

Are we ready for SERENA-6? Real-world benchmarking of ctDNA NGS assays for ultra-sensitive *ESR1* testing in HR+/HER2– metastatic breast cancer (MBC)

Konstantinos Venetis¹, Lucia Palumbo¹, Arianna Borghi¹, Alberto Ranghiero¹, Davide Vacirca¹, Mariano Lombardi¹, Marianna D’Ercole¹, Antonio Marra^{2,3}, Elisabetta Munzone⁴, Giuseppe Curigliano^{2,3}, Elena Guerini Rocco^{1,2}, Nicola Fusco^{1,2}

¹Division of Pathology, European Institute of Oncology IRCCS Milan, Italy, ²Department of Oncology and Hemato-Oncology, University of Milan, Italy, ³Division of Early Drug Development for Innovative Therapies, European Institute of Oncology IRCCS, Milan, Italy, ⁴Division of Medical Senology, European Institute of Oncology IRCCS Milan, Italy



BACKGROUND AND AIM OF THE STUDY

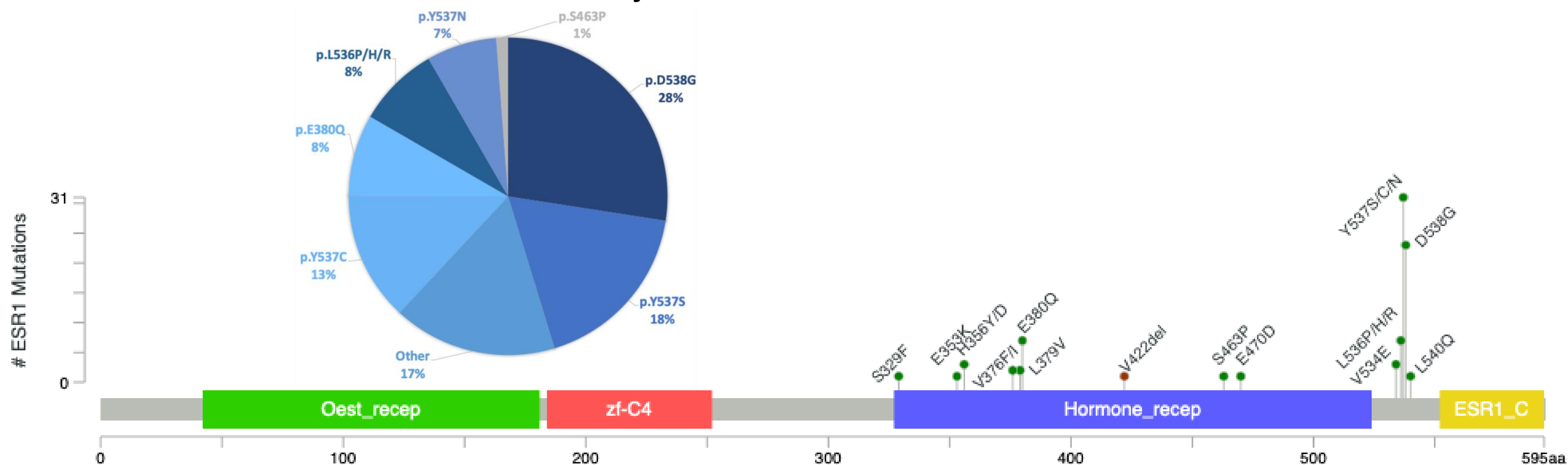
METHODS

- SERENA-6 highlights that ctDNA-guided molecular surveillance can enable early therapeutic intervention with camizestrant in *ESR1*-mutant HR+/HER2– MBC¹.
 - Therefore, there is need for ultra-sensitive *ESR1* testing at very low VAFs².
 - The clinical readiness of commercial ctDNA NGS panels remains uncertain³.

AIM: To evaluate the fitness-for-purpose of two NGS assays for *ESR1* surveillance in routine clinical samples.
- We retrospectively analyzed residual plasma ctDNA from patients with HR+/HER2– MBC progressing on ET plus CDK4/6 inhibitors tested at the European Institute of Oncology.
 - A routine NGS assay (Panel 1: Avenio ctDNA Expanded v2®) and a second assay (Panel 2:oncoReveal® Essential LBx) were used. Average DNA input was 45ng and 26ng, respectively.
 - ESR1* variants with VAF ≥0.05% were considered valid after internal validation.
 - Clinically actionable *ESR1* variants were defined as oncogenic or likely oncogenic variants predictive of response to FDA-approved therapies for breast cancer (OncoKB Level 1). Samples were classified as clinically actionable (positive) if ≥1 such variant was detected, and negative otherwise.
 - Variant-level concordance was restricted to *ESR1* variants that were reportable and located within regions targeted by both assays.
 - Concordance was evaluated at variant and clinical levels using statistical parameters (OPA, PPA, and NPA).

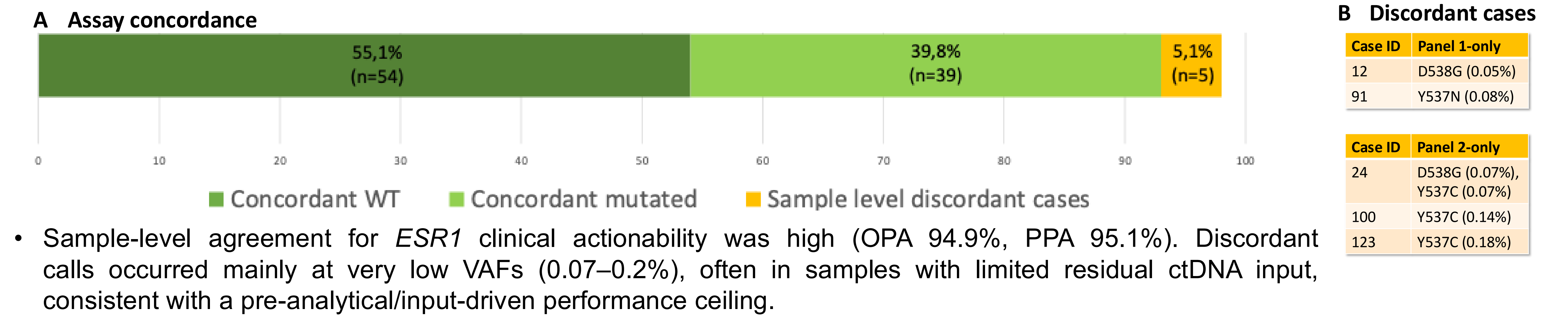
RESULTS

Result 1. *ESR1* mutation distribution as identified by Pillar NGS method



- A total of 100 samples were collected for analysis. Two samples failed quality control criteria for the Pillar NGS method due to insufficient sequencing depth and were excluded from the analysis. The evaluable population (n=98) was used for the analyses.
- N=84 mutations in *ESR1* were found. The most frequent alterations were p.D538G (28%), p.Y537S/C/N (38%), and p.E380Q (8%).

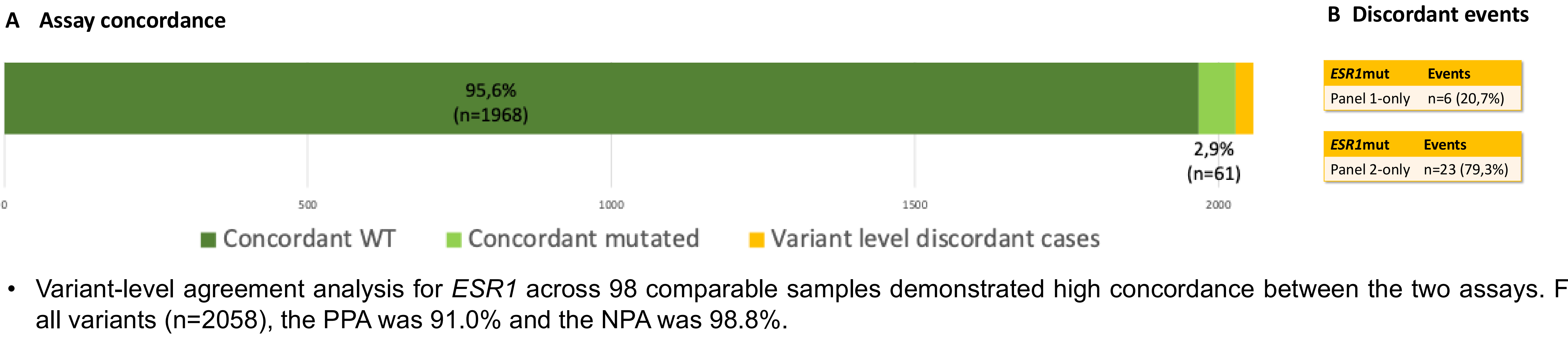
Result 2. *ESR1* sample-level clinical agreement at the clinical actionability tier



CONCLUSIONS

These results showed high concordance for both NGS panels for clinically actionable *ESR1* status and detected variants at ultra-low VAFs. Discordant cases were observed in limited residual ctDNA input samples highlighting ctDNA quantity as a critical determinant of assay performance. For reliable *ESR1* surveillance, labs should define minimum input thresholds and consider confirmation of ultra-low VAF variants by adopting orthogonal methods.

Result 3. *ESR1* variant level agreement analysis



Result 4. Prevalence and co-occurrence analysis of oncogenic mutations in *ESR1* and PI3K/AKT pathway genes

