Validation of MODEPLEX technology for the determination of POLE hotspot mutations in endometrial carcinoma samples.

Eduard Dorca [1], Ana Velasco [2,3] , Dolors Cuevas [3], Marta Vaquero [3], Sonia Gatius [2,3], Zaida Ramos[1] , Esther Guerra [1] , August Vidal [1], Xavier Matias-Guiu [1,2,3].
Pathology Department, Bellvitge Universitary Hospital [1]. Pathology Department, Arnau de Vilanova Universitary Hospital [2]. IRB Lleida (Biomedical Research Institute Lleida) [3].

INTRODUCTION:

Molecular classification of endometrial cancer based on TCGA data recognizes 4 subtypes with histological, prognostic and therapeutic implications: 1) Copy Number High or “Serous Like” (aneuploidy and mutations in TP53), 2) Hypermutated (harboring Microsatellite instability), 3) Ultramutated (with mutations in POLE gene) and 4) Copy Number Low (with an unspecific molecular profile).

To extrapolate this classification into clinical practice, some authors have proposed a simplified scheme using three immunohistochemical markers (p53, MSH6, and PMS2) and a molecular test (POLE mutation analysis).

The determination of POLE mutations can be carried out through massive sequencing techniques or using Single-Gene strategies with traditional methods such as Sanger sequencing.

The lack of a cost-effective Gold-Standard has prompted the development of new techniques so that this determination can be carried out routinely in most centers.

ABOUT MODAPLEX (BIOTYPE®):

MODAPLEX is a novel technology that combines multiplex PCR amplification and Capillary Electrophoresis with subsequent fragment analysis, allowing to identify the most frequent mutations in POLE and POLD1 genes in a fast (3.5 hours), economic and user friendly way, available for fresh and FFPE tissues .

POLE	V411L(G>C)
T278M	V411L(G>T)
P286H	H422N
P286R	L424V
P286L	P436R
P286S	M444K
S297A	A456P
S297F	S459F
F367S	A465V

Table 1: POLE Hotspot mutations covered by MODAPLEX

MATERIALS AND METHODS:

258 endometrial cancers samples with available histological and molecular classification, were selected (**Figure 2**). For each case a tumoral block was chosen, obtaining eight sections of 10 µm thickness and subsequently isolating DNA with “Cobas DNA Sample Preparation Kit”. DNA concentration was measured by Qubit, excluding those cases with a concentration under 10 ng / µl. Positive results were reconfirmed using Sanger sequencing. The design of this study encompassed 4 different steps: 1) a retrospective pilot phase, with 80 tumors, balancing the four molecular subgroups.

2) A retrospective phase of 25 tumors obtained between 2016 and 2020; and 30 tumors obtained between 2000 and 2015, to check test performance in cases with different storage time. 3) A selected set of 29 cases of groups 1 and 2, was analyzed in a different center to check inter-laboratory consistency of the test. 4) A prospective cohort 3 of 123 tumors, of unknown POLE status, with simultaneous validation by Sanger sequencing.

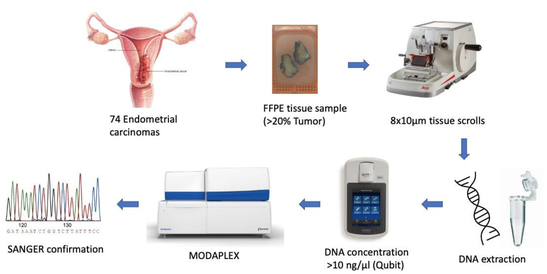

Figure 2: Study workflow

RESULTS:

A total of 258 samples were finally submitted to the test, 2 of them were discarded for obtaining an invalid result.

A total of 258 samples were analyzed; two of them discarded for showing an invalid result. In the first phase, the test showed positive and negative predictive values of 100% and 100%, by identifying correctly POLE mutation status in 79 out of 79 cases, after excluding a case of Lynch syndrome with two non-pathogenic POLE mutations.

In phase 2, the test correctly detected 12 positive and 43 negative cases, independently of storage time. Phase 3 showed 100% of inter-laboratory consistency.

Finally, Phase 4 showed 16 positive samples out of the 123 prospective cases. Overall, the test have revealed sensitivity and specificity values of 100% and 100% respectively, with a total of 47 POLE mutated tumors (20 V411L, 20 P286R, 3 S297F, 3 A456P and 1 L424V).

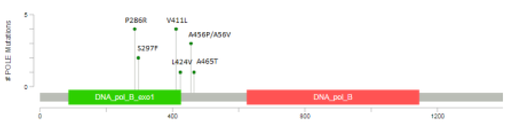

Figure 3: Mutational map of the reported mutations in POLE gene.

CONCLUSIONS:

1) We have shown that this standardized technique allows faster and easier identification of multiple pathogenic POLE mutations.

2) Following a Single-Gene approach and in this clinical context, this technology could compete with Sanger sequencing for the study of POLE mutations.

3) This test could emerge as a valid and fast alternative to Next – Generation Sequencing, especially in those centers where they do not have access to massive sequencing techniques.