Molecular characterization of 361 cases of uterine carcinosarcomas reveal alterations in the DNA repair and PI3K pathways as potential therapeutic targets

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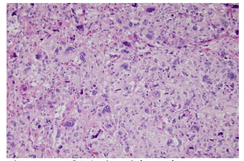
Abstract

Background: Uterine carcinosarcomas (malignant mixed Müllerian tumors or MMMT) are rare endometrial cancers (EC) composed of epithelial and mesenchymal components. MMMTs exhibit aggressive behavior with poor prognosis. We aim to evaluate patterns of molecular, genomic and protein changes in a large cohort of uterine carcinosarcomas and identify potential treatment options.
Methods: 361 out of 3133 (11.5%) of EC submitted to Caris Life Sciences from March 2011 to July 2014, were identified as MMMT. A combination of sequencing (Sanger or next generation sequencing), protein expression (immunohistochemistry), and /or gene amplification (FISH/CISH) was performed.
Results: Of 47 genes sequenced 26 genes were mutated, including TP53 (69%) and PIK3CA (22%). Within the PI3K pathway, there was 12% PTEN mutation, 56.7% PTEN loss, and only 0.7% of tumors had AKT mutation. Other significant findings within tyrosine kinase growth factor pathways include: 13.5% KRAS mutation, 1.7% Her2 overexpression, 4.3% Her2 amplification, and 0.7% mutation rate for ERBB2 and EGFR. Of interest, of the patients who had BRCA genes sequenced, 18.2% (4/22) and 27.3% (6/22) had BRCA1 and 2 mutation, respectively. Also implicated in DNA repair are the loss of ERCC1 (84%) and MGMT (68%) protein expression, suggesting benefit of alkylating agents in a subset of MMMT tumors. Hormonal receptors including ERα, PR, and AR were expressed in 25%, 21%, and 12% of tumors, respectively. Evaluation of the immunomodulatory pathway shows 25% PDL1 and 84% PD1 expression. There is an extremely low rate of cMET pathway alteration (cMET mutation 1.4%, amplification 1.2%, and 4.7% overexpression). Increased TOPO2A expression, associated with anthracycline efficacy, was seen in over 87% of cases.
Conclusions: Our findings contribute to increased understanding of the molecular drivers within this heterogeneic and rare endometrial carcinoma. Within a large cohort of 361 MMMT tumors, we identified potential pathways that warrant further clinical exploration, including those targeting DNA repair, PI3K, and PD1/PDL1 pathways. In addition, alkylating agents and anthracyclines may have benefit in a selected subset of patients.

Background



Firure 1a. H&E Stain, High-grade malignant cells in epithelial and mesenchymal components

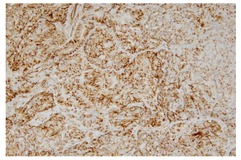


Figure 1b. PD-L1 positive IHC staining.

- Uterine carcinosarcomas (previously known as malignant mixed Müllerian tumor or MMMT) are a rare and aggressive subtype of endometrial cancer that account for less than 5% of all uterine malignancies.[1-2]
- Carcinosarcomas are composed of malignant epithelial (carcinoma) and mesenchymal (sarcoma) components. While traditionally thought to be a sarcoma, it is now categorized as a uterine epithelial carcinoma based on a growing fund of knowledge.
- Uterine carcinosarcomas occur in older women with a median age of diagnosis of 62-67 years and are more common in African American women.[3-4]
- Carcinosarcomas exhibit aggressive behavior with poor prognosis. Despite advancements and aggressive management with surgery and adjuvant therapy, little improvement has been made in terms of overall survival.
- Additional understanding of the molecular and genomic characteristics of MMMTs could offer insight into the pathogenesis of this disease and provide additional treatment options with targeted therapies.

Methods

- 361 out of 3133 (11.5%) of endometrial cancers submitted to Caris Life Sciences from March 2011 to July 2014 were identified as MMMT.
- Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), gene amplification (CISH or FISH), and/or RNA fragment analysis.
- IHC analysis was performed on formalin-fixed paraffin-embedded tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, and AutostainerLink 48, Dako), and commercially available antibodies.
- Fluorescent in-situ hybridization (FISH) was used for evaluation of the HER-2/neu [HER-2/CEP17 probe], EGFR [EGFR/CEP7 probe], and cMET [cMET/CEP7 probe] (Abbott Molecular/Vysis). HER-2/neu and cMET status were also evaluated by chromogenic in-situ hybridization (INFORM HER-2 Dual ISH DNA Probe Cocktail; commercially available cMET and chromosome 7 DIG probe; Ventana). The same scoring system was applied as for FISH.
- Direct sequence analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina MiSeq platform. Specific regions of 47 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel.
- Mutation analysis by Sanger sequencing included selected regions of BRAF, KRAS, NRAS, c-KIT, EGFR, and PIK3CA genes and was performed by using M13-linked PCR primers designed to amplify targeted sequences.
- Retrospective data analysis; Statistical analysis (unpaired t-tests used to compare biomarker expression across histologic subtypes) performed using Prism™ v6. Biomarker associations were calculated by two-tailed Fisher Exact tests.

Results

Figure 2. Pie chart representing the histology of all 3133 cases of endometrial cancer reviewed.

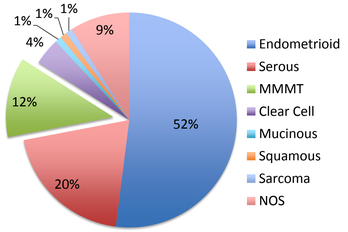
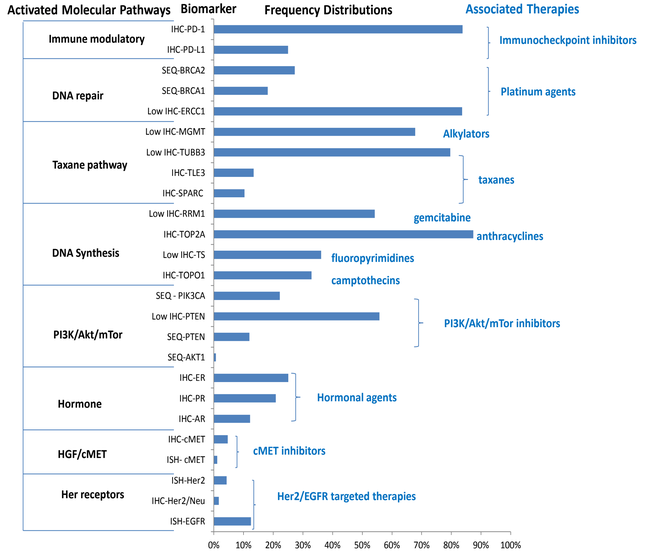


Figure 3. Patient and Tumor Characteristics

Patient Age	N
20-40	N=4
41-60	N=75
61-80	N=254
81-90	N=28
Average	66.9 yrs

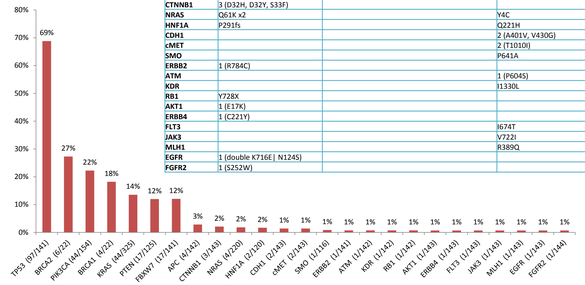
Specimen Site	N
Uterus/endometrium	246
Peritoneal tissue	25
Vagina	14
Pelvis, NOS	13
Lymph Nodes	13
Lung	11
Ovary, Colon, liver and others	39
Total	361

Figure 4. Percentage of molecular and genomic aberration organized by pathway and associated potential therapeutic options.



Results (continued)

Figure 5. Mutation Prevalence in Carcinosacoma



	Pathogenic	Presumed Pathogenic	Variant of unknown significance
TP53	65	24 (C135W x3, D281E x3, K132R x3, 35Y, C277F, G244S, I195T, M237K, M246R, N239S, P278L, P278R, Q144P, R213L, S241F, V157F, Y234C, Y236D)	9 (E171_V172dup, F109_R110del, I195dup, K139_Q144del, S127P, S227C, V143G, V73M, Y234D)
BRCA2			6 (C1290Y, D1920N, Q2384K, S1674G, T3374I, V2728I)
PIK3CA	38 (exon 9 x20; exon 20x18)	4 (C420R x3; N345K)	2 (Y1021H, double R93Q|D350G)
BRCA1	2 (L1561I, R1203X)		2 (R1347G, S1860Y)
KRAS	44 (Codon 12 x36; codon 13 x5; codon 61x3)		
PTEN	14 (truncating/frameshift)	D24Y	4 (C250del, L70del, T321I, V255E)
FBXW7	12 (R465C|H x5, R505C|Gx7)	2 (R505L)	3 (R278G, R505P, R505S)
APC		3 (A1474T, I1307Kx2)	1 (E1317Q)
CTNNB1	3 (D32H, D32Y, S33F)		
NRAS	Q61K x2		Y4C
HNF1A	P291fs		Q221H
CDH1			2 (A401V, V430G)
cMET			2 (T1010I)
SMO			P641A
ERBB2	1 (R784C)		
ATM			1 (P604S)
KDR			I1330L
RB1	Y728X		
AKT1	1 (E17K)		
ERBB4	1 (C221Y)		
FLT3			I674T
JAK3			V722I
MLH1			R389Q
EGFR	1 (double K716E| N124S)		
FGFR2	1 (S252W)		

No mutations found in: ABL1, ALK, CSF1R, FGFR1, GNA11, GNAQ, , NAS, HRAS, IDH1, JAK2, MPL, NOTCH1, NPM1, PDGFRA, PTPN11, RET, SMAD4, SMARCB1, STK11, VHL, cKIT, BRAF

Conclusions

- Our retrospective data analysis of a large cohort (n=361) of uterine carcinosarcomas contributes to the increased understanding of the molecular drivers within this heterogeneic and rare cancer.
- We identified potential pathways that warrant further clinical exploration, including DNA repair, PI3K, and PD1/PDL1 pathways.
- In addition, alkylating agents and anthracyclines may have benefit in a selected subset of patients.
- Prospective studies and additional clinical data are needed to further explore the role of these biomarkers and identify prognostic and therapeutic significance.

References

1. Arend R, Doneza JA, Wright JD. Uterine carcinosarcoma. Curr Opin Oncol 2011; 23:531.
2. Kernochan LE, Garcia RL. Carcinosarcomas (malignant mixed Mullerian tumor) of the uterus: advances in elucidation of biologic and clinical characteristics. J Natl Compr Canc Netw. 2009;7:550-556; quiz 557.
3. Gadducci A, Cosio S, Romanini A, Genazzani AR. The management of patients with uterine sarcoma: a debated clinical challenge. Crit Rev Oncol Hematol 2008; 65:129.
4. Sherman ME, Devesa SS. Analysis of racial differences in incidence, survival, and mortality for malignant tumors of the uterine corpus. Cancer 2003; 98:176.