SUPPLEMENTARY MATERIALS

Integration of clinical sequencing and immunohistochemistry for the molecular classification of endometrial carcinoma

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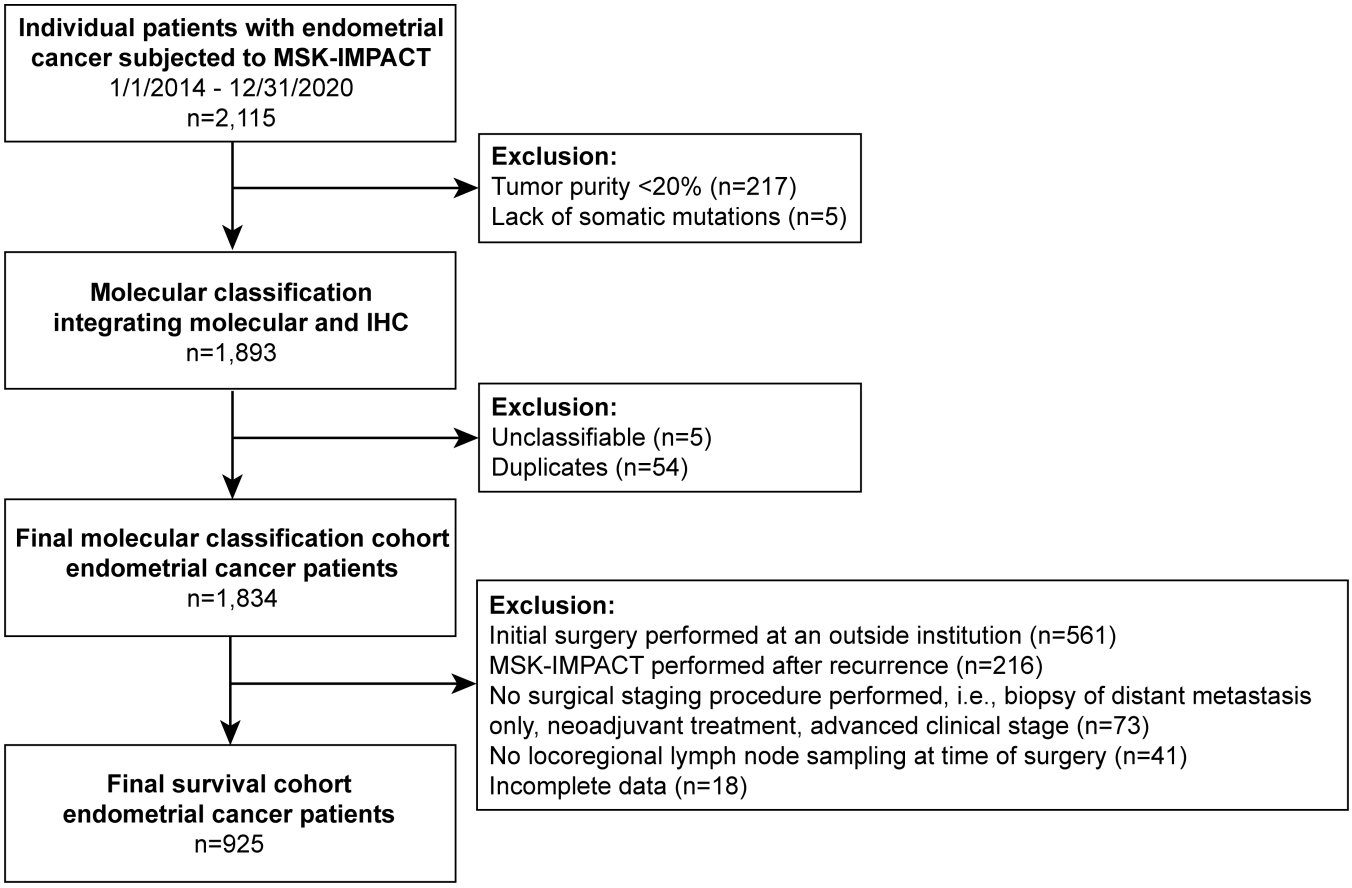
Supplementary Figures S1 – S4

Supplementary Tables S1 - S5

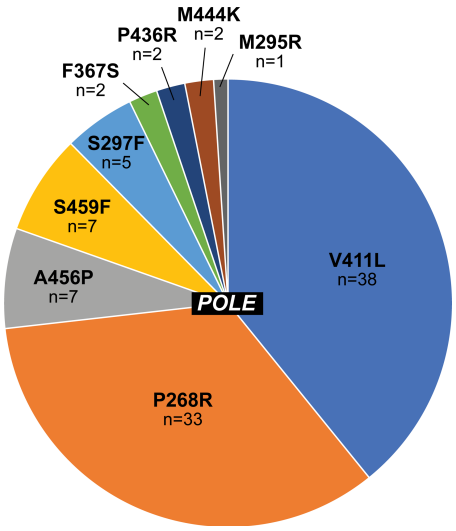
Supplementary Figure S1



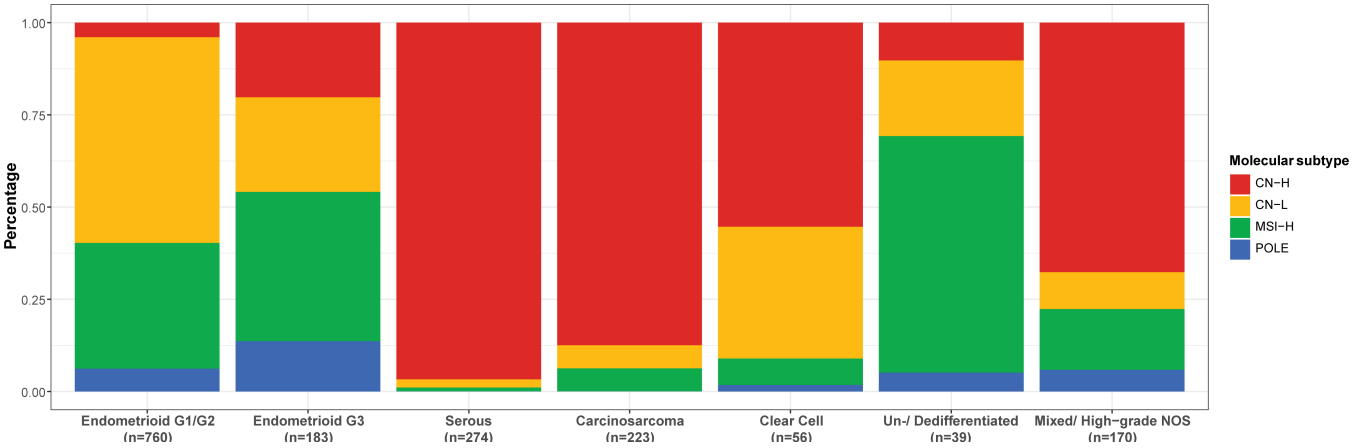
Supplementary Figure S1. CONSORT diagram summarizing the endometrial cancer patients included in the survival analyses.
Cases were eligible for inclusion in the analyses of clinical outcomes if MSK-IMPACT was performed prior to a documented recurrence and if treatment planning with upfront surgical staging was performed at our institution.

Supplementary S2

A

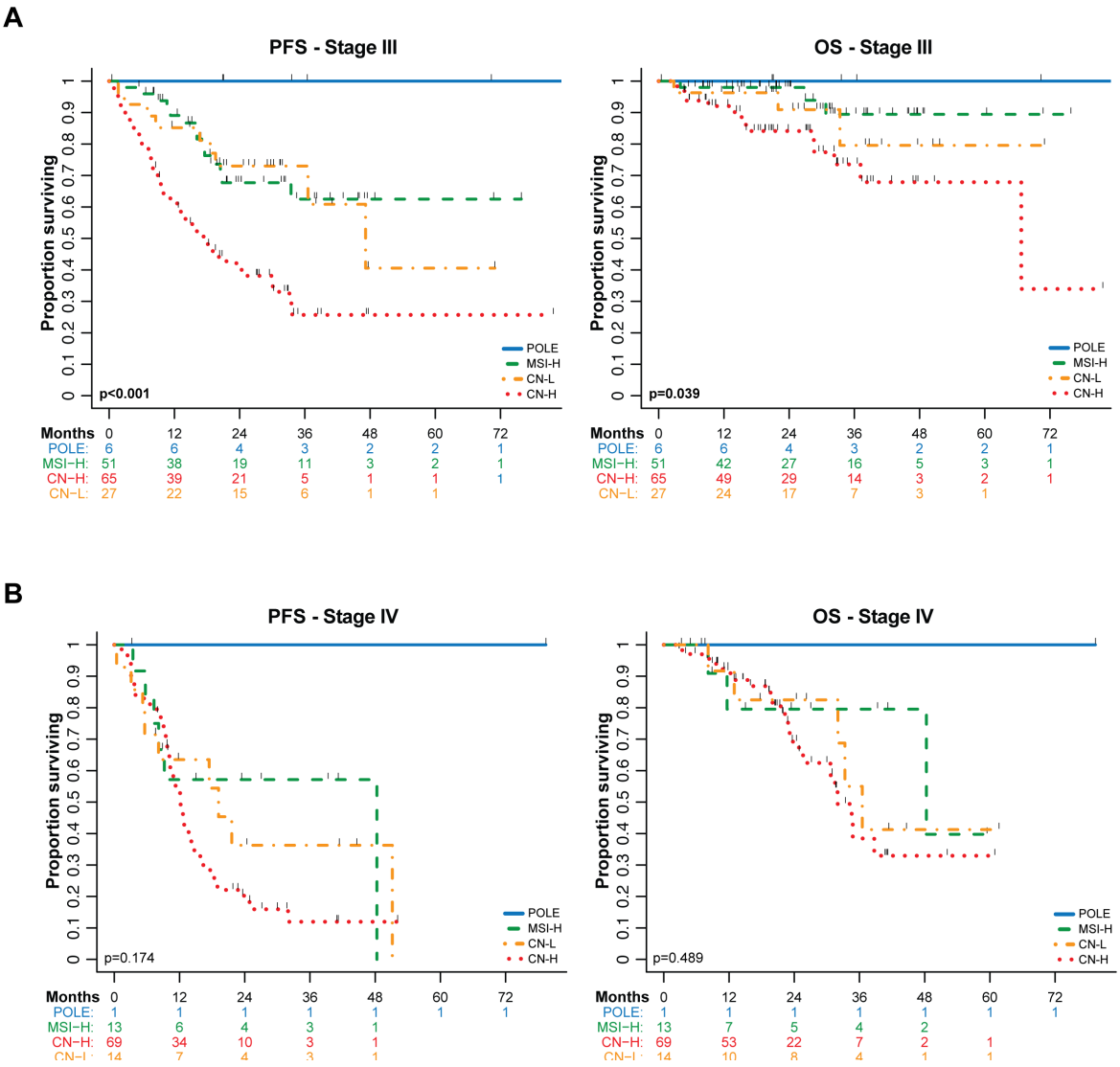


B



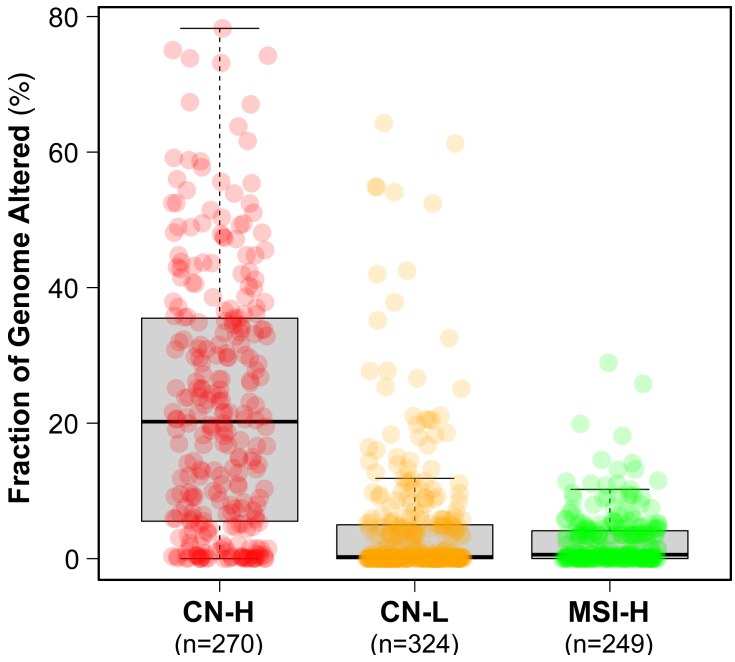
Supplementary Figure S2: Distribution of *POLE* mutations and of molecular subtypes across endometrial cancer histologic types defined using an integrated clinical sequencing - immunohistochemistry approach.
A, Prevalence of somatic *POLE* exonuclease domain hotspot mutations identified in endometrial cancers subjected to clinical tumor-normal sequencing; **B**, Distribution of molecular subtypes across histologic subtypes of endometrial cancer.
CN-H, copy number-high; CN-L, copy number-low; MSI-H, microsatellite instability-high; NOS, not otherwise specified.

Supplementary Figure S3



Supplementary Figure S3. Survival outcomes of endometrial cancer patients with stage III and IV disease by molecular subtype.
Survival was assessed in patients with endometrial cancer of all histologic types whose tumors were subjected to MSK-IMPACT prior to recurrence, had upfront surgery, were surgically staged, and had surgery performed at our institution. **A.** Kaplan-Meier curves comparing progression-free survival (PFS) and overall survival (OS) in all endometrial cancer patients with stage III disease by molecular subtype. **B.** PFS and OS of endometrial cancer patients with stage IV disease by molecular subtype. Survival compared with log-rank test. p-values defined excluding the *POLE* molecular subtype due to the lack of events. CN-H, copy number-high; CN-L, copy number-low; MSI-H, microsatellite instability-high.

Supplementary Figure S4



Supplementary Figure S4. Fraction of genome altered in endometrial cancers of different molecular subtypes.
The fraction of genome altered inferred from MSK-IMPACT sequencing of copy number-high (CN-H), copy number-low (CN-L) and microsatellite instability-high (MSI-H) endometrial cancers of the survival cohort (n=925) is shown.

Supplementary Table S1. Agreement between our integrated clinical sequencing – immunohistochemistry-based approach and the ProMisE algorithm for molecular classification of endometrial cancers.

	Integrated clinical sequencing and immunohistochemistry					
ProMisE	***CN-H***	***CN-L***	***MSI-H***	***POLE***	***Unclassified***	**Total**
CN-H	365	0	2	0	0	367
CN-L	25	324	6	0	0	355
MSI-H	0	0	389	0	0	389
POLE	0	0	0	97	0	97
Unclassified	344	251	31	0	0	626
Total	734	575	428	97	0	**1,834**
					Cohen's Kappa 0.555, 95% CI 0.531-0.579	
ProMisE (without unclassified)	***CN-H***	***CN-L***	***MSI-H***	***POLE***	**Total**	
CN-H	365	0	2	0	367	
CN-L	25	324	6	0	355	
MSI-H	0	0	389	0	389	
POLE	0	0	0	97	97	
Total	390	324	397	97	**1,208**	
					Cohen's Kappa 0.962, 95% CI 0.949-0.975	

CI, confidence interval; CN-H, copy number-high; CN-L, copy number-low; MSI-H, microsatellite instability-high.

Supplementary Table S2. Distribution of histology, stage, and molecular subtype amongst primary and recurrent endometrial cancers subjected to MSK-IMPACT sequencing and molecular subtyping.

	Primary Tumor n=1,501	**Recurrent Tumor n=323**	**p value**
Histology, n (%)			0.24
Endometrioid	815 (54)	161 (50)	
Serous	216 (14)	58 (18)	
Clear Cell	43 (3)	13 (4)	
Carcinosarcoma	181 (12)	42 (13)	
Dediff./ Undiff.	35 (2)	4 (1)	
Mixed/ high-grade NOS	144 (10)	26 (8)	
Other	67 (5)	19 (6)	
Endometrioid Grade, n (%)	418 (53)	55 (37)	**<0.001**
1	224 (28)	63 (43)	
2	153 (19)	30 (20)	
3			
Stage, n (%)			0.09
I	804 (59)	152 (54)	
II	53 (4)	19 (7)	
III	275 (20)	66 (23)	
IV	241 (18)	47 (17)	
Molecular Subtype, n (%)			**<0.001**
POLE	93 (6)	3 (1)	
MSI-H	355 (24)	73 (23)	
CN-H	590 (39)	139 (43)	
CN-L	463 (31)	108 (33)	

CN-H, copy number-high; CN-L, copy number-low; MSI-H, microsatellite instability-high; NOS, not otherwise specified.

Supplementary Table S3. Clinico-pathologic and molecular characteristics of endometrial cancers according to molecular subtype for the survival cohort (n=925).

	Total	POLE n=82	MSI-H n=249	CN-H n=270	CN-L n=324	p value*
Histology, n (%)						**<0.001**
Endometrioid	595	68 (83)	200 (80)	32 (12)	295 (91)	
Serous	85	0	2 (0.8)	82 (30)	1 (0.3)	
Clear Cell	24	1 (1.2)	3 (1.2)	11 (4.1)	9 (2.8)	
Carcinosarcoma	90	0	9 (3.6)	78 (29)	3 (0.9)	
De-/ Undifferentiated	21	1 (1.2)	16 (6.4)	1 (0.4)	3 (0.9)	
Mixed/ high-grade NOS	85	8 (9.8)	18 (7.2)	49 (18)	10 (3.1)	
Other	25	4 (4.9)	1 (0.4)	17 (6.3)	3 (0.9)	
Endometrioid Grade, n (%)						**<0.001**
1	333	23 (35)	84 (43)	12 (40)	214 (73)	
2	166	20 (30)	77(39)	2 (7)	67 (23)	
3	87	23 (35)	36 (18)	16 (53)	12 (4)	
Stage, n (%)						**<0.001**
I	643	72 (88)	173 (69)	121 (45)	277 (85)	
II	36	3 (3.7)	12 (4.8)	15 (5.6)	6 (1.9)	
III	149	6 (7.3)	51 (20)	65 (24)	27 (8.3)	
IV	97	1 (1.2)	13 (5.2)	69 (26)	14 (4.3)	
Adjuvant Treatment†, n (%)						**<0.001**
None	353	30 (37)	89 (36)	32 (12)	202 (62)	
Chemo +/- RT	381	18 (22)	95 (38)	210 (78)	58 (18)	
RT	191	34 (41)	65 (26)	28 (10)	64 (20)	
Surgical Approach, n (%)						**<0.001**
MIS	758	69 (84)	212 (85)	185 (69)	292 (90)	
Open	167	13 (16)	37 (15)	85 (31)	32 (10)	
Age at diagnosis, n (%)						**<0.001**
< 60	343	54 (66)	93 (37)	51 (19)	145 (45)	
≧ 60	582	28 (34)	156 (63)	219 (81)	179 (55)	
BMI kg/m^2, (median, range)	30.4 (16.9-67.6)	26.3 (17.7-41.1)	30.3 (16.9-58.0)	29.8 (17.7-51.1)	32.5 (17.8-67.6)	**<0.001**
TMB (mutations/Mb), median (range)	7.0 (0.0-558.2)	135.2 (19.3-558.2)	29.8 (0.0-397.9)	4.4 (0.9-74.6)	6.1 (0.0-57.1)	**<0.001**
Tumor Purity (%), median (range)	40 (20-90)	40 (20-85)	40 (20-90)	50 (20-90)	30 (20-90)	**<0.001**
Somatic Mutations (n)**,** median (range)	8 (1-634)	150 (22-634)	34 (1-483)	5 (1-85)	7 (1-65)	**<0.001**
Fraction genome altered (%), median (range)	1.5 (0.0-78.3)	0.1 (0.0-10.9)	0.6 (0.0-28.9)	20.2 (0.0-78.3)	0.2 (0.0-64.3)	**<0.001**
MSIsensor score, median (range)	0.2 (0.0-41.9)	0.2 (0.0-20.1)	13.1 (0.0-41.9)	0.3 (0.0-4.2)	0.0 (0.0-7.7)	**<0.001**

* Kruskal-Wallis rank sum test; Fisher's exact test; Fisher's Exact Test for Count Data with simulated p-value (based on 2000 replicates)

† Adjuvant treatment: none, chemotherapy with or without radiation therapy, radiation therapy alone

BMI, body mass index; Chemo, chemotherapy; CN-H, copy number-high; CN-L, copy number-low; MIS, minimally invasive surgery; MSI-H, microsatellite instability-high; RT, radiotherapy; TMB, tumor mutational burden.

Supplementary Table S4. Univariate and multivariate analysis on progression-free survival for patients meeting survival criteria (n=925).

	Total	Univariate HR[1] (95% CI)	p-value[2]	Multivariate HR (95% CI)	p-value
Molecular Classification			**<0.001**		**0.005**
POLE	82	1		-	
MSI-H	249	7.25 (1.75-30.06)		4.76 (1.14-19.9)	
CN-H	270	25.96 (6.42-104.94)		7.87 (1.85-33.4)	
CN-L	324	4.7 (1.12-19.63)		3.84 (0.91-16.2)	
Age (years)			**<0.001**		**0.002**
< 60	343	1		-	
≥ 60	582	2.47 (1.76-3.47)		1.75 (1.22-2.50)	
Stage			**<0.001**		**<0.001**
I	643	1		-	
II	36	1.43 (0.57-3.56)		1.13 (0.44-2.86)	
III	149	4.82 (3.4-6.84)		4.23 (2.77-6.44)	
IV	97	11.09 (7.85-15.66)		7.13 (4.63-11.0)	
Histology			**<0.001**		**0.014**
Endometrioid	595	1		-	
Non-Endometrioid	330	4.69 (3.46-6.36)		1.78 (1.12-2.82)	
Adjuvant Treatment[3]			**<0.001**		0.44
None	348	1		-	
Chemo +/- RT	379	4.03 (2.72-5.96)		0.74 (0.45-1.19)	
RT	191	1.04 (0.58-1.84)		0.92 (0.51-1.65)	
Surgical Approach			**<0.001**		0.85
Minimally Invasive	758	1		-	
Open	167	2.15 (1.59-2.91)		1.03 (0.74-1.44)	
BMI, kg/m^2 (5 unit increase)		0.99 (0.91-1.08)	0.884	-	-

[1] HR = Hazard ratio, CI = confidence interval

[2] p-value obtained by applying Log-rank Test for categorical variables and Wald test based on Cox-Proportional Hazard model for continuous variables; p-value obtained by applying permutation Log-Rank test with 5000 permutation times if events count < 3.

BMI, body mass index; Chemo, chemotherapy; CN-H, copy number-high; CN-L, copy number-low; MSI-H, microsatellite instability-high; RT, radiotherapy.

Supplementary Table S5. Clinico-pathologic characteristics by molecular subtypes for stage I/II endometrioid endometrial carcinoma.

	Total	POLE n=61	MSI-H n=152	CN-H n=23	CN-L n=266	p value
Endometrioid Grade, n (%)						**<0.001**
1	302	21 (36)	71 (47)	12 (57)	198 (75)	
2	130	18 (31)	53 (35)	2 (10)	57 (22)	
3	63	20 (34)	26 (17)	7 (33)	10 (4)	
Stage, n (%)						0.028
I	456	58 (95)	145 (95)	20 (87)	261 (98)	
II	18	3 (5)	7 (5)	3 (13)	5 (2)	
LVSI, n (%)						**<0.001**
Negative	399	40 (66)	111 (73)	18 (78)	230 (86)	
Positive	94	21 (34)	37 (24)	5 (22)	31 (12)	
Suspicious/ Unknown	9	0	4 (3)	0	5 (2)	
Cytology, n (%)						0.78
Negative	430	50 (82)	130 (86)	20 (87)	230 (86)	
Positive	34	4 (6.6)	13 (9.6)	1 (4)	16 (6)	
Suspicious/ Unknown	38	7 (11)	9 (5.9)	2 (9)	20 (8)	
Depth of Invasion, n (%)						**<0.001**
None	253	18 (30)	57 (38)	11 (48)	167 (63)	
< 50%	169	33 (54)	66 (43)	7 (30)	63 (24)	
$\geq$ 50%	80	10 (16)	29 (19)	5 (22)	36 (14)	
Adjuvant Treatment, n (%)						**<0.001**
Chemo +/- RT	47	6 (10)	22 (14)	4 (17)	15 (6)	
RT	144	27 (44)	54 (36)	6 (26)	57 (21)	
None	311	28 (46)	76 (50)	13 (57)	194 (73)	
Surgical Approach, n (%)						0.14
Minimally invasive	456	51 (84)	139 (91)	20 (87)	246 (92)	
Open	46	10 (16)	13 (9)	3 (13)	20 (8)	
Age, n (%)						**<0.001**
< 60 years	234	41 (67)	56 (37)	10 (43)	127 (48)	
$\geq$ 60 years	268	20 (33)	96 (63)	13 (57)	139 (52)	
Recurrence Site, n (%)						0.90
Locoregional	19	2 (100)	7 (64)	4 (80)	6 (86)	
Distant	6	0	4 (36)	1 (20)	1 (14)	

Chemo, chemotherapy; CN-H, copy number-high; CN-L, copy number-low; LVSI, lymphovascular space invasion; MSI-H, microsatellite instability-high; RT, radiotherapy.