Uterine leiomyosarcomas exhibit distinct drug resistance molecular profiles compared to extrauterine leiomyosarcomas: A comprehensive analysis of 1,023 leiomyosarcomas

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ABSTRACT

Objectives: Controversy exists as to whether uterine leiomyosarcomas (ULMS) and extrauterine leiomyosarcomas (ELMS) represent distinct pathological and molecular entities. We aim to evaluate molecular, genomic, and protein expression patterns in a large cohort of leiomyosarcomas (LMS) in hopes of identifying novel treatment strategies.

Methods: A total of 1,023 cases of LMS were submitted for molecular analysis from 2009 to 2015, including 635 ULMS and 388 ELMS. Testing included a combination of sequencing (Sanger or next-generation sequencing [NGS]), protein expression (immunohistochemistry), and gene amplification (fluorescence in situ hybridization [ISH]/chromogenic ISH).

Results: The mean age in the LMS cohort was 56.8 years, with 34% of ELMS occurring in men. Figure 1 summarizes molecular and sequencing alterations in ULMS and ELMS. Of the LMS samples evaluated using NGS, *TP53* was most commonly altered (41%), followed by *BRCA2* (6.3%) and *RB1* (4.5%). Evaluating markers of drug resistance, RRM1 expression, associated with gemcitabine resistance, was seen in 36% of ULMS and higher than in EMLS ($P < .0001$). On subanalysis, RRM1-expressing LMS had higher expression of TOP2A ($P < .0001$) and TOPO1 ($P = .0039$), suggesting a potential role for anthracyclines and topotecan in these patients. Significantly more ULMS expressed TUBB3, a marker correlated with taxane resistance (33% ULMS vs 17% ELMS, $P < .0001$). Lower ERCC1 expression was seen in ULMS ($P=.0352$). Hormone receptor expression was frequent in LMS overall (45.2% ER, 34.2% PR and 24% AR), but much more common in ULMS than ELMS: AR ($P = .0014$), ER ($P < .0001$), PR ($P < .0001$). In ER/PR negative LMS, epidermal growth factor receptor overexpression, via immunohistochemistry and ISH, were significantly elevated ($P = .04$, $P = .001$, respectively). Of interest, 28.6% of LMS expressed PDL1 on tumor cells, and 48.6% PD1 protein on tumor-infiltrating lymphocytes. Table 1 summarizes statistically significant differences in biomarker expression profiles between ULMS and ELMS.

Conclusions: Our findings highlight the molecular heterogeneity in LMS, and distinct differences between ULMS and ELMS. Uterine LMS display significantly more biomarkers implicating drug resistance than extrauterine LMS. Of interest, one-third of ULMS expressed proteins associated with gemcitabine and docetaxel resistance. Alternate strategies such as anthracyclines, hormonal therapy, PD-1 inhibitors, and tyrosine kinase inhibitors may be considered as adjuvant therapy.

RESULTS

1,023 cases of LMS were submitted for molecular analysis from 2009 to 2015
- 635 Uterine LMS
- 388 Extrauterine LMS

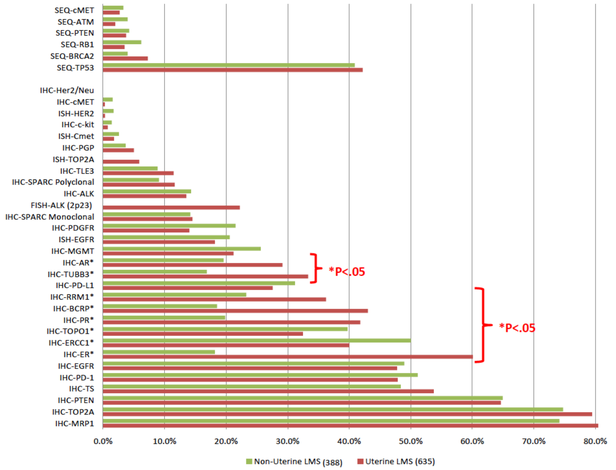
Patent Demographics
- Mean age of LMS cohort 56.8
- 34% of ELMS occurred in men

Sequence Alterations
- No statistical difference between sequencing alterations of ULMS and ELMS
- Most common sequencing alterations among entire cohort:
  - TP53 alterations in 41%
  - BRCA2 alterations in 6.3%
  - RB1 alterations in 4.5%

Table 1: Molecular profile distinctions between Leiomyosarcoma of uterine and extra-uterine origin

	Uterine LMS (n=635)	Extra-Uterine LMS (n=388)	p-values
Hormone Receptors			
IHC-ER	60.1%	18.2%	<0.0001
IHC-PR	41.8%	19.8%	<0.0001
IHC-AR	29.1%	19.6%	0.0014
DNA Repair			
IHC-ERCC1	40.0%	50.0%	0.0352
DNA Replication			
IHC-TUBB3	33.3%	16.9%	<0.0001
IHC-TOPO1	32.5%	39.7%	0.0412
Drug Resistance Associated Proteins			
IHC-BCRP	43.0%	18.5%	0.0032
Other			
IHC-RRM1	36.2%	23.3%	<0.0001
Immunomodulatory Checkpoints			
IHC- PDL1	27.5%	31.2%	0.5692
IHC-PD1	47.9%	51.1%	0.6923

Figure 1: Biomarkers and Sequence Alteration Frequencies



Hormone receptor expression more common in ULMS than ELMS
- In ER/PR negative LMS, epidermal growth factor receptor overexpression noted

ULMS with lower ERCC1 expression than ELMS
- ERCC1 part of nucleotide excision repair pathway
  - Suggests ULMS may have increased sensitivity to platinum agents

Higher expression of TUBB3 in ULMS compared to ELMS
- Class III beta-tubulin - hypothesize TUBB3 may increase dynamic instability of microtubules
  - Correlated with taxane resistance

Higher RRM1 expression in ULMS compared to ELMS
- Gemcitabine diphosphate functions by inhibiting ribonucleotide reductase, enzyme required for synthesis of dNTPs
  - Increased expression associated with gemcitabine resistance
- On subanalysis, RRM1-expressing LMS had higher expression of TOP2A and TOPO1
  - Suggesting a potential role for anthracyclines and topotecan in these patients

Among entire LMS cohort, 28.6% expressed PDL1 on tumor cells, and 48.6% PD1 protein on tumor-infiltrating lymphocytes
- Potential role for PD-1 inhibitors

INTRODUCTION

- Leiomyosarcomas (LMS) comprise 25% of all soft tissue sarcomas with the uterus, retroperitoneum, and extremities representing the most common primary anatomical sites.
- Only recently ULMS has been studied uniquely in clinical trials separate from other gynecological sarcomas or soft tissue sarcomas of other sites
- Controversy exists as to whether uterine LMS and extrauterine LMS represent distinct biological and clinical entities.
- Observational studies have demonstrated that ULMS responds differently to adjuvant treatment compared to ELMS

METHODS

- Retrospective data analysis was done on uterine leiomyosarcoma (ULMS) and extrauterine leiomyosarcoma (ELMS) cases that were submitted to a commercial referral diagnostic laboratory (Caris Life Sciences, Phoenix, AZ) for molecular profiling aimed to provide therapeutic information based on tumor biomarkers.
- Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS), protein expression (IHC)and gene amplification (CISH or FISH).
- 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 insitu hybridization (INFORM HER-2 Dual ISH DNA Probe Cocktail; commercially available cMET and chromosome 7 DIG probe; Ventana).
- 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

CONCLUSIONS

- LMS demonstrate considerable molecular heterogeneity
- Although there are similarities in the molecular, genomic, and protein expression patterns of ULMS and ELMS, there are important and potentially clinically significant differences between the two.
- Uterine LMS display significantly more biomarkers implicating drug resistance than extrauterine LMS.
- Of interest, one-third of ULMS expressed proteins associated with gemcitabine and docetaxel resistance.
- Alternate strategies such as anthracyclines, hormonal therapy, PD-1 inhibitors, and tyrosine kinase inhibitors may be considered as adjuvant therapy.