# Analytical validation of HRD calculation performed with the OncoDEEP® Kit Comprehensive Genomic Panel

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**Introduction :**

- Homologous Recombination Repair (HRR) pathway restores DNA double-strand breaks and deficiency in this pathway is called **Homologous recombination deficiency** (HRD) which leads to genomic instability
- HRD biomarker can **predict response** to specific therapies, such as PARP inhibitors, and helps clinician to determine **the best treatment option** for the patients
- OncoDEEP® kit is a Comprehensive Genomic Panel allowing the analysis of both **DNA and RNA** combined with **Bio-IT analysis** and **clinical reporting**.
- Complete tumor characterization **through 638 genes, genomic signatures and fusions (Figure 1)** that helps **reducing the costs** of testing and deliver **faster results** in the selection of appropriate cancer treatment options.

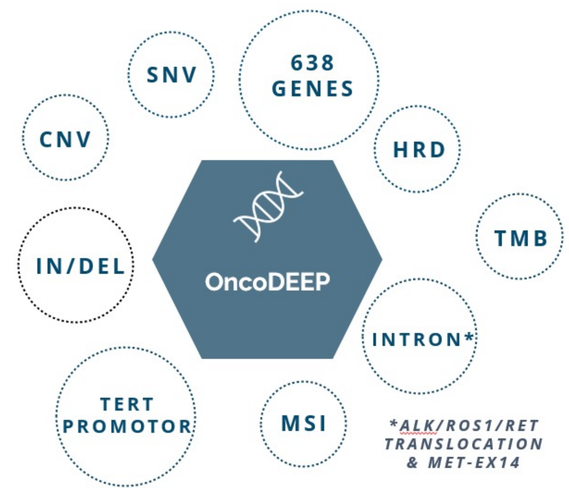



**Figure 1**: Alterations and biomarkers analzed by the OncoDEEP panel at the DNA level

**Material and Methods:**

- 76 ovarian carcinoma FFPE samples were selected from 4 Spanish Hospitals according to study criteria (**Figure 2**) and OncoDEEP kit was performed in the laboratory facilities of those 4 centers.

| Criteria | Value |
|---|---|
| % of tumoral cells | >20% |
| DV900 | ≥50% |
| DNA input | 30-100 ng |
| Characterization | Myriad MyChoice DX |

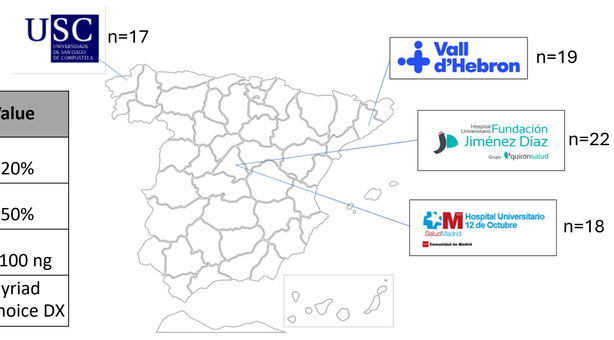



**Figure 2**: Selection criteria of 76 samples for the HRD OncoDEEP analytical validation and distribution of those samples in the 4 spanish centers

- The OncoDEEP kit allowed the full characterization, from the DNA extraction to the final report, of the samples in less than 5 working days (**Figure 3**). After extraction, libraries were constructed (**3h**), enriched (**hands on time: 4h**) based on Twist Biosciences Technology and sequenced (**20h**) on an Illumina NextSeq 500 or NextSeq 2000, depending on the center. Finally, FastQ files were uploaded and analyzed through OncoDNA dedicated BioIT pipeline.

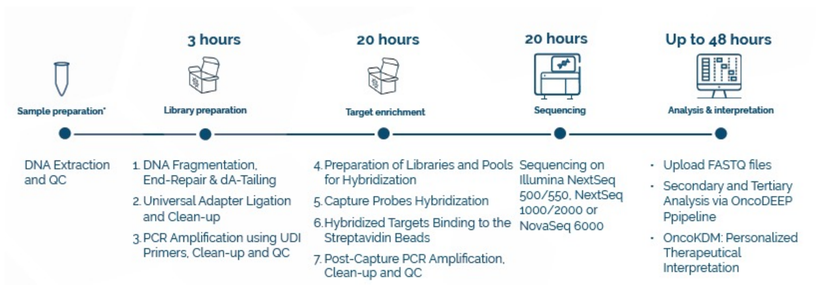



**Figure 3**: Full OncoDEEP workflow from the wetlab part (DNA extraction to sequencing) to FASTQ files upload and secondary/tertiary analysis.

- HRD results were obtained combining the BRCA1/2 status and the Genomic Scar (GS) (**Figure 4**). The three biomarkers Loss Of Heterozygosity (LOH), Allelic Disparity on Telomere (ADT) and Large-scale Rearrangements (LR) were used for GS calculation. Then, those results were compared with the one obtained with Myriad MyChoice DX. An overall concordance **>80%** and BRCA1/2 status concordance **>90%** were to be reached.


| Results | Characterization |
|---|---|
| HRD positive | GS≥40 and/or BRCA1/2 mutated |
| HRD negative | GS<40 and BRCA1/2 wild-type |
| HRD inconclusive | GS<40 and conflicting BRCA1/2 |
| HRD failed | Coverage<150x and/or Uniformity<85% |

**Figure 4**: HRD determination by OncoDEEP combining both BRCA1/2 status and GS and results criteria.

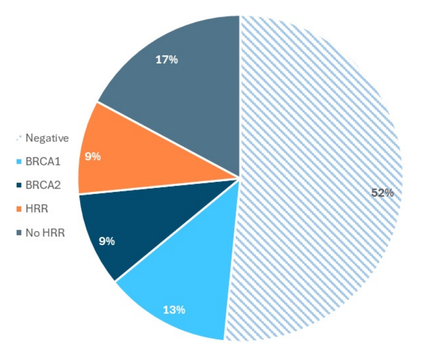
**Conclusion**: OncoDEEP® kit is an end-to-end solution allowing the detection of variants in 638 genes and the calculation of genomic signatures such as Tumor Mutational Burden (TMB), Microsatellite Instability (MSI) and HRD. OncoDEEP® HRD analysis showed an overall concordance of **88.8%** with a sensitivity and specificity of **90%** and **88%**, respectively, compared to the gold standard and is therefore applicable to the identification of ovarian cancer patients eligible for PARPi therapy.

**Results**

- From the 76 samples (**Table 1**), 5 were rejected due to low coverage and/or uniformity. This led to a failure rate of **6%**. In comparison, 8 samples failed with Myriad MyChoice DX, representing a failure rate of 11%. Eliminating those failed samples, 63 left for the comparison.

**Table 1**: Distribution per center of samples and results used for the comparison study.

| Center | Sequenced samples | Published samples (good QC) | Not Valuable from Myriad | Samples usable for the correlation analysis | Concordant HRD | Conflicting HRD |
|---|---|---|---|---|---|---|
| Hospital U. 12 de Octubre | 18 | 18 | 3 | 15 | 14 | 1 |
| Hospital Vall d'Hebron | 19 | 17 | 2 | 15 | 15 | 0 |
| Fundación Jimenez Díaz | 22 | 19 | 2 | 17 | 14 | 3 |
| Hospital Clínico U. Santiago | 17 | 17 | 1 | 16 | 13 | 3 |
| TOTAL | 76 | 71 | 8 | 63 | 56 | 7 |




- Of the 63 samples (**Figure 5**), 31 (**48.4%**) were **HRD positive** and 32 (**51.6%**) were **HRD negative**. Of the 31 HRD positives, 8 had alteration in BRCA1 (**13%**), 6 in BRCA2 (**9%**), 6 in other HRR genes (**9%**) and **17%** without HRR variant, which is in line with literature. Overall, the most mutated gene was TP53, followed by HRR genes (BRCA1, BRCA2, CCNE1,...).

**Figure 5**: Distribution of negative and positive (BRCA1, BRCA2 or HRR altered or HRR wild-type) HRD samples.

- 56 out of the 63 samples showed concordant results (**88,8%**) (**Figure 6**). The remaining 7 samples with conflicting results; 4 positives and 3 negatives and 5 fell in the grey zone (40+-5). All 14 BRCA1/2 alterations were detected (**100%**). Both expected **concordance scores** were passed and the HRD analysis showed a sensitivity of **90%** and a specificity of **88%.**

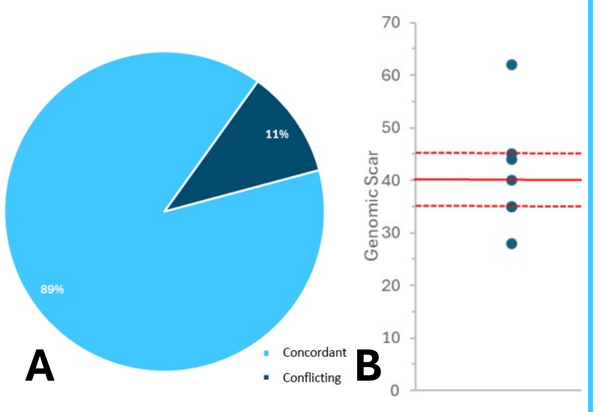
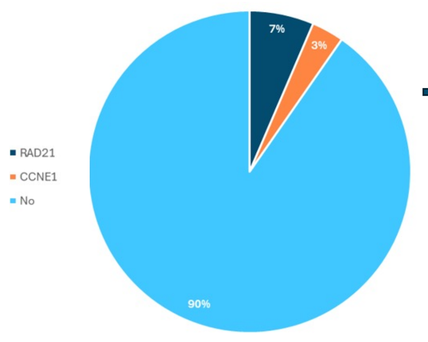



**Figure 6**: Distribution of samples showing concordant or conflicting HRD results compared to Myriad MyChoice DX (A) and distribution of the conflicting GS (B).




- OncoDEEP panel is **more than just a HRD test** (**Figure 7**). 3 HRD positive samples showed alterations possibly impacting PARP inhibitors response and allowing the identification of patients who **need closer monitoring**.

**Figure 7**: Additional information given by OncoDEEP in HRD positive patients highlighting alterations linked to shorter respons to PARP inhibitos.

- Therapeutical options in 27/32 HRD **negative** patients (**85%**)(**Figure 8**): 2 patients elligible for **immunotherapy** and 25 patients harboring **alterations matching to recruiting clinical trials**

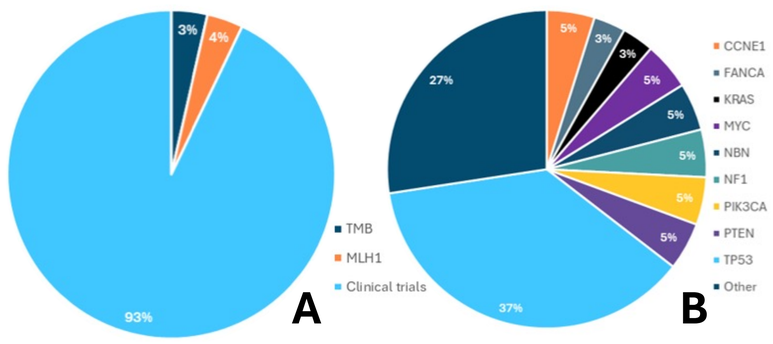



**Figure 8**: Therapeutical alternatives given by OncoDEEP in HRD negative patients showing patients elligible for immunotherapy (A) or directly enrollable to clinical trilas through detected alterations (B).