Next Generation sequencing (NGS) : attuali applicazioni nella pratica clinica

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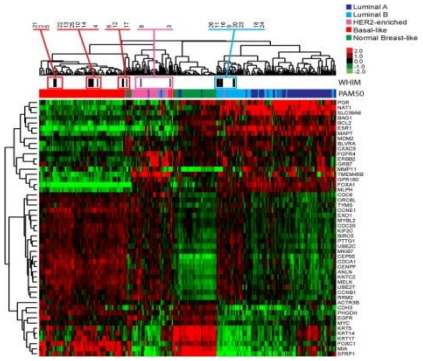
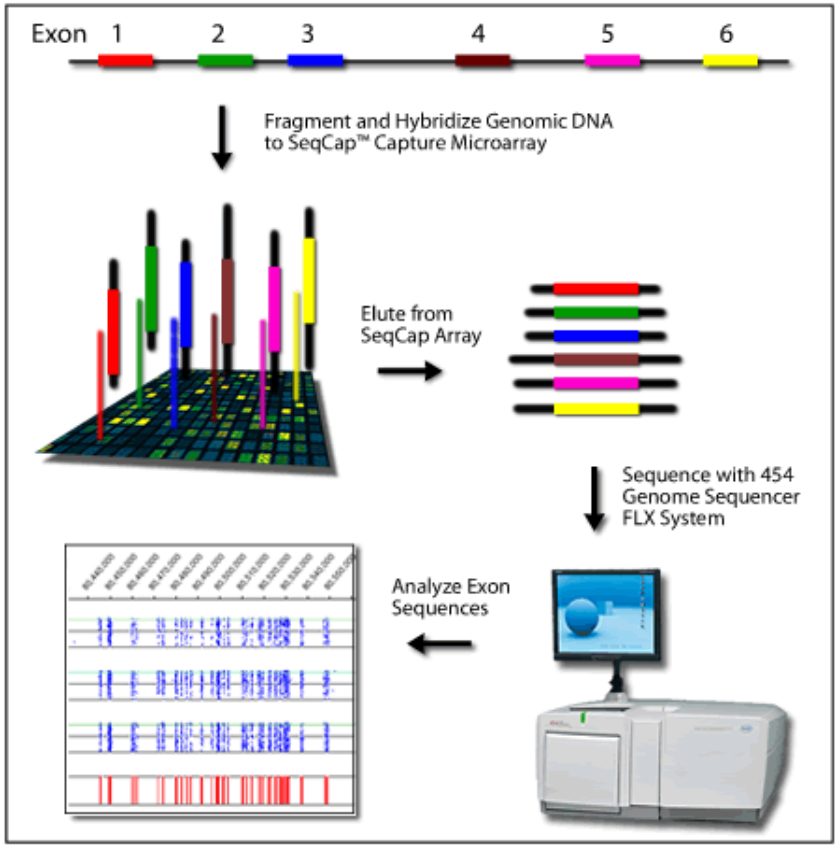
A decade ago, ‘Sanger’ sequencing of the human reference genome and the subsequent development of microarrays revolutionized breast cancer research.

Microarrays enabled the interrogation of cancer genomes for DNA-copy number changes and loss-of-heterozygosity events, as well as entire cancer transcriptomes for changes in gene expression level.

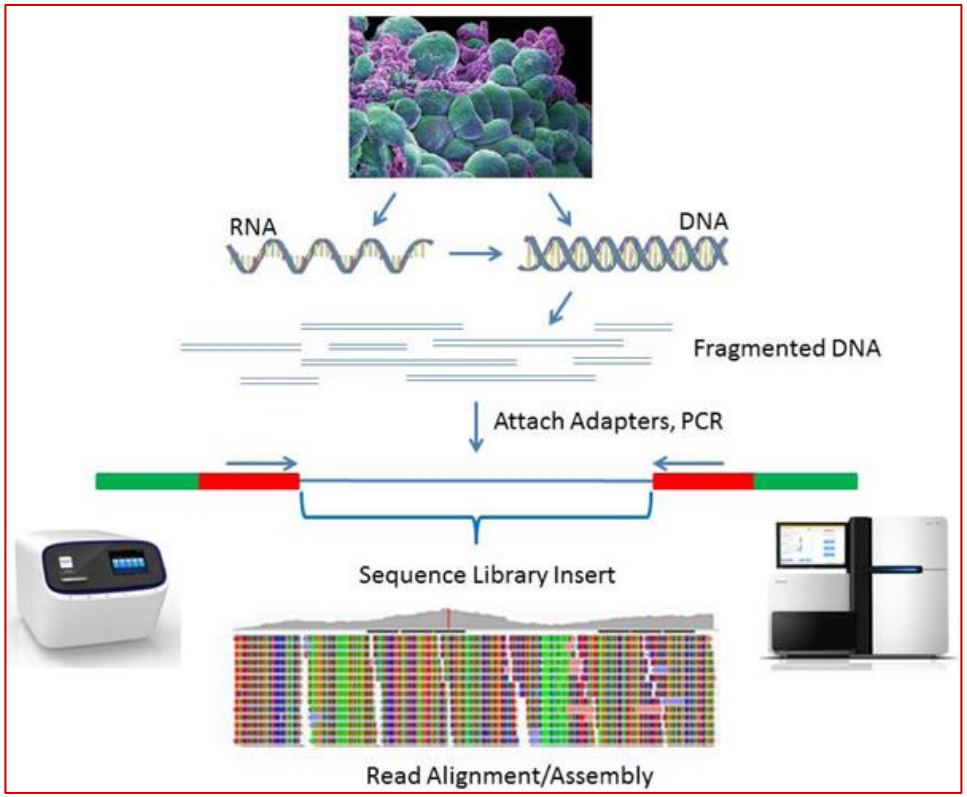


- ❖a better understanding of the biology of breast cancer
- ❖Proposal for a new molecular classification system for the disease
- ❖a refinement of breast cancer prognosis
- ❖identification of predictive markers for response to anticancer treatments

- a better understanding of breast cancer heterogeneity
- identification of distinct molecular breast cancer subtypes underpinned by a number of genetic and epigenetic aberrations presenting different prognosis and response to therapy governed by distinct molecular pathways and networks



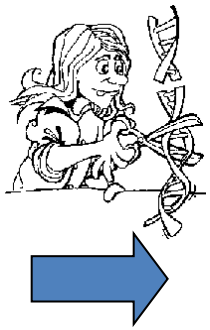
Currently, we are at the doorstep of yet another revolution in cancer research based around **next generation DNA sequencing (NGS).**

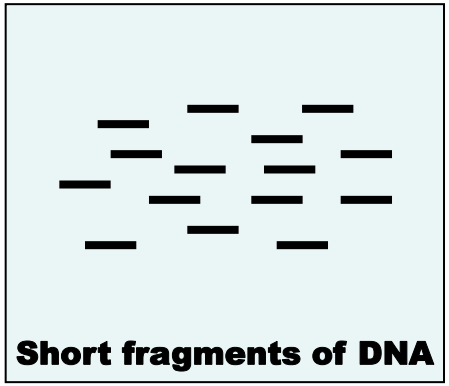


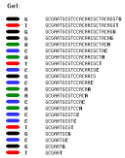
An ideal tool for the genetic characterisation of cancers is one that , in a single experiment, could provide information about

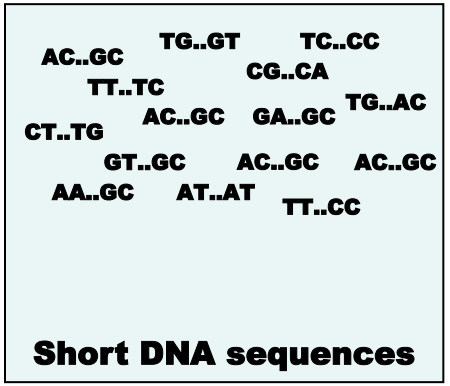
- copy number aberrations
- allelic information
- somatic rearrangements
- base pair mutations.

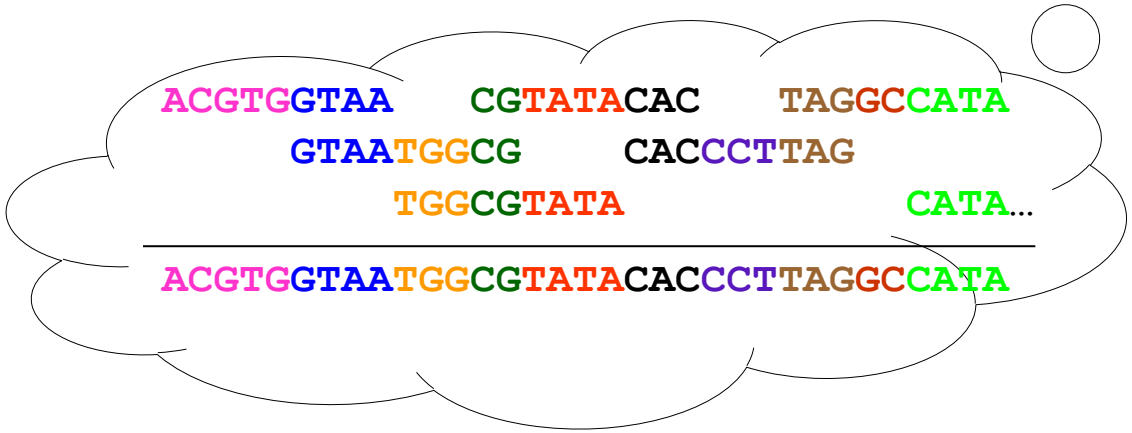
Genome Sequencing or second-generation sequencing technologies have the advantage that enormous numbers of sequencing reactions can be performed in parallel in a time- and cost-effective manner.

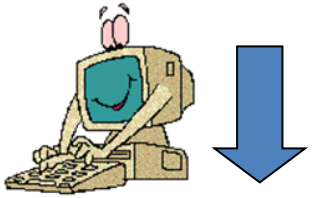












Genome sequencing projects that previously required decades of Sanger 'chaintermination' sequencing can now be accomplished in a matter of days at 10.000 to 100.000 times cost reduction.

Review

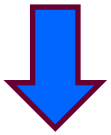
Opportunities and challenges of next-generation DNA sequencing for breast units

S. M. Pilgrim[1], S. J. Pain[4] and M. D. Tischkowitz[2,3]

BJS 2014; **101**: 889–898

the whole *BRCA1/BRCA2n* testing process currently takes approximately a minimum of **10–12weeks.**

Introduction of NGS could cut this to 4 weeks, with turnaround times continuing to decrease as the volume of tests increases.



This will have a significant impact on clinical practice, as it will mean that *BRCA1/BRCA2* mutation status can be established at approximately the same time as treatment decisions are being made in women with newly diagnosed breast cancer

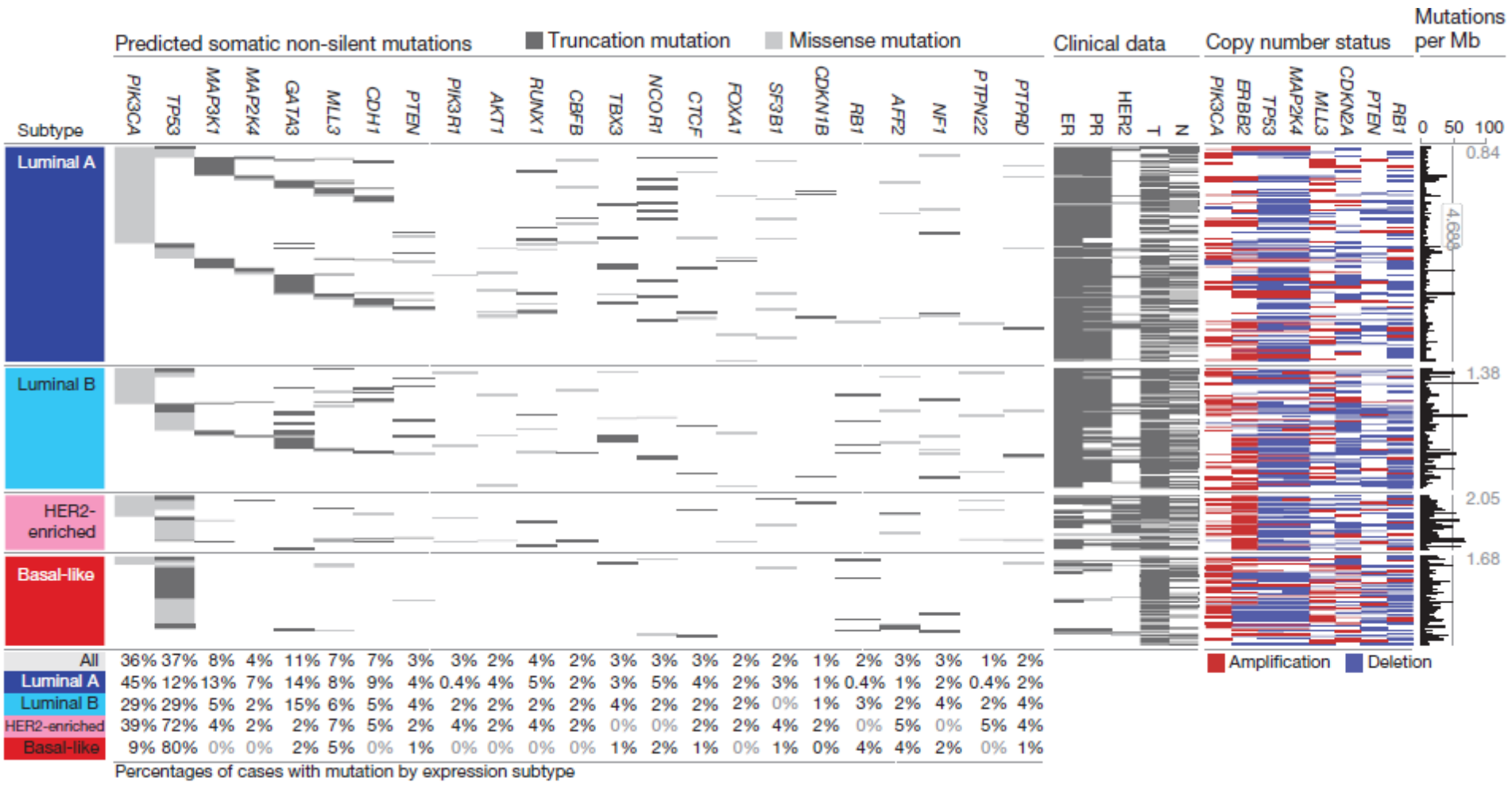
ARTICLE

doi:10.1038/nature11412

Comprehensive molecular portraits of human breast tumours

The Cancer Genome Atlas Network*

510 primary breast cancers was analysed by genomic DNA copy number arrays, DNA methylation, exome sequencing, messenger RNA arrays, microRNA sequencing and reverse-phase protein arrays.



ARTICLE

doi:10.1038/nature11412

Comprehensive molecular portraits of human breast tumours

The Cancer Genome Atlas Network*

- Somatic mutations in only three genes (TP53, PIK3CA and GATA3) occurred at more than 10% incidence across all breast cancers
- there were numerous subtype-associated and novel gene mutations including the enrichment of specific mutations in GATA3, PIK3CA and MAP3K1 with the luminal A subtype
- two novel protein-expression-defined subgroups, possibly produced by stromal/microenvironmental elements
- Integrated analyses identified specific signalling pathways dominant in each molecular subtype including a HER2/phosphorylated HER2/EGFR/phosphorylated EGFR signature within the HER2-enriched expression subtype.
- Comparison of basal-like breast tumours with high-grade serous ovarian tumours showed many molecular commonalities, indicating a related aetiology and similar therapeutic opportunities.

ARTICLE
doi:10.1038/nature11412

Comprehensive molecular portraits of human breast tumours

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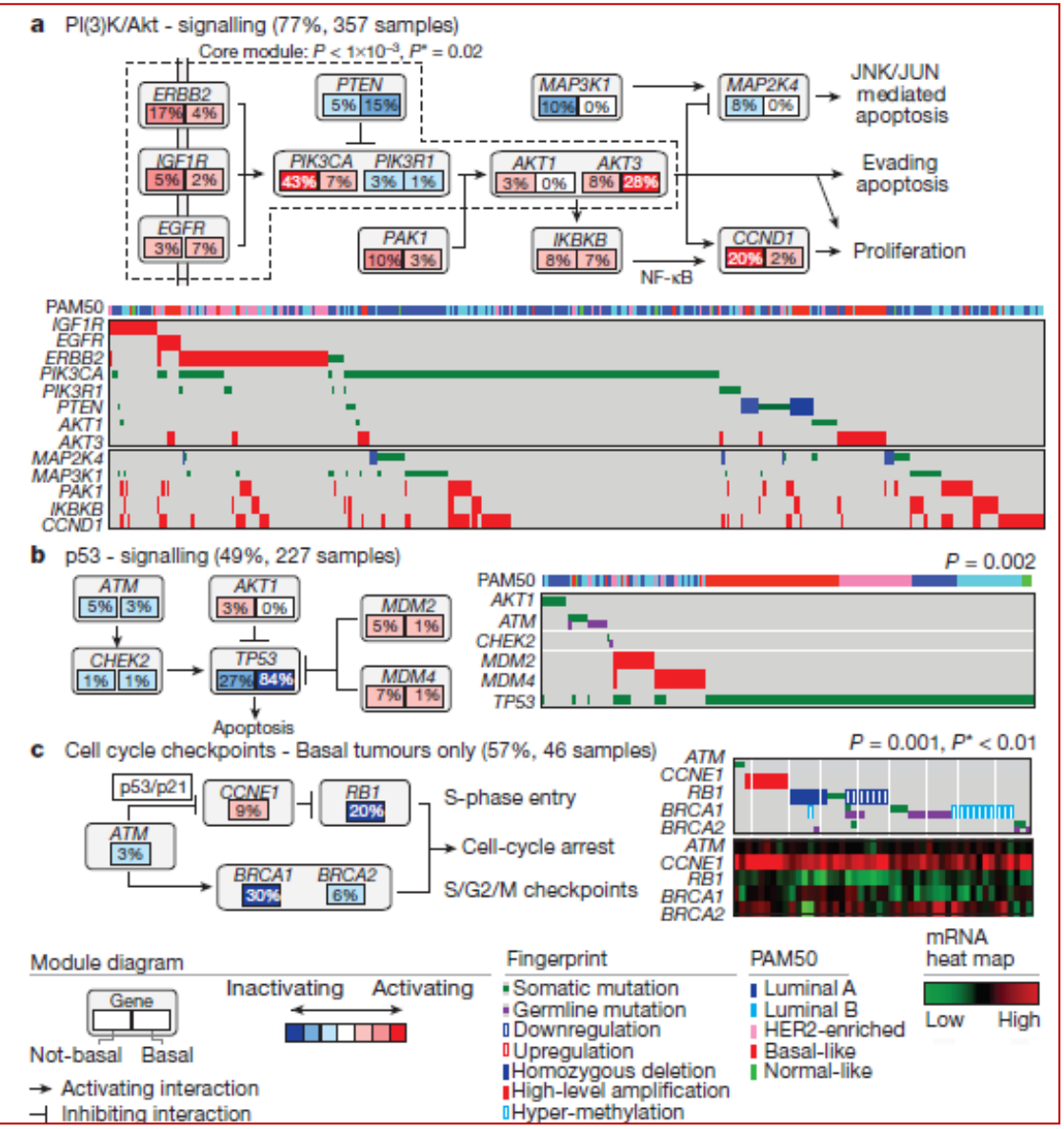
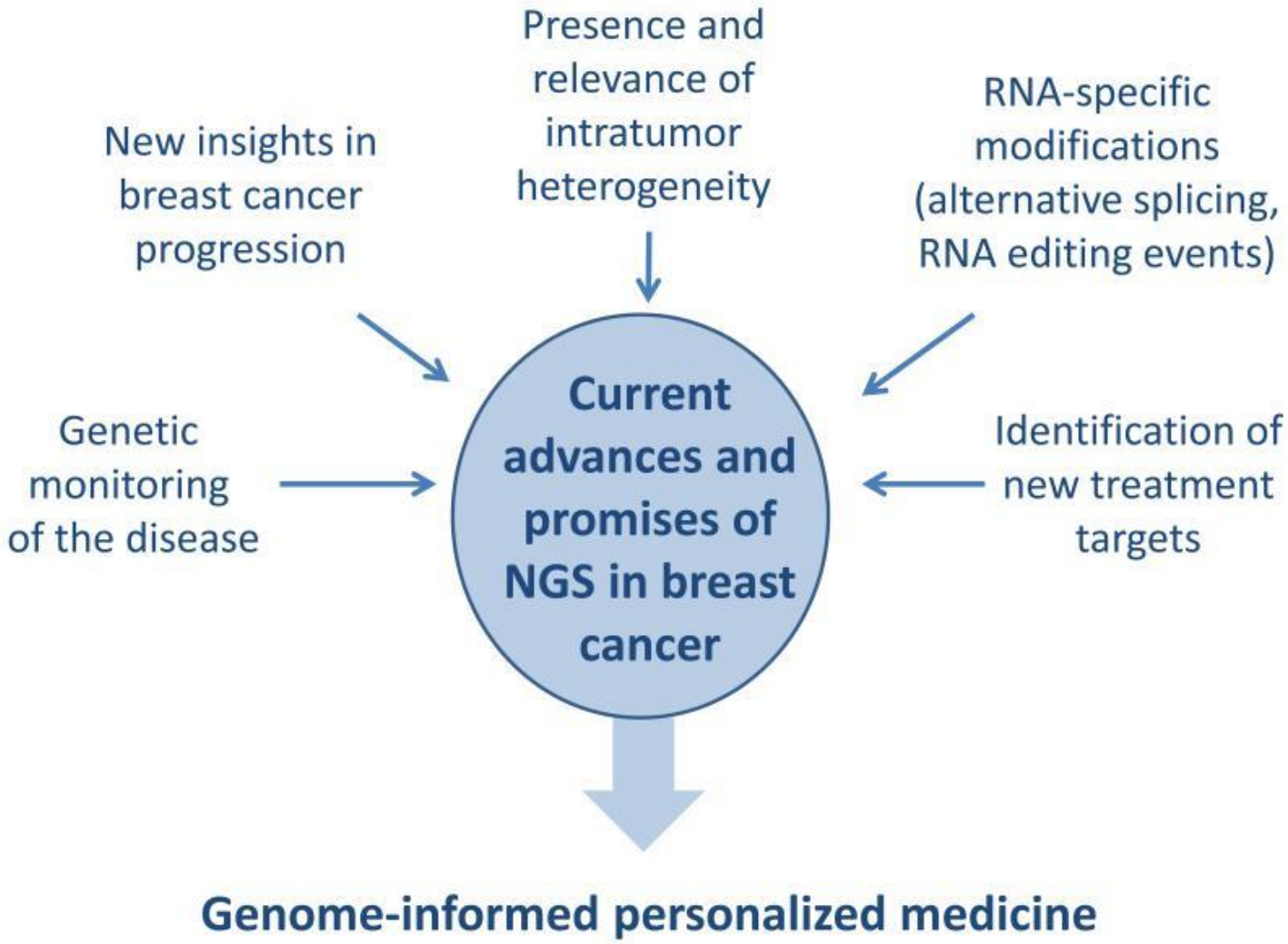


The biological finding of the four main breast cancer subtypes caused by different subsets of genetic and epigenetic abnormalities raises the hypothesis that much of the clinically observable plasticity and heterogeneity occurs **within, and not across,** these major biological subtypes of breast cancer.

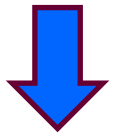
Next generation sequencing a new avenue for cancer research



Identification of new cancer genes

By sequencing both the tumor and the germline DNA, NGS may reveal the somatic genetic alterations in a cancer genome. These somatic genetic changes can be classified in two ways.

- Driver mutations contributing to tumor development
- Passenger mutations which may be the product of the genomic instability of the tumor.



the distinction between driver and passenger mutations is dynamic and can change throughout the course of the disease

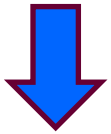
For example a passenger mutation could become a driver mutation after anticancer treatment by providing clonal advantage to the resistant clone.

Identification of new cancer genes

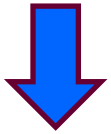
Although new cancer (driver) genes were identified, there were no new frequently mutated cancer genes.

Unlike the known cancer genes **P53 and PIK3CA which are mutated in >30% of breast cancer** patients (P53 preferentially in ER-negative and PIK3CA preferentially in ER-positive tumors), **most newly identified cancer genes are mutated in less than 10% of the patients**

There is a very large genetic diversity among different breast cancer which are also heterogeneous with regard to mutated genes, the mutated genes can be grouped into the deregulation of similar pathways.

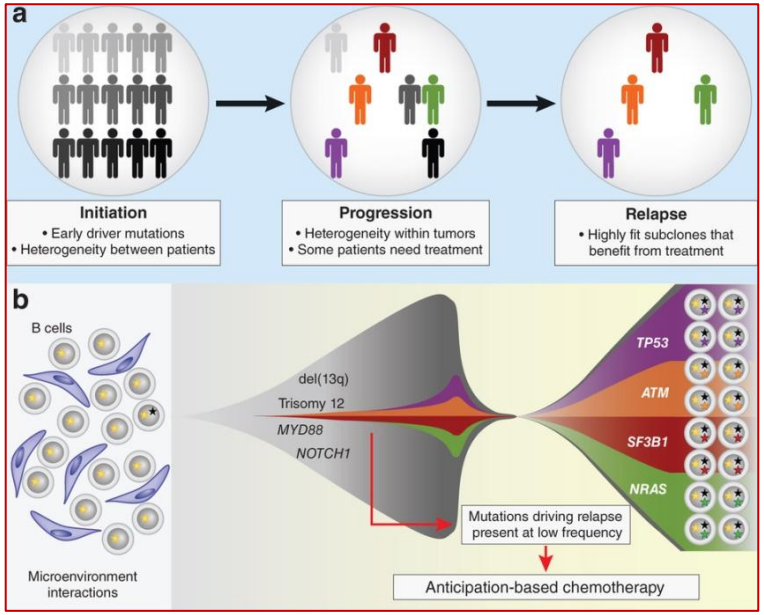


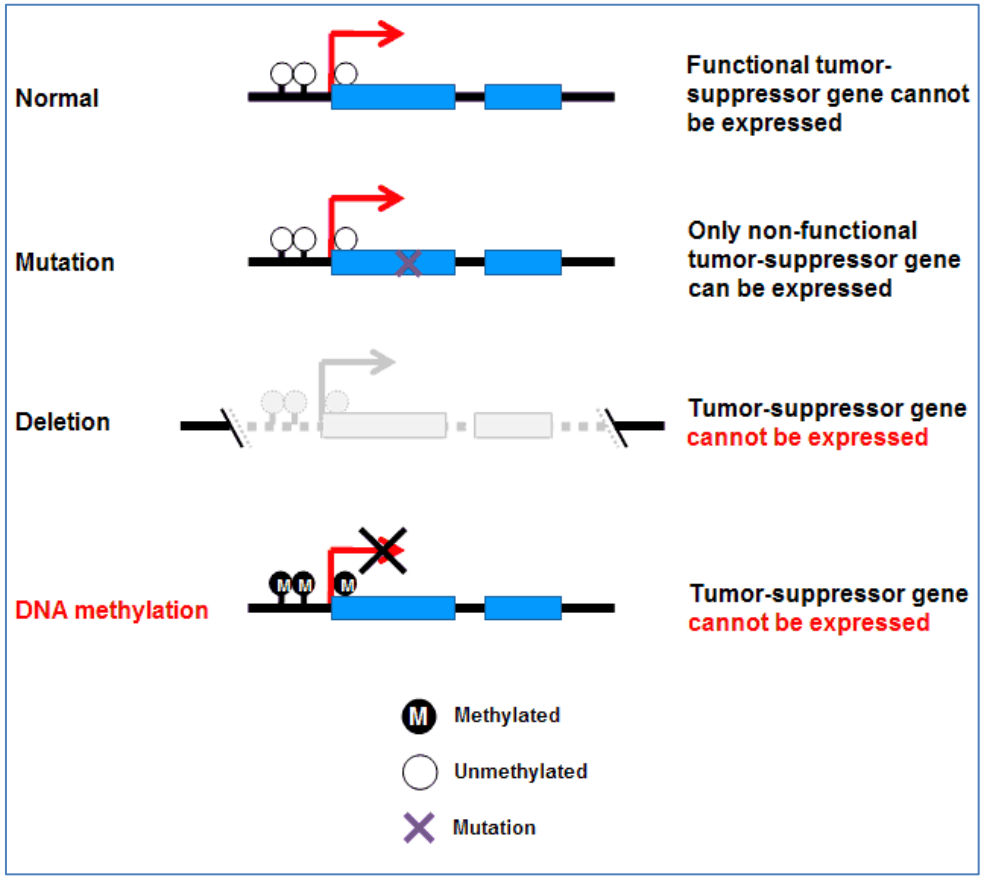
This means that although the tumors are genetically different, some could be phenotypically similar due to mutations in the same pathway



very important in terms of treatment

No driver mutation was obvious. This may suggest that a different mechanism is driving tumor development in breast cancer, such as for example DNA methylation.

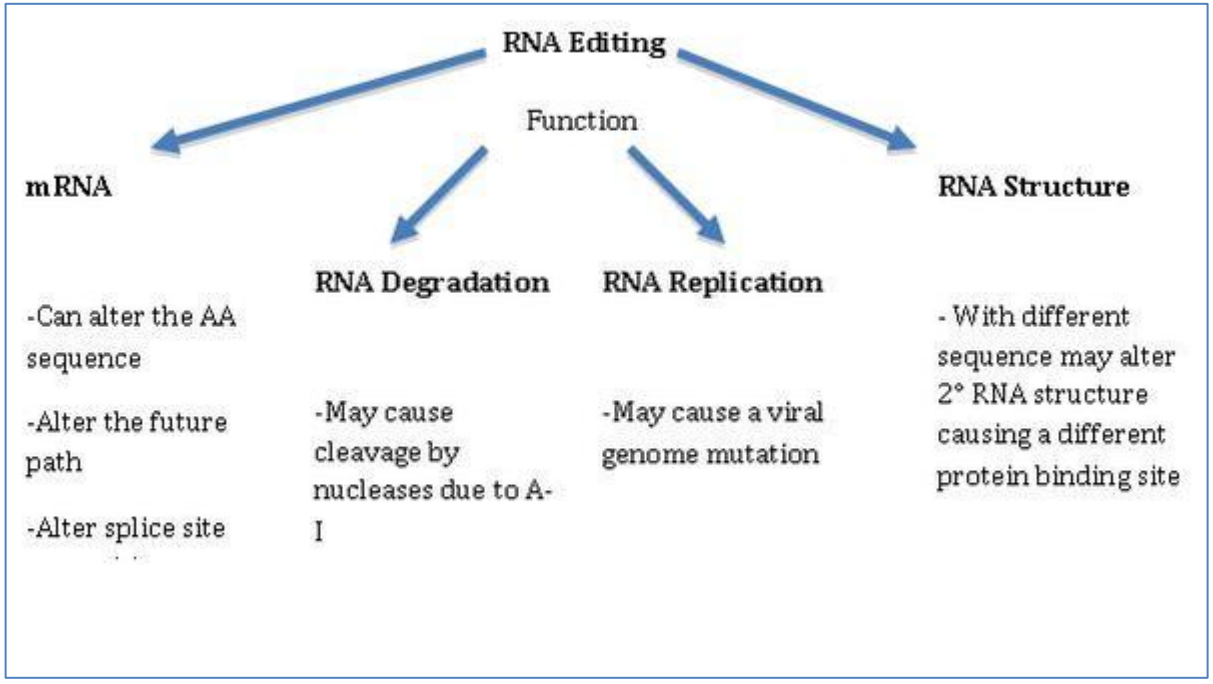




some mutations might be associated with the response/resistance to anti-cancer treatment.

RNA-seq can do more than quantifying gene expression

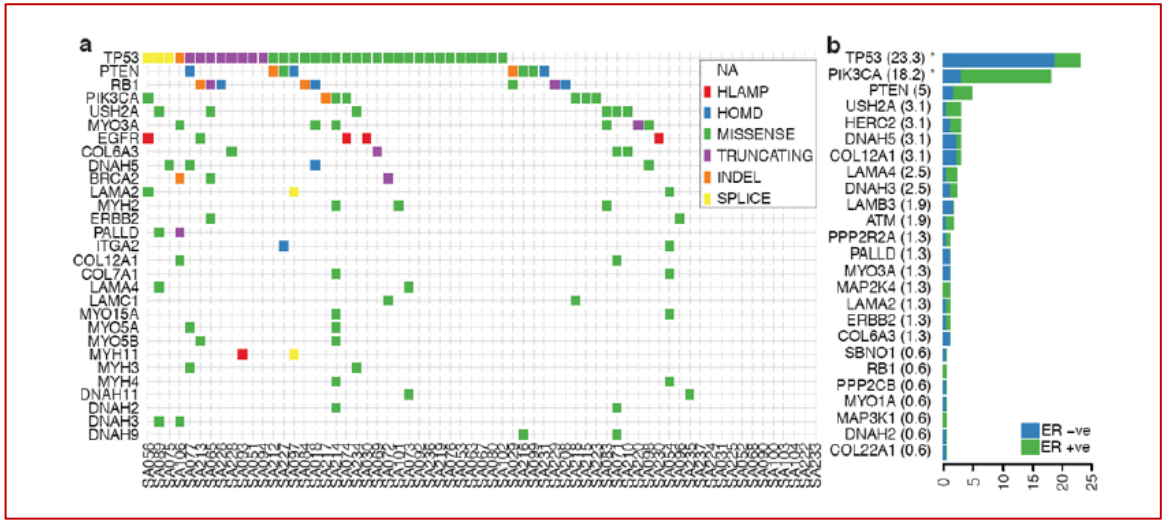
RNA editing and alternative splicing are mechanisms which modify the transfer of genetic information from the nuclear genome to proteins and represent an important additional source of the biological complexity of a cancer.



Although very few papers have used RNA-seq to investigate breast cancer until now, the application of RNA-seq to breast cancers has great promises to deliver better understanding of the complexity of breast cancer disease.

Published in final edited form as:
Nature. ; 486(7403): . doi:10.1038/nature10933.

The clonal and mutational evolution spectrum of primary triple negative breast cancers



RNA and DNA sequencing on 80 and 65 cases of triple negative breast cancers

TNBC vary widely and continuously in their clonal frequencies at the time of diagnosis, with basal subtype TNBC exhibiting more variation than non-basal TNBC.

p53 and PIK3CA/PTEN somatic mutations appear clonally dominant compared with other pathways

Shaah et al 2013

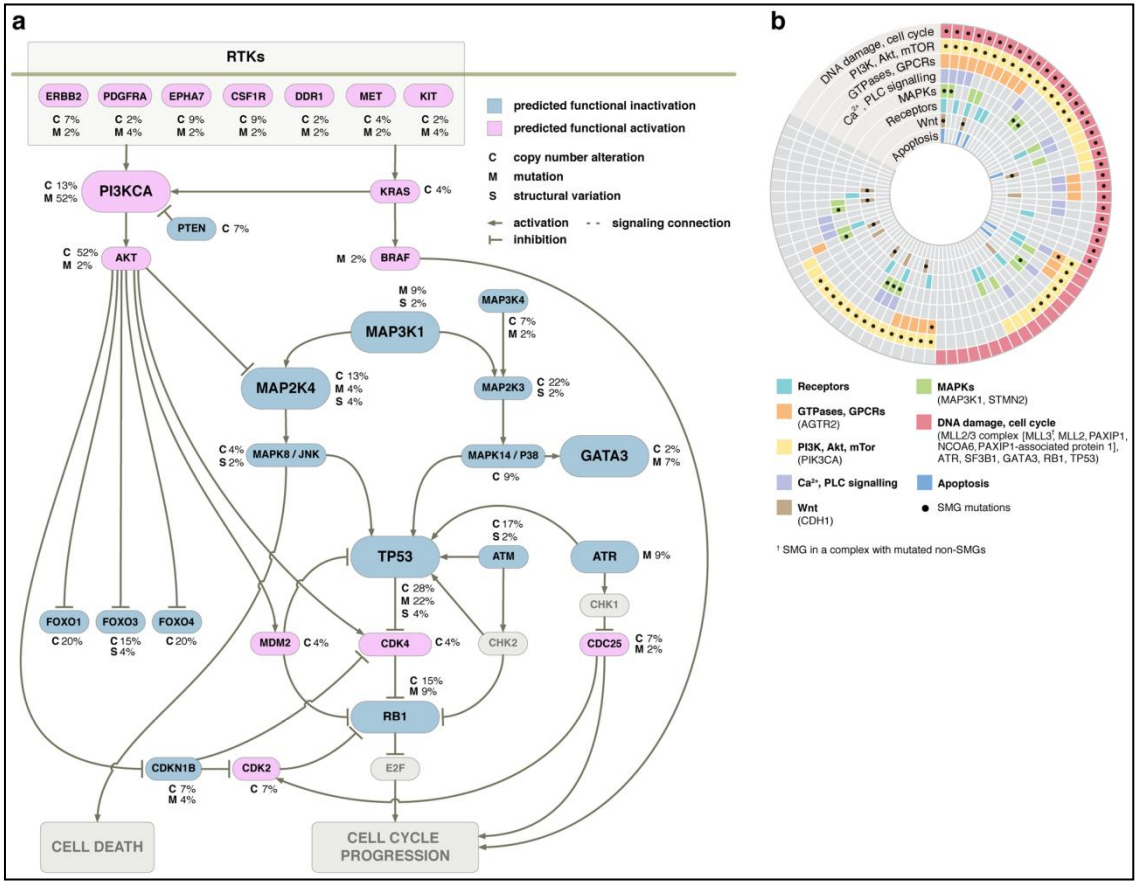
Whole Genome Analysis Informs Breast Cancer Response to Aromatase Inhibition

Published in final edited form as:
Nature. ; 486(7403): 353–360. doi:10.1038/nature11143.

Ellis et al 2012

Clinical trial :

1. preoperative letrozole phase 2 study (NCT00084396) 2 that investigated effect of letrozole for 16 to 24 weeks on surgical outcomes
2. American College of Surgeons Oncology Group (ACOSOG) Z1031 study (NCT00265759) 3 that compared anastrozole with exemestane or letrozole for 16 to 18 weeks before surgery



somatic mutations affect a number of pathways:

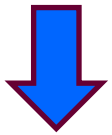
- caspase cascade/apoptosis
- ErbB signaling
- Akt/PI3K/mTOR signaling
- TP53/RB signaling
- MAPK/JNK pathways

several pathways were enriched in the AI resistant group, including the TP53 signaling pathway

- DNA replication
- mismatch repair.

38% of the AI resistant group have mutations in the TP53 pathway with three having double or triple hits involving TP53, ATR, APAF1, or THBS1.

16.6% of the Ki67 low group had mutations in the TP53 signaling pathway, each with only a single hit in genes TP53, ATR, CCNE2, or IGF1



mutant GATA3 correlated with suppression of proliferation upon aromatase inhibitor treatment

Published in final edited form as:
Nature. ; 486(7403): 405–409. doi:10.1038/nature11154.

Sequence analysis of mutations and translocations across breast cancer subtypes Shantanu Banerji

Published in final edited form as:
Nature. ; 486(7403): 400–404. doi:10.1038/nature11017.

The landscape of cancer genes and mutational processes in breast cancer Philip J. Stephens

The common messages from these papers are the following:

- although new cancer (driver) genes were identified there were no new frequently mutated cancer genes.
- unlike the known cancer genes P53 and PIK3CA which are mutated in >30% of breast cancer patients (P53 preferentially in ER-negative and PIK3CA preferentially in ER-positive tumors), most newly identified cancer genes are mutated in less than 10% of the patient
- there is a very large genetic diversity among different breast tumors.

The Oncologist®

Breast Cancer

NEIL VASAN,

A Targeted Next-Generation Sequencing Assay Detects a High Frequency of Therapeutically Targetable Alterations in Primary and Metastatic Breast Cancers: Implications for Clinical Practice

Conclusion. Overall, 84% of cancers harbored at least one genomic alteration linked to potential treatment options. Systematic evaluation of the predictive value of these genomic alterations is critically important for further progress in this field. ***The Oncologist*** 2014;19:453–458

Alkner et al. Breast Cancer Research (2015) 17:102
DOI 10.1186/s13058-015-0608-x

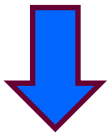
Sara Alkner

Breast Cancer RESEARCH

RESEARCH ARTICLE Open Access

Contralateral breast cancer can represent a metastatic spread of the first primary tumor: determination of clonal relationship between contralateral breast cancers using next-generation whole genome sequencing

Using NGS authors show that a contralateral BC2 can represent a metastatic spread of BC1.



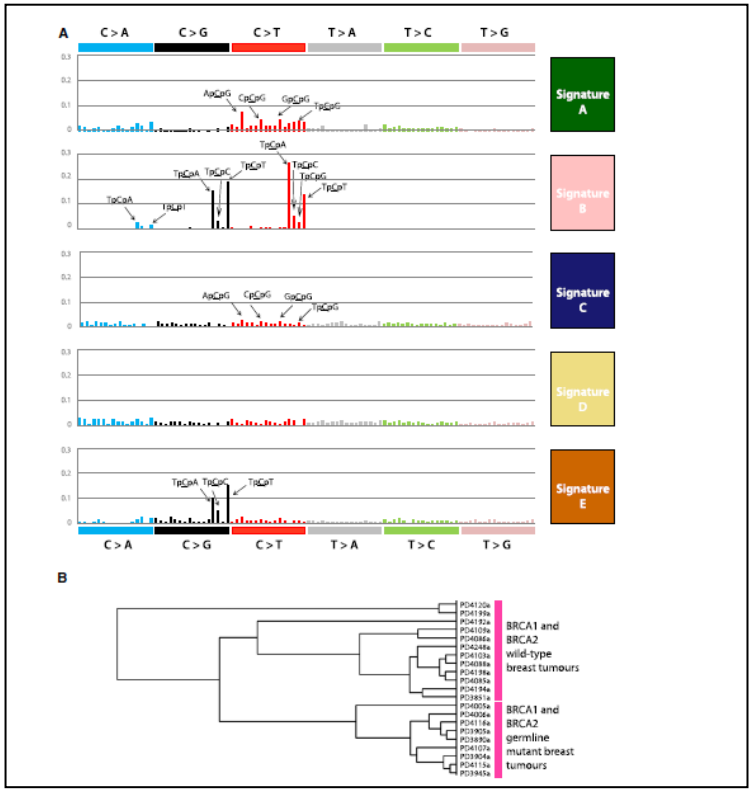
appropriate determination of treatment.

Intratumor heterogeneity

clonal mutations, which are the ones appearing early in tumor development, comprised mutations in many cancer genes such as PIK3CA and P53, as well as all HER2, MYC and CCND1 amplifications and the somatic loss of the wild-type BRCA1 and BRCA2 alleles in hereditary breast cancers.

Mutational Processes Molding the Genomes of 21 Breast Cancers

Nik Zainal et al. Cell 2012



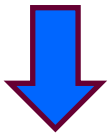
although breast tumors are heterogeneous with regard to mutated genes, **the mutated genes can be grouped into the deregulation of similar pathways.**

TAKE HOME MESSAGES

NGS sequencing in breast cancer revealed

- new cancer genes which are not frequently mutated

- subclonal mutations in all tumors and a dominant clone comprising at least 50% of the tumor cells.



could be used to personalize the monitoring of the disease by interrogating tumor-specific DNA rearrangements in the patient's plasma.

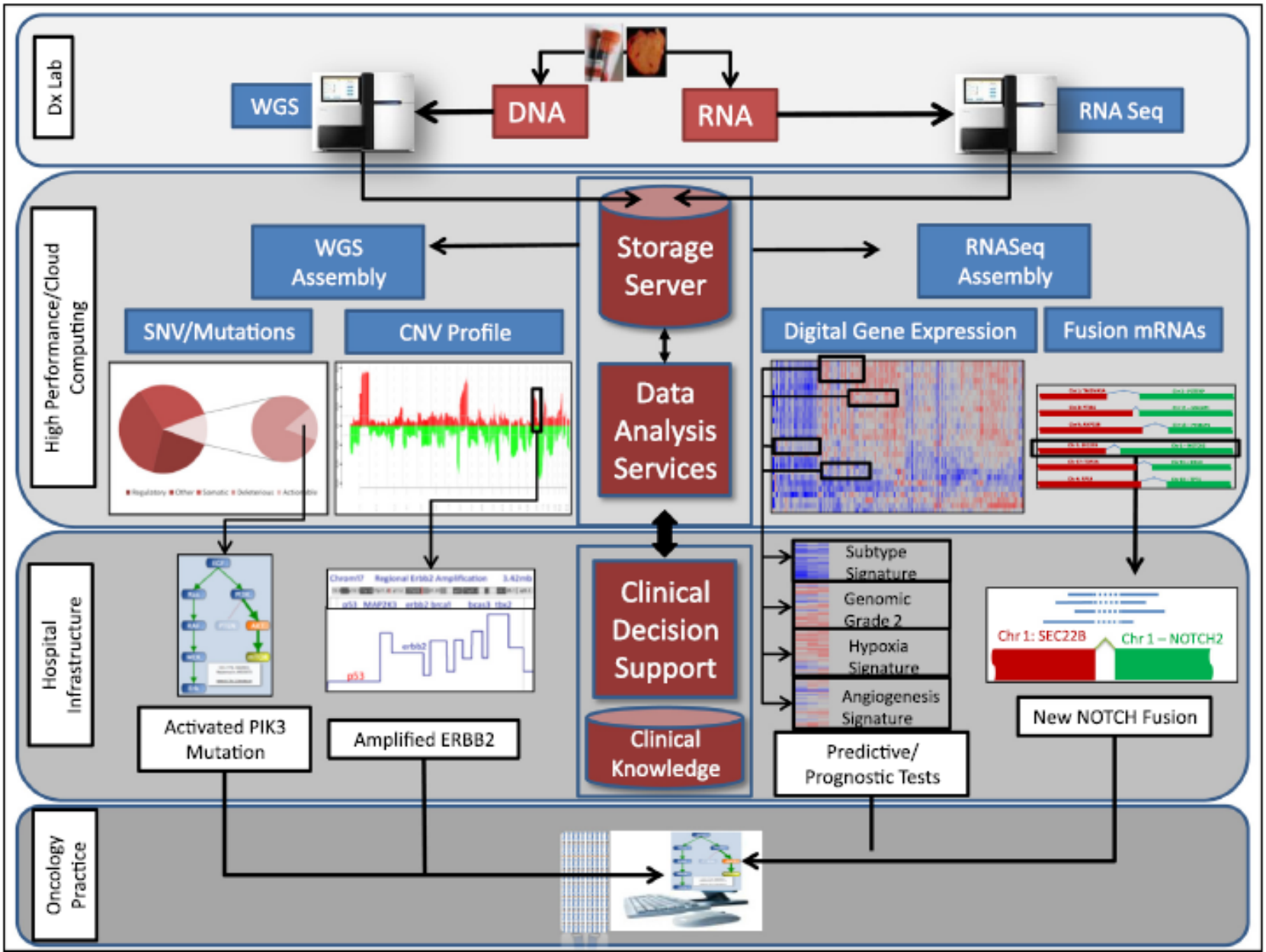
TAKE HOME MESSAGES

- NGS technologies provide important knowledge on
  - epigenetic changes
  - tumor microenvironment
  - germline genetic variation

Altogether, the promises for NGS in breast cancer may allow:

- to increase our understanding of the disease
- to identify new treatment targets
- to move towards genome-informed personalized medicine

A model for enabling sequencing based personalized oncology.



Grazie per l'attenzione