Predictive biomarker profiling of > 1,900 sarcomas: Identification of potential novel treatment modalities

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Abstract #10509

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Introduction

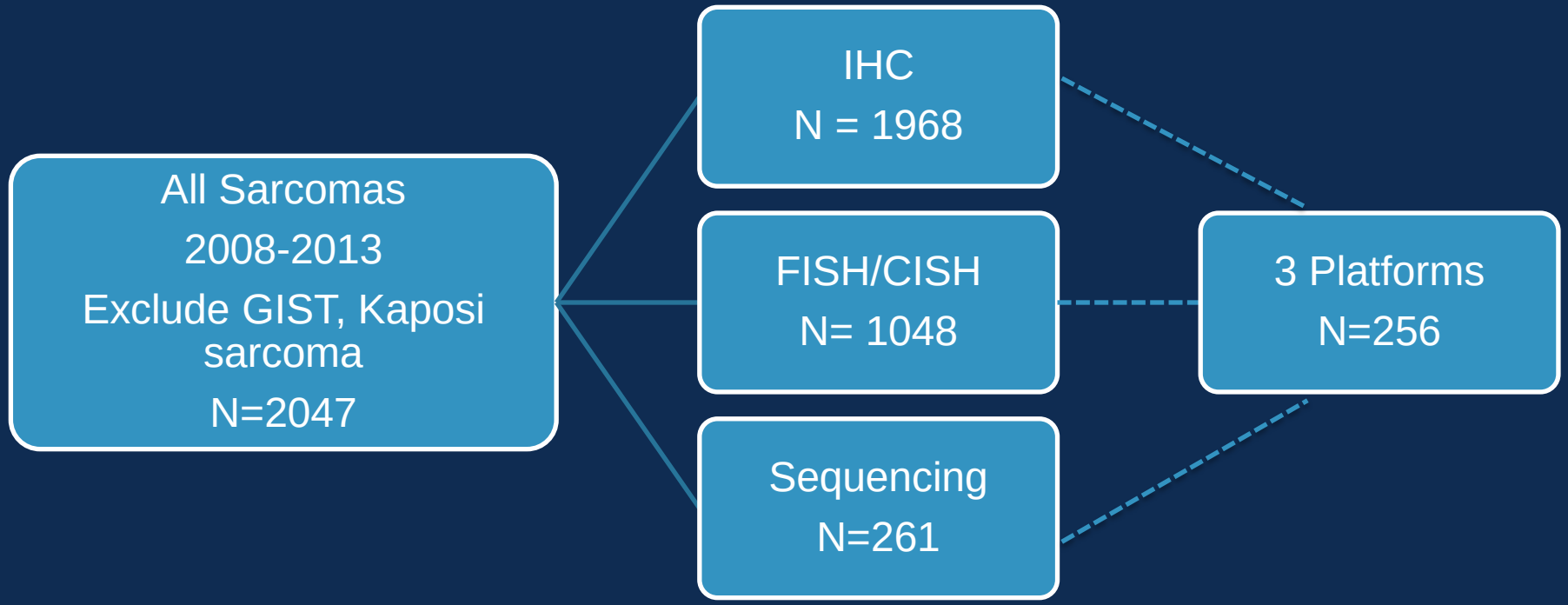
- Sarcomas are rare, heterogeneous tumors
- Predictive biomarkers may help direct the optimal selection of therapy
- Identification of new therapeutic targets is needed

Methods

- Multiplatform profiling at Caris Life Sciences, CLIA certified, specimen reviewed by Board certified pathologists
- Formalin-fixed paraffin-embedded samples
  - Immunohistochemistry
    - 21 protein panel
    - Standard thresholds specific to each antibody
  - Fluorescence/Chromogenic *in situ hybridization* (FISH/CISH)
    - Detect gene amplifications
    - 7 gene panel
    - Standard scoring systems applied
  - DNA Sequencing (Next generation sequencing or Sanger)
    - Somatic mutations
    - 45 genes
    - Next generation sequencing
      - Illumina MiSeq platform (Illumina TruSeq Amplicon Cancer Hotspot panel)

Results

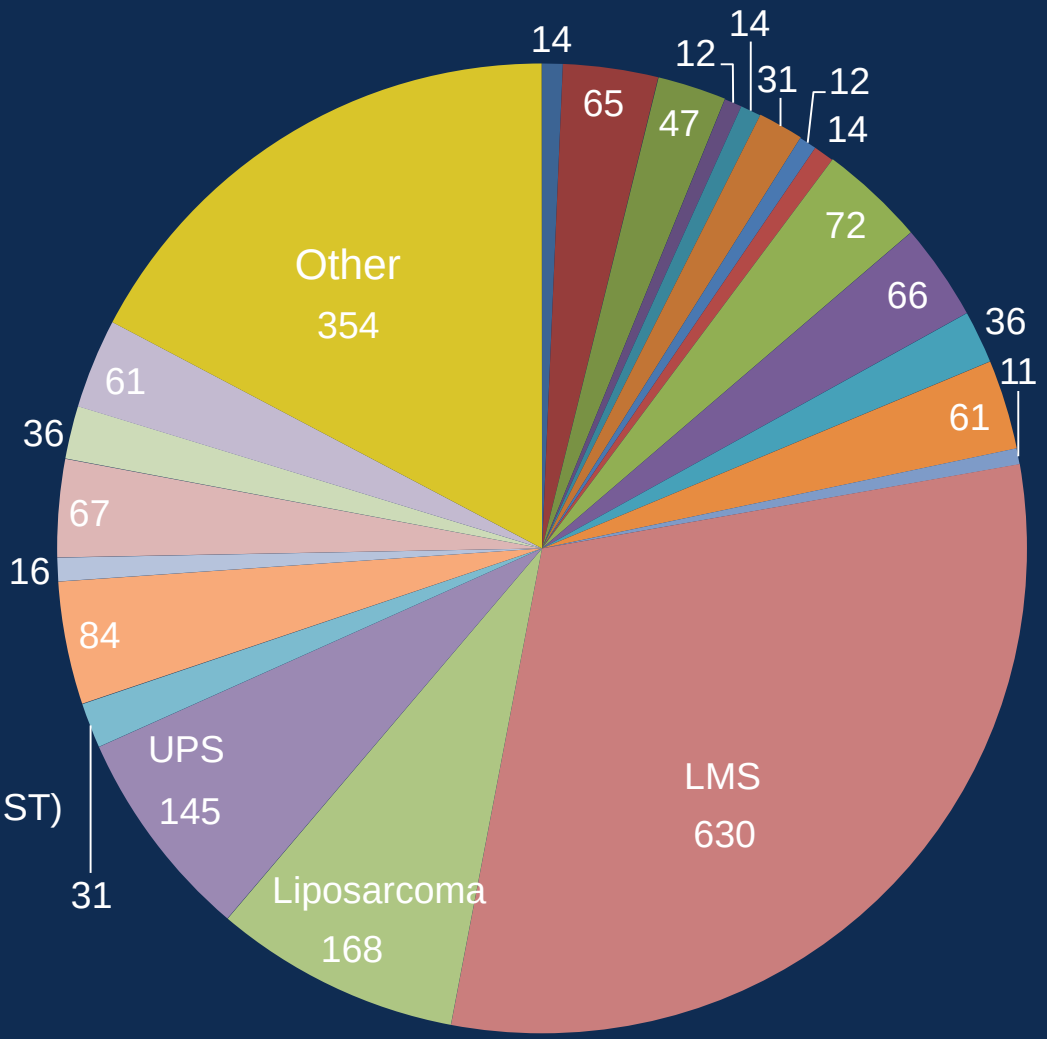
- 713 samples known to be from a metastatic site
- Median age: 55 (range: 1-92)
- 62% female



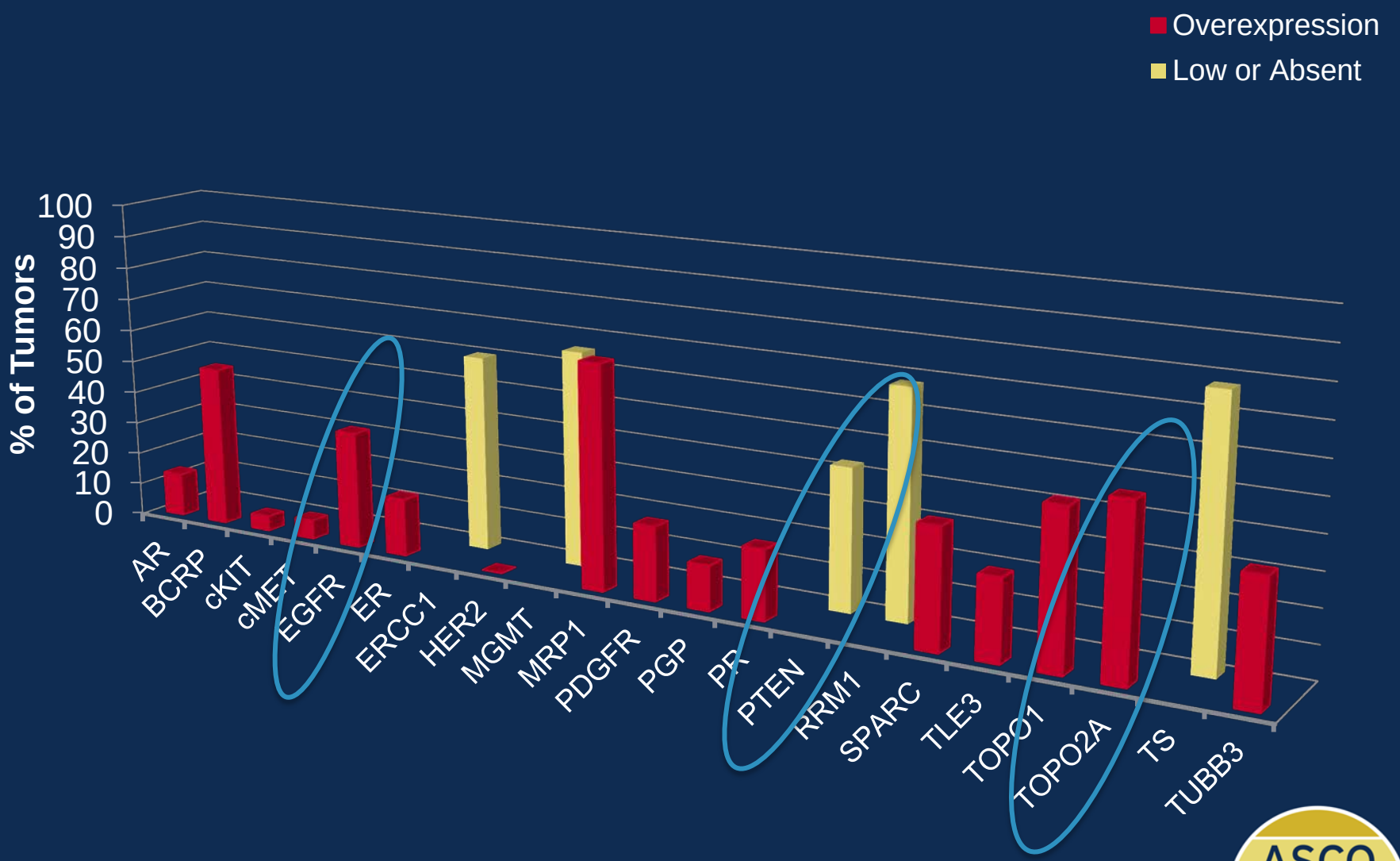
Over 6000 physicians submitted specimens from 59 countries

Results

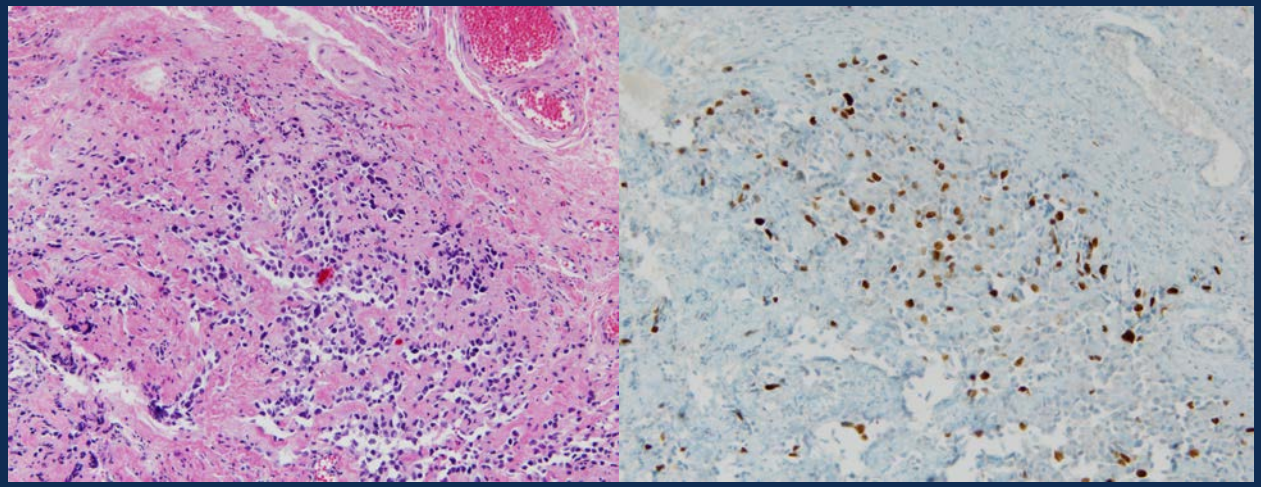
- Alveolar soft part sarcoma (ASPS)
- Angiosarcoma (11=breast)
- Chondrosarcoma
- Chordoma
- Clear cell sarcoma
- Desmoplastic small round cell tumor (DSRCT)
- Epithelioid hemangioendothelioma (EHE)
- Epithelioid sarcoma
- Endometrial stromal sarcoma (ESS)
- Ewing sarcoma
- Fibromatosis
- Fibrosarcoma
- Giant cell tumour
- Leiomyosarcoma (355=uterine)
- Liposarcoma
- Malignant fibrous histiocytoma (MFH/UPS)
- Malignant peripheral nerve sheath tumor (MPNST)
- Osteosarcoma
- Perivascular epithelioid cell tumor (PEComa)
- Rhabdomyosarcoma
- Solitary fibrous tumor (SFT)
- Synovial sarcoma
- Other



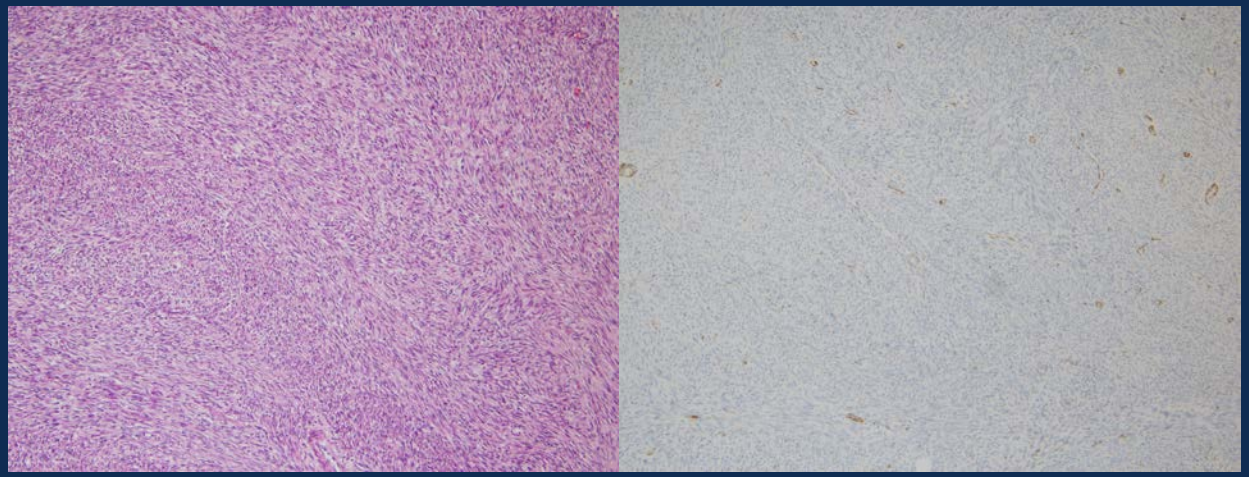
Results (IHC)


Overexpression
Low or Absent
% of Tumors
100
90
80
70
60
50
40
30
20
10
0
AR
BCRP
cKIT
cMET
EGFR
ER
ERCC1
HER2
MGMT
MRP1
PDGFR
PGP
PR
PTEN
RRM1
SPARC
TLE3
TOPO1
TOPO2A
TS
TUBB3

Results



TOPO2A overexpression



PTEN loss

Results, % IHC+, by histology

Histology	N	MGMT*	RRM1*	SPARC	TOPO2A
Angiosarcoma	64	48.4	39.0	53.1	63.8
Chondrosarcoma	47	70.2	20.5	51.1	14.3
EHE	12	16.7	27.3	66.7	0.0
Epithelioid sarcoma	14	46.2	38.5	30.8	15.4
Fibromatosis	34	3.2	10.7	48.5	0.0
LMS	610	22.8	33.3	30.7	62.0
Liposarcoma	158	40.0	15.8	35.4	31.4
MFH/UPS	140	24.8	25.6	36.6	63.9
Osteosarcoma	80	29.1	38.2	47.6	48.6

*Expression of the biomarker below the threshold is considered predictive of a positive response to therapy
Rates were tested using resampling tests (10,000 permutations)

Results, % IHC+, by histology

Histology	N	AR	cKIT	cMET	ERα	PDGFRA	PTEN*
Angiosarcoma	64	0.0	28.6	9.5	0.0	46.7	50.8
Clear cell sarcoma	12	0.0	0.0	50.0	0.0	50.0	63.6
Chondrosarcoma	47	23.9	4.3	9.1	0.0	40.0	63.8
DSRCT	30	40.0	19.0	11.1	0.0	7.7	50.0
EHE	12	8.3	0.0	0.0	0.0	33.3	75.0
Epithelioid sarcoma	14	0.0	0.0	0.0	0.0	25.0	23.1
ESS	71	28.2	1.8	5.9	46.5	40.0	78.9
Ewing sarcoma	63	3.6	37.3	25.0	5.4	31.8	41.7
LMS	610	22.4	1.1	3.9	43.2	15.4	59.2
Osteosarcoma	80	2.6	0.0	0.0	0.0	27.8	29.6
PEComa	16	12.5	0.0	0.0	25.0	0.0	81.3
Rhabdomyosarcoma	64	8.5	9.3	15.0	3.3	17.4	41.0

*Expression of the biomarker below the threshold is considered predictive of a positive response to therapy
NT=not tested; Lowlighted cells - less than 10 cases tested
Rates were tested using resampling tests (10,000 permutations)

Results, PD-1/PD-L1 expression

Sarcoma subtype	N (33)	PD-1 expression/hpf (TILs)	PD-L1 (tumor cells)	Concurrent PD-1 and PD-L1 expression
Liposarcoma	20	45%	100%	45%
Chondrosarcoma	9	11%	100%	11%
Extraskeletal myxoid chondrosarcoma	3	0%	67%	0%
Uterine sarcoma	1	0%	100%	0%

Results (FISH/CISH)

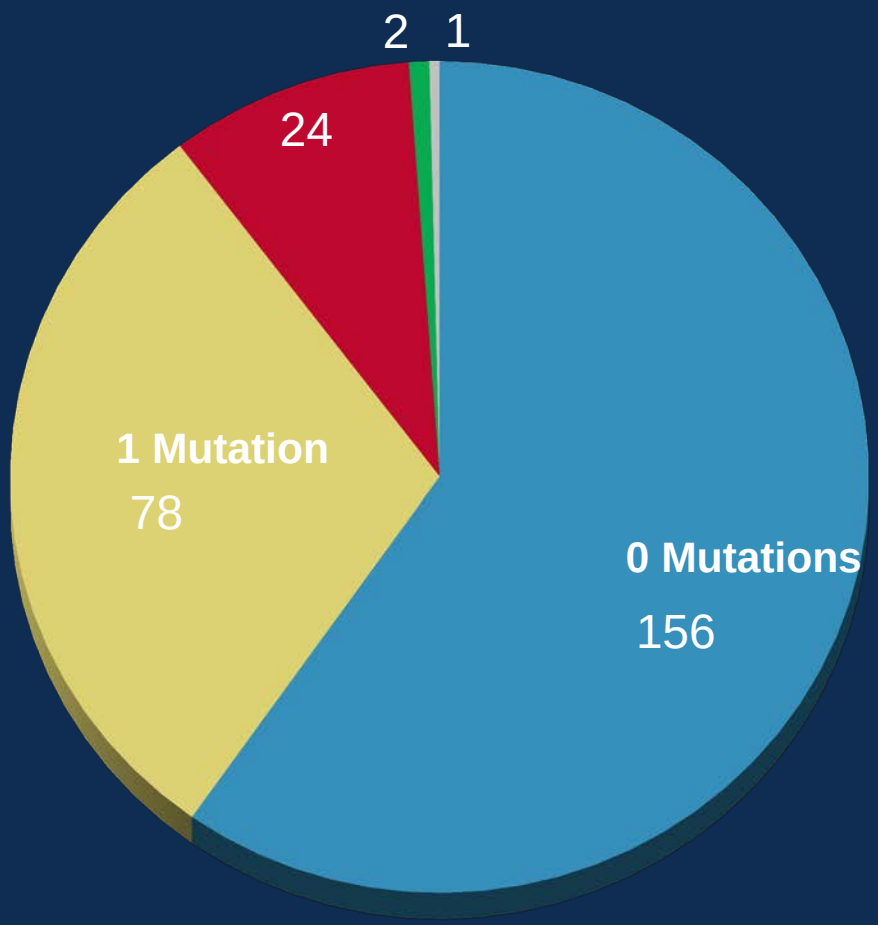
Assay	Total	Normal	Amplified	% Amplified
cMET	431	414	17	3.9
cMYC	18	17	1	5.6
EGFR	1048	872	176	16.8
HER2	573	565	8	1.4
TOP2A	107	105	2	1.9

Results, FISH, EGFR amplification

EGFR Amplification	Histology
> 5%	Chondrosarcoma ESS Ewing sarcoma
> 10%	Fibrosarcoma Liposarcoma Rhabdomyosarcoma
≥ 20%	LMS MPNST Osteosarcoma UPS

Results (Sequencing)

N = 261



Results (Sequencing)

Gene	APC	ATM	BRAF	cKIT	cMET	CTNNB1	IDH1	JAK3	KRAS	NRAS	PIK3CA	PTEN	RB1	STK11	TP53
Total Tested	261	258	542	394	260	261	261	260	1473	365	333	249	258	247	254
WildType	254	252	534	389	254	255	257	257	1454	362	323	241	252	243	197
Mutated	7	6	8	5	6	6	4	3	19	3	10	8	6	4	57
% Mutated	2.7	2.3	1.5	1.3	2.3	2.3	1.5	1.2	1.3	0.8	3.0	3.2	2.3	1.6	22.4

Only 1 mutant found: ABL1, AKT1, AKT1, FGFR2, FLT3, GNA11, KDR, MLH1, SMARCB1, SMO
No mutations found: ALK, CDH1, CSF1R, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, GNAQ, GNAS, HRAS, JAK2, MPL, NOTCH1, NPM1, PDGFRA, PTPN11, SMAD4, VHL

Results, % mutated by histology

Histology	N, NGS	APC	ATM	BRAF	cKIT	cMET	CTNNB1	IDH1	JAK3	KRAS	NRAS	PIK3CA	PTEN	RB1	STK11	TP53
Angio (all)	15	13.3	6.7	10.0	0.0	6.7	0.0	0.0	0.0	5.8	13.3	0.0	6.7	0.0	0.0	26.7
Chondro	12	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	16.7	0.0	0.0	25.0
LMS (all)	44	2.3	0.0	0.0	0.0	4.5	0.0	0.0	0.0	0.0	0.0	1.6	7.1	7.0	2.5	41.5
Liposarcoma	30	0.0	3.3	2.1	0.0	3.3	0.0	0.0	3.3	0.0	0.0	5.6	3.6	0.0	3.7	13.3
UPS	24	0.0	0.0	2.4	3.1	0.0	0.0	4.2	0.0	2.7	0.0	3.8	0.0	4.2	0.0	34.8
Synovial sarcoma	10	0.0	10.0	0.0	11.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

BRAF, KIT, KRAS, NRAS, PIK3CA include Sanger and NGS test results

Results, % mutated, by histology, rare sarcomas

Histology	N, NGS	APC	ATM	BRAF	cKIT	cMET	CTNNB1	IDH1	JAK3	KRAS	NRAS	PIK3CA	PTEN	RB1	STK11	TP53
ESS	4	NT	NT	0.0	0.0	NT	NT	NT	NT	6.1	0.0	0.0	NT	NT	NT	NT
Fibromatosis	7	14.3	0.0	0.0	0.0	0.0	85.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.3	0.0
Fibrosarcoma	7	0.0	14.3	0.0	0.0	0.0	0.0	0.0	0.0	6.1	0.0	6.7	16.7	0.0	0.0	28.6
MPNST	9	0.0	0.0	7.1	0.0	0.0	0.0	0.0	0.0	3.8	0.0	0.0	0.0	0.0	0.0	11.1
Giant cell tumor	3	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhabdo	9	0.0	0.0	4.2	0.0	0.0	0.0	0.0	0.0	2.4	0.0	10.0	0.0	0.0	0.0	11.1

BRAF, KIT, KRAS, NRAS, PIK3CA include Sanger and NGS test results
NT=not tested

Results - Sequencing Summary

- Mutations with frequency ≥ 5%
  - Synovial sarcoma and ATM, cKIT
  - Angiosarcoma and BRAF, APC, NRAS, ATM, cMET, KRAS, PTEN
  - Chondrosarcoma and IDH1, PTEN
  - Liposarcoma and PIK3CA
  - LMS and PTEN, RB1

Results

	N positive concordance/N	IHC+(%)	FISH amplified (%)	Mutation (%)
CKIT	0/22	60/1393 (4.3)	NA	5/394 (1.3)
CMET	0/424 (IHC/ISH) 1/260 (IHC/NGS)	33/561 (5.9)	17/431 (3.9)	6/260 (2.3)
EGFR	2/43 (IHC/ISH) 0/195 (IHC/NGS)	70/195 (35.9)	176/1048 (16.8)	0/280 (0)
HER2	0/561(IHC/ISH) 0/243 (IHC/NGS)	1/1950 (0.05)	8/573 (1.4)	0/250 (0)
PDGFR	NA	128/571 (22.4)	NA	0/260 (0)
PTEN	3/243	Loss 816/1907 (42.8)	NA	8/249 (3.2)
TOPO2A	0/34	844/1666 (50.7)	2/107 (1.9)	NA

Results

	PTEN Loss IHC	TOPO2A IHC+	PTEN MT	cMET MT	IDH MT	CTNNB1 MT	APC MT	KRAS MT
TP53wt	24/197 (12.2%)	98/182 (53.8%)	5/192 (2.6%)	2/201 (1.0%)	1/202 (0.5%)	6/202 (3.0%)	5/202 (2.5%)	6/201 (3.0%)
TP53 mutated	10/51 (19.6%)	41/50 (82%)	3/52 (2.6%)	4/52 (7.7%)	3/52 (5.8%)	0/52 (0)	2/52 (3.8%)	0/52 (0)
P value	0.17	0.0003	0.37	0.03	0.03	0.35	0.63	0.35

	TP53 MT	PTEN Loss IHC	TOPO2A IHC+	PTEN MT
PIK3CA mutated	3/7 (42.9%; 1LMS, 1 lipo)	1/10 (10.0%)	7/8 (87.5%)	0/6 (0)
PIK3CA WT	54/243 (22.2%)	39/316 (12.3%)	136/229 (59.4%)	2/240 (0.8%)
P value	0.40	1.0	0.15	1.0

Limitations

- Subtype of sarcoma extracted from paperwork submitted by treating physician
  - "Sarcoma, NOS"
- Limited clinical information regarding:
  - Site of tumor (primary vs. metastatic)
  - Treatment history

Conclusions

- TOPO2A is overexpressed in approximately 50% of sarcomas, without associated gene amplification
  - Most commonly in angiosarcoma, LMS, UPS
- SPARC is overexpressed in angiosarcoma, chondrosarcoma, EHE and osteosarcoma

Conclusions

- PTEN loss was found in up to 80% of sarcomas, without a high frequency of PTEN mutations noted
- Concordance between EGFR overexpression and EGFR gene amplification was low
  - Gene amplification was highest in LMS, MPNST, osteosarcoma and UPS
- PD-L1 expression was noted in 100% of liposarcomas (mostly dedifferentiated) and 100% of chondrosarcomas

Conclusions

- Profiling through protein expression, gene copy variations and mutations identified alterations in 99% of sarcoma samples
- Future clinical trials are needed to determine the predictive and/or prognostic nature of these findings

Acknowledgements

All of the clinicians and patients who submitted tumor specimens from around the world!