Whole Genome Sequencing and Cancer Therapy: What is too much?

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Summary

- Precision medicine
- Have we learnt all we need to know?
- How much do we need to sequence?

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

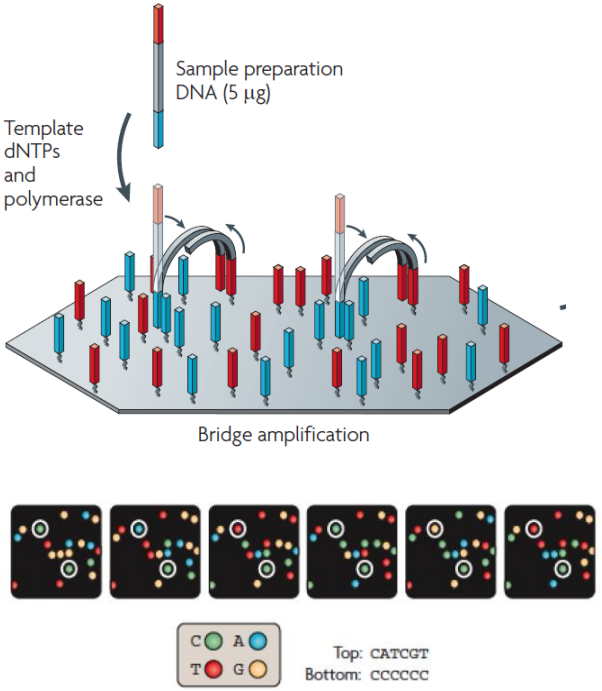
Precision medicine is now possible

Development of targeted treatments

- Small molecule inhibitors
- Monoclonal antibodies

Massively Parallel Sequencing

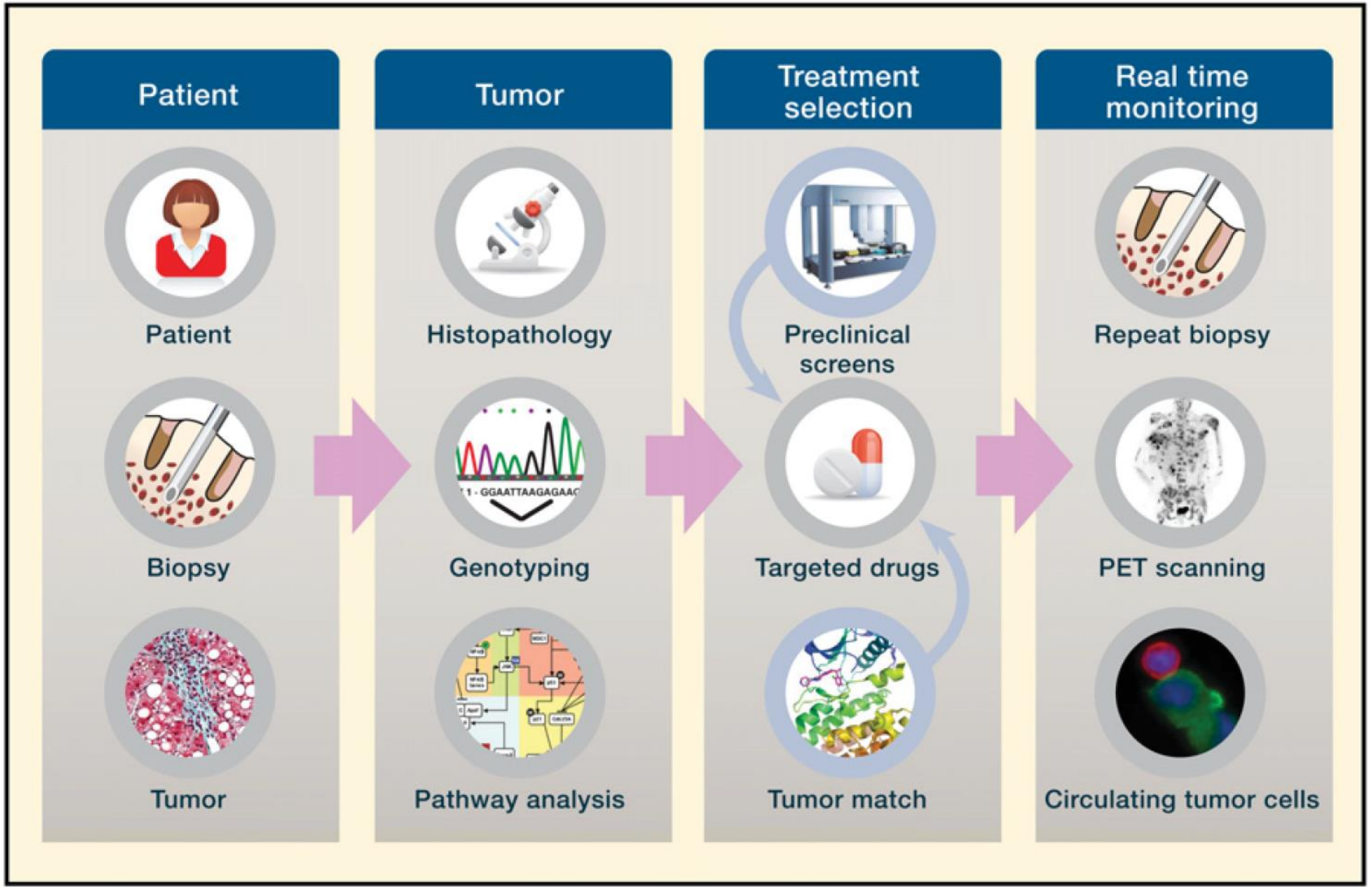
- Tumour genomes



Metzker et al. Nat Rev Genet 2010

Breast Cancer Patient Management

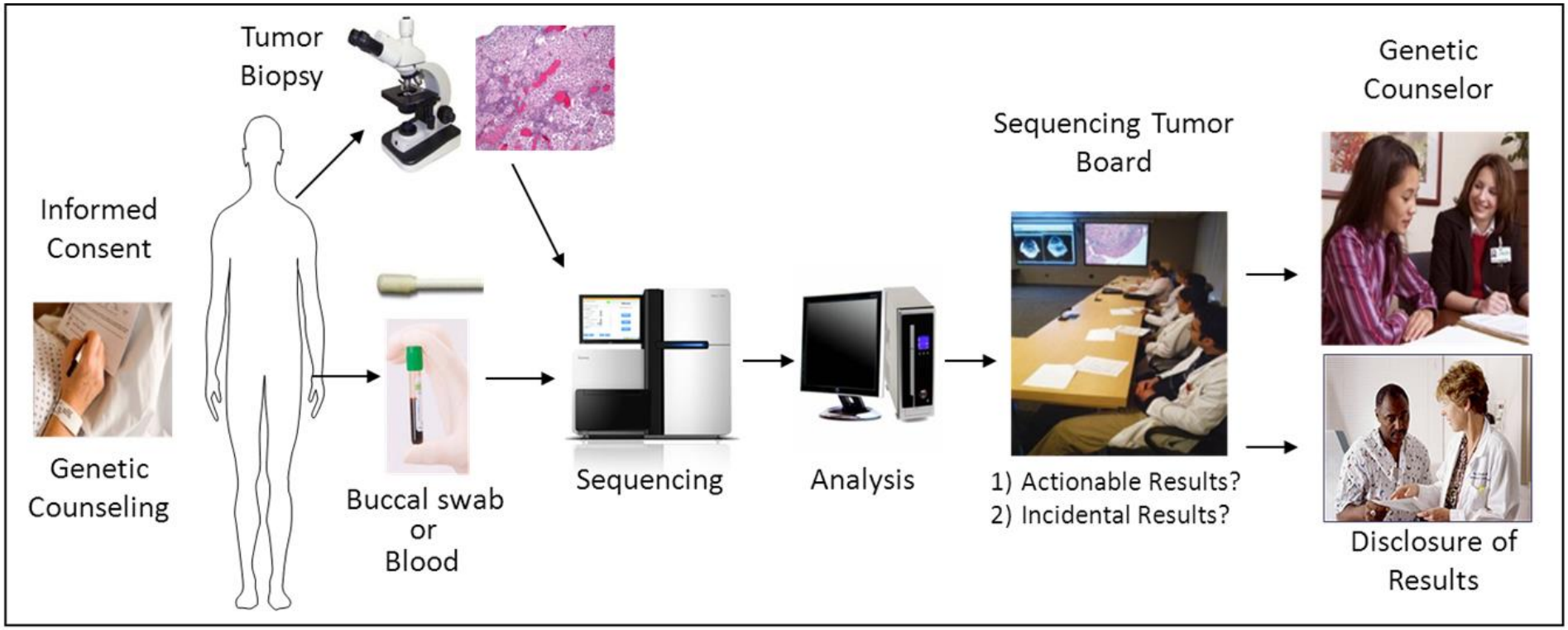
“Precision medicine”-based breast cancer patient therapy



Haber DA, Gray NS, Baselga J. Cell 2011

Systematic massively parallel sequencing analysis of tumours for clinical decision making

Mi-OncoSeq (PI: Arul Chinnaiyan)



Have we learnt all we need to know?

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

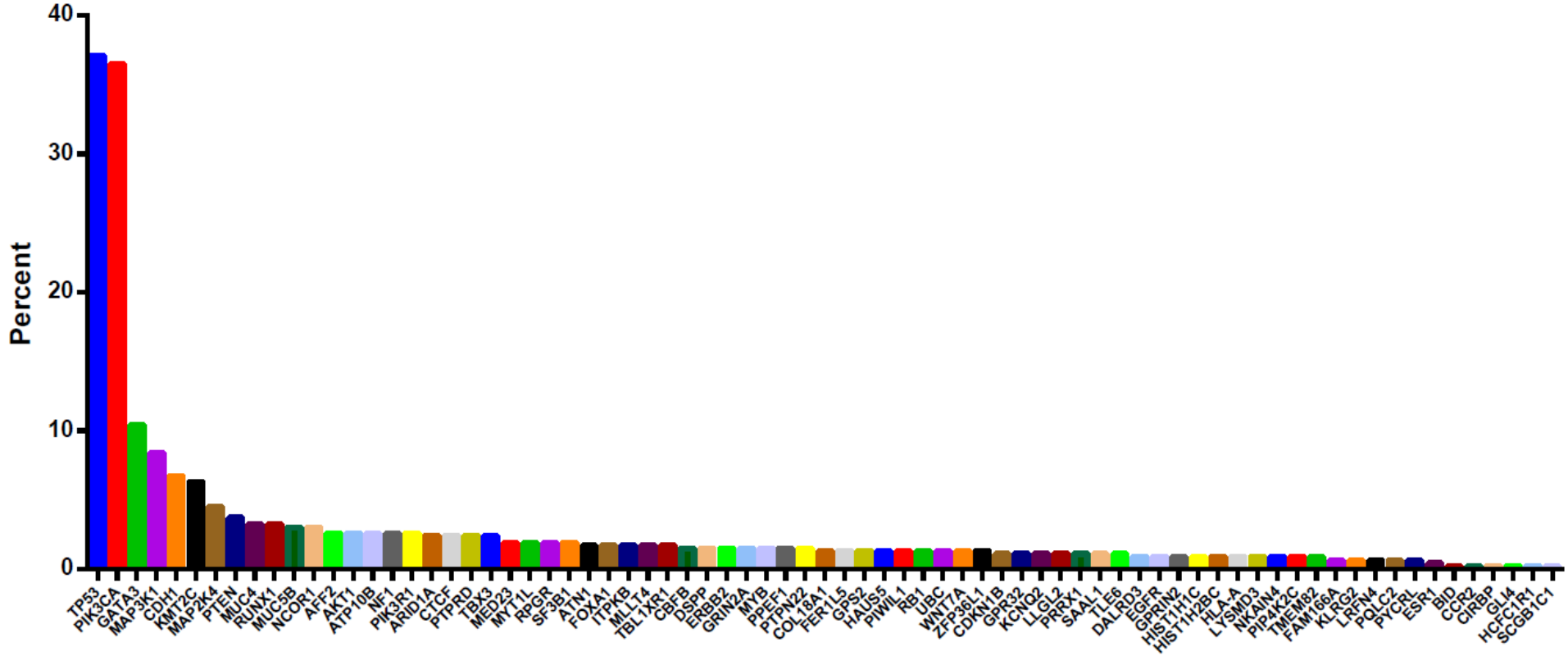
Oncogene ‘addiction’

- *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

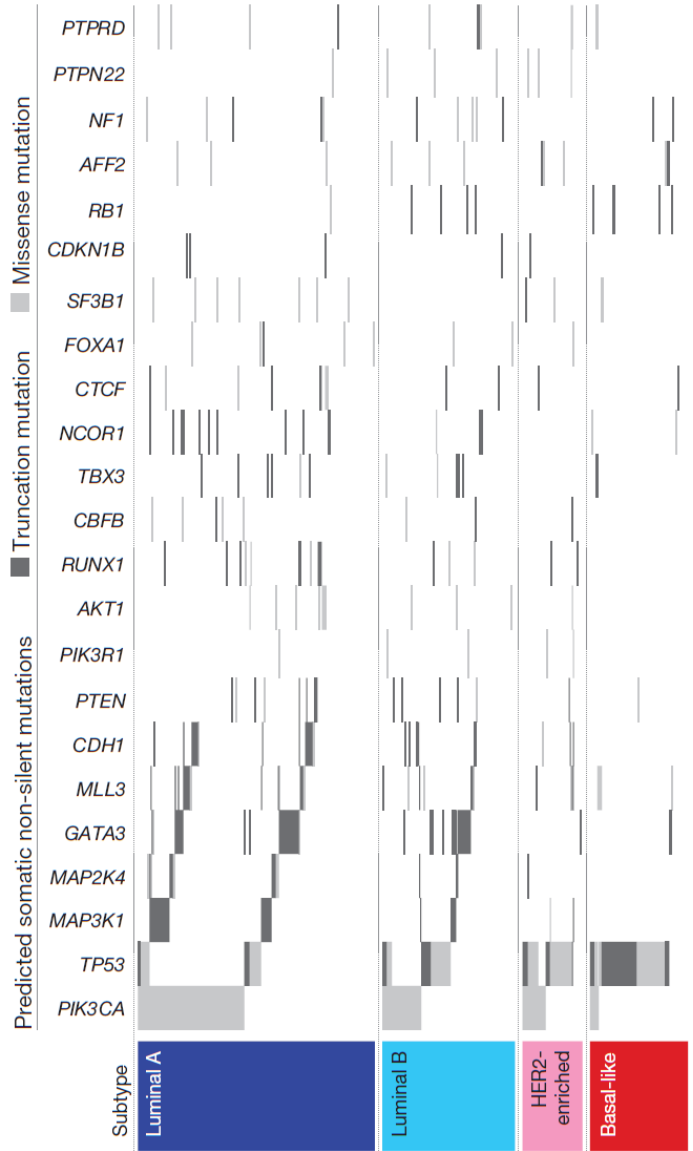
Inhibition is selectively lethal

Few highly recurrently mutated driver genes…



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

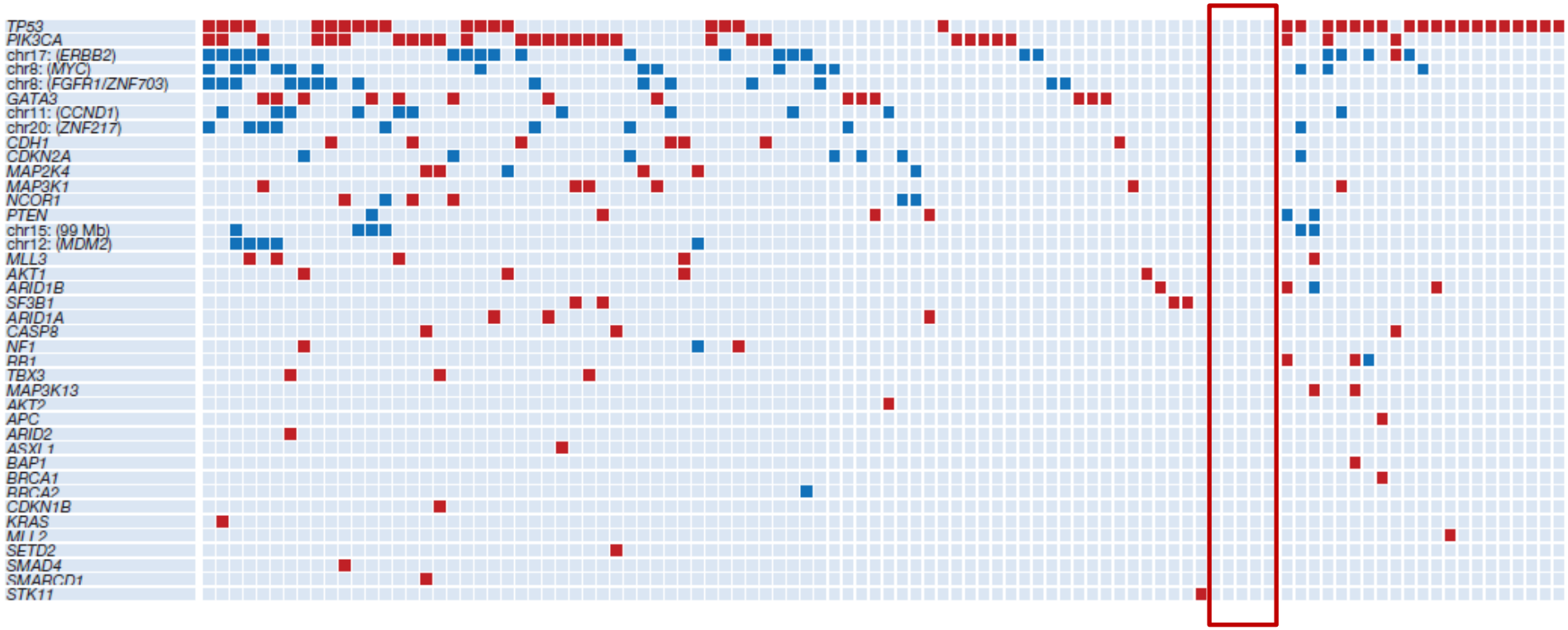
Genes identified as significantly mutated in breast cancer



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

TCGA. Nature 2012

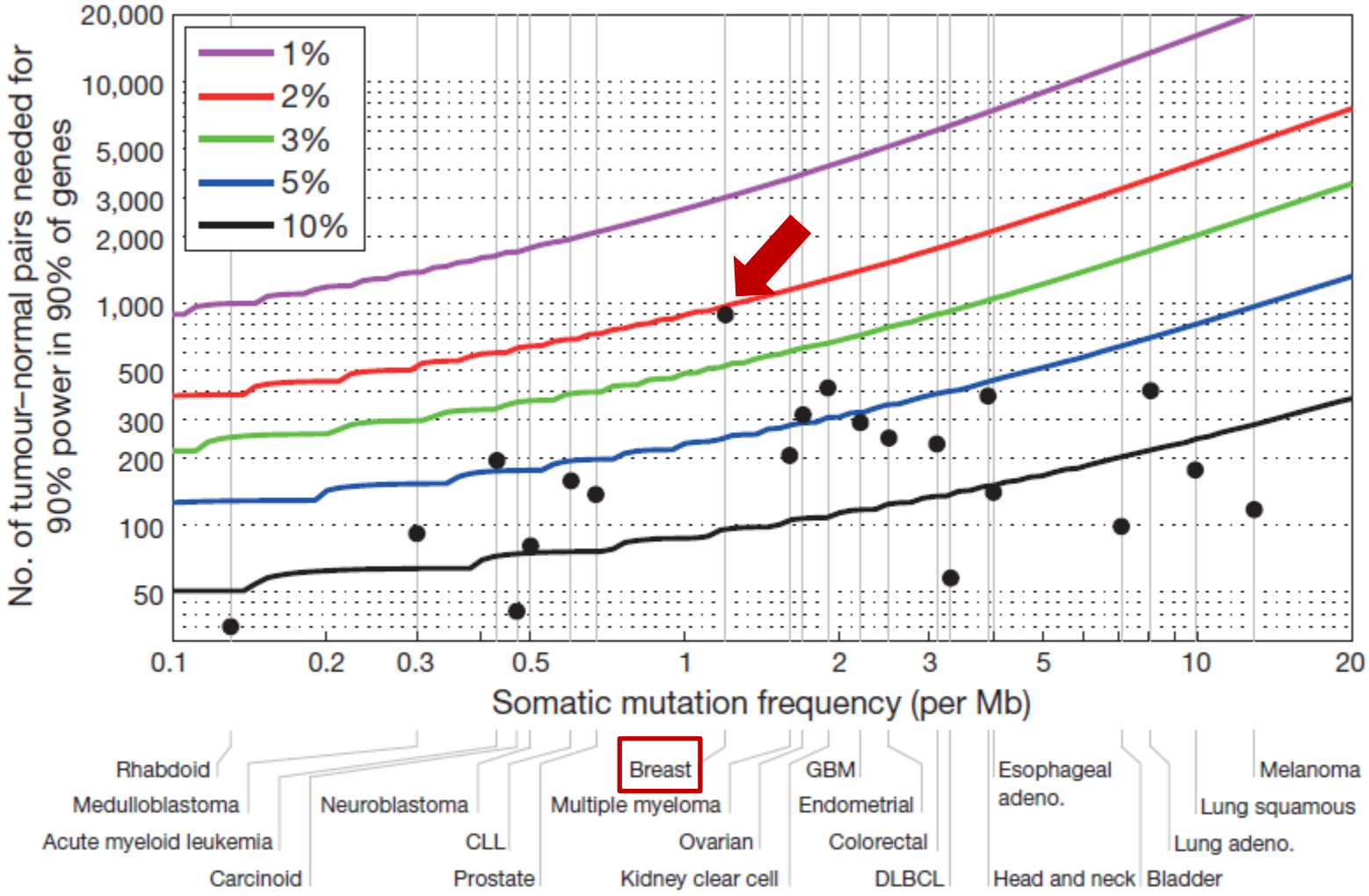
Exome analysis of 101 breast cancers



No driver genetic aberrations in a subset of breast cancers

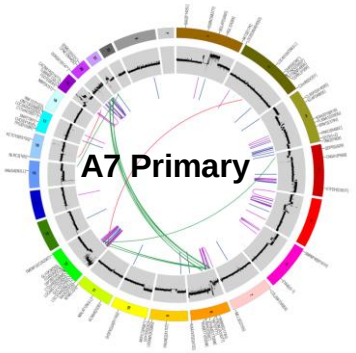
Stephens et al. Nature 2012

Have we found all drivers in breast cancers?



Lawrence et al. Nature 2014

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

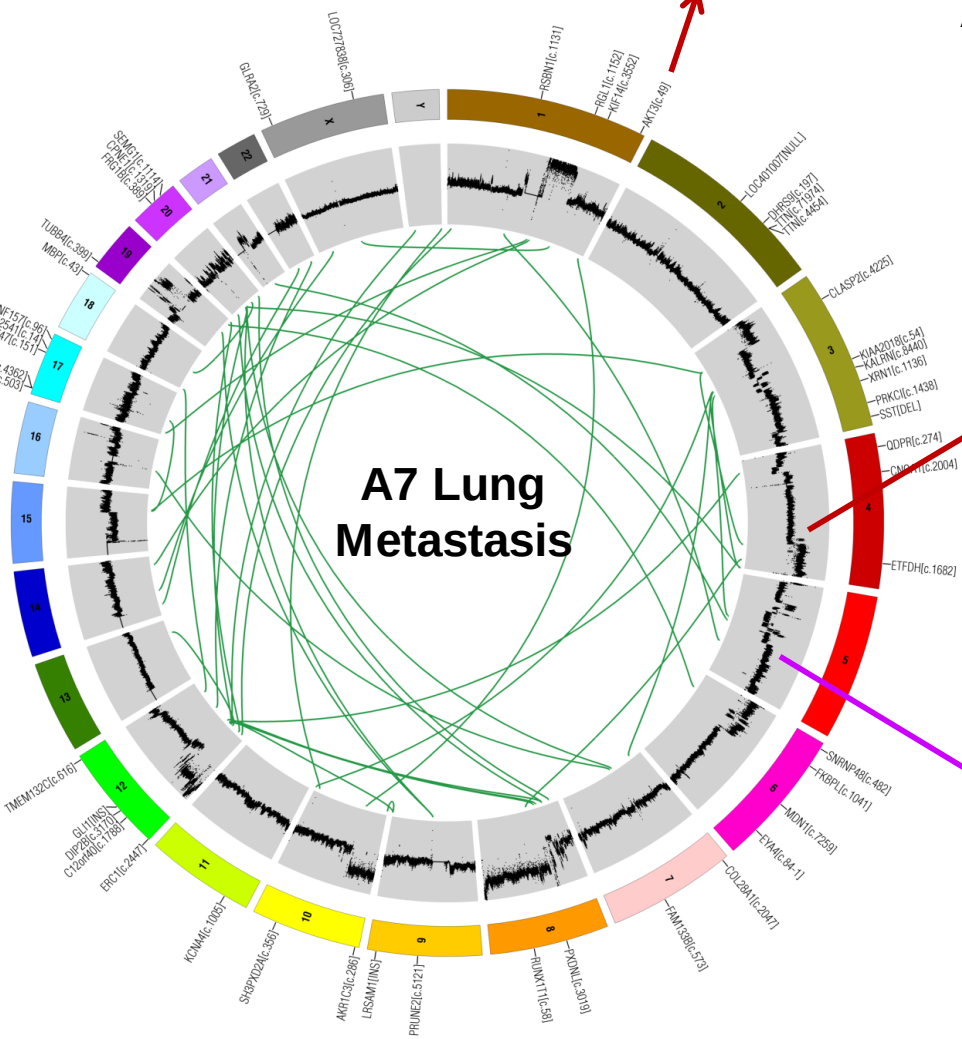


AKT3 E17K activating mutation

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

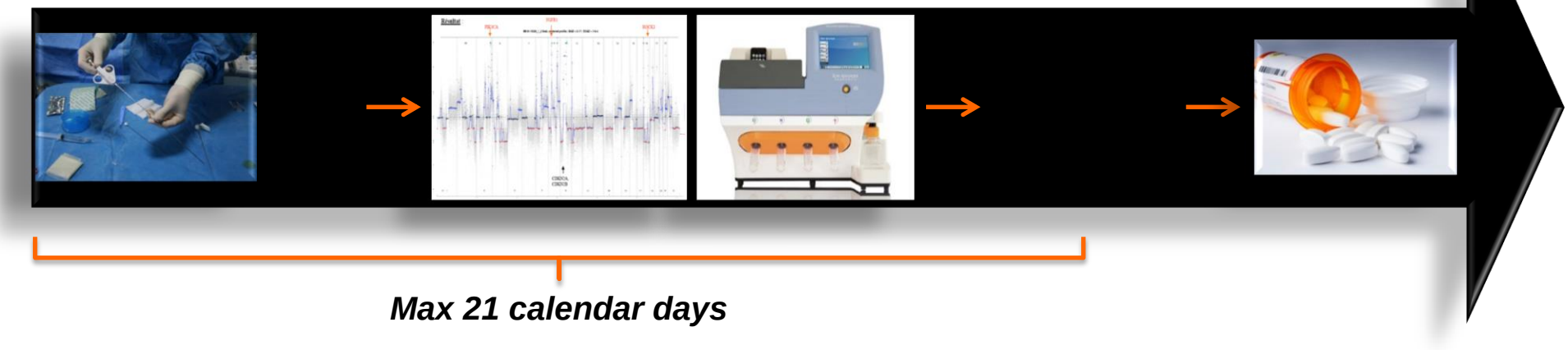
MOSCATO trial: implementation of Next Generation Sequencing in high volume phase I center

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

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

BIOPSY → **PATHOLOGICAL CONTROL**

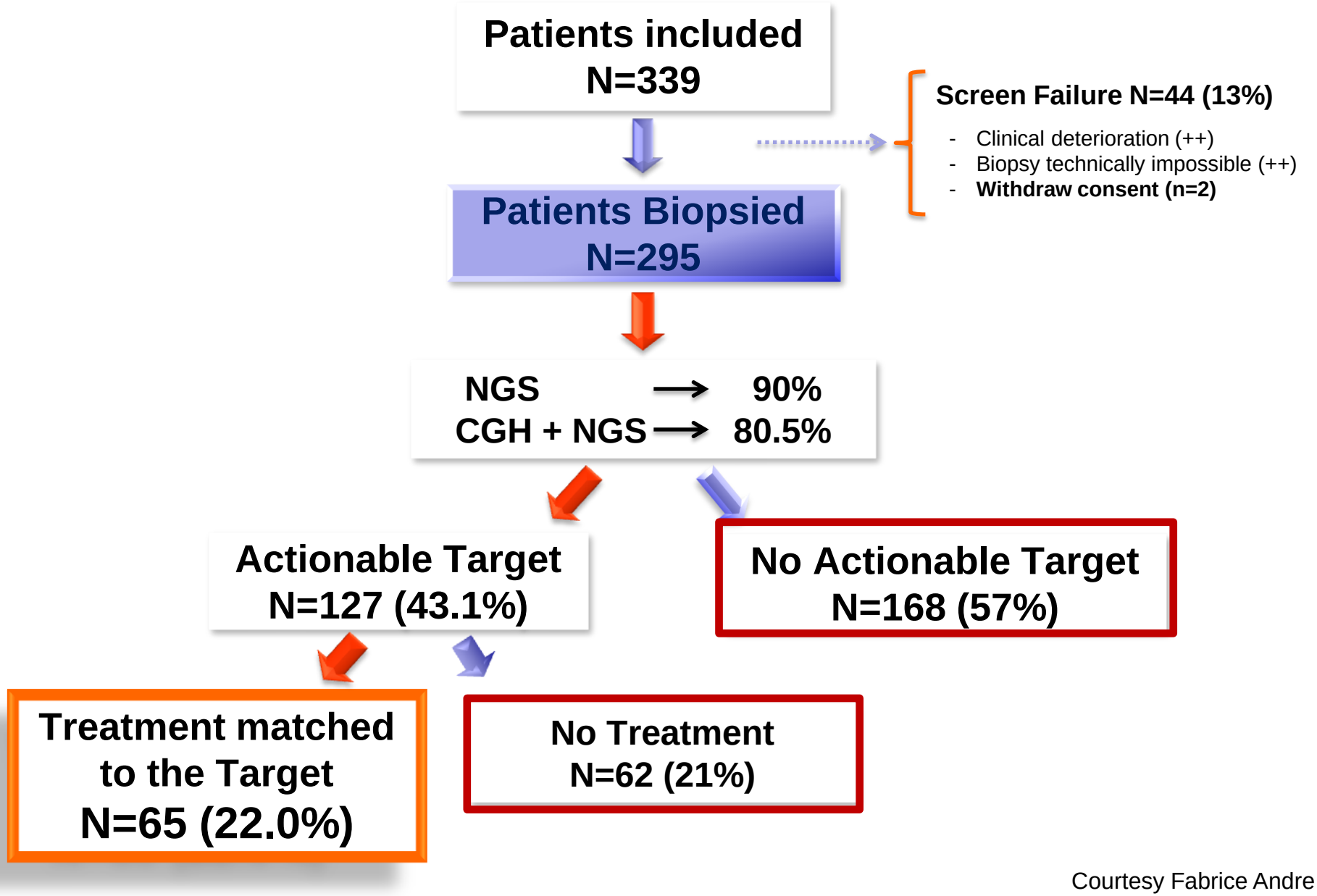
CGH Array & NGS



Max 21 calendar days

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

Update September 2013



Courtesy Fabrice Andre

Factors to consider

- Not all tumours have identifiable driver mutations
- Not all drivers have been identified
- Incomplete characterisation of drivers
  - Drivers of metastatic disease
  - Drivers of resistance to specific agents
- Limited availability of therapeutic agents
- Beginning of understanding of epistatic interactions
  - Mutation A + Mutation B results in a different phenotype

How much do we need to sequence?

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

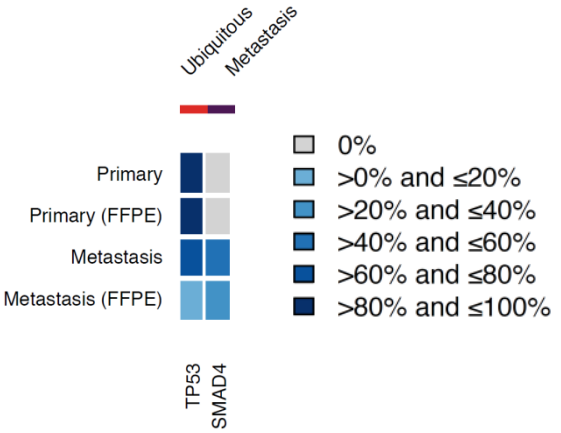
What are we trying to achieve?

- 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

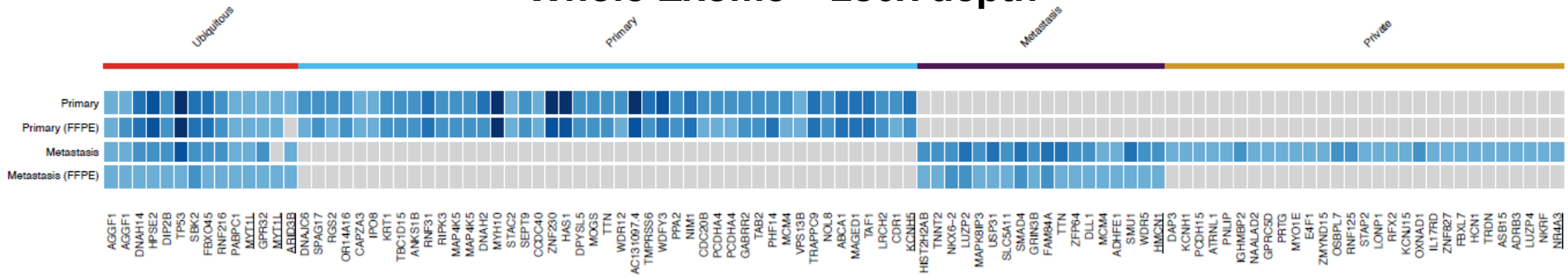
What do we miss by looking only at what we know?

ER-/ PR-/ HER2+, grade III breast cancer with liver metastasis at presentation
2 biopsies of the **primary tumour** and **2 biopsies** of the **liver metastasis**

Targeted capture massively parallel sequencing
300 known **cancer genes** and potential therapeutic targets
400x depth

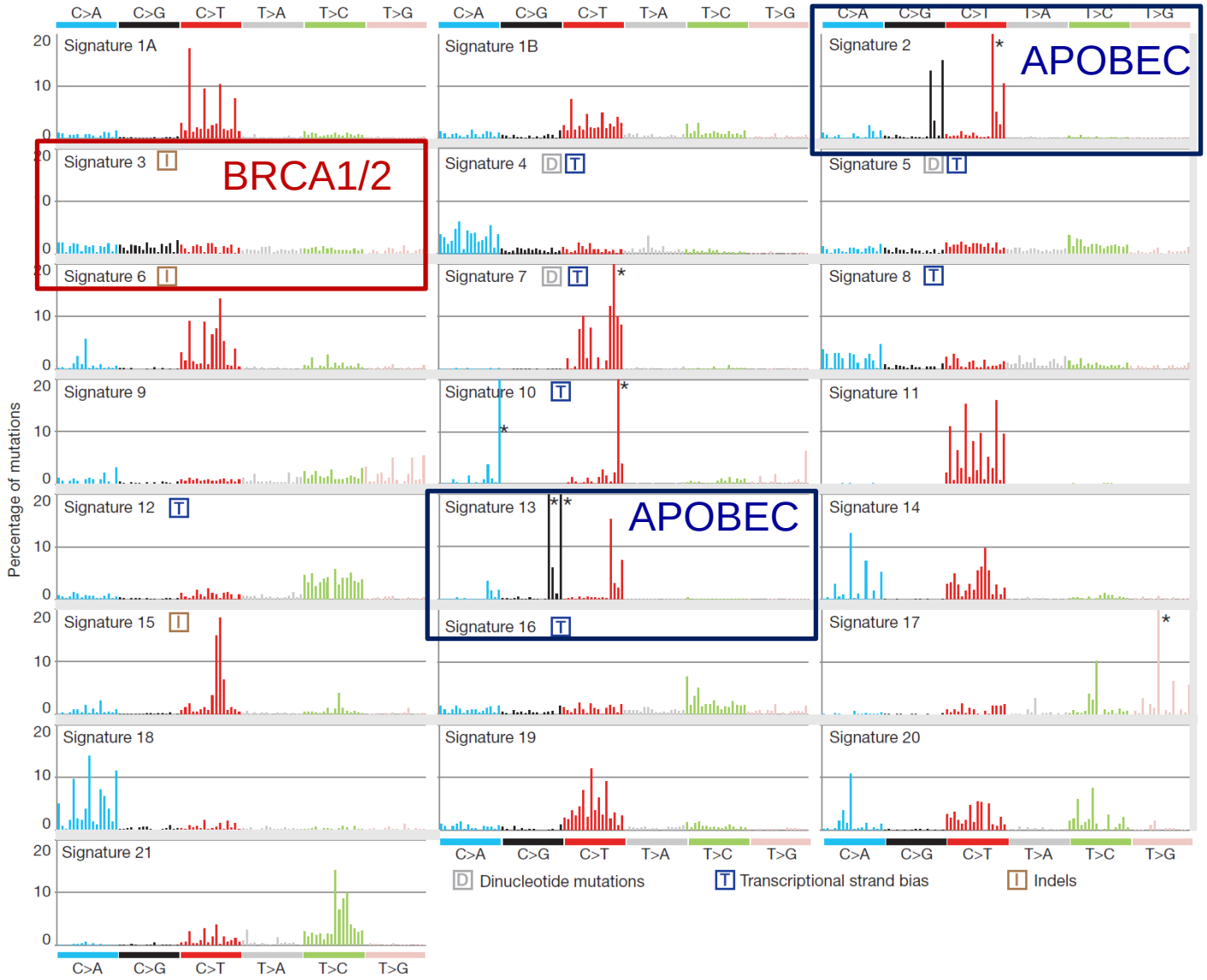


Whole Exome – 250x depth



Ng, Bidard, Weigelt, Reis-Filho, unpublished

Mutation signatures and genomic scars are not identified

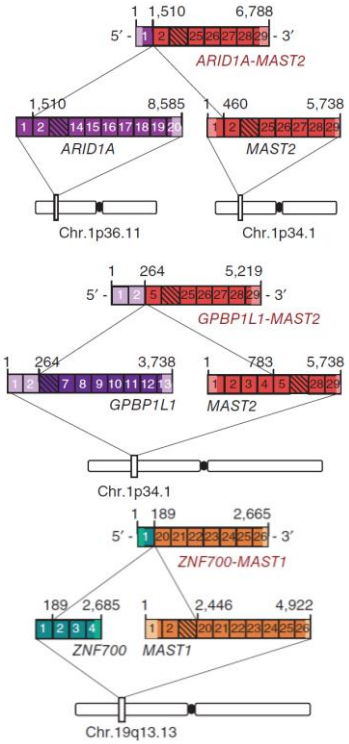


Alexandrov et al. Nature 2013

If we go with exome sequencing instead

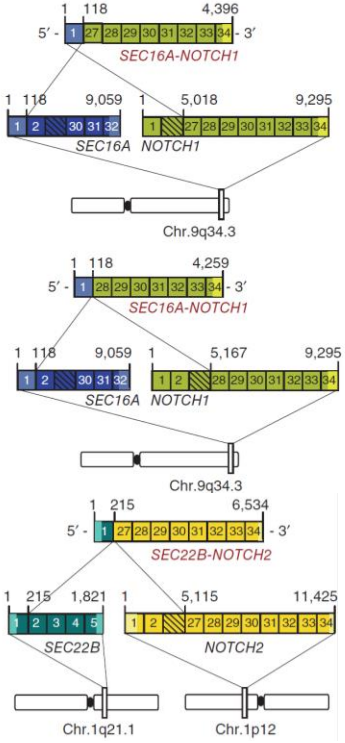
- Mutations in coding regions and some 3’ and 5’ UTRs

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



~6% of all breast cancers

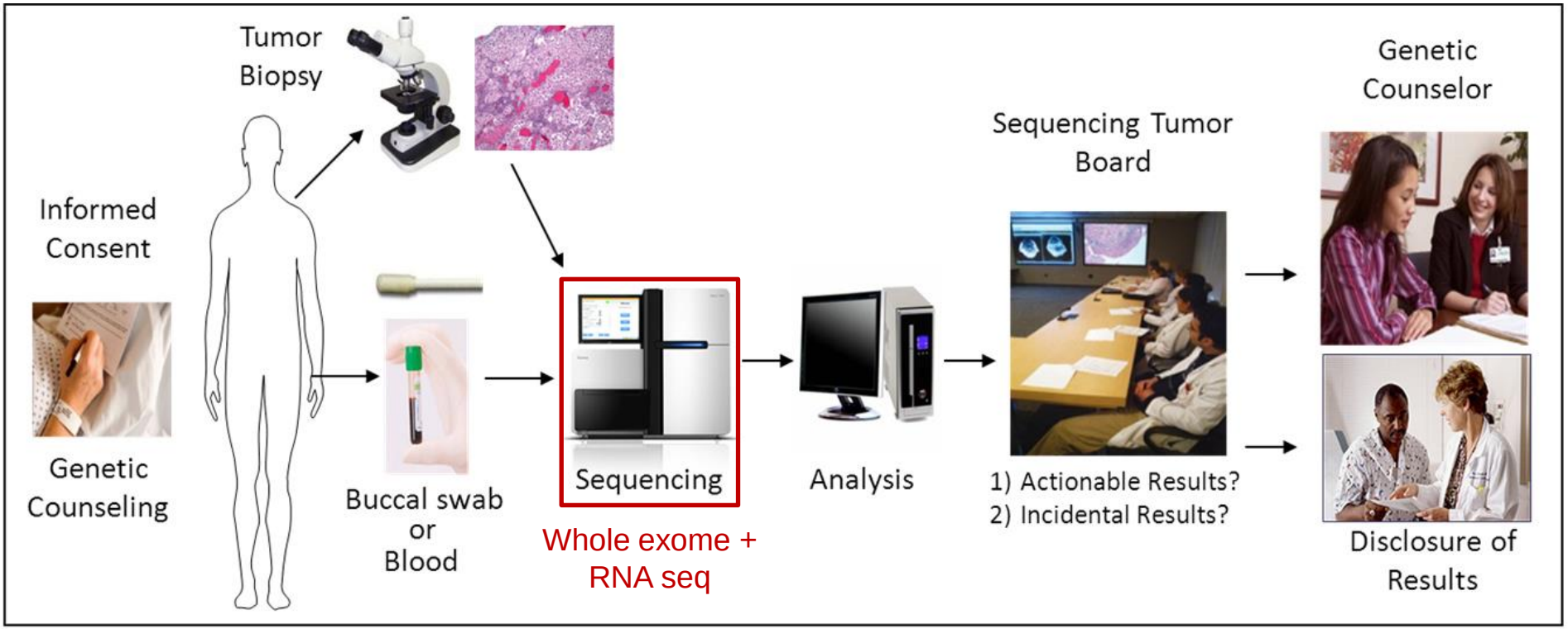
NOTCH1 and *NOTCH2*
Robinson et al. Nat Med 2011



~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