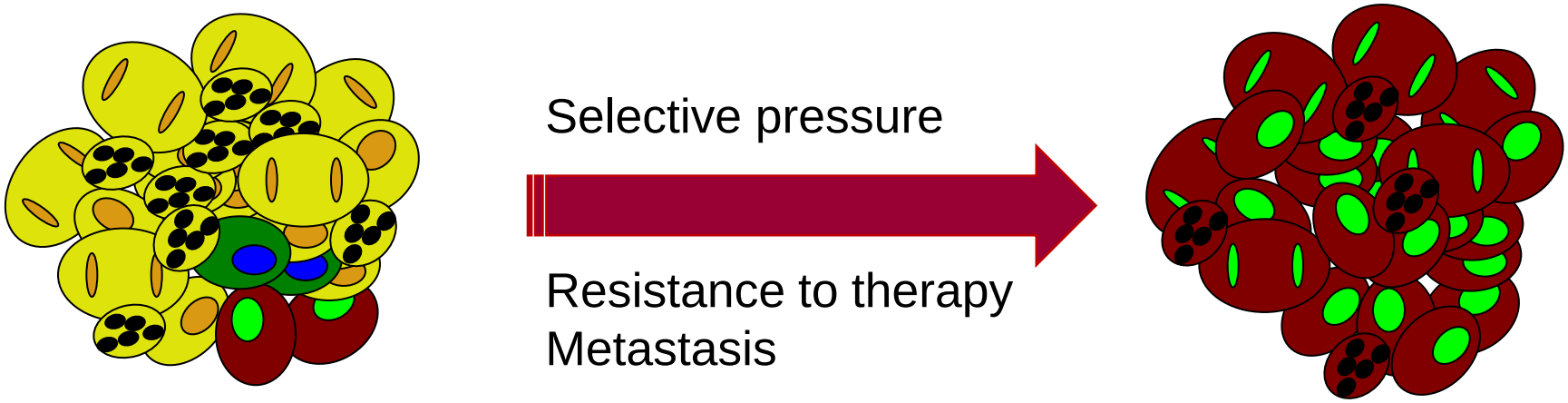
2 Tumour sampling bias

3 Drivers of heterogeneity

4 Drivers of disease – actionable mutations

Adapted from Swanton C, Cancer Res 2012

Intra-tumour genetic heterogeneity: Darwinian evolution model



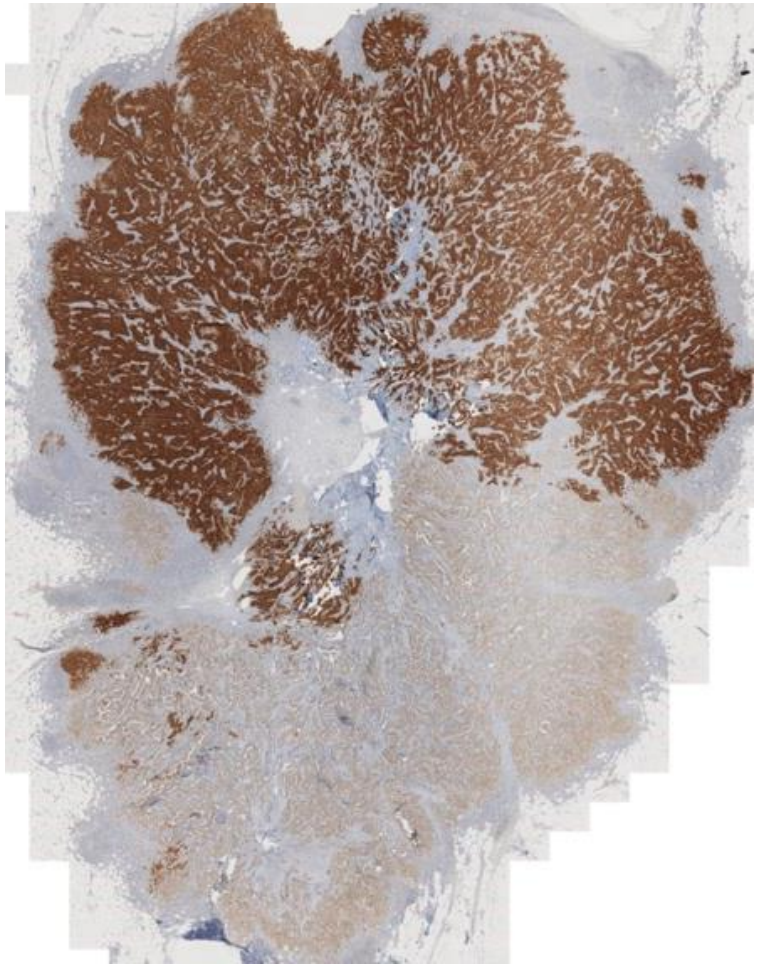
Tumour cell with mutation 1

Tumour cell clone with mutations 1+2

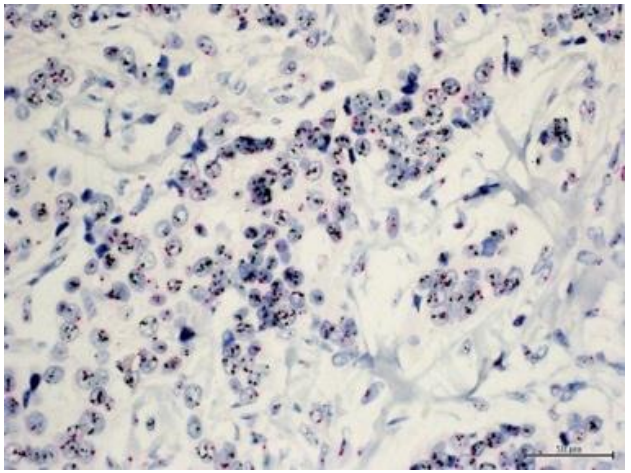
Tumour cell clone with mutations 1+3

HER2 intra-tumour heterogeneity
2%-3% of HER2+ cancers

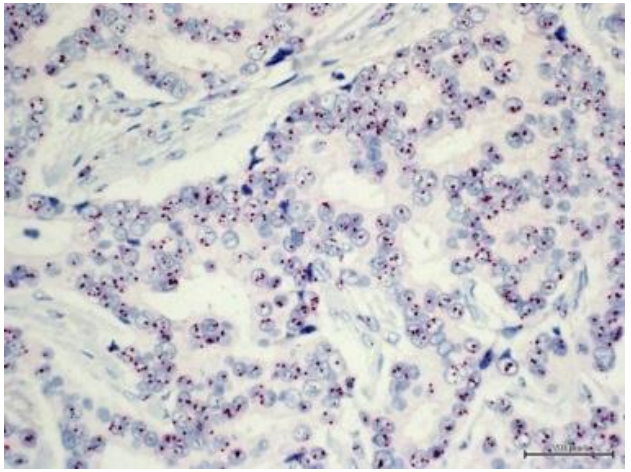
HER2 Immunohistochemistry



Dual colour CISH

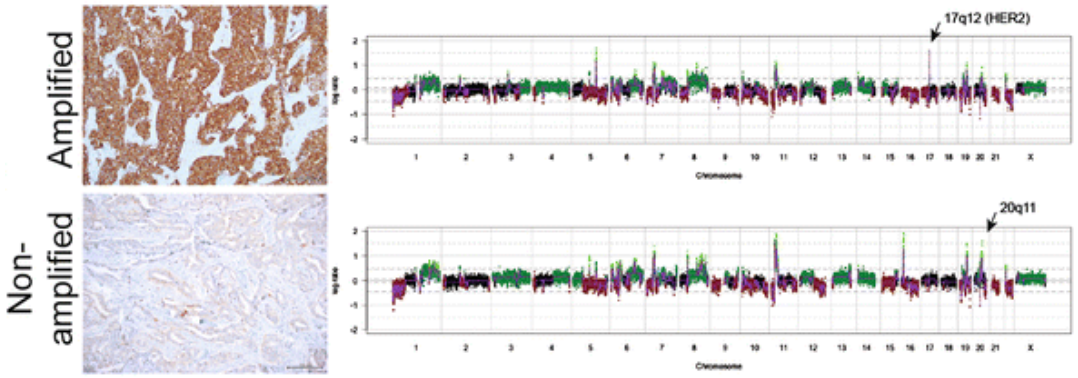


Amplified



Non-Amplified

Somatic mutations associated with HER2 intra-tumour heterogeneity



Sample ID	Potential driver mutations present in both HER2-negative and HER2-positive components	Potential driver mutations restricted to the HER2-negative component	Potential drivers within regions whose amplification was restricted to the HER2-negative component
T1	*TP53* (P152L)		*FAM83A, MDM4*
T2	NP	NP	*BRF2, FGFR1, ZNF703, RAB11FIP1, LSM1, DDHD2, WHSC1L1, PPAPDC1B, EEF1A2, ERLIN2, BAG4*
T3	*TP53* (E258D)	*ATRX* (splice site dinucleotide substitution)	*YWHAZ, MYC, FAM83A*
T4	*ARID1A* (R1446*)		*BRF2, ZNF703, RAB11FIP1, ERLIN2*
T5	*TP53* (E286D)	NP	*IKBKB, CAMK1D*
T6	*TP53* (R273H), *PIK3CA* (H1047R)	*HER2* (I767M), *ETV5* (E60K)	*PHGDH*
T8	*PIK3CA* (H1047R), *CBFB* (splice site)	*BRAF* (P403S), *XRCC1* (S236F)	
T9	*TP53* (R282G), *PIK3CA* (H1047R), *MAP2K4* (R110G), *MED12* (R2015M)		*LMX1B*
T10	*TP53* (S94fs)	NP	*CBX3, RAD21*
T11	*TP53* (G187_E192delLAPPQ)	*NRP1* (R767H)	*MYC, RAD21*
T12	*TP53* (T195N), *KIT* (A755T)	*FANCD2* (L1394F)	*DSN1*
T13	*TP53* (S240I)	NP	*PIK3CA*

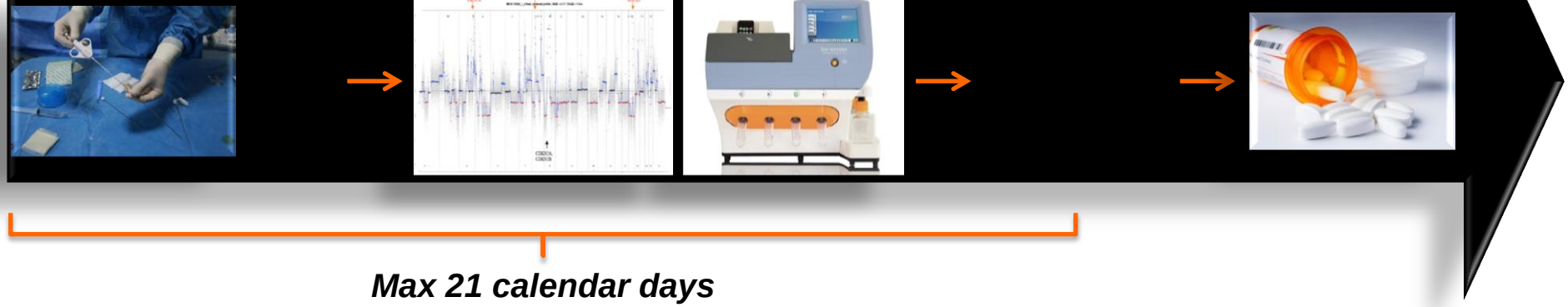
MOSCATO trial: implementation of Next Generation Sequencing in high volume phase I center

- **Monocentric**
- **Target Accrual = 900 patients**

FRESH TUMOR → **MOLECULAR SCREENING** → **CLINICAL DECISION** → **TREATMENT**

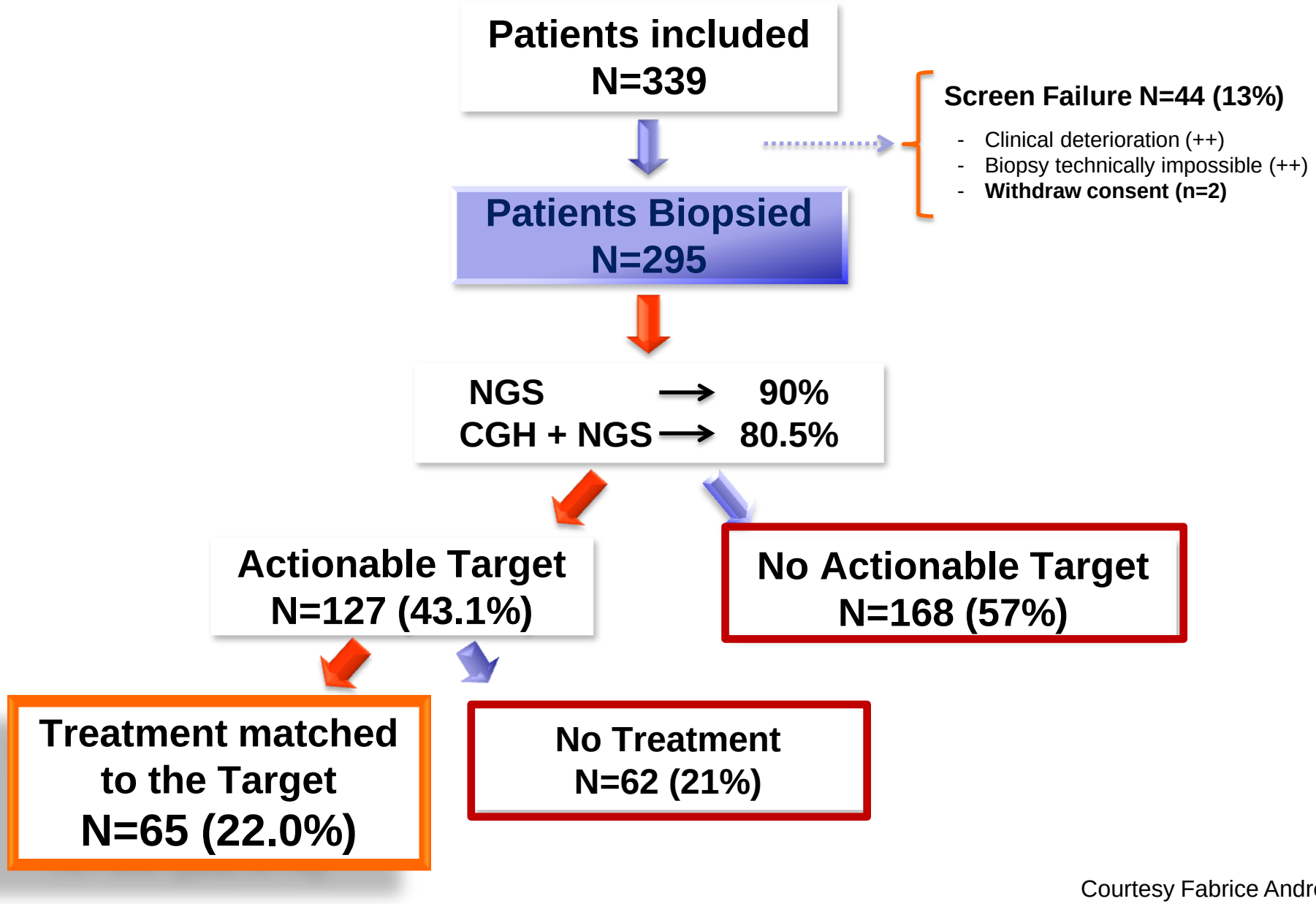
BIOPSY → **PATHOLOGICAL CONTROL**

CGH Array & NGS



Max 21 calendar days

Presented by: Antoine Hollebecque et al., ASCO 2013;
Courtesy Fabrice Andre



Courtesy Fabrice Andre

Take Home Messages

- Breast cancers display complex genomes
- Few highly recurrently mutated genes
- Large number of genes rarely mutated
- No common denominator for each subtype
- Highly recurrent drivers have been identified
- Drivers of rare subtypes and of metastasis and resistance yet to be fully characterised

Take home messages

- Not all drivers have been identified
  - Drivers of metastatic disease
  - Drivers of resistance to specific agents
- Beginning to understand
  - Intra-tumour genetic heterogeneity

Approaches for the delivery of precision medicine

Approaches for massively parallel sequencing and therapy decision making

- Whole genome sequencing
- Targeted capture sequencing
- Whole exome sequencing
- Whole exome sequencing + RNA sequencing

How deep should we sequence in clinical decision making?

- Higher depth – greater accuracy
- Mutations found in at least 10% of cancer cells
  - Typical sample: approx 50% of tumour cell content
  - At least 5 reads supporting a mutation

	Pure sample 100% tumour cells Heterozygous SNV	Sample with 50% stroma 100% of tumour cells Heterozygous SNV	Sample with 50% stroma 10% of tumour cells Heterozygous SNV
100x	50 reads	25 reads	2 – 3 reads
200x	100 reads	50 reads	5 reads
500x	250 reads	125 reads	12 – 13 reads

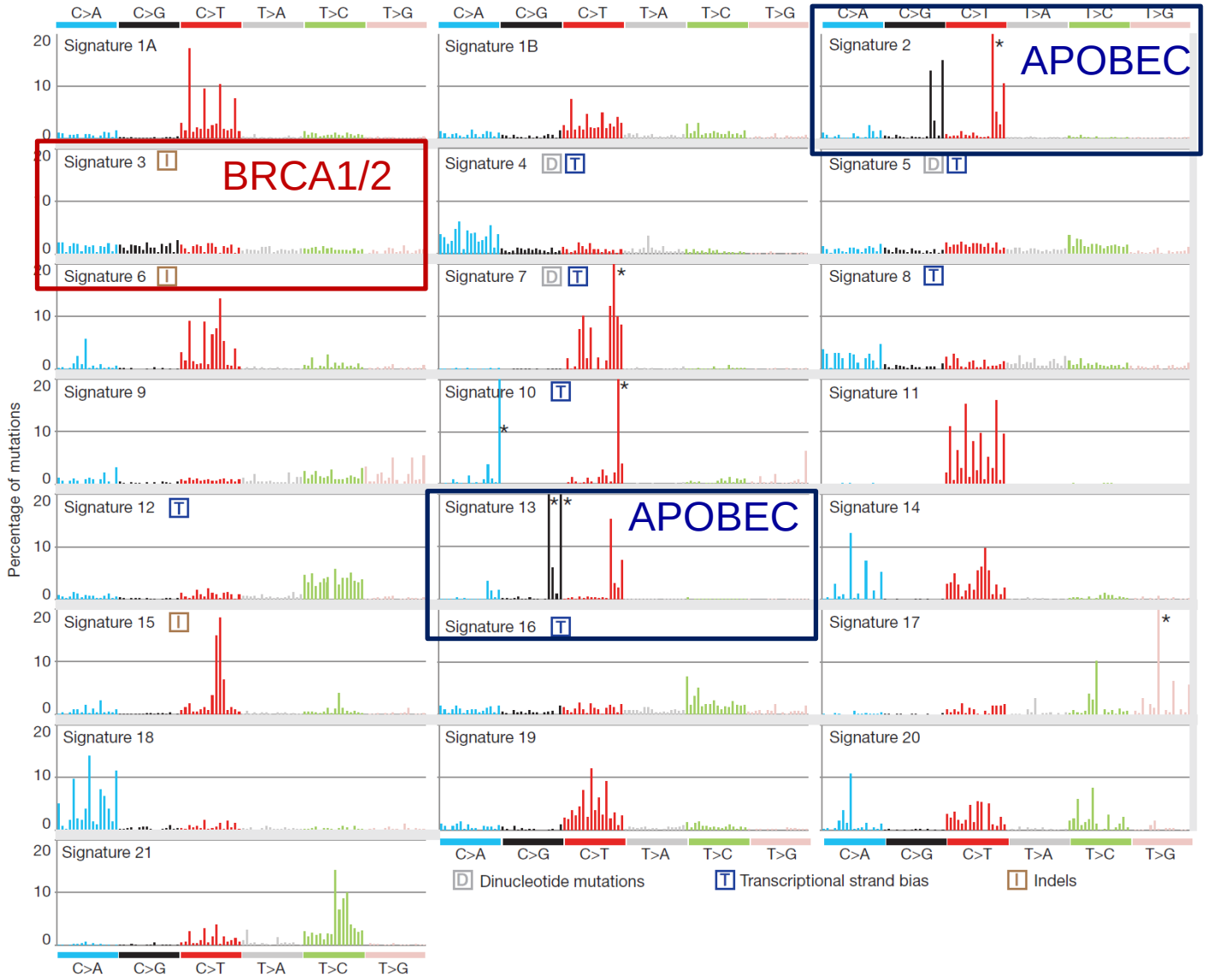
Whole genome sequencing

- All somatic genetic aberrations
  - Mutation calls
    - some uncertainty for SNVs
    - still problematic for indels
  - Fusion gene identification: not trivial
  - Validation with orthogonal methods is required
- Still expensive
  - Usually low depth: 30x to 100x
- Computer power and army of bioinformaticians

What are we trying to achieve?

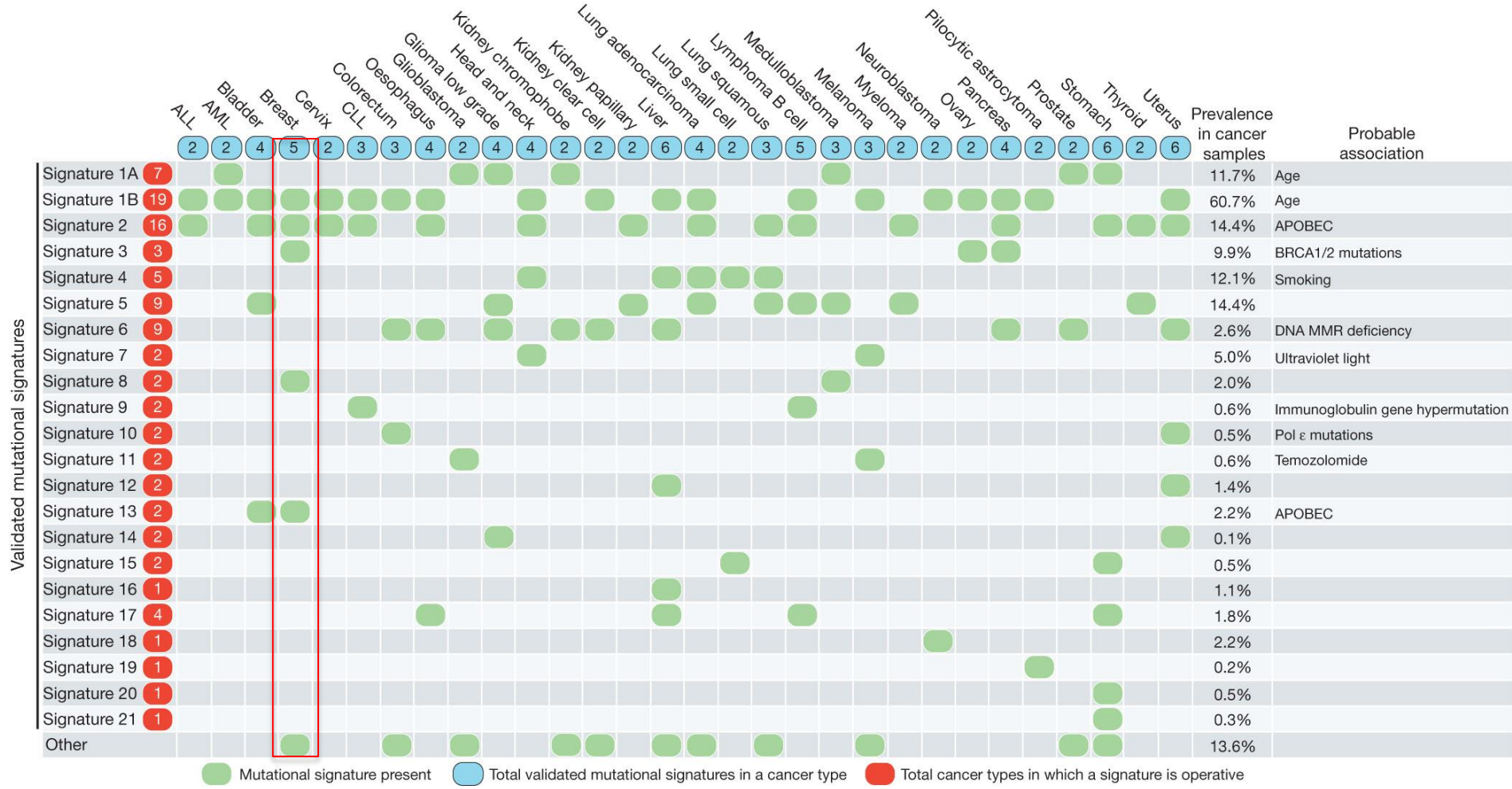
- Targeted capture sequencing is an excellent option
- If we believe that
  - i) breast cancers are driven by a limited constellation of known driver mutations, fusion genes and copy number aberrations
  - ii) we can target the functional impact of each mutation

Mutation signatures and genomic scars are not identified



Alexandrov et al. Nature 2013

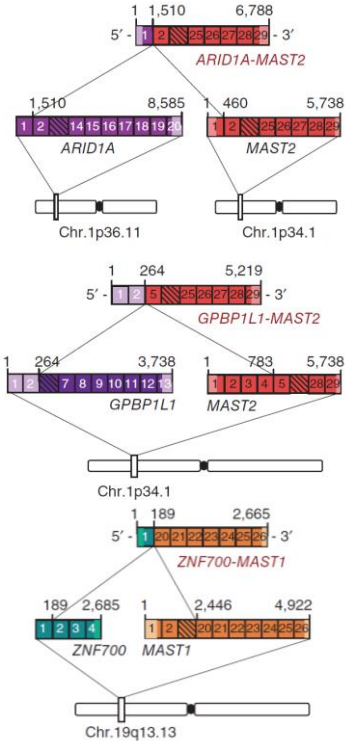
Mutation signatures and genomic scars are not identified



If we go with exome sequencing instead

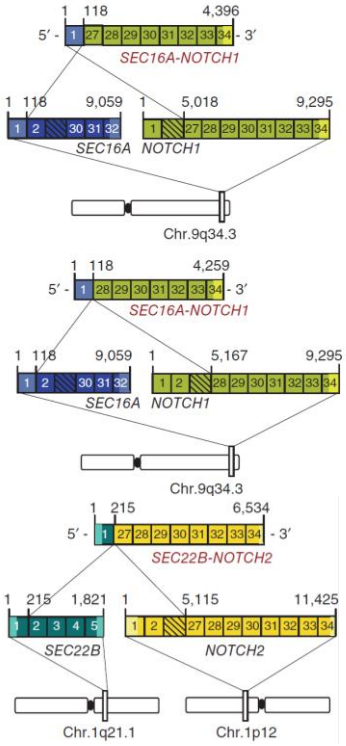
- Mutations in coding regions and some 3’ and 5’ UTRs

MAST1 and *MAST2*
Robinson et al. Nat Med 2011



~6% of all breast cancers

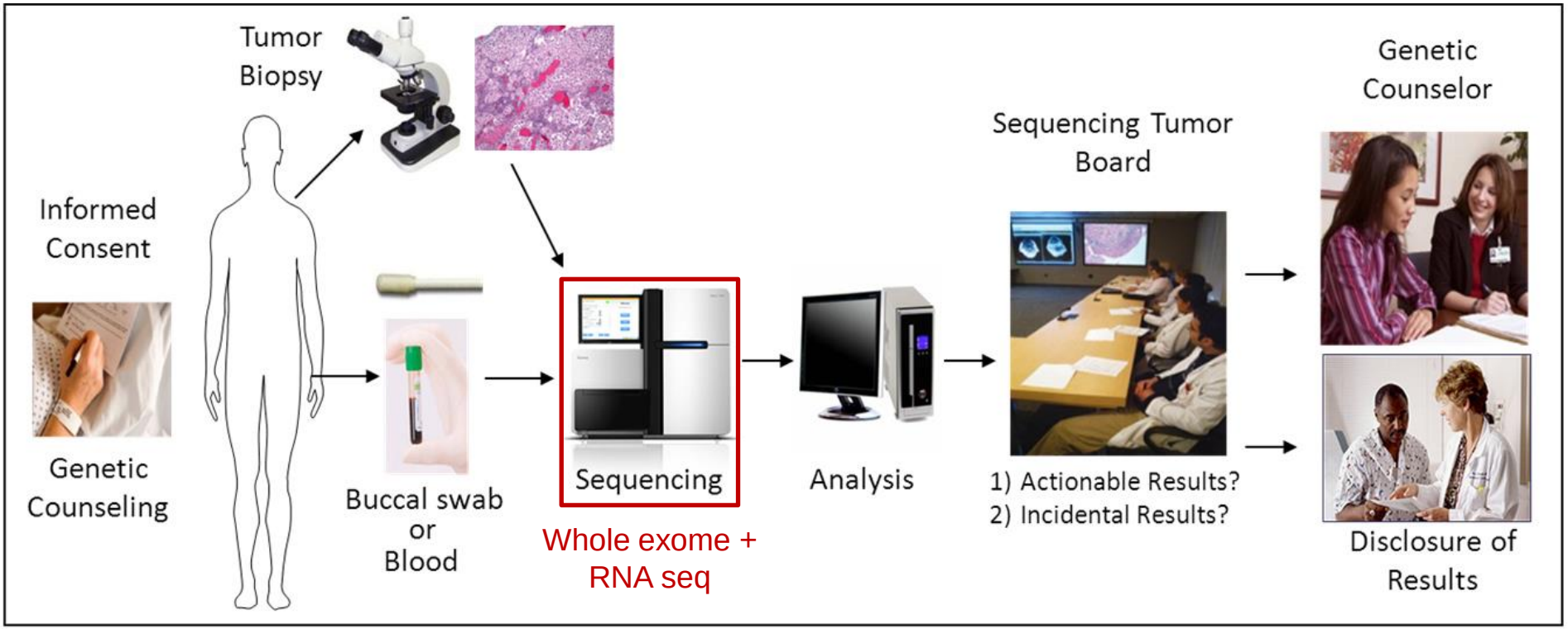
NOTCH1 and *NOTCH2*
Robinson et al. Nat Med 2011



~25% of TNBCs

Fusion genes cannot be identified reliably

Whole exome + RNA seq



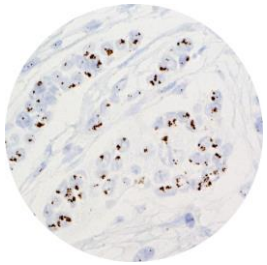
- Excellent approach, but...
- What do we do with the incidental findings?

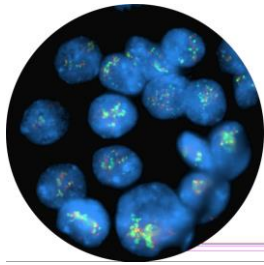
Take Home Messages

- Sequencing for therapy decision making
  - Dependent on the use intended
  - For enrollment in clinical trials
    - Targeted capture sequencing (including selected intronic regions)
  - For patients in the metastatic setting after multiple lines of therapy
    - Targeted capture sequencing (including selected intronic regions)
    - Exome + RNA seq
  - Whole genome sequencing – unjustified at present

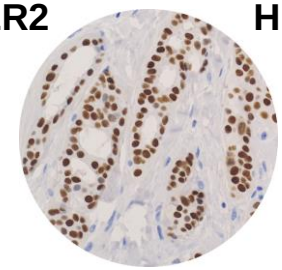
Breast Cancer Patient Management

Size
Grade
Type
Lymph Node metastasis
Vascular Invasion

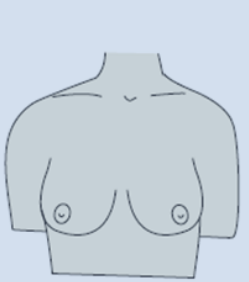




HER2 HER2



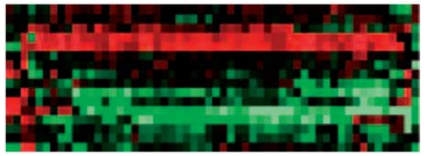
ER, PR and HER2



Clinical

Pathology

Proteomics/metabolomics



Precision medicine-based breast cancer patient therapy

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Memorial Sloan Kettering Cancer Center™