Animal Models and DNA sequencing technologies to aid in personalized cancer care and research

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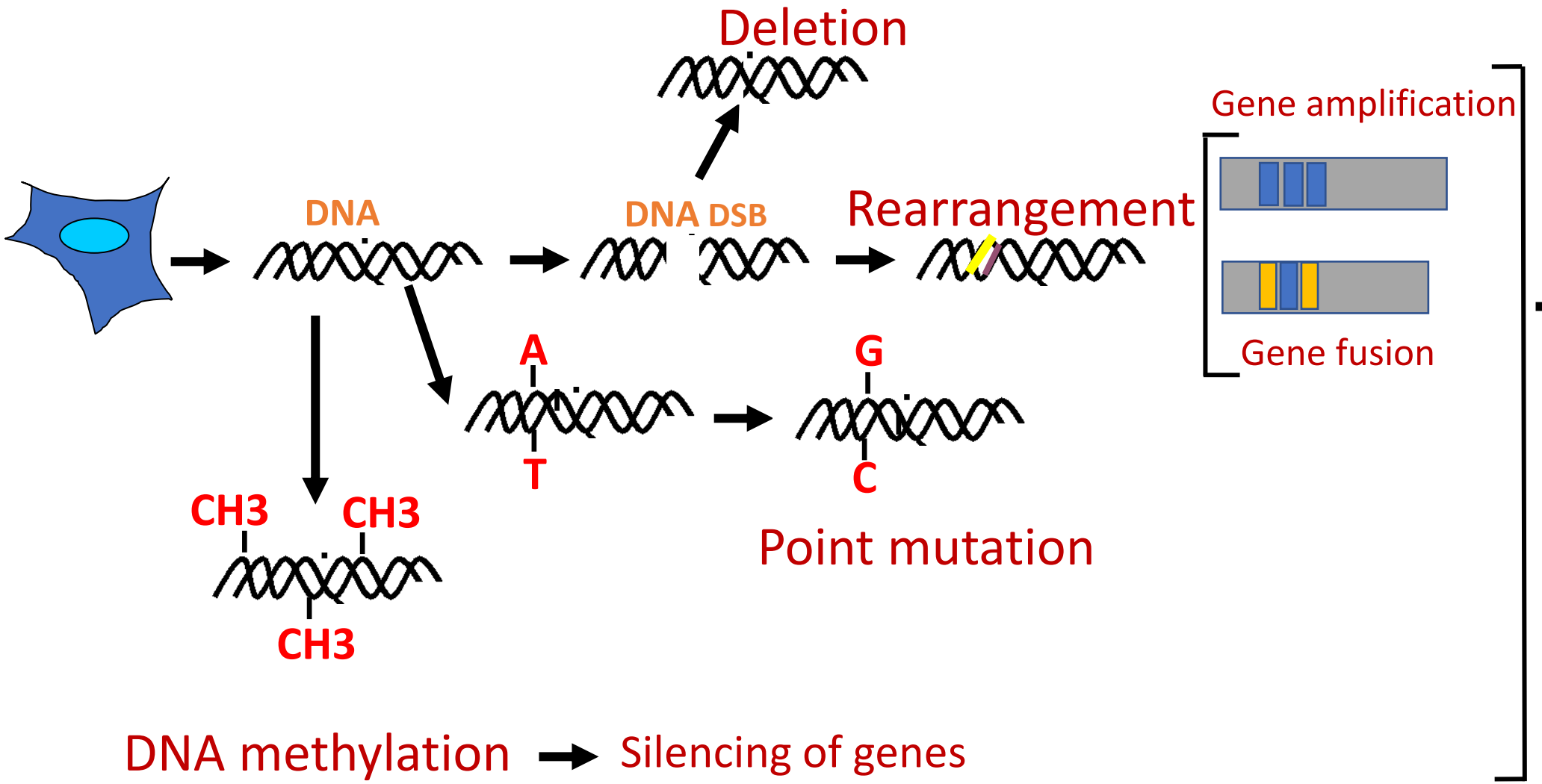
Examples of DNA alterations



Next generation DNA sequencing (NGS) technologies

Single gene deep sequencing	Identification of all changes in a gene-driver (BRCA1 and 2 in breast and ovarian cancers).
Targeted gene panel	Cancer type specific, includes a number of genes frequently mutated in a specific cancer.
Whole exome sequencing (WES)	Identification of all DNA alterations concerning gene regions.
RNA sequencing	Identification of alterations in the expressed genes, consequential.
Mate-pair sequencing	Identification of structural alterations, i.e. gene amplifications, big deletions and gene fusions

Genomic profiling of tumors by NGS in Research

- Improve understanding of biology of cancer and mechanisms of disease progression
- Identify biomarkers of early disease detection and treatment sensitivity
- Identify new therapy targets for further drug development

Preclinical testing of genomically-guided therapies

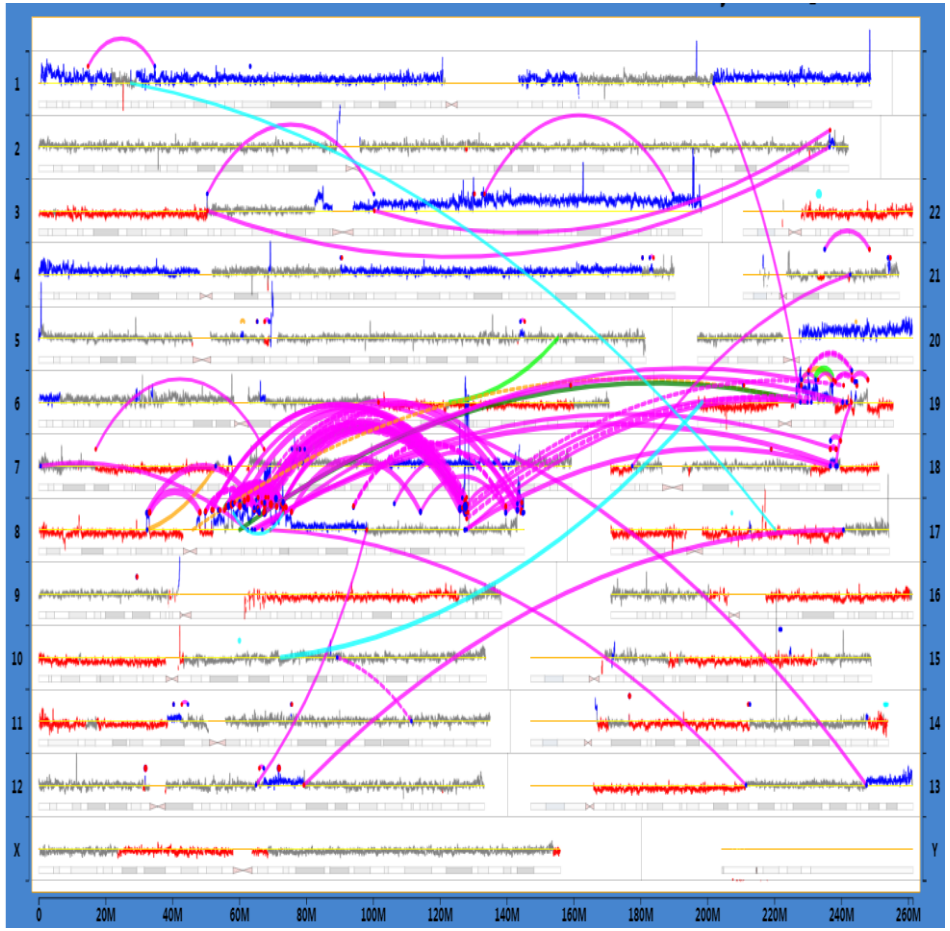
- Generation of cell lines expressing identified alteration to follow progression and study treatment sensitivity
- Testing therapies in high throughput platform in vitro
- Testing of therapies in mouse models in vivo

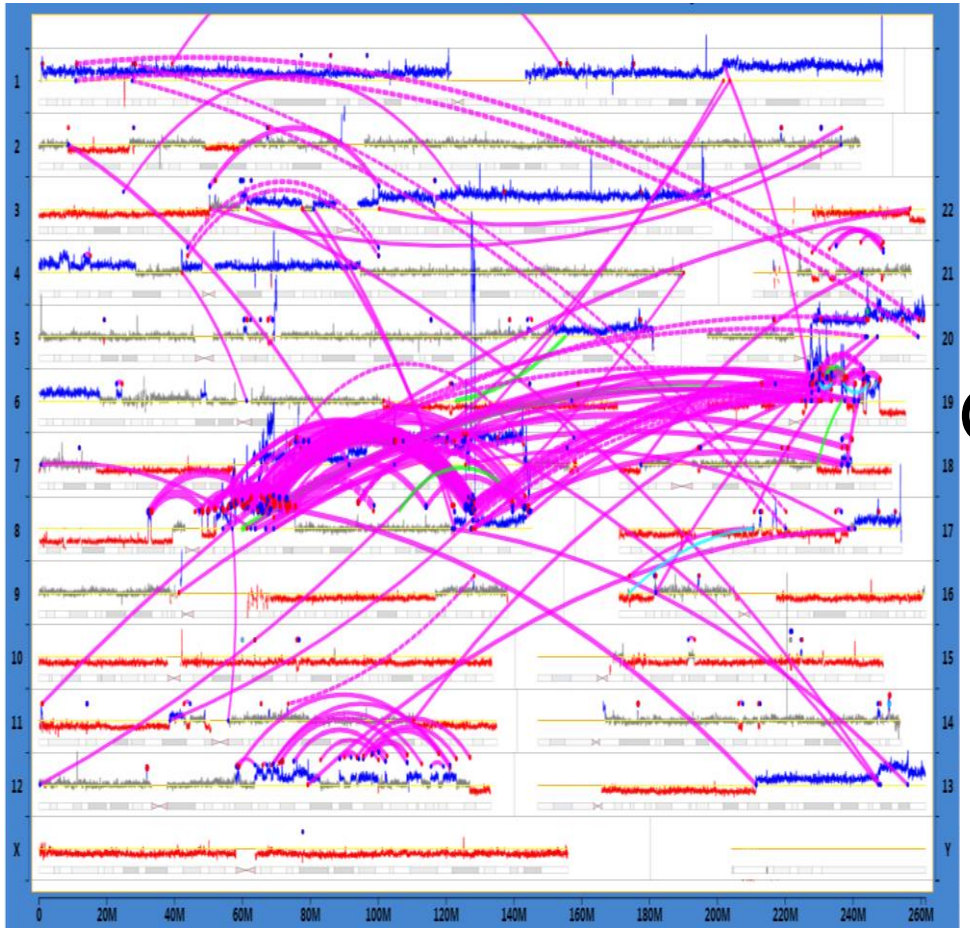
Tumor evolution

Patient 1, resected tumor

Patient 1, liver metastases, 1yr later

Genome plot





Genome plot

Accumulation of total DNA alterations over time

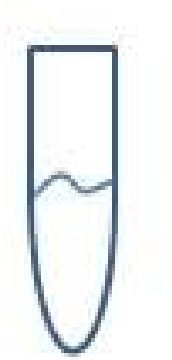
Appearance of new changes to revert benign changes

Appearance of new changes impacting treatment resistance

Harris FR……Kovtun IV et al. *Mol Oncol*. 13: 132-152, 2019

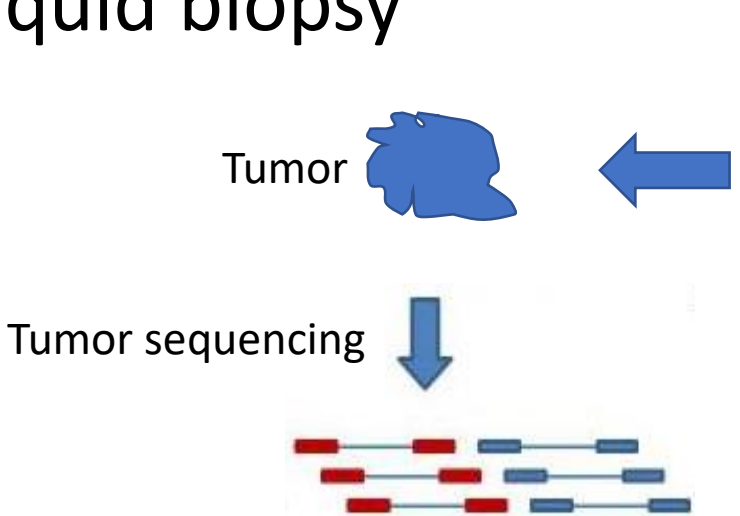
Clinical applications of NGS

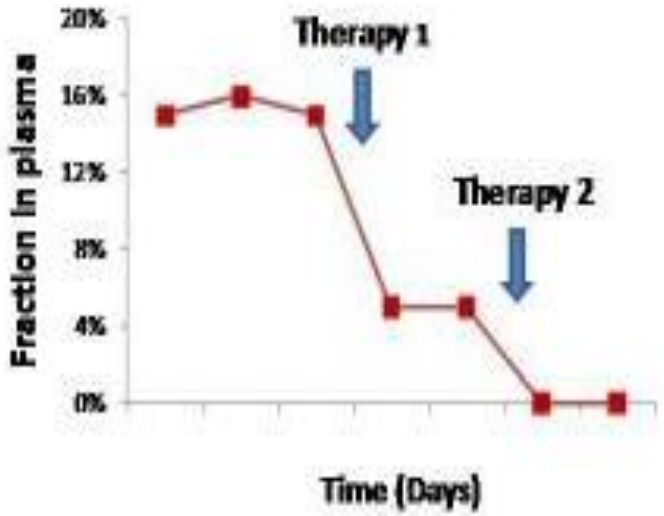
I. Liquid biopsy



Plasma or other bodily fluids

Early disease screening





Monitoring of disease progression and treatment sensitivity

Adopted from Leary, Vogelsten,. Velculescu et al 2010.

Liquid biopsy

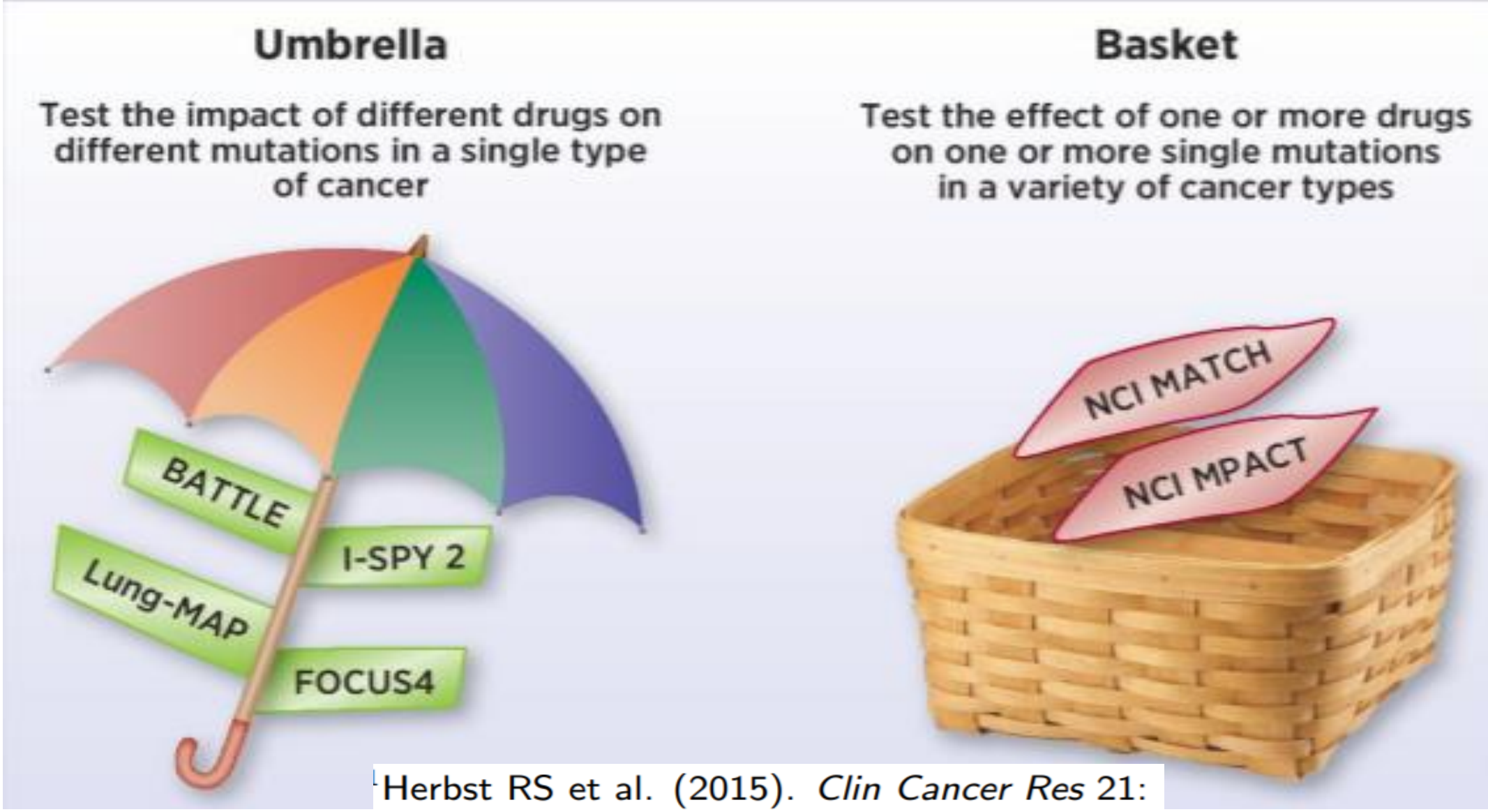
Advantages

- Non- invasive nature of the procedures
- Allow more frequent and serial samplings over time
- Useful in case tissue biopsy is not available

Challenges

- The need for an initial histologic diagnosis to be obtained by tissue biopsy.
- Low variant frequency in the blood may => higher false negative rates
- Sensitivity and specificity

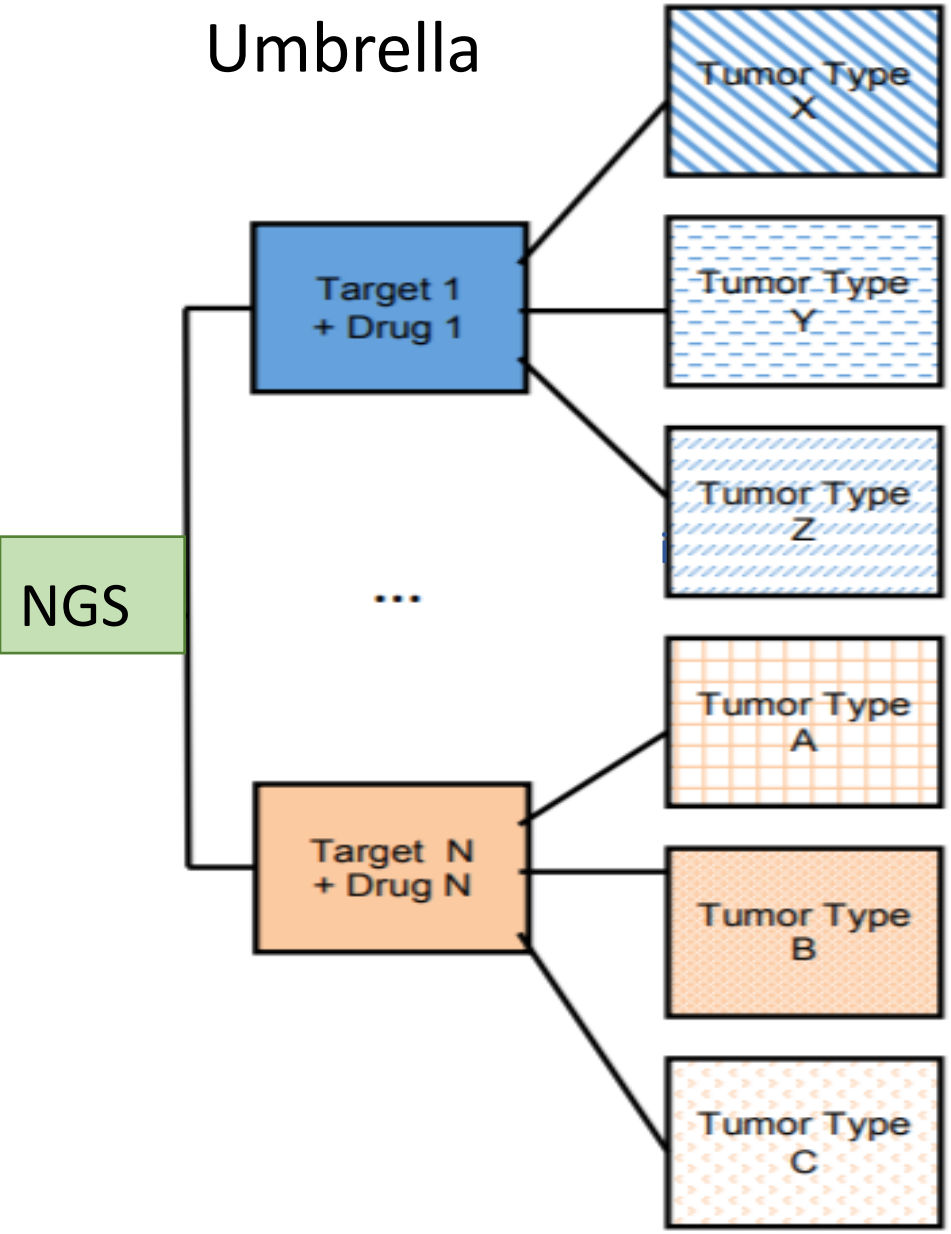
Clinical applications of NGS
II. Clinical trials



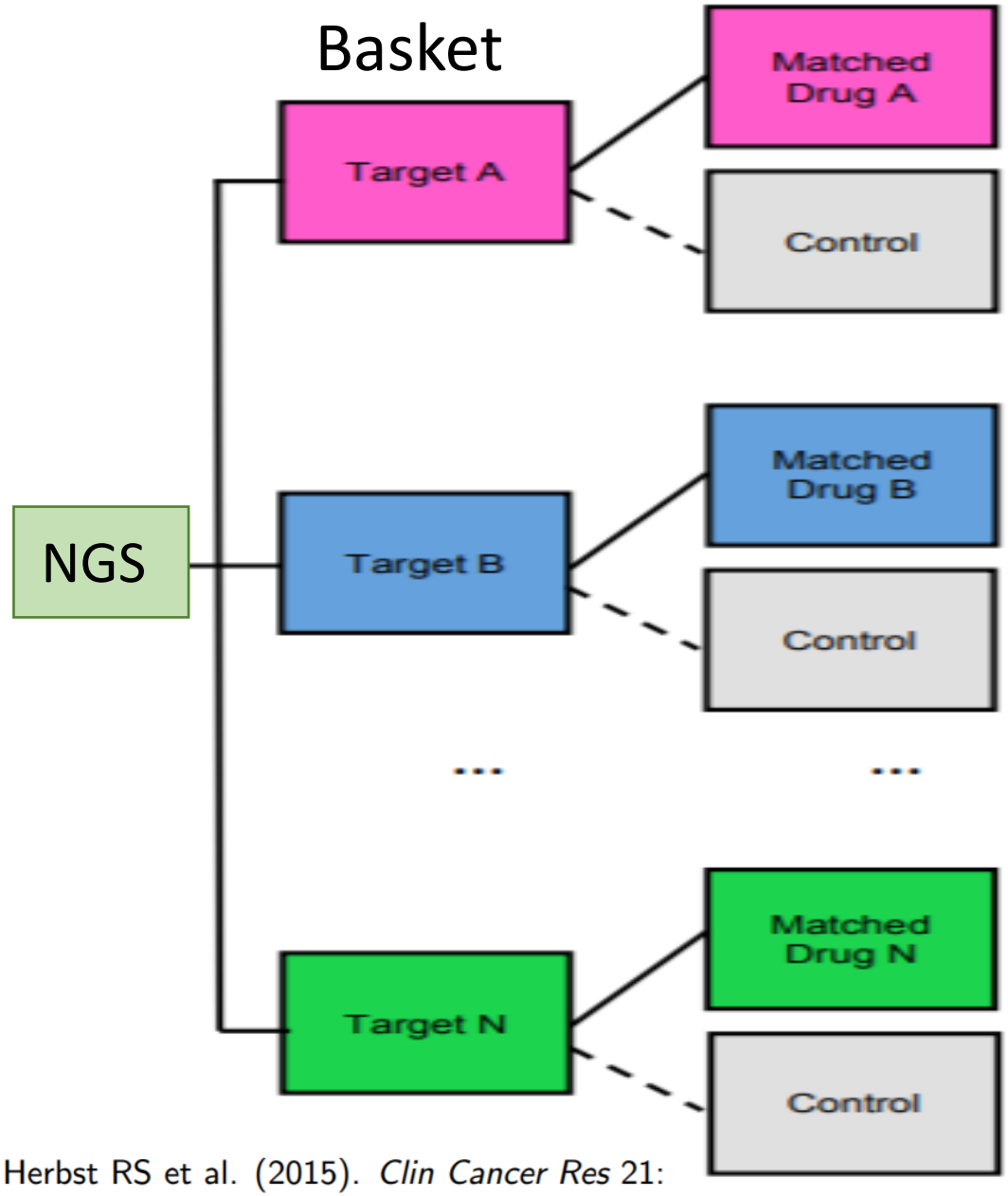
Herbst RS et al. (2015). *Clin Cancer Res* 21:

Clinical trials

Umbrella



Basket



Herbst RS et al. (2015). *Clin Cancer Res* 21:

Off-label use

NGS

↓

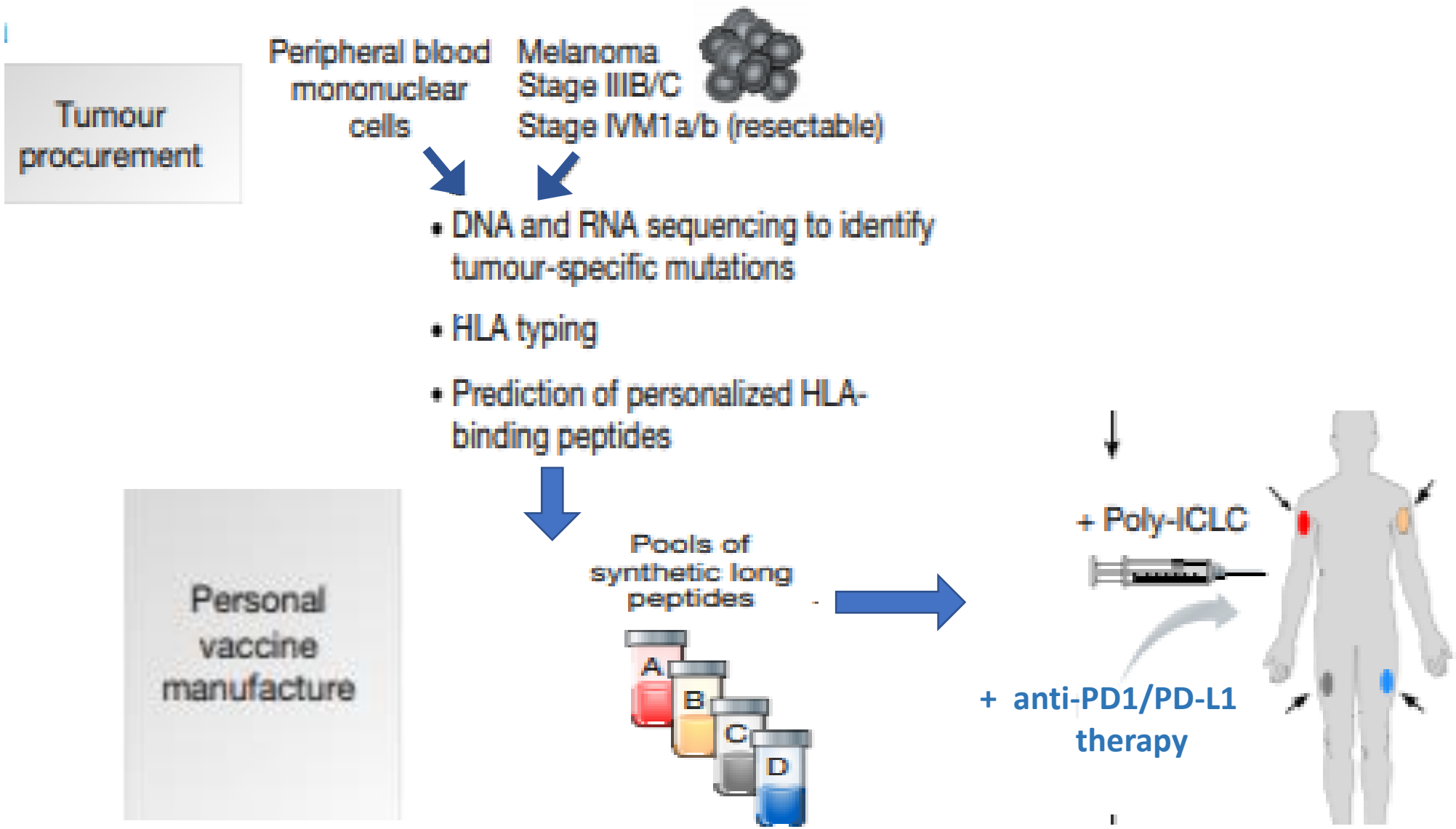
Selection of targets

↓

Treatment with FDA approved drug

Clinical applications of NGS, III

Tailored Immunotherapy

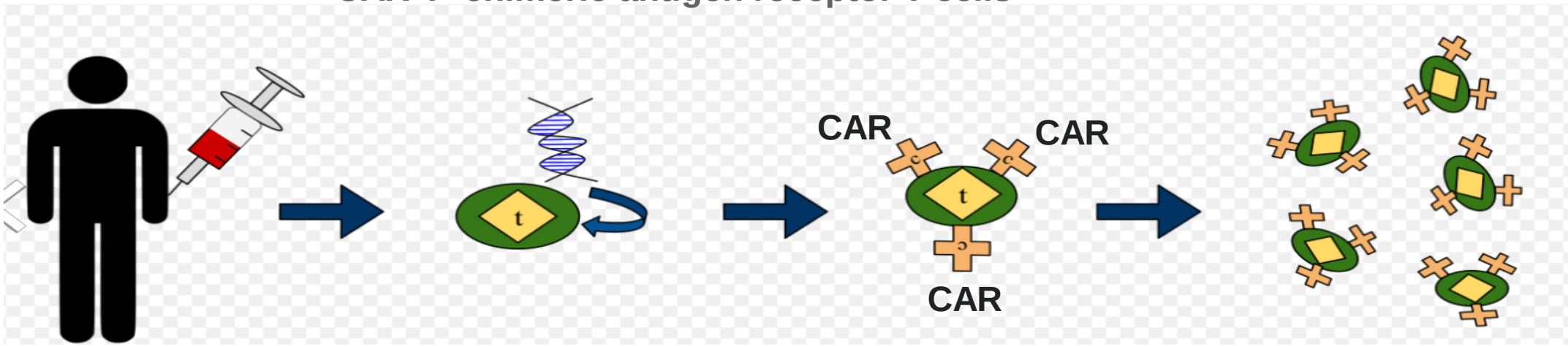


Adopted from Ott et al., Nature, 547, p 217–221, 2017

Clinical applications of NGS, III

Development of CAR-T therapies for solid tumors

CAR-T=chimeric antigen receptor T cells



T-cells removed from the patient's blood

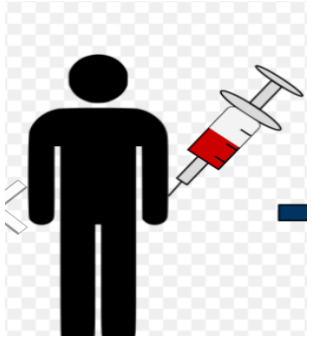
gene encoding specific antigen receptors incorporated into the T-cells

CAR receptors expressed on the surface of T cells

CAR-T cells grown

NGS to identify solid tumor antigens

Challenge



Infusion of CAR-T cells

Cancer models

Genetic mouse models

Common DNA alteration in tumor subtype

gene knockdown, mutation knock in

Treatment in vivo
Study of tumorigenesis

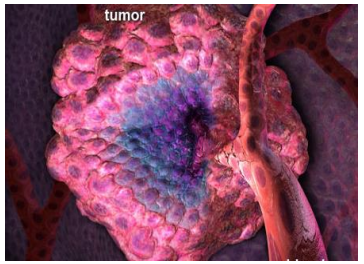
Manipulation using established cell line (e.g. gene knockdown, mutation knock in)

Treatment sensitivity to appropriate drug (known or novel)

Implantation of cells

Treatment in vivo

In vitro models for treatment sensitivity



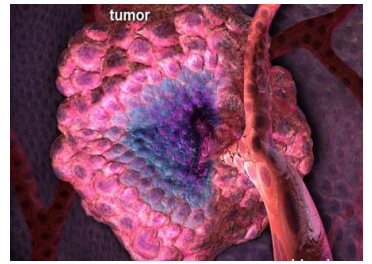
Primary tumor

Resection

Tumor maintenance on a dish

Treatment with array of drugs

Patient-derived xenografts



Primary tumor

Resection,
Genomic profiling

Implantation of primary tumor

Patient-derived xenografts

Tumor propagation

Treatment with appropriate drug

3D cell models, organoids

- Very amendable
- Small amount of material needed
- Multiple drug testing
- Time-efficient

Patient-derived xenografts (PDXs)

- Biomarker discovery
- Drug testing
- Elucidation of drug resistance mechanisms and
- Strategies to overcome it
- Toxicity can be assessed

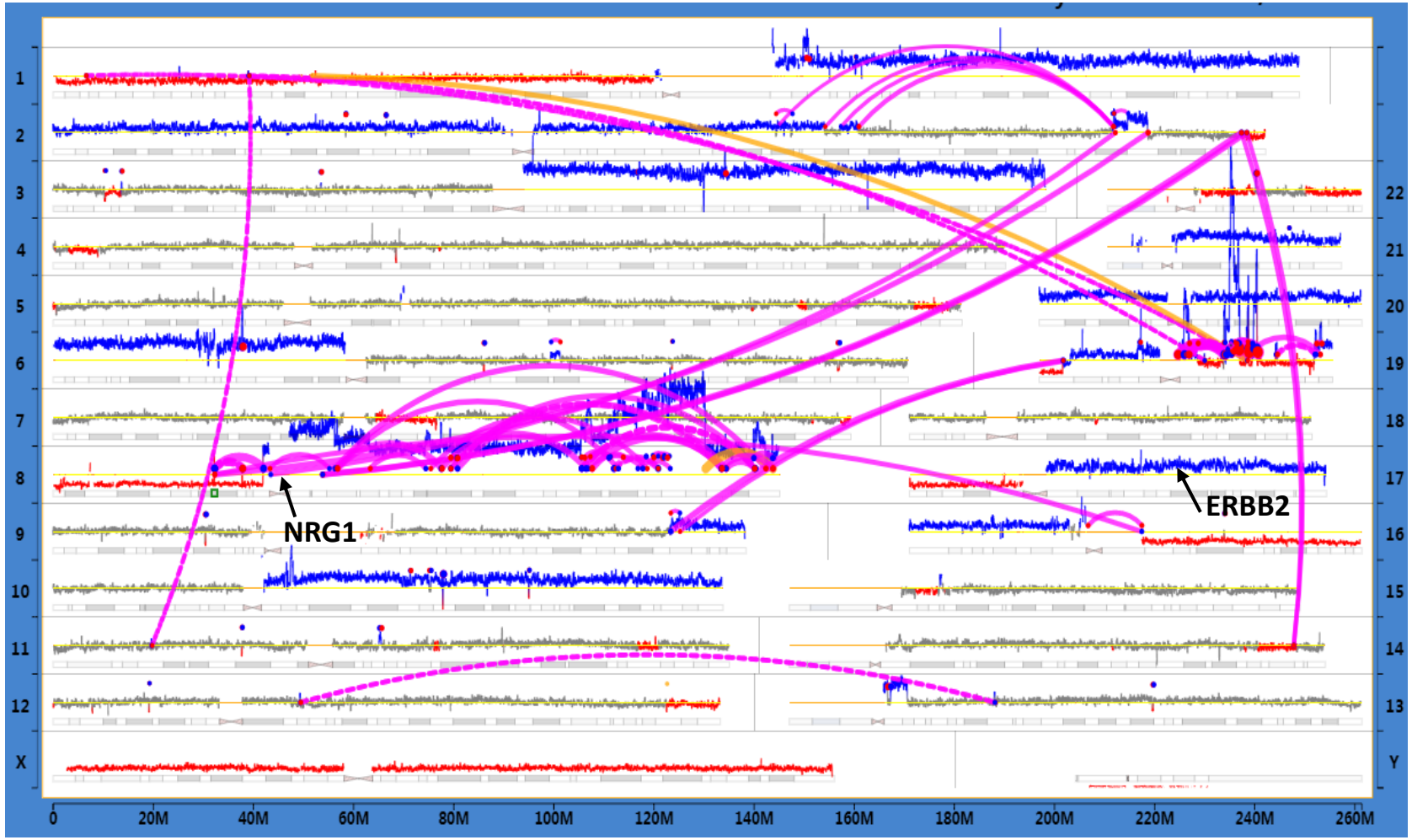
Challenges

- Does not address toxicity overall
- Does not capture tumor heterogeneity

- Time consuming
- Expensive
- Poor rates of implantation for less aggressive tumors

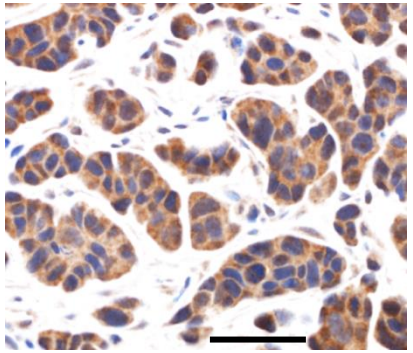
Selection of treatment target

Genome plot, ovarian tumor

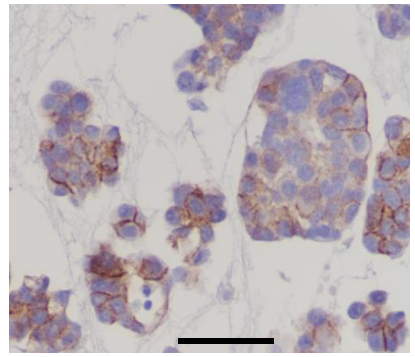


Staining of corresponding proteins for the altered genes

NRG1

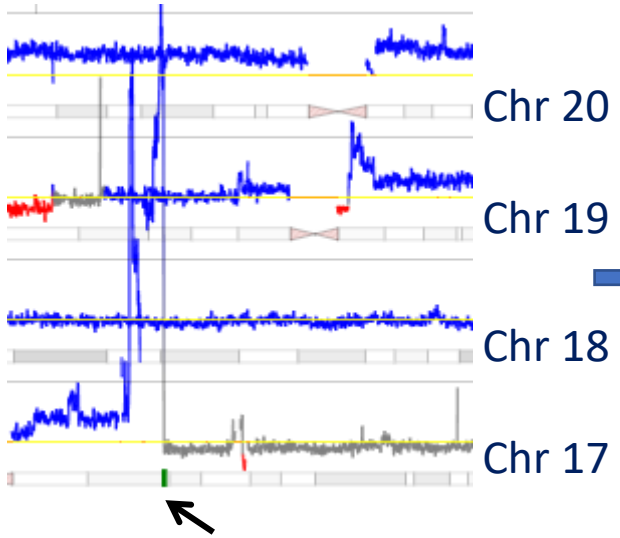


HER2



Combination of NGS and cancer models

NGS

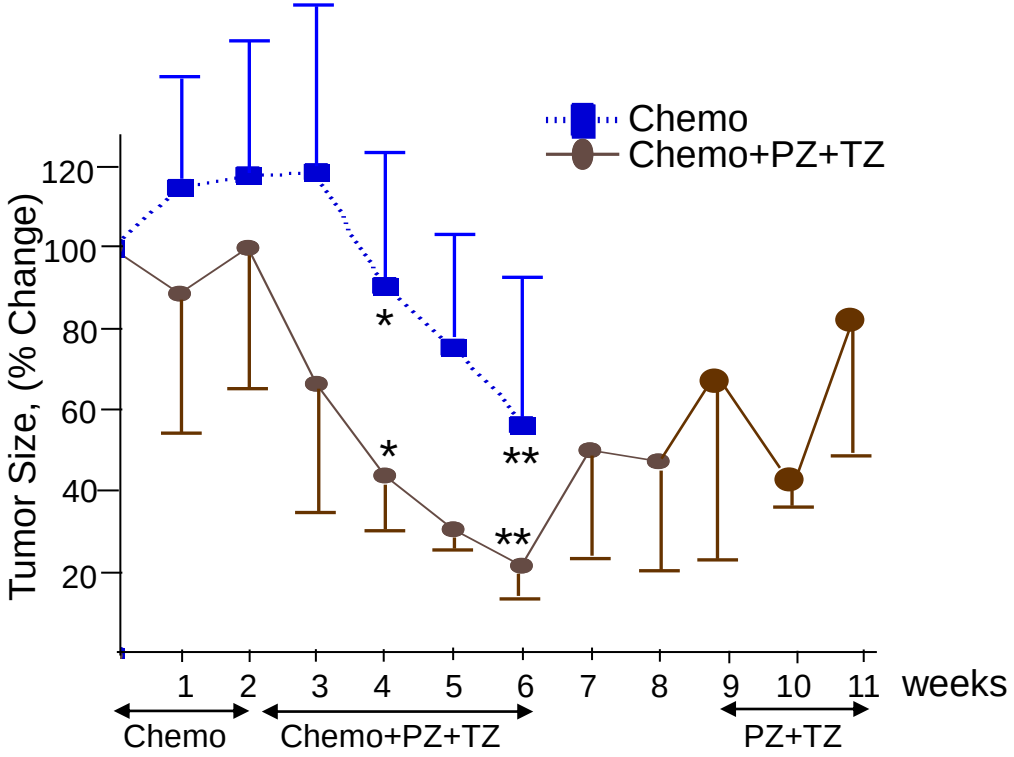


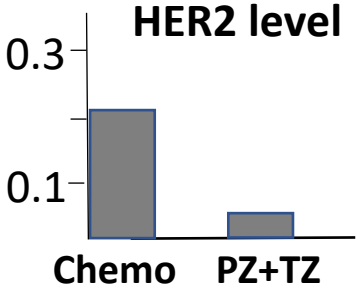
ERBB2, gene coding for HER2

PDX model

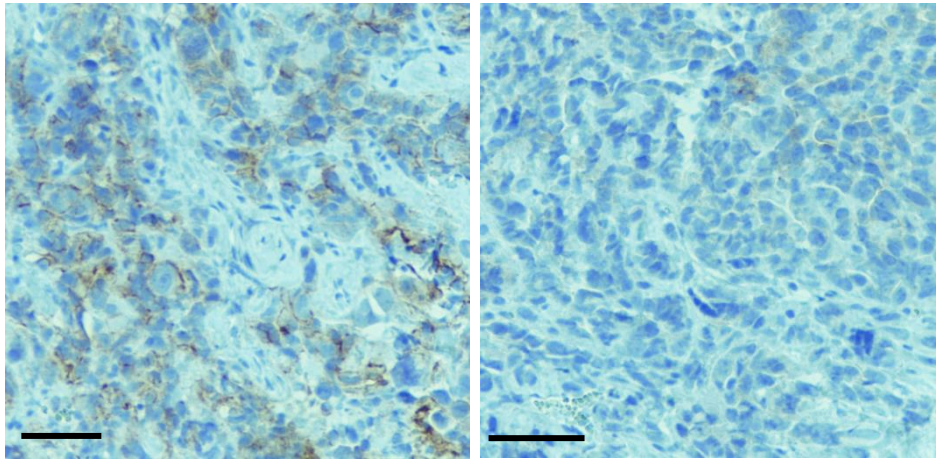
treatment

Pertuzumab and Trastuzumab (PZ+TZ) target HER2





Chemo **PZ+TZ**



Tissue analyses

Zhang P and Kovtun IV J Vis Exp. 2020 , v. 161

Conclusions

Integrative approach combining

1) NGS technologies,
2) Therapeutic target analyses and
3) Drug response monitoring using appropriate cancer model

Has become an established practice in modern oncology