In-silico analysis of TCGA data showing multiple POLE-like favourable subgroups overlapping with TP53 mutated endometrial cancer: Implications for clinical practice in low and middle-income countries

Damayanti Das Ghosh [a,d,*], Rahul Roy Chowdhury [b,d], Rajeswari Dutta [a], Indranil Mukhopadhyay [c], Asima Mukhopadhyay [d], Susanta Roychoudhury [a,d,1]

[a] *Department of Basic and Translational Research, Saroj Gupta Cancer Centre and Research Institute, Kolkata, India*
[b] *Department of Gynecological Oncology, Saroj Gupta Cancer Centre and Research Institute, Kolkata, India*
[c] *Human Genetics Unit, Indian Statistical Institute, Kolkata, India*
[d] *Kolkata Gynecological Oncology Trials and Translational Research Group, Kolkata, India*

A B S T R A C T

Introduction: The Cancer Genome Atlas cohort of endometrial carcinoma (TCGA-UCEC) includes almost 40% TP53-mutants encompassing missense and truncated variants. TCGA revealed 'POLE', characterized by POLE gene bearing exonuclease domain mutation (EDM), as the prognostically best molecular profile. The worst profile was characterized by TP53-mutated Type 2 cancer requiring adjuvant therapy having cost implications in low-resource settings. We aimed to find more 'POLE-like' favourable subgroups by searching TCGA cohort, especially within TP53 mutated risk group, that could eventually avoid adjuvant treatment in resource-poor settings.
Method: Our study was an in-silico survival analysis performed on the TCGA-UCEC dataset using SPSS statistical package. TP53 and POLE mutations, microsatellite instability (MSI), time-to-event and clinicopathological parameters were compared among 512 endometrial cancer cases. Deleterious POLE-mutations were identified by Polyphen2. Progression free survival was studied using Kaplan-Meier plots keeping original 'POLE' as comparator.
Result: In presence of wild type (WT)-TP53, other deleterious POLE-mutations behaved like POLE-EDM. Only truncated and not missense TP53 benefitted from POLE/MSI overlap. However, TP53 missense mutation, Y220C, was found to be as favourable as 'POLE'. Overlapping POLE, MSI and WT-TP53 also performed favourably. Truncated TP53 overlapped with POLE and/or MSI, TP53 Y220C alone and, WT-TP53 overlapped with POLE and MSI both, were named 'POLE-like' for prognostically behaving like the comparator 'POLE'.
Conclusion: Obesity being a lesser frequent event in low and middle-income countries (LMICs), relative proportion of women with lower BMI and Type 2 endometrial cancers may be high. Identification of 'POLE-like' groups may facilitate therapeutic de-escalation in some TP53-mutated cases - a novel option. Instead of 5% (POLE-EDM), potential beneficiary would then comprise 10% (POLE-like) of TCGA-UCEC.

1. Introduction

Uterine corpus endometrial carcinoma (UCEC) accounts for 16,413 new cases and 6385 deaths annually among Indian women (https://gco.iarc.fr/today/data/factsheets/populations/356-india-fact-sheets.pdf). UCEC can be classically divided into Type 1 and Type 2 carcinomas. Majority of type 1 cancers are low grade (G1 or G2) with good prognosis.

Type 2 cancers are typically high grade (G3) having serous histology, TP53-mutations and worse prognosis (Bell and Ellenson, 2019). Stage I endometrioid cancers are generally treated with surgery and/or radiation. Fertility-sparing treatment can be considered for young women (Signorelli et al., 2009). Chemotherapy remains the standard adjuvant therapy for high grade and advanced stage endometrial cancer (Nout et al., 2010; de Boer et al., 2018; Colombo et al., 2016). Advanced/

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recurrent disease is treated with surgery, chemotherapy and/or radiation therapy. Therapy for recurrent disease depends on the site of recurrence as well as prior treatments and may include immunotherapy or targeted therapy among additional options (Mittica et al., 2017; Makker et al., 2019). Global incidence of UCEC has increased by 132% in last 30 years (Sung et al., 2021; Crosbie et al., 2022). The recent introduction of molecular classification has increased the scope for precision in treatment stratification. However, women with endometrial cancer residing in low and middle-income countries (LMICs) face additional challenges of either over or under-treatment due to lack of access to these facilities. This will have impact on survival and quality-of-life in these women (Crosbie et al., 2022; Suarez et al., 2017).

TP53, also called 'guardian of the genome', is a tumor suppressor involved in DNA-repair, cell-cycling and apoptosis. However, TP53 is infamous to have a vast mutational spectrum reflected across human cancers (Sabapathy and Lane, 2018). Broadly, TP53 mutations can be grouped into missense mutations, truncation mutations (nonsense/frameshift/splice-variant/large deletions) and others (silent/intronic). Truncation mutations result in loss of tumor suppressor function (LOF) of TP53. Missense mutations result in LOF or gain-of-function (GOF). Oncogenic GOF mutants include missense mutations at 'hotspots' in the DNA-binding domain of TP53 namely R175H, G245S, R248Q/W, R249S, R273H/C and R282W and other frequently occurring mutations like Y220C, V157F, C176F and many more (Sabapathy and Lane, 2018; Kim and Lozano, 2018; Baugh et al., 2018; Muller and Vousden, 2014). Some of these are 'contact mutations' hindering DNA-contact (R248Q, R273H) while some are 'conformation mutants' altering the structure of DNA-binding domain (R249S, R282W) and some lie far from the DNA-binding interface (Y220C) (Sabapathy and Lane, 2018; Baugh et al., 2018).

The Cancer Genome Atlas (TCGA) portrayed UCEC cases as four integrated molecular clusters, 'POLE', 'MSI', 'copy number low' and 'copy number high' on the basis of somatic nucleotide substitutions, microsatellite instability and somatic copy number alterations (Cancer Genome Atlas Research Network et al., 2013). 'POLE' and 'copy number high' clusters were depicted as having the best and worst prognoses, respectively. The molecular phenotype of Type 1 endometrioid UCEC strictly belonged to the first three clusters while that of Type 2 serous UCEC mainly belonged to the 'copy number high' cluster characterized by TP53-mutation (Cancer Genome Atlas Research Network et al., 2013). However, 'copy number high' cluster also included many endometrioid UCECs. On the other hand, some cases belonging to 'POLE' cluster also harbored TP53-mutations and high grade endometrioid cases. (Cancer Genome Atlas Research Network et al., 2013). Presently, TCGA-UCEC consists of 548 cases with 512 having data on somatic mutations, of which about 40% harbour TP53-mutations, which is almost double the number as in original TCGA article on molecular profiles of UCEC (Cancer Genome Atlas Research Network et al., 2013). The POLE cluster represented only 5% of the TCGA cohort that could avoid adjuvant therapy, which is commonly indicated for TP53 mutant Type 2 cases. However, aggressive Type 2 cases are increasingly seen in clinical practice in LMICs and among many ethnic groups commonly found in LMICs (Maheshwari et al., 2016; Mullins and Cote, 2019). Besides, obesity is less frequently found in LMICs. Therefore, the relative proportion of women with lower BMI and Type 2 endometrial cancers may be high. Adjuvant therapy remains a challenge in resource-poor setting owing to high cost, long waiting list and radiation-induced morbidity (Varughese and Richman, 2010). This represents an unmet clinical need to investigate whether any favourable subgroups exist within TP53 mutated cases that behave like POLE and may forgo adjuvant treatment.

We were thus prompted to explore possibilities of other 'POLE-like' prognostically better groups by studying three additional aspects not considered in original TCGA article (Mittica et al., 2017). Those were, (i) presence of deleterious POLE-mutations additionally to the known pathogenic POLE-mutations (ii) TP53 mutational heterogeneity, and,

(iii) overlaps among molecular profiles (León-Castillo et al., 2020b).

2. Methods

The original TCGA paper considered 373 cases which were categorized into 4 integrated clusters or molecular profiles, (a) hypermutated POLE (b) ultramutated MSI, (c) TP53 wild type 'copy number low' and, (d) TP53-mutated 'copy number high'. Our study design was based on mutation-analysis from the present TCGA-UCEC cohort in GDC data-portal (https://portal.gdc.cancer.gov/exploration), which provided detailed mutational status of 512 cases having simple somatic mutation (SSM) data. We found out cases having TP53-mutations and POLE-mutations. TP53-mutations were primarily categorized into missense and truncated subtypes, leaving out the cases with both subtypes as well as those with synonymous/UTR/intronic mutations. POLE missense mutations were tested *in silico* using Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) to find deleterious mutations in addition to the known exonuclease domain mutations (EDM) (Adzhubei et al., 2010). Only deleterious POLE-mutations, either missense or truncated, having a damaging effect on POLE were considered. MSI-status was obtained from another report in TCGA Pan-Cancer Atlas (Berger et al., 2018). Other than mutational subtypes of TP53 mutation, overlaps with other molecular profiles (POLE/MSI) and, the most frequent TP53 mutations (missense) were also recorded. Overlap among molecular profiles was termed 'mixed' without which each profile was termed 'only'. Time-to-event data were collected from the report on Clinical Data Resource (CDR) in TCGA Pan-Cancer Atlas (Liu et al., 2018). Time-to-event data was used to analyze progression free survival (PFS) using Kaplan-Meier (KM) plots and log-rank test. Survival (PFS) was compared among (i) TP53-missense only, TP53-truncated only, TP53-missense mixed, TP53-truncated mixed, and wild type (WT)-TP53 overlapped with both POLE and MSI, (ii) most frequent TP53-missense mutations. Prognostically favourable groups were compared with original TCGA-based 'POLE' as a comparator, to identify POLE-like survival-groups in the context of TP53 mutation. Clinicopathological parameters including histopathology (H/P), stage, grade and age-at-diagnosis were also collected from the CDR (Liu et al., 2018). H/P included endometrioid, serous and mixed (both endometrioid and serous) types, the last being clubbed with serous (Cancer Genome Atlas Research Network et al., 2013). Grade 3 was high grade and stages III/IV were considered as advanced. Clinicopathological parameters were compared among different survival-groups by Chi-square tests. Data on receiving adjuvant therapy was collected from TCGA-based Pan-Cancer Atlas available through cBioPortal (https://www.cbioportal.org/study/clinicalData?id=ucec_tcga_pan_can_atlas_2018). PFS was compared between adjuvant therapy treated and untreated cases belonging to POLE-like groups, using KM plots. The plan of analysis has been provided in Fig. 1. KM plots, log-rank test and Chi-square tests were done by SPSS statistical package (version 22).

3. Results

3.1. Increase in number of POLE pathogenic/deleterious variants

Pathogenic POLE-mutations will restrict POLE from DNA repairing, giving rise to ultramutated DNA. Presently, only 11 POLE-EDM has been considered pathogenic, although TCGA accounted for over hundred POLE-mutations within coding region, majority being missense and few truncated (Makker et al., 2019). Out of the missense mutations, 69 were found to be deleterious including the 11 known pathogenic variants (62 with highest scores of "probably damaging" and 7 with close scoring of "possibly damaging") by Polyphen-2 (electronic Table S1). Out of 87 POLE-mutated cases, 75 harbored at least one of the 69 deleterious missense mutations or any truncation mutation. In presence of WT-TP53, cases bearing any of the 11 known pathogenic POLE-mutations showed similar PFS as those with other deleterious POLE-mutations ($p_{log-rank}$ = 0.948) (Fig. 2a). Henceforth, we considered all the 69
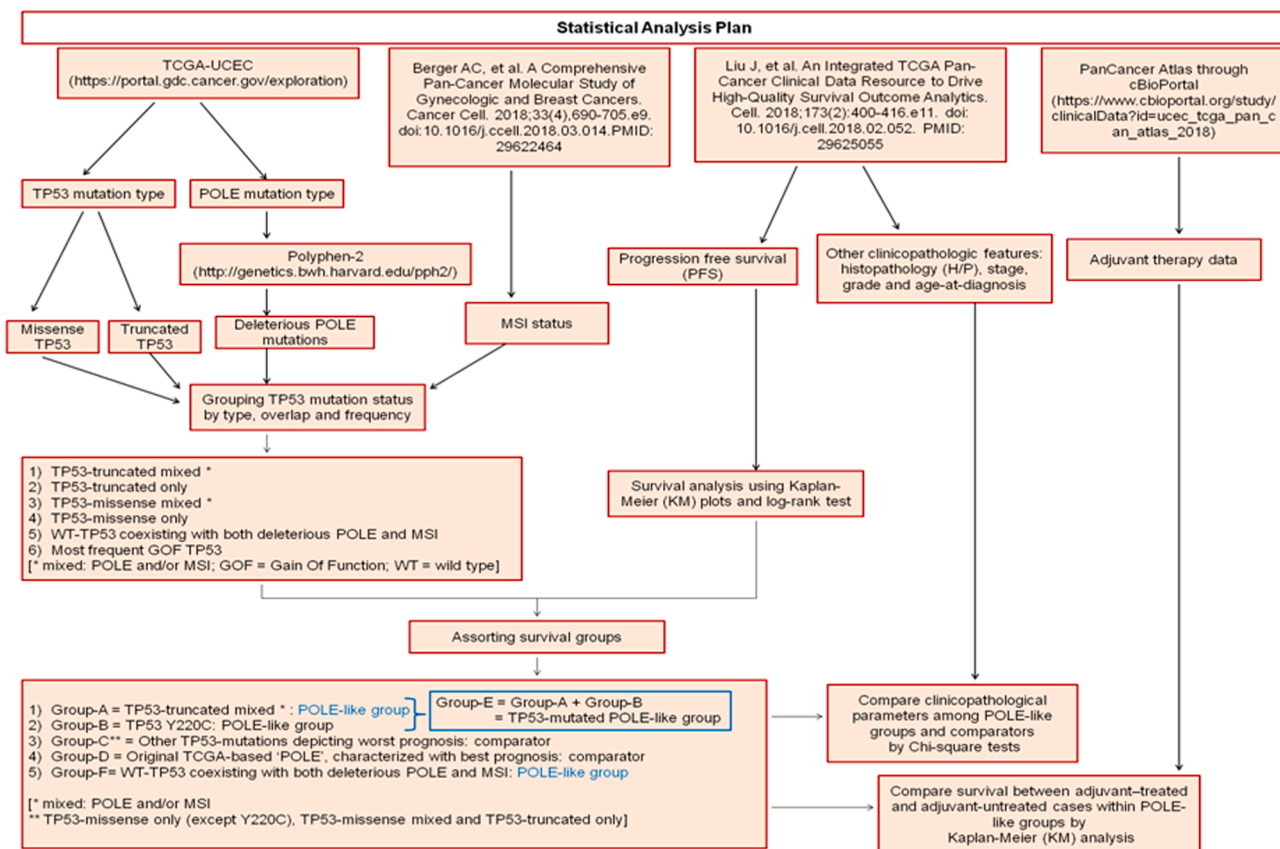
Fig. 1. Statistical analysis plan. Analysis has been done in statistical package SPSS. [Relevant clinical names for survival groups: Group-A = truncated TP53 with POLE and/or MSI; Group-B = TP53 Y220C; Group-C = mutated TP53; Group-D = POLE; Group-E = Group-A + Group-B = POLE-like mutated TP53; Group-F = WT-TP53 with POLE and MSI].
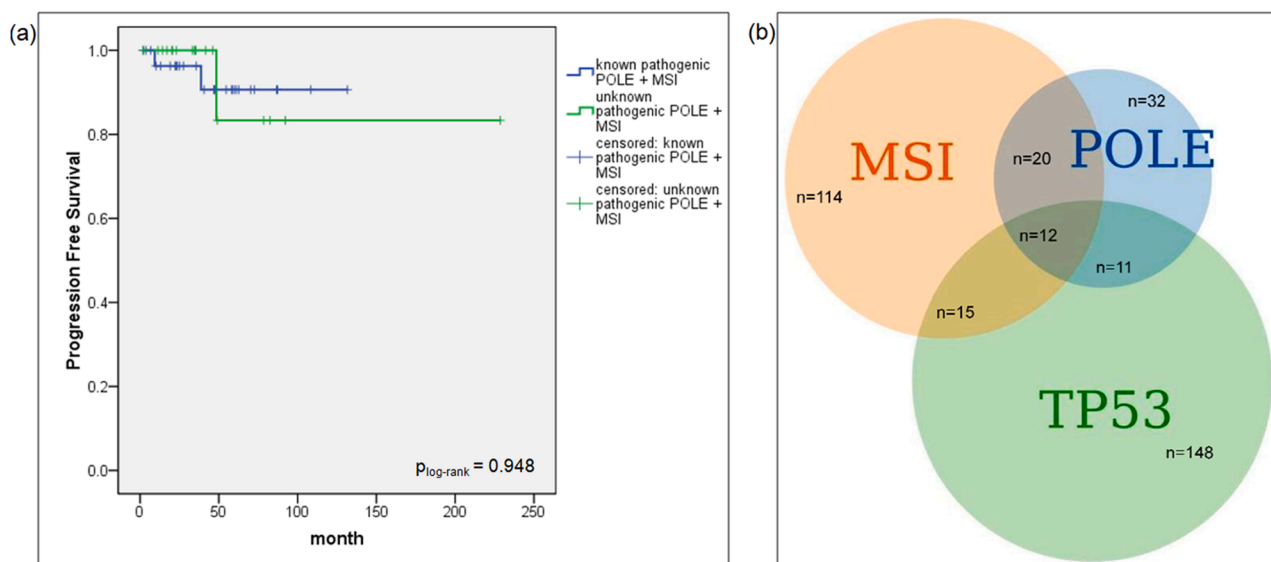
Fig. 2. Mutational profiles. (a) Comparison of PFS between cases bearing POLE-EDM specific acclaimed mutations and those bearing other unknown functionally deleterious POLE mutations, under wild type TP53 background. (b) Cases from TCGA-UCEC cohort showing mutations in TP53, POLE and presence of MSI. (Functionally inert synonymous/intronic/UTR-specific mutations were not considered for TP53; only functionally deleterious missense and truncated mutations of POLE were considered; MSI: microsatellite instability; PFS: Progression Free Survival; n: sample size; TCGA: The Cancer Genome Atlas; UCEC: Uterine corpus endometrial carcinoma).
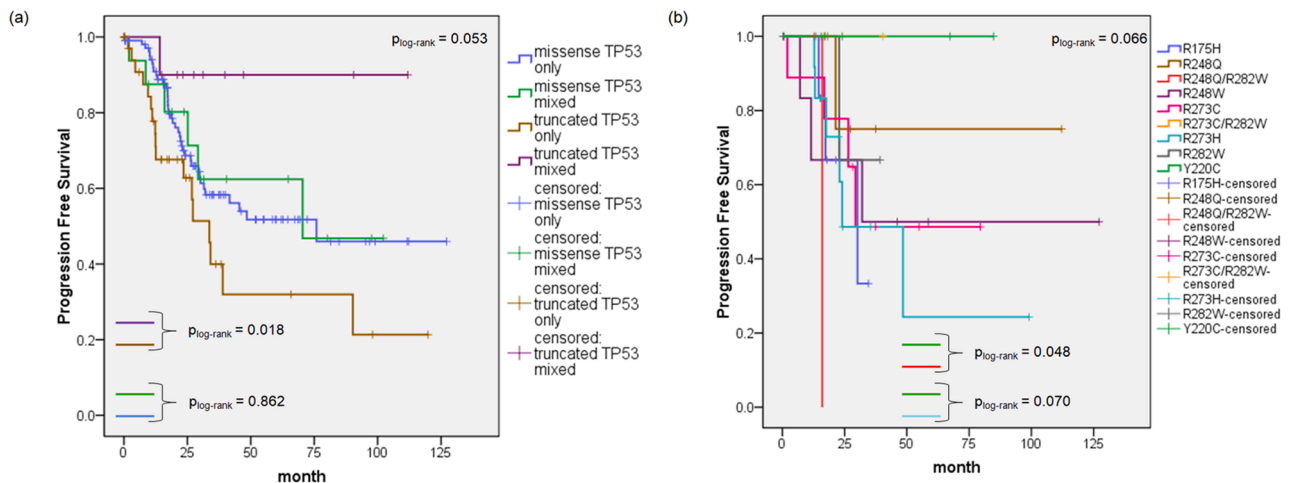
Fig. 3. Differential effect of TP53 mutation subtypes: identification of favourable subtypes. (a) Identification of truncated mixed TP53 (Group-A; n = 10) by comparing PFS of TP53 missense and truncated cases with ('mixed') or without ('only') overlapping deleterious POLE and/or MSI (microsatellite instability). (b) Identification of GOF-TP53 Y220C (Group-B; n = 6) by comparing PFS among cases with most frequent GOF mutant TP53. (PFS: Progression Free Survival; GOF: gain of function; n: sample size; MSI = microsatellite instability; Relevant clinical names for survival groups: Group-A = truncated TP53 with POLE and/or MSI; Group-B = TP53 Y220C).

deleterious/pathogenic missense as well as truncated POLE-variants in determining POLE-status and analyzing its influence on overlapping TP53-mutation subtypes. We excluded synonymous, missense but benign and, intronic POLE-mutations.

3.2. Categorization of TCGA-UCEC cases with respect to TP53- and POLE-mutations, MSI-status and their overlaps

Classification based on TP53-mutations (excluding synonymous/intra-UTR/intronic), pathogenic/deleterious POLE-mutations and MSI-status, showed 186 TP53-mutated, 75 POLE-mutated and 161 MSI-high cases (electronic supplementary Table S2). Out of these, 11, 15 and 20 cases respectively had coexisting TP53- and POLE-mutations, TP53-mutation and MSI, and POLE-mutation and MSI, while 12 cases had all the three (Fig. 2b). Therefore, out of 186 TP53-mutated cases, 148, 32 and 114 respectively only had, TP53-mutations, POLE-mutations and MSI, with no overlap. The 186 TP53-mutated cases included 127 TP53-missense only, 48 TP53-truncated only (loss-of-function due to frameshift/nonsense-mutations) and 11 with coexisting TP53-missense and –truncated (were removed from downstream analyses). Cases with synonymous/UTR/intronic mutations were not functionally considered as TP53-mutated.

3.3. TP53 truncated-mixed mutations depicted better prognosis than TP53 truncated-only, TP53 missense-mixed, and TP53 missense-only mutations

We performed multi-gene sub-classification analysis to assess the prognostic contribution of TP53-mutation subtypes in patients harboring deleterious POLE and/or MSI. PFS was compared among TP53-missense only (n = 109), TP53-truncated only (n = 36), TP53-missense mixed, (n = 17) and TP53-truncated mixed (n = 10) ($p_{log-rank}$ = 0.053). When compared pair-wise, there was a significant difference in PFS between TP53-truncated only and TP53-truncated mixed ($p_{log-rank}$ = 0.018), but not between TP53-missense only and TP53-missense mixed ($p_{log-rank}$ = 0.869) (Fig. 3a). There was no significant difference in PFS among TP53-missense only, TP53-truncated only, and TP53-missense mixed ($p_{log-rank}$ = 0.167) (Fig. 3a). It might be inferred that the disease outcome of POLE/MSI-positive patients could be influenced by the presence of TP53-mutation subtypes. In other words, truncated TP53 complied with coexisting favourable POLE-effect

whereas missense TP53 completely overrode it.

3.4. TP53 missense mutation subtypes show bad prognoses irrespective of POLE/MSI co-occurrence except Y220C GOF mutation

It is known that all TP53-missense mutations do not occur in equal frequency in different cancers (Sabapathy and Lane, 2018). Few of about 100 reported TP53-missense mutations from TCGA-UCEC result in gain-of-function (GOF) in addition to loss-of-function (LOF) (Baugh et al., 2018). These GOF-TP53 mutations occur at high frequencies in different cancers. The most frequent (defined as those at ≥ 2.50% frequency) GOF-TP53 mutations in TCGA-UCEC cohort were R273C (5.7%), R273H (5.7%), R248Q (5.18%), R248W (3.63%), R282W (3.11%), Y220C (3.11%) and R175H (2.59%). There were 56 cases that harbored at least one of these mutations. The rare overlaps between GOF-TP53 and POLE-mutation/MSI included (i) R273C and POLE-mutation (n = 1), (ii) R273H and MSI (n = 1), (iii) double GOF R248Q/R282W and MSI (n = 1), (iv) R273C with POLE-mutation and MSI (n = 1) and (v) double GOF R273C/R282W with POLE-mutation and MSI (n = 1).

When compared among cases harboring the most frequent GOF-TP53, irrespective of overlaps with deleterious POLE and/or MSI, Y220C suggested better survivability than the rest. Y220C was exclusively found not to overlap with other molecular phenotypes. It was particularly favourable compared to the double GOF R248Q/R282W which showed poor survival even with MSI ($p_{log-rank}$ = 0.048) and R273H (although not statistically significant; $p_{log-rank}$ = 0.070) (Fig. 3b).

There was no significant difference ($p_{log-rank}$ = 0.29) in PFS between the most frequent GOF-TP53 only (excluding Y220C; n = 40) and GOF-TP53 mixed (n = 4) cases (electronic supplementary Fig. S1a). PFS was also similar among the most frequent GOF-TP53 only (excluding Y220C), TP53-truncated only and TP53-missense only ($p_{log-rank}$ = 0.201) cases (electronic supplementary Fig. S1b).

3.5. Assorting mutational groups of differential and comparable prognoses: Group-E (POLE-like mutated TP53) and Group-F (WT-TP53 with POLE and MSI) were similar to Group-D (POLE) and distinct from Group-C (mutated TP53)
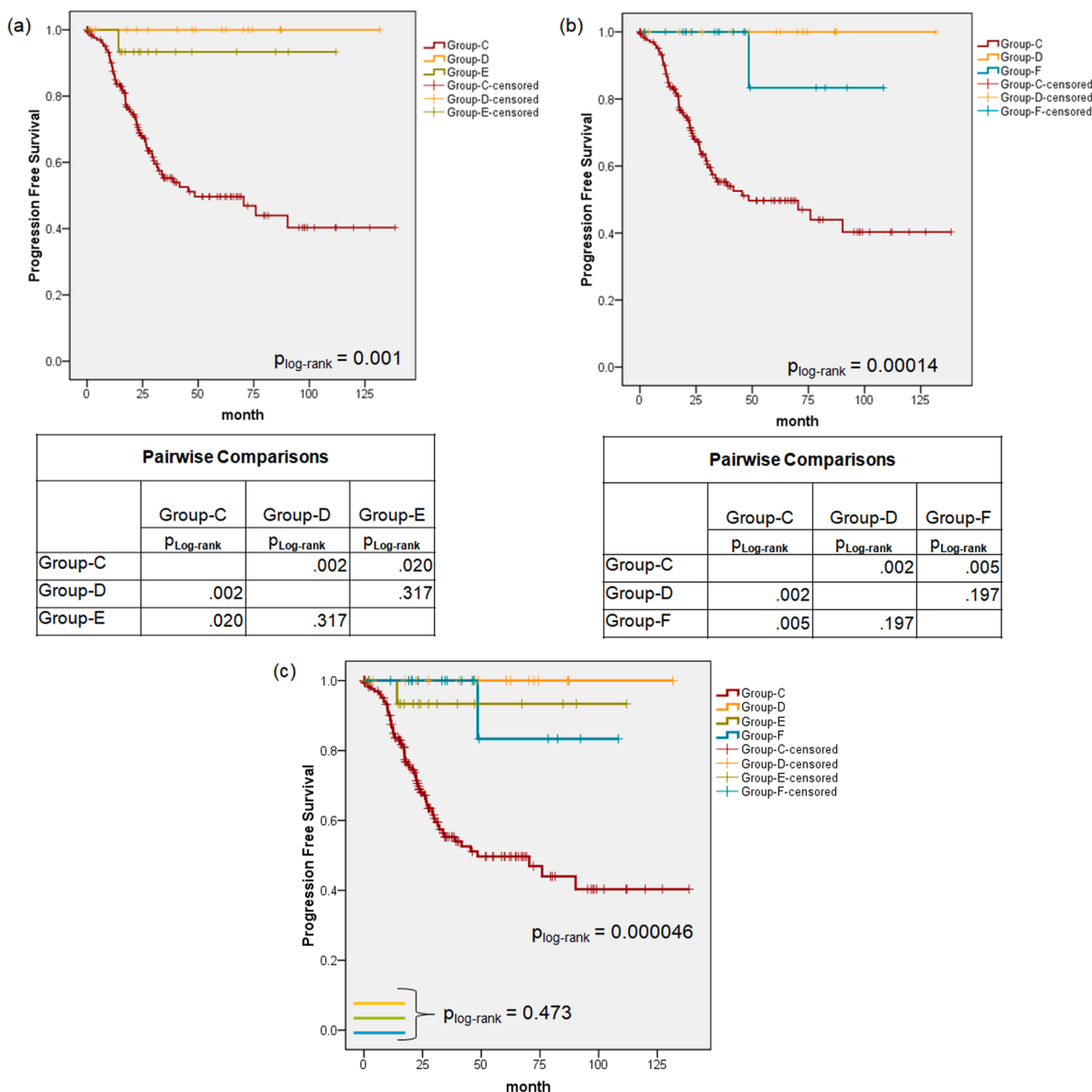
We found three categories of molecular status depicting favourable prognosis: TP53-truncated mixed (Group-A; n = 10), GOF-TP53 Y220C (Group-B; n = 6) and WT-TP53 coexisting with deleterious POLE and

MSI (Group-F; n = 19). Other TP53-mutations namely, TP53-missense only (except Y220C), TP53-missense mixed and TP53-truncated only, were grouped together as separate comparator category, Group-C (n = 174), depicting worst prognosis. Original TCGA-based integrated cluster, 'POLE', characterized with best prognosis, formed another comparator, Group-D (n = 17) (Cancer Genome Atlas Research Network et al., 2013).

The TP53-mutant Groups-A and -B being similar in prognoses ($p_{\text{log-rank}}$ = 0.480) were clubbed as Group-E (n = 16). Group-E and Group-F were separately compared with the prognostically extreme comparators, Group-C and Group-D. Group-E ($p_{\text{log-rank}}$ = 0.317) and Group-F ($p_{\text{log-rank}}$ = 0.197) behaved like Group-D. Conversely, both Groups-E ($p_{\text{log-rank}}$ = 0.020) and –F ($p_{\text{log-rank}}$ = 0.005) were significantly better than Group-C (Fig. 4a and 4b). Group-D, Group-E and Group-F showed

identical PFS ($p_{\text{log-rank}}$ = 0.473) (Fig. 4c).

If only 11 pathogenic POLE-mutations were acknowledged as beneficial, overlooking other pathogenic/deleterious POLE-mutations and overlaps with MSI and/or specific TP53-mutations, a small prognostically favourable group would be identified from present TCGA-UCEC (n = 26; 26/512; 5.08%). However, by considering 'POLE-like' groups, higher proportion of cases (Group-E + Group-F + Group-D; (16 + 19 + 17)/512 = 52/512; 10.16%) revealed better disease-outcome. The above mentioned groups can be clinically named as 'truncated TP53 with POLE and/or MSI' (Group-A), 'TP53 Y220C' (Group-B), 'POLE-like mutated TP53' (Group-E), 'mutated TP53' (Group-C), 'WT-TP53 with POLE and MSI' (Group-F) and 'POLE' (Group-D).

Pairwise Comparisons (a)

	Group-C $p_{\text{Log-rank}}$	Group-D $p_{\text{Log-rank}}$	Group-E $p_{\text{Log-rank}}$
Group-C		.002	.020
Group-D	.002		.317
Group-E	.020	.317	

Pairwise Comparisons (b)

	Group-C $p_{\text{Log-rank}}$	Group-D $p_{\text{Log-rank}}$	Group-F $p_{\text{Log-rank}}$
Group-C		.002	.005
Group-D	.002		.197
Group-F	.005	.197	

Fig. 4. 'POLE-like' groups. Comparisons of PFS (a) among Group-E, Group-D and Group-C, (b) among Group-F, Group-D and Group-C, (c) among 'POLE-like' groups. [Relevant clinical names for survival groups: Group-C (n = 155; worst prognoses) = mutated TP53; Group-D (n = 17; original TCGA-POLE with best prognosis) = POLE; Group-E (n = 16; favourable prognosis) = POLE-like mutated TP53; Group-F (n = 17; favourable prognosis) = WT-TP53 with POLE and MSI; PFS: Progression Free Survival; MSI = microsatellite instability].

3.6. Group-E (POLE-like mutated TP53) and Group-F (WT-TP53 with POLE and MSI) were prognostically favourable despite some adverse clinicopathological features

Group-E (POLE-like mutated TP53) showed similar representation of < 60 years (y) (n = 8) and ≥ 60y (n = 8) aged cases, like Group-D (POLE) (<60y: n = 10 and ≥ 60y: n = 7) ($p_{Chi-square}$ = 0.61), but significantly different from Group-C (mutated TP53), which was predominated by older cases (<60y: n = 22 and ≥ 60y: n = 132) ($p_{Chi-square}$ = 0.002). However, ≥60y aged cases were significantly more represented within Group-F (WT-TP53 with POLE and MSI) (<60y: n = 4 and ≥ 60y: n = 11) than Group-D (POLE) ($p_{Chi-square}$ = 0.0002) but less than Group-C (mutated TP53) ($p_{Chi-square}$ = 0.002) (Fig. 5a, Table 1).

Group-E (POLE-like mutated TP53) showed somewhat greater representation, of advanced stage (stages III/IV: 5/16 = 31.25%) and higher grade (Grade 3: 12/16 = 75%) than Group-D (POLE) (stages III/IV: 4/17 = 23.51%; Grade 3: 8/17 = 47.05%) (E_{stage} vs D_{stage}: $p_{Chi-square}$ = 0.41; E_{grade} vs D_{grade}: $p_{Chi-square}$ = 0.167). Group-E was similar to Group-C (mutated TP53) in stage (stages III/IV: 69/155 = 44.52%) (E_{stage} vs C_{stage}: $p_{Chi-square}$ = 0.62) but not grade (Grade 3: 143/174 = 82.18%; E_{grade} vs C_{grade}: $p_{Chi-square}$ = 0.034) (Fig. 5b, 5c, Table 1).

Group-F (WT-TP53 with POLE and MSI) also had a sizeable representation of high grade disease (13/17; 76.47%) midway between Group-D (POLE) (8/17; 47.06%; F_{grade} vs D_{grade}: $p_{Chi-square}$ = 0.135) and Group-C (mutated TP53) (143/155; 92.26%; F_{grade} vs C_{grade}: $p_{Chi-square}$ = 0.047) (Fig. 5b, Table 1). However, advanced stage was much less represented among Group-F (WT-TP53 with POLE and MSI) (1/17; 5.88%), even lower than Group-D (POLE) (4/17; 23.53%; F_{stage} vs D_{stage}: $p_{Chi-square}$ = 0.344) and, in sharp contrast to Group-C (mutated TP53) (69/155; 44.52%; F_{stage} vs C_{stage}: $p_{Chi-square}$ = 0.013) (Fig. 5c, Table 1).

Group-E (POLE-like mutated TP53) differed from Group-D (POLE) in having a substantial share of unfavourable serous histopathology (H/P) (Group-E: 5/16; 31.25%; Group-D: 0/17; 0.00%; $E_{H/P}$ vs $D_{H/P}$: $p_{Chi-square}$ = 0.126). However, Group-E (POLE-like mutated TP53) differed from

Group-C (mutated TP53) in having a higher proportion of endometrioid H/P (Group-E: 11/16; 68.75%; Group-C: 60/155; 38.71%; $E_{H/P}$ vs $C_{H/P}$: $p_{Chi-square}$ = 0.02) (Fig. 5d). Group-F (WT-TP53 with POLE and MSI) had similar representation of endometrioid H/P (17/17; 100%) as Group-D (POLE) (17/17; 100%) with no serous representation unlike Group-C (mutated TP53) (95/155; 61.29%; $F_{H/P}$ vs $C_{H/P}$: $p_{Chi-square}$ = 0.0000014) (Fig. 5d, Table 1).

3.7. Group-E (POLE-like mutated TP53), Group-F (WT-TP53 with POLE and MSI) and Group-D (POLE) were prognostically favourable irrespective of adjuvant therapy

Out of the total number of cases having favourable prognosis (n = 52) belonging to Group-E (POLE-like mutated TP53), Group-F (WT-TP53 with POLE and MSI) or Group-D (POLE), 40.38% (21/52) received adjuvant radiation therapy, while 59.62% (31/52) did not. There was no difference in PFS between the adjuvant-treated and adjuvant-untreated cases within these groups ($p_{log-rank}$ = 0.251) (Fig. 6).

4. Discussion

We found multiple 'POLE-like' groups within present TCGA-UCEC cohort suggesting favourable prognosis of a larger number of patients than hitherto identified.

We tested the deleterious effect of each POLE mutation on POLE protein by in-silico tool, Polyphen2 (http://genetics.bwh.harvard.edu/pph2/). More than 50% of mutations were found to be equally pathogenic as the well-established 11 POLE exonuclease domain mutations (EDM). Moreover, cases with these mutations were also found to have similar prognoses as those with POLE-EDM in the presence of wild-type TP53. Hence we considered all these mutations in our study. It indicated existence of a larger prognostically favourable POLE-mutation pool, which might be shadowed by TP53-overlap. We further analyzed the effect of deleterious POLE-mutations overlapped with different
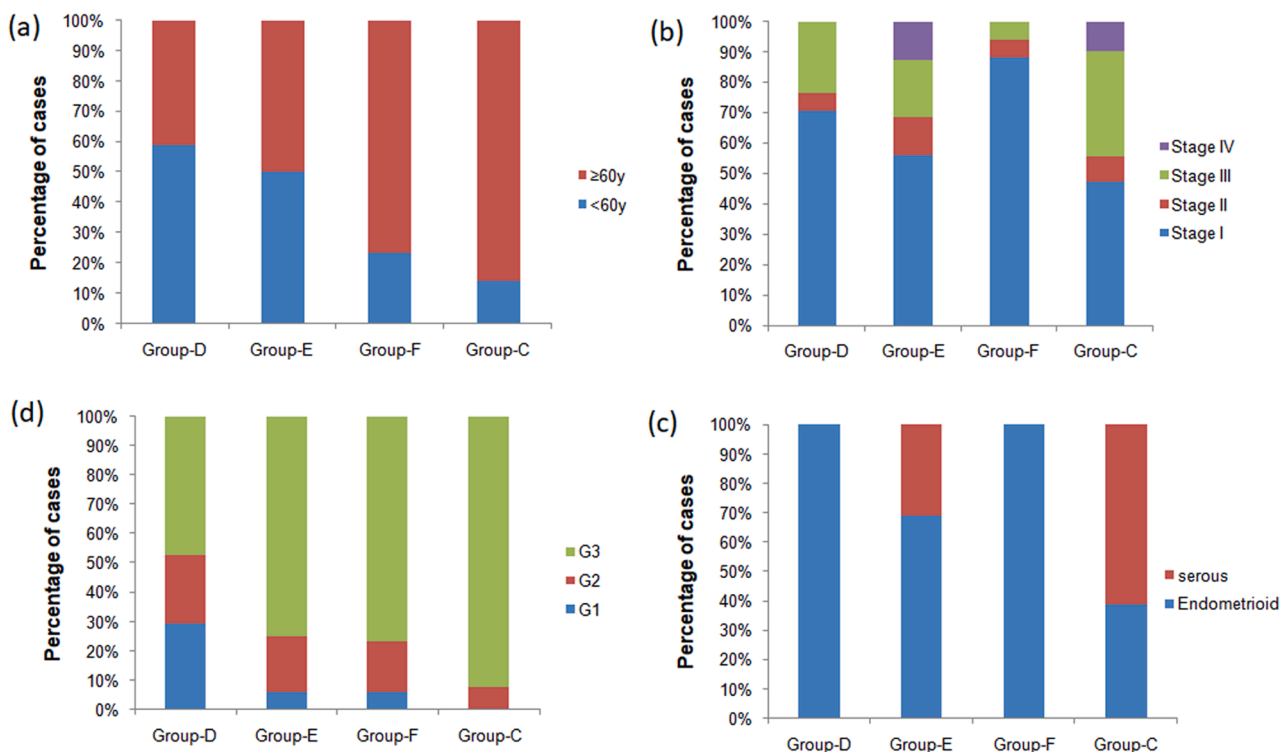

Fig. 5. Comparison of frequencies of Group-E, Group-F, Group-D and Group-C among different clinicopathological parameters like (a) age (b) stage (c) grade and (c) histopathological types. [Relevant clinical names for survival groups: Group-C (worst prognoses) = mutated TP53; Group-D (original TCGA-POLE with best prognosis) = POLE; Group-E (favourable prognosis) = POLE-like mutated TP53; Group-F (favourable prognosis) = WT-TP53 with POLE and MSI].

Table 1

Comparison of clinicopathological parameters of the different molecular groups.

	Number of cases				Statistical significance ($p_{Chi-square}$)			
	Group-D (n = 17)	Group-E (n = 16)	Group-F (n = 17)	Group-C (n = 155)	Group-E vs Group-D	Group-E vs Group-C	Group-F vs Group-D	Group-F vs Group-C
Histopathology								
endometrioid	17	11	17	60	0.126	0.02	–	0.0000014
serous	0	5	0	95				
Stage								
I	12	9	15	73	0.41	0.62	0.344	0.013
II	1	2	1	13				
III	4	3	1	54				
IV	0	2	0	15				
Grade								
G1	5	1	1	1	0.167	0.034	0.135	0.047
G2	4	3	3	11				
G3	8	12	13	143				
Age[†]								
< 60 years	10	8	4	22	0.61	0.002	0.00023	0.002
≥ 60 years	7	8	11	132				

[†] 1 sample of Group-C and 2 samples of Group-F had missing data for age; [Relevant clinical names for survival groups: Group-A = truncated TP53 with POLE and/or MSI; Group-B = TP53 Y220C; Group-C = mutated TP53; Group-D = POLE; Group-E = Group-A + Group-B = POLE-like mutated TP53; Group-F = WT-TP53 with POLE and MSI].
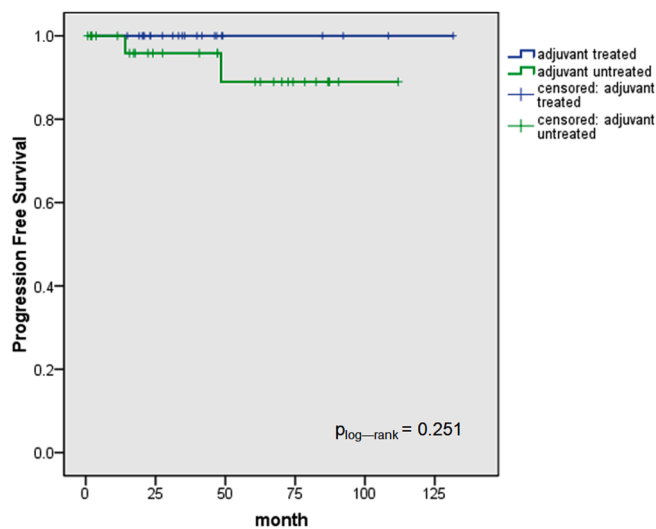
Fig. 6. Comparisons of PFS between adjuvant therapy-treated and –untreated cases belonging to POLE-like survival groups with favourable prognosis.

TP53-mutation subtypes. We also reclassified the TCGA cohort according to available MSI status from Berger et al., Cancer cell 2018, and ran the analysis. We got a similar result as before, validating our results. Furthermore, our results focused on TP53 mutated cases, most of which were not MSI+. Hence, changes in MSI status did not affect the result. Clinical implications of TP53 mutational spectrum is well-reported (Sabapathy and Lane, 2018; Xu et al., 2014). We found that TP53-truncated 'mixed' (Group-A; relevant clinical name: Truncated TP53 with POLE and/or MSI) significantly outperformed TP53-truncated 'only'. However, TP53-missense showed unfavourable prognosis irrespective of overlaps with POLE-mutation and/or MSI. Not all TP53-GOF mutants behaved equally supporting a growing repertoire of literature (Baugh et al., 2018; Xu et al., 2014; Padmanabhan et al., 2018). Y220C (Group-B; relevant clinical name: TP53 Y220C) showed superior prognosis to other frequent GOF-TP53 mutations, like R273H and double-mutant R248Q/R282W. GOF-TP53 mutants (excluding Y220C), TP53-missense only and TP53-truncated only behaved similarly. Like Group-D or original TCGA-POLE (relevant clinical name: POLE), TP53-mutated Groups-A and -B (clubbed as Group-E; relevant clinical name:

POLE-like mutated TP53) exhibited significantly better prognosis than Group-C (relevant clinical name: mutated TP53) comprising of other TP53-mutated cases. 'POLE-like' groups, Group-E (POLE-like mutated TP53) and Group-F (WT-TP53 with POLE and MSI) showed favourable prognoses despite occasional apprehensive clinicopathological features like high grade, late-presentation or serous histopathology.

"Multiple-classifiers", POLE with TP53-mutation, or, MSI with TP53-mutation, were earlier reported to behave like "single-classifiers", 'POLE' or 'MSI' (León-Castillo et al., 2020a). We added more granularity by splitting TP53-mutations further into truncated and missense with LOF/GOF (Muller and Vousden, 2014). Previously, transPORTEC study validated original TCGA findings in high-risk endometrial cancer and ProMisE study devised molecular risk classifier system based on TCGA (Stelloo et al., 2015; Talhouk et al., 2017). Subsequently, clinical trials (RAINBO) have started on different treatments for different molecularity [https://clinicaltrials.gov/ct2/show/NCT05255653]. However, those studies considered TP53-mutation as uniformly unfavourable and did not focus on overlapping profiles. TCGA documented above 100 POLE-mutations within coding region though only 11 POLE-EDM had been considered pathogenic (Cancer Genome Atlas Research Network et al., 2013; León-Castillo et al., 2020a). We considered the unclaimed deleterious POLE-mutations also, while deciphering the effects of overlapping POLE/MSI on PFS of WT-TP53, TP53-truncated and TP53-missense cases. Deleterious POLE and MSI impair DNA-repair increasing sensitivity to DNA-damaging treatments. MSI-status has been associated with POLE/POLD-mutations and immunotherapeutic success (Wang et al., 2019; Li et al., 2020).

'POLE-like' Y220C is a conformational mutant residing far from the DNA-contact interface of TP53 creating a destabilizing crevice on the protein surface (Baugh et al., 2018; Boeckler et al., 2008). It is reported in Li-Fraumeni Syndrome and in somatic/germline DNA of other cancers (https://cancer.sanger.ac.uk/cosmic/mutation/overview; https://www.ncb\i.nlm.nih.gov/clinvar/variation/127819). Y220C was earlier associated with better prognosis in a breast cancer case-report (Meißner et al., 2017). It was reported to be tolerated as germline-mutation before being appended by driver TP53-mutation (Meißner et al., 2017).

Endometrioid and serous UCEC comprise of distinctly treated low and high grade tumors, respectively. Generally, unlike endometrioid UCEC, serous cancers bear TP53-mutations. However, TP53-mutated high grade endometrioid tumors have been justified to behave and be treated as serous (Colombo et al., 2016). We found high grade endometrioid tumors bearing truncated TP53 with POLE and/or MSI (Group-

A) indicated favourable prognosis unlike missense TP53 (except Y220C). In other words, we identified, for the first time, that TP53-truncated but not TP53-missense cases could prognostically benefit from overlapping deleterious POLE and/or MSI. Additionally, GOF-TP53 Y220C had 'POLE-like' prognosis even in absence of POLE-mutation. POLE- and MSI-overlap with WT-TP53 also behaved 'POLE-like'. Our findings might help to identify 'POLE-like' molecular profiles as well as those overriding the 'POLE-effect', potentially increasing treatment precision. Nevertheless, our study included only *in silico* analyses of TCGA-UCEC dataset which was based only on cases from USA and may not be generalized to LMIC. Hence, further validations by studying LMIC-based cohorts are essential. Around 40% of the cases within prognostically favourable POLE-like groups were adjuvant-treated and 60% were adjuvant-untreated. No difference was found in their prognosis supporting that adjuvant therapy did not confound prognostic behaviour of POLE-like groups.

Clinically, patients from 'POLE-like' groups may not require adjuvant therapy despite TP53-mutation. Our findings may have major implications in devising treatment protocols for TP53-mutated cases in LMICs whereby some proportion of TP53 mutated women may have favourable prognosis and may not need adjuvant therapy. Indication of TP53 sequencing at around 1/5th of treatment cost may prove to be cost-beneficial with reduced morbidity for the individual patient vis-à-vis challenges of adjuvant therapy in LMIC (Varughese and Richman, 2010). The subset availing de-escalated treatment would be 10.156% (Group-E = 3.125%; Group-F = 3.711%; Group-D = 3.320%), as opposed to the acclaimed 5.08% POLE-EDM mutants without overlapping molecular phenotypes. Our findings may open newer avenues for clinical trials and translational research.

The cost of performing molecular profiling based on Next Generation Sequencing might be seen as an impediment for adopting the molecular classification/subgroups in clinical practice. However, a balanced argument or justification may be made for reduction in toxicity from unnecessary treatment, atleast in some women who are paying out-of-pocket for their adjuvant therapy. Ultimately, it depends on individual patient preference whether they would want to pay for a costly test in order to avoid toxicity or complications from an unnecessary treatment.

5. Conclusion

We found that, in WT-TP53 background, deleterious POLE-mutations outside POLE exonuclease domain conferred similarly favourable prognosis as the acclaimed POLE-EDM, and we included both in defining POLE-mutants. We found, for the first time, that TP53-truncated cases with deleterious POLE and/or MSI (Group-A; relevant clinical name: truncated TP53 with POLE and/or MSI), GOF-TP53 Y220C without POLE/MSI (Group-B; relevant clinical name: TP53 Y220C) and, WT-TP53 with overlapping POLE and MSI (Group-F; relevant clinical name: WT-TP53 with POLE and MSI) were prognostically superior to all other TP53-mutated cases irrespective of POLE/MSI (Group-C; relevant clinical name: mutated TP53). Group-A, Group-B (together called Group-E; relevant clinical name: POLE-like mutated TP53) and Group–F were named as 'POLE-like' groups owing to their favourable prognoses like original TCGA-POLE (Group-D; relevant clinical name: POLE). This would double the number of cases eligible for de-escalated treatment. Our findings might tell apart more cases having 'POLE-like' molecular profiles from those with unfavourable profiles, reducing over/under treatment.

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CRediT authorship contribution statement

Damayanti Das Ghosh: Conceptualization, Data curation,

Methodology, Software, Visualization, Investigation, Writing – original draft. **Rahul Roy Chowdhury:** Conceptualization. **Rajeswari Dutta:** Data curation. **Indranil Mukhopadhyay:** Methodology, Software. **Asima Mukhopadhyay:** WrIting and language editing. **Susanta Roychoudhury:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2023.101209.

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