MaNGO
Mario Negri Gynecologic Oncology

XVIII ASSEMBLEA MANGO

Ricerca Clinica e Traslazionale in Ginecologia Oncologica

MILANO, 2-3 LUGLIO 2021

Con il Patrocinio di:

AIOM Associazione Italiana di Oncologia Medica

FICOG | Federation of Italian Cooperative Oncology Groups

SOCIETA' ITALIANA DI CANCEROLOGIA

SIGO SOCIETA' ITALIANA DI GINECOLOGIA E OSTETRICIA

ACTO ALLEANZA CONTRO IL TUMORE OVARICO

HUMANITAS
RESEARCH HOSPITAL

Aggiornamento sui protocolli traslazionali **MaNGO** per l'analisi della "**Genomic Scar**" e della "**biopsia liquida**"

Milan, July 2 2021

A cura di:
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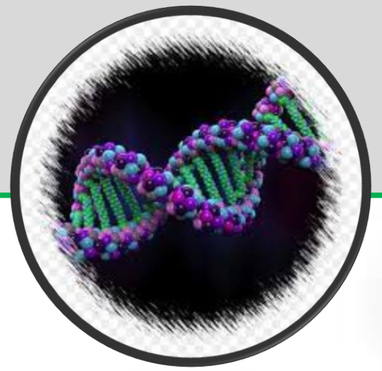
Head Molecular Pharmacology Lab/Cancer Pharmacology Group
IRCCS Humanitas Research Hospital,

Translational Projects

The new paradigms

- ✓ **There are currently two different genomics tools available within the MaNGO team:**
  - ✓ **The HRD assay**
  - ✓ **The ctDNA analysis**.

The idea is to provide to all the translational arms of the clinical trials developed by MaNGO team the optimum set of genomic workflow to achieve the aims of the studies.



Molecular Signatures of HRD

The footprint

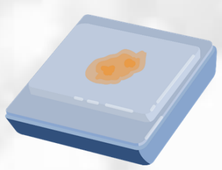
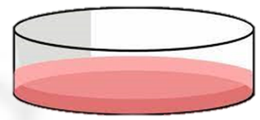
The philosophy is to select patients eligible for PARPi therapy independent of the underlying causes that impair HR activity.

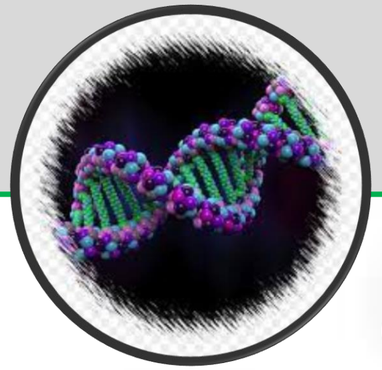
The loss of specific DDR pathways in cancer, in contrast to other cellular ‘‘hallmarks’’, generates stable—and thus more readily interpretable—‘‘footprints’’ in cancer genomes, detected as an increased mutation burden (**TMB**), altered mutational signatures (**S3, SR3, SR5**) or structural changes in the genome architecture (*e.g.*, **LOH/TAI/LST/MMEJ/TD**)

The HRD assays

An overview

Analysis of
altered HR activity

	CAUSES	EFFECTS	FUNCTIONS
Specimens	 *Single gene*	 *Genes panel*	 *Mutational Signatures*  *Structural alterations*	
Variables	*gBRCA1* *gBRCA2*	*tBRCA1* *tBRCA2* *PALB2* *RAD51D* *RAD51C*	*S3* *SR3+SR5*	*LOH* *TAI* *LST* *MMEJ* *TD*	*RAD51 foci* *mRNA analysis*



The Genomic Scar

GENOMIC SCARS as reporters of **HR** deficiency and drug response

Defects in the mechanisms of HR causes a stable alteration in the DNA structure currently known as "**GENOMIC SCAR**".

The use of the **GENOMIC SCAR** arises from the need to identify genomic aberrations with a known origin- defects in the DNA repair pathway- independently of the causes.

The major challenge in the identification of **GENOMIC SCARS** has been to distinguish HR defect (**HRD)-related genomic aberrations** from the **wide-ranging complexity** inherent to cancer genomes.

This can prevents **the accurate** measurement of HRD-related scars

The Genomic Scar

In the absence of HR, the DNA is characterized by specific structural changes due to the fact that other "error prone" DNA repair systems are active.

- Extensive Loss of Heterozigosity (**LOH**)
- Large Scale Transitions (**LSTs**) increase
- Telomeric Allelic Imbalance (**TAI**)

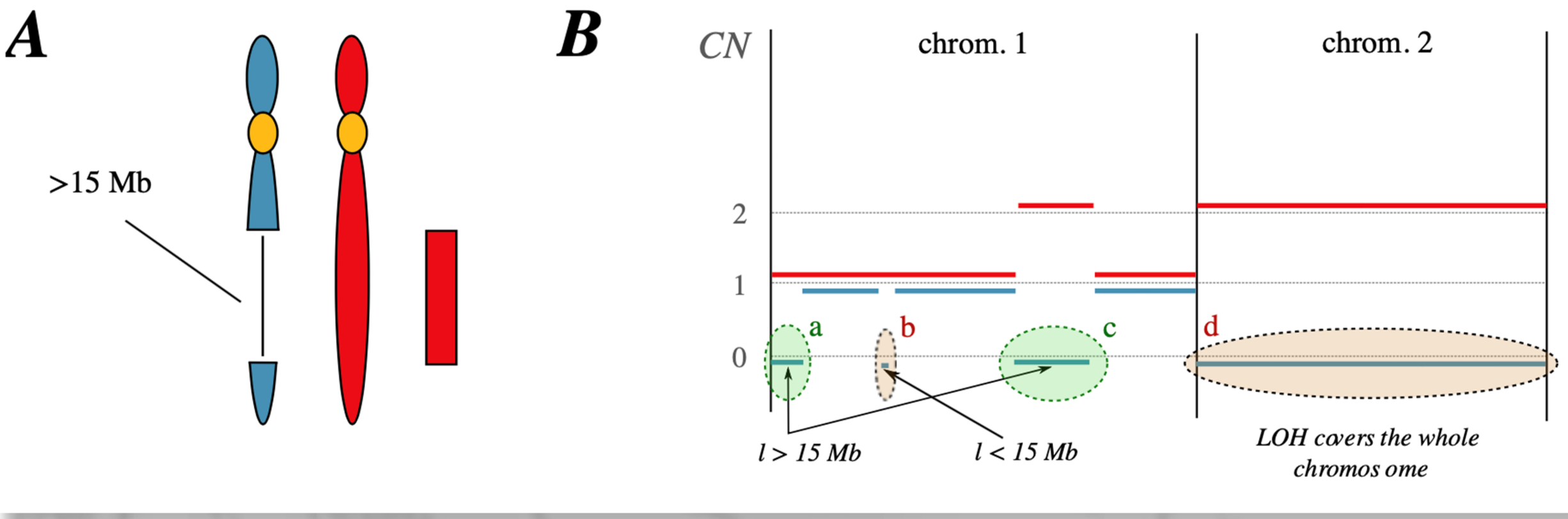
HRD-score

- Mutational Signature 3
- Rearrangements Signatures (Tandem duplication <10kb; Deletions <100kb)
- Microhomology mediated small InDels (<25bp)

HRDetect

Knijnenburg et al., 2018 CellReports 23,239-254

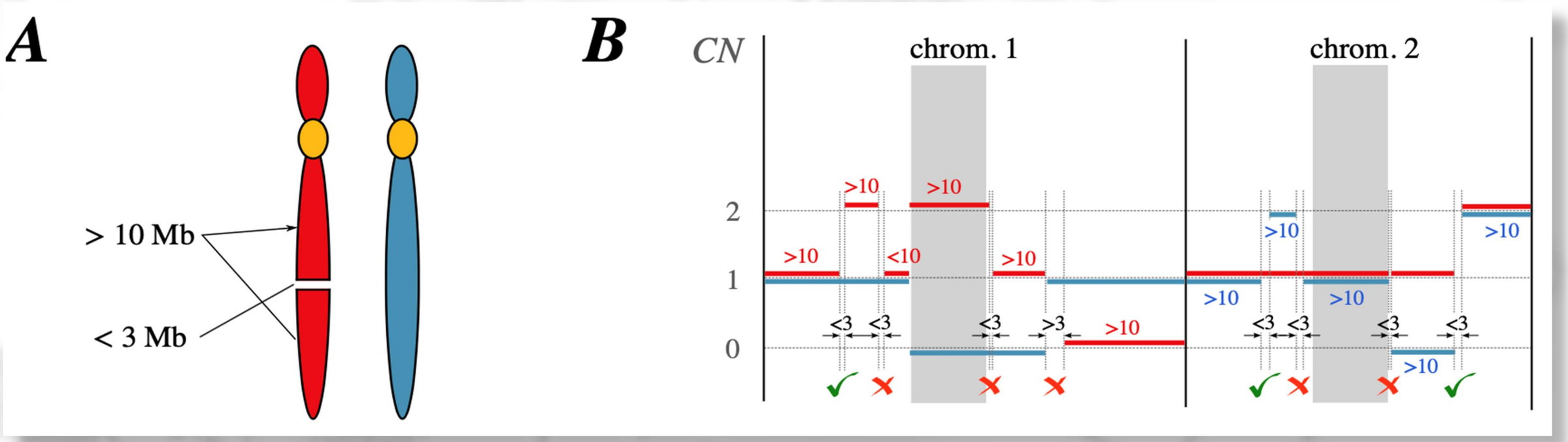
Loss Of Heterozygosity



https://github.com/sztup/scarHRD

The number of 15 Mb exceeding LOH regions which do not cover the whole chromosome.

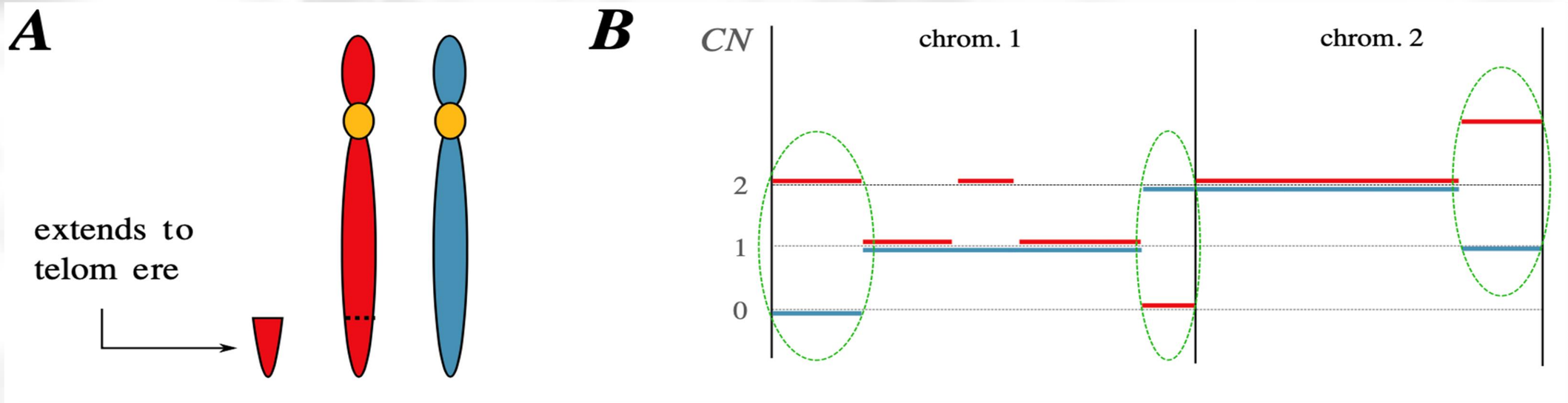
Large Scale Transition



https://github.com/sztup/scarHRD

LST is a chromosomal break between adjacent regions of at least 10 Mb, with a distance between them not larger than 3Mb.

Telomeric Allelic Imbalance

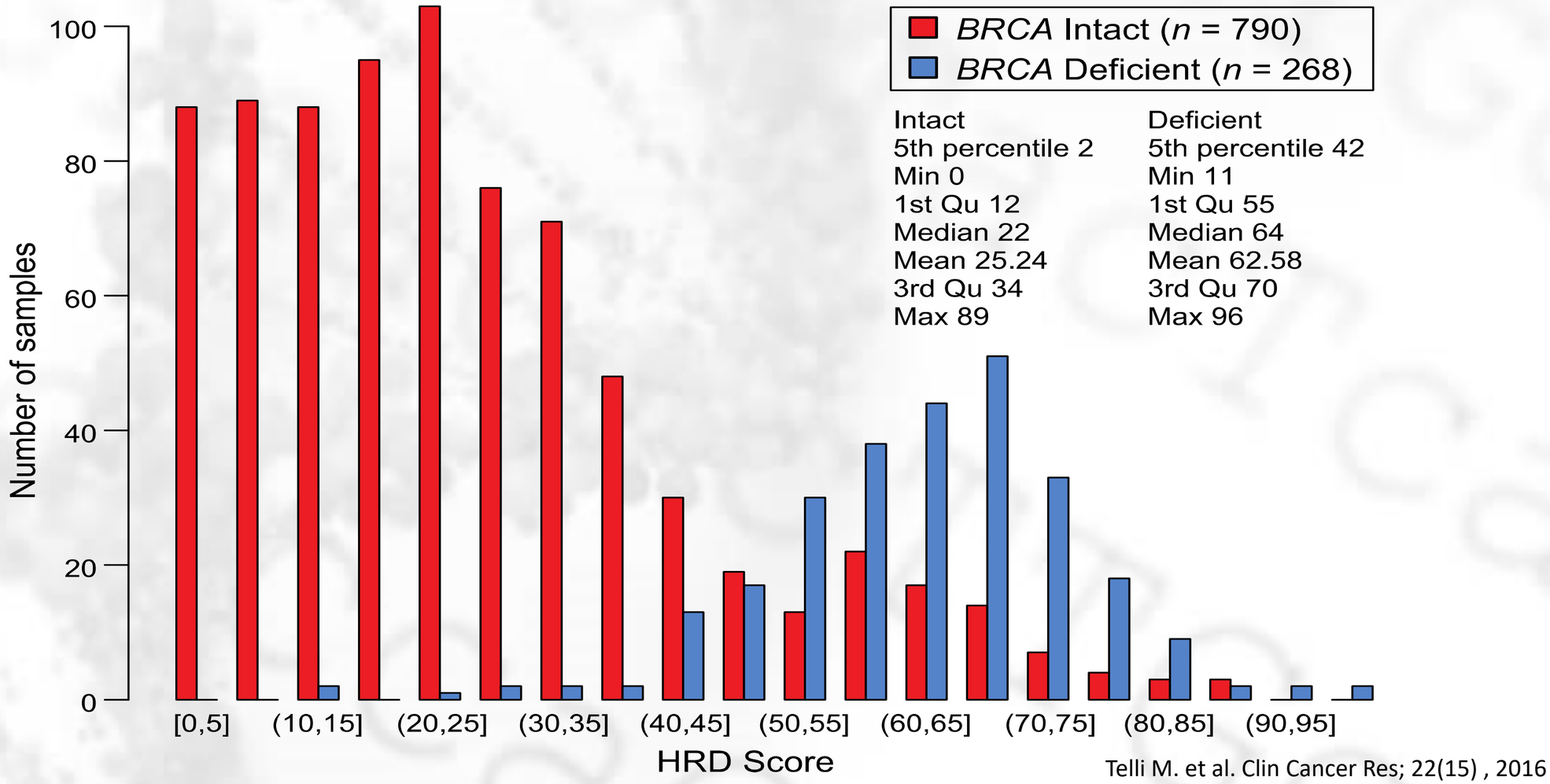


https://github.com/sztup/scarHRD

The number Allelic Imbalances of at least 1Mb that extend to the telomeric end of a chromosome.

The HRD

The metric



Telli M. et al. Clin Cancer Res; 22(15) , 2016

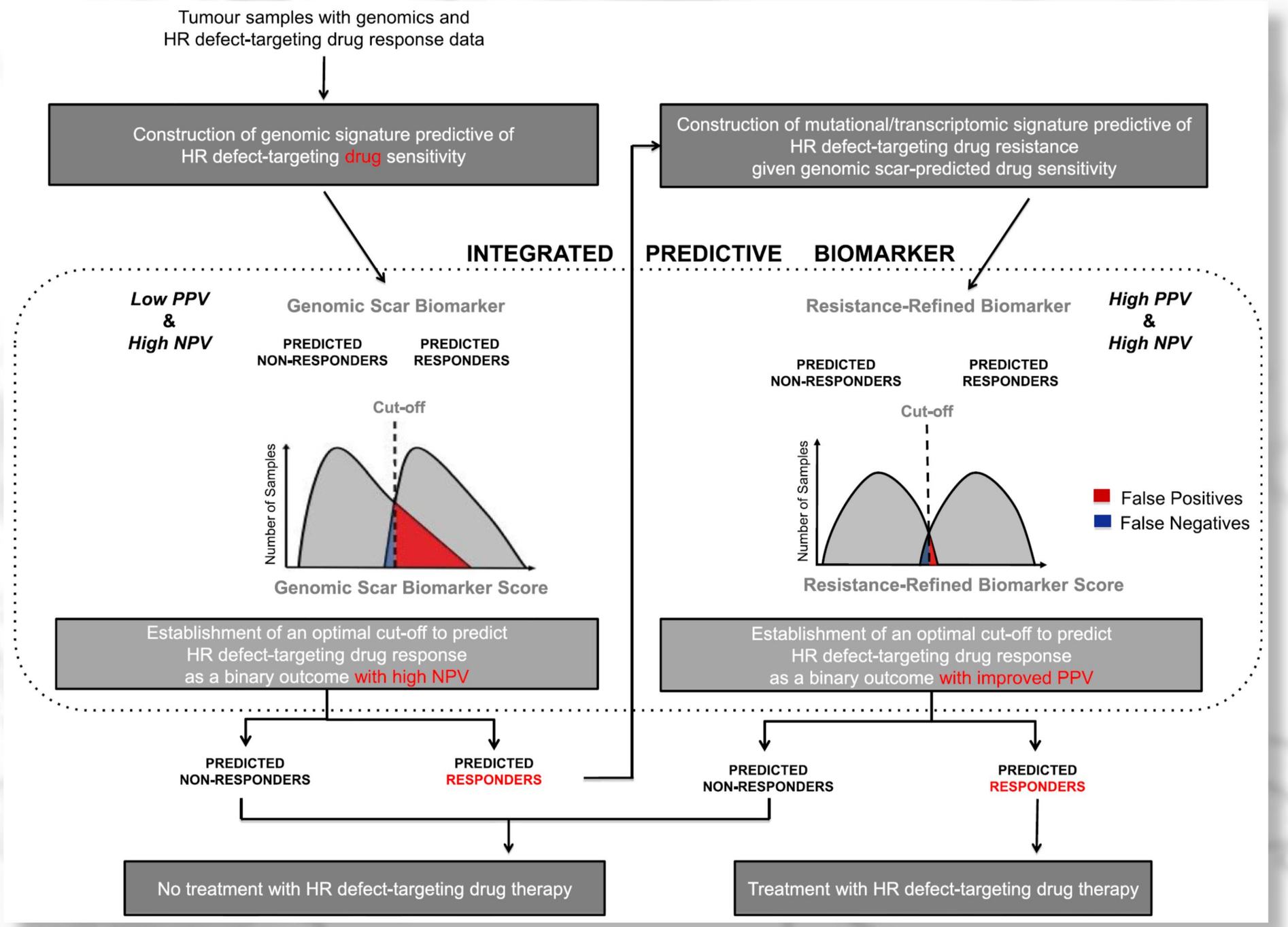
HRD is calculated as the sum of three metrics (**LOH + LST + TAI**) and the cut off is settled **to 42**, which represents the **5° percentile with 95% of sensitivity**:

>42 HR DEFICIENT SAMPLE = eligible for PARPi

<42 HR PROFICIENT SAMPLE-= not eligible for PARPi

The HRD

The limit



Watkins et al., Breast Cancer Res. 2014; 16:211

The HRD

The weakness

EFFECTS

- ✓ By chronicling the past but not documenting the present, genomic scar measures report whether or not a defect in HR has been operative at some point in tumorigenesis and not whether it remains operative at the point of treatment. **Tumor cells restore HR functions but retain the "Genomic Scar" value indicatives of an HRD**
- ✓ A variety of mechanisms could restore HR or compensate for its loss in the aftermath of genomic scarring. Loss of ***53BP1*** and **reversion mutations to *BRCA1* and *BRCA2*** have both been demonstrated to confer resistance to platinum agents and **PARPi** through the restoration of HR.
- ✓ Other pharmacological mechanisms such as over expression of **MDR1 protein** or **desmoplastic reactions** that limit drug access, can confer a resistance mechanism not intercepted by HRD value.

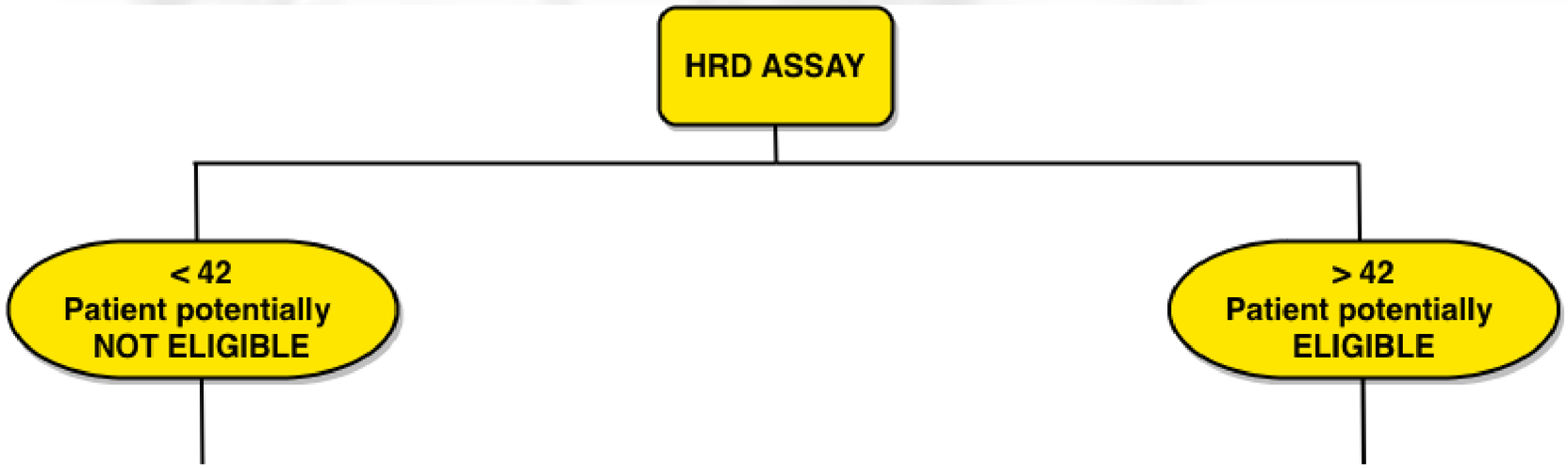
The HRD

- ✓ MaNGO group developed an in house NGS-based HRD assay for its own translational studies.
- ✓ Initially developed on the commercially available OneSeq constitutional panel (CCP 17), the design was then modified.
- ✓ It works with both snap frozen and FFPE tumor biopsies with low tumor purity (< 30%).
- ✓ Bioinformatic pipeline was developed on the metrics described in Telli et al. (LOH+TAI+LST) plus SNV across a list of almost 300 genes.

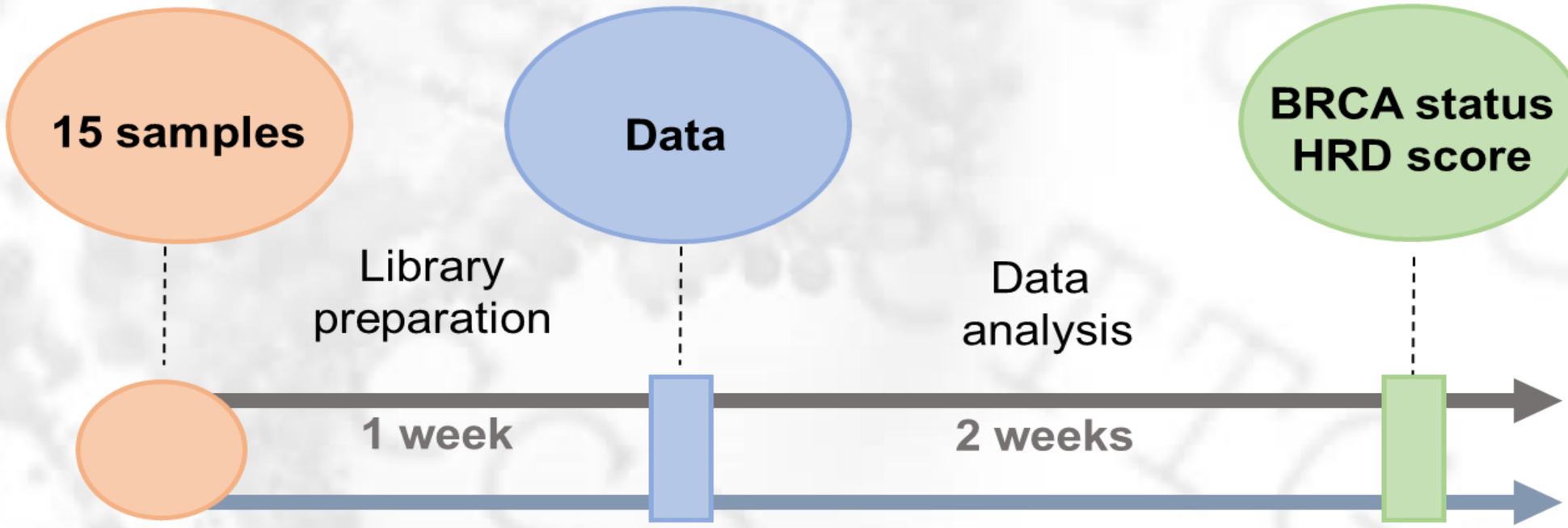
The main aims of the assay are to:

1. reduce **FP cases** by intercepting patients with primary resistance to PARPi, due to somatic mutations in a selection of genes known to drive PARPi resistance.
2. Identify driving mutations with potential therapeutic relevance, in order to suggest additional therapeutic approach for PARPi resistant cases.

The Workflow



The workflow



3 weeks to have complete results

The assay validation

- ✓ Our team has been included within seven different countries in the development process of an «**European academic HRD test**», under the supervision of the ENGOT group.
- ✓ Experiments have been performed on a blind selection of **PAOLA-1** clinical trial samples (85 FFPE samples) .
- ✓ Data were compared by ENGOT group to published "MyChoice Miriad" results.
  Results demonstrated that our assay has:
  I. Sensitivity: **83%**
  II. Specificity: **94%**
  III. F-score: **89%**
  IV. Kappa score: **0.78** (good concordance)

- ✓ These results made our test suitable for the clinical validation (Phase 3 study) on the entire cohort of FFPE samples recruited within the PAOLA -1 study (almost 350 samples).

Watkins et al., Breast Cancer Res. 2014; 16:211

Study Work-Flow

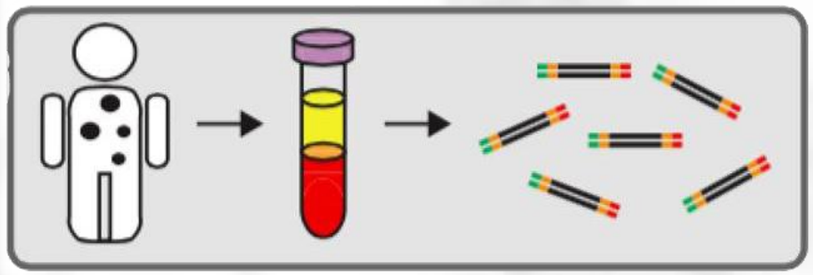
Technical view

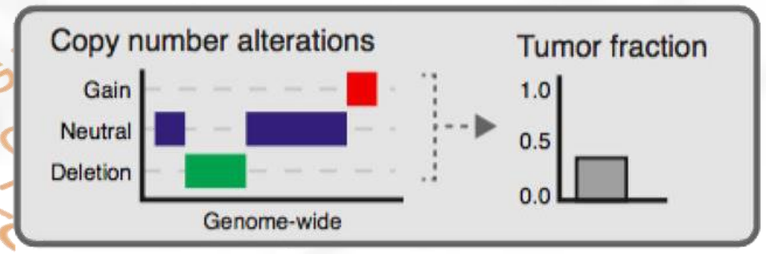
Amplifying the Voice of the Whispering Cancer

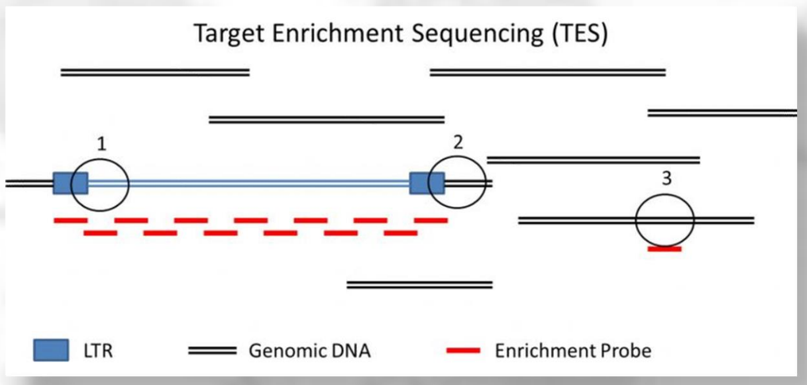
Library Prep
(KAPA HyperPlus Library Prep KIT)

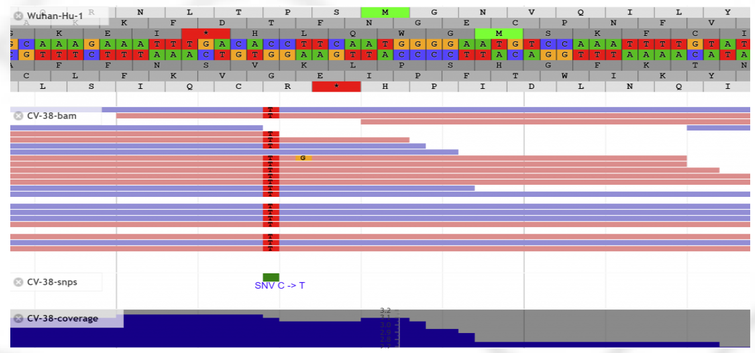
sWGS
(**0,2x coverage**)

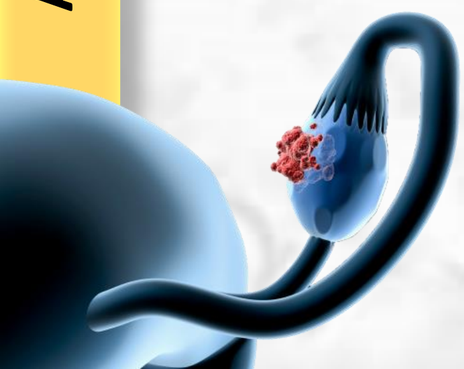
SCNA Analysis











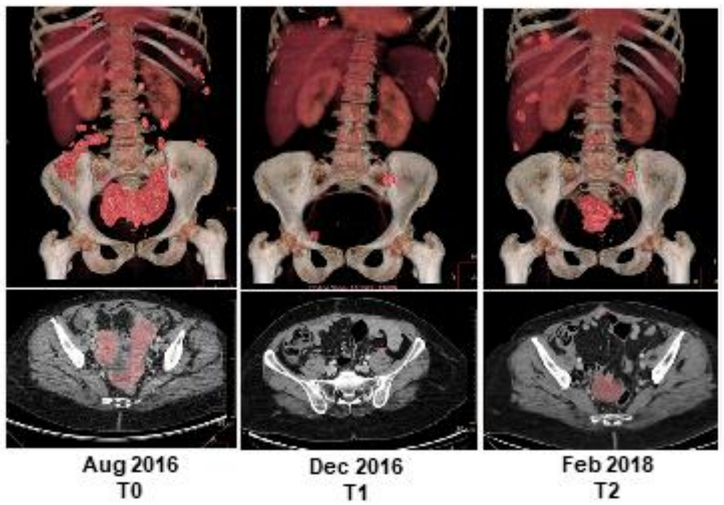
Target enrichment 65 genes
(SeqCAP EZ Prime Choice probes)

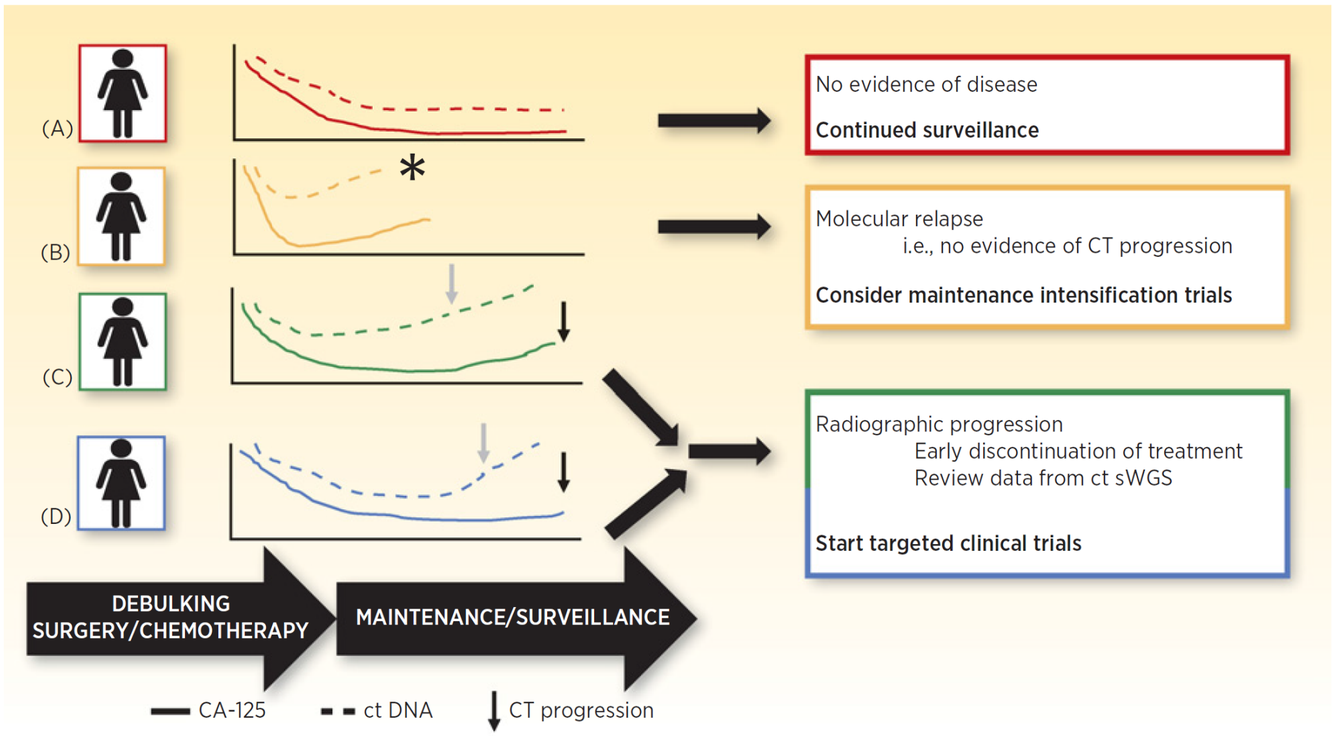
Targeted Sequencing
(**3000x coverage**)

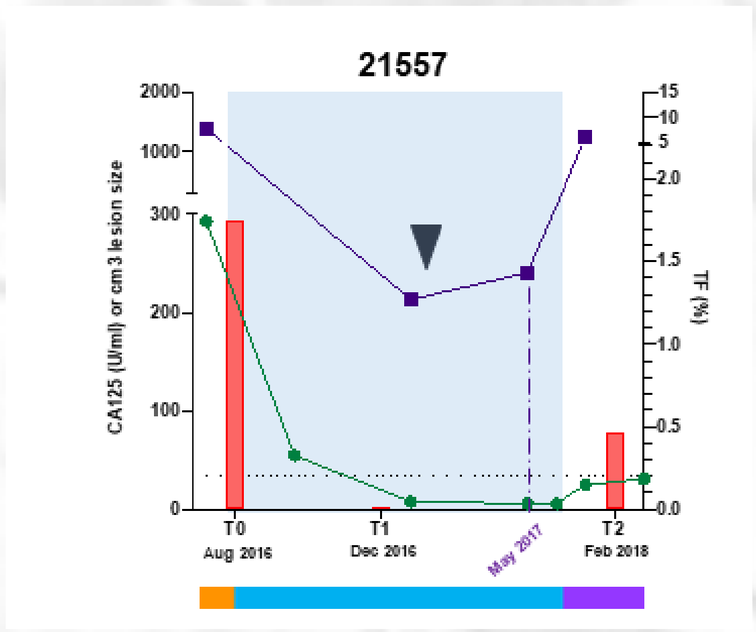
SNV Analysis

The Tumor Fraction

The results







Dhani NC et al., Comment on CCR 2021

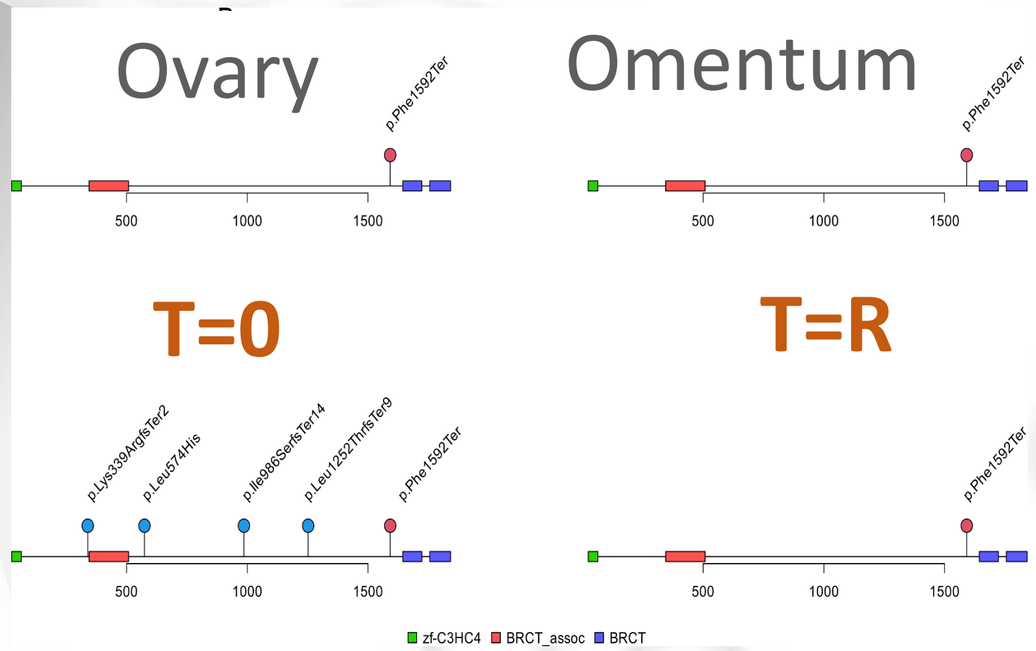
As **TF o**utperformed CA-125 in anticipating clinical and radiological progression by **240 days , we have time enough to get information on the biology of relapsed disease**

The SNV Landscape

Targeted resequencing

BRCA1
21543

Solid biopsy



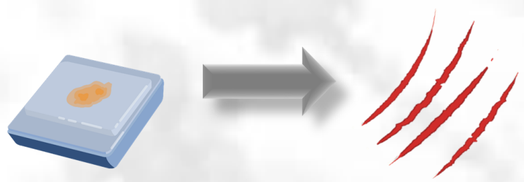
Liquid biopsy

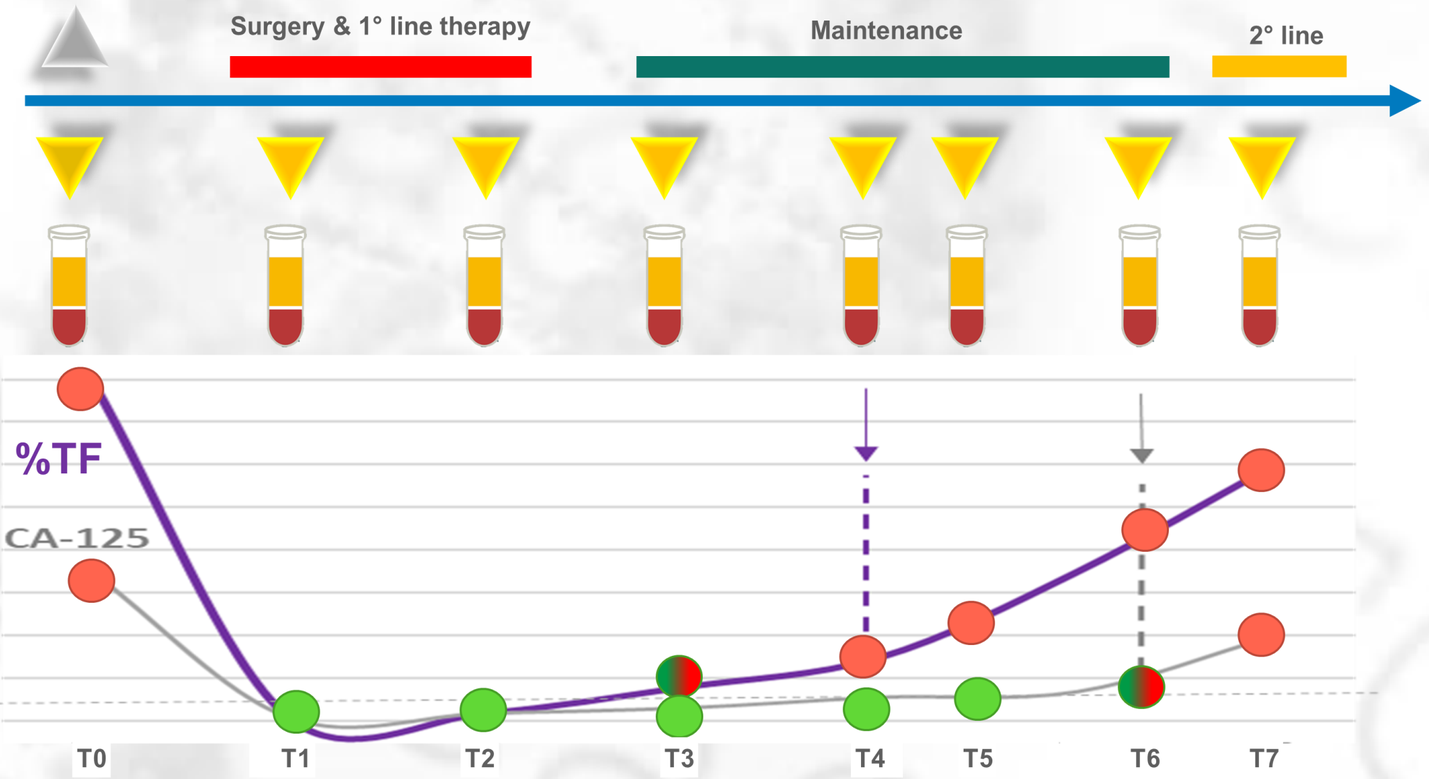
Take Home Message

HRD Analysis

sWGS

Targeted Seq.





Solid biopsy

Select patients eligible for PARPi

Liquid biopsy

Predict the timing of biological relapse

Infer the biology of relapsed disease

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Marta Jaconi
Maria C Paderno
Marco Adorni

Thanks!

To go fast go alone, to go far go together

Padova University Computational Biology Group

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Angelo Velle
Enrica Calura

Harvard Medical School & MGH Cancer Center

Giulia Siravegna

CLOUD 4 CARE

Alessandra Bono FONDAZIONE ONLUS

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AIRC

Thank you for your attention