an NGS panel, identifying challenges in case classification and possible solutions.

**Methodology** We performed the FoundationOne CDx NGS panel on 60 EC and assigned molecular subtype: POLE mutated (POLEmut), mismatch repair deficient (MMRd), p53 abnormal (p53abn) or no specific molecular subtype (NSMP).

**Result(s)** In 55 patients the molecular classification was successful. A pathogenic POLE mutation was detected in 9 cases (POLEmut). 20 were MMRd (12 MSI-high, 8 MSI-indeterminate based on the NGS panel MSI classifier) and a known or likely MMR gene mutation was found in 7 of these. Of the remaining 26 cases, 17 carried a TP53 mutation (p53abn) and the remaining 9 were considered to be NSMP. The tumor mutation burden (TMB) was significantly different (p<0.001) in the molecular subtypes (A) and high TMB (>55 mut/MB) was 100% specific for POLEmut EC (p<0.001). High TMB was specific for known pathogenic POLE mutations and was not elevated in cases with solely non-pathogenic POLE mutations (B).

In non-POLEmut cases, TMB was higher in MSI-high and MSI-indeterminate than microsatellite stable (MSS) cases (C), and a TMB of >7 mut/MB was 100% specific for MMRd EC. There was one MSS EC with a TMB of 18 mut/MB but it showed loss of MLH1 and PMS2 proteins on immunostaining and was classified as MMRd.

**Conclusion** An NGS panel can be used in the molecular classification of EC when there is sufficient tumor cellularity. TMB can be used as an adjunct in molecular subtype diagnosis for tumors that are difficult to classify. Additionally, TMB can potentially serve as a diagnostic adjunct in cases with POLE mutation of unknown significance.