ential therapeutic Genomic alterations in Desmoplastic Small Round Blue Cell Tumor

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Abstract

Background: Desmoplastic Small Round Blue Cell Tumor (DSRCT) originates from a cell with multilineage potential. A molecular hallmark of DSCRT is the EWS-WT1 reciprocal translocation. Ewing's and DSRCT are treated similarly due to similar oncogene activation pathways and DSRCT has been represented in very limited numbers in sarcoma studies.

Methods: Thirty five DSRCT tumors were tested with a multiplatform profiling service (Caris Life Sciences, Phoenix, AZ). Specific tests performed included sequencing (NextGen), protein expression (IHC) and gene amplification (CISH or FISH). Tumor mutational load (TML) was calculated as somatic nonsynonymous missense mutations sequenced with a 592gene panel. Molecular alterations were compared to 88 Ewing sarcomas (ES). Chisquare tests were used for comparison and statistical significance was determined as $p < 0.05$.

Results: In the 35 DSRCT tumors, high expression of TOP2A were seen in 63%, TOPO1 in 63%, PTEN in 62%, androgen receptor (AR) in 59%, EGFR in 42% of tumors; low expression of TUBB3 was seen in 44%, MGMT in 45%, TS in 48%, RRM1 in 57% and ERCC1 in 76% of tumors. When compared to ES, no significant difference was seen in protein expressions with the exception of a significantly higher overexpressson of AR in DSRCT (59% vs. 3%, p = 1.7E10) and TUBB3 (56% vs. 29%, p = 0.03). Tumor expression of PDL1 (Ab: SP142) was not seen in the 4 DSRCT and 10 ES tested. NextGen revealed a TP53 mutation (7%) and a FOXO3 mutation (L382fs) in DSCRT, while 6 TP53 mutations (13%), 2 APC mutations (L1129S and I1307K), 1 BRCA1(c.301+1G > A) and 1 CTNNB1 (T41A) mutation were identified in ES. Tumor mutational load evaluated in the 3 DSRCT and 11 ES tumors averaged 6 and 5 mutations per megabase, respectively.

Conclusions: Molecular profiling on 35 DSRCT tumors and comparison with Ewing's sarcoma revealed low immunogenicity (< 10 Mutations/MB) and low frequency of actionable mutations including PDL1 in both tumor types. High AR expression

Background

- Desmoplastic small round cell tumor (DSRCT) is a highly aggressive and rare mesenchymal tumor, around 200-450 cases have been so described so far [1,2]
- Despite aggressive therapy, median survival ranges from 17 to 25 month[2,8] a 5-year survival rate remains around 15%[8] with higher survival reported among those undergoing removal of at least 90% of tumor absence of extraperitoneal metastasis[7]
- Almost 100% of these tumors contain t(11;22) (p13;q12) translocation it is likely that EWS/WT1 functions as a transcription factor, possibly through WT1 targets [1,2,3,5]
- It has a predilection of developing in the abdominal and pelvic cavity of young adult men in 88-97% of cases [2,3]
- Despite aggressive therapy, median survival ranges from 17 to 25 months [2,5]
- While there is no standard protocol for this aggressive disease, treatment usually includes Neoadjuvant HD P6 regimen (High-dose cyclophosphamide, doxorubicin, and vincristine (HD-CAV) alternating with ifosfamide and etoposide (IE) chemotherapy combined with aggressive attempted R0 resection [6,7]
- We aimed to investigate the molecular characteristics of 35 DSRCT tumors tested by IHC, ISH and mutational tests and compare that with Ewing's sarcoma in order to explore therapeutic opportunities for this extremely rare and aggressive cancer type.

Method

- IHC, ISH and NextGen sequencing were performed on full slides of formalin-fixed paraffin-embedded (FFPE) tumor samples.
- All tests were optimized and validated, and met the standards and requirements of the Clinical Laboratory Improvement Amendments/College of American Pathologists and the International Organization for Standardization.
- The primary antibody used against PD-L1 was SP142 (Spring Biosciences). The staining was regarded as positive if its intensity on the membrane of the tumor cells was >=2+ (on a semiquantitative scale of 0–3: 0 for no staining, 1+ for weak staining, 2+ for moderate staining, or 3+ for strong staining) and the percentage of positively stained cells was >5%.
- Antibody used for AR is AR27 and for TUBB3 is polyclonal. Cutoff used is 1+, 10% and 2+, 30%, respectively.
- DNA from formalin-fixed paraffin-embedded samples was sequenced using the Illumina NextSeq (Agilent SureSelect XT, 592 gene selected based on COSMIC database) and MiSeq (TruSeq, 47 gene) to evaluate mutation and gene amplification.

Result

Table1: Distribution of specimen sites that the DSRCT and ES tumors were taken from. A total of 35 and 88 tumors were tested with molecular profiling, respectively

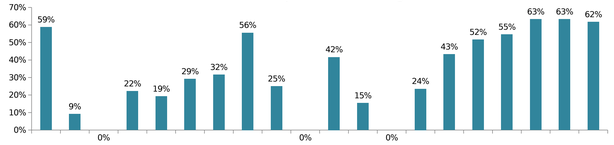
		DSRCT		Ewing's Sarcoma
Specimen Site (n)	Connective tissue	7	Bone	30
	Peritoneum	5	Lung	25
	Abdomen	5	Connective tissue	15
	Liver	4	CNS	5
	N/A	3	Intestine	3
	Lymph Nodes	2	Muscle	2
	Omentum	2	Abdomen	1
	Colon	2	Chest	1
	Lung	2	Kidney	1
	Uterus	1	Liver	1
	Small Intestine	1	Lymph Node	1
	Pelvis	1	Other	3
Total		35		88

Result

Table 2: Basic demographic information of patients included in the study. While 86% of DSRCT tumors were taken fro male patients, only 57% of ES tumors were taken from male patients (p=0.003). Average age of DSRCT and ES patients were not significantly different.

	DSRCT		Ewing's Sarcoma	
Gender	Female	Male	Female	Male
N	5	30	38	50
Total N	35		88	
Average Age (years old)	33.8	29.9	28.0	28.0
	30.4		28.0	

Figure 1: Results of 20 immunohistochemistry markers seen in the 35 DSRCT tumors tested. Sample size for each test is indicated in the preference. Bars shown indicate the incidence of positive staining found.



- Among hormone receptors, androgen receptor shows the highest expression frequency.
- PD-L1 expression on tumor cells is not seen (0 of 4)while PD-1 staining on tumor-infiltrating lymphocytes is seen in 25% tumors tested (2 of 8)

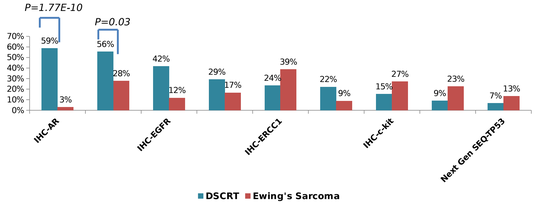
Table 3: Additional molecular findings in DSRCT tumors.

		Positive N	Total N tested	Percent
NextGen Sequencing	TP53	1 (G245G)	15	7%
	FOXO3	1 (L382fs)	3	33%
Copy Number Variation	CCND1	1	3	33%
in situ hybridization	EGFR	0	6	0%
	cMET	0	17	0%
	Her2	0	22	**0%**

- 12 tumors were sequenced with 45-gene panel and 3 tumors were sequenced with 592-gene panel.
- Only pathogenic or presumed pathogenic mutations seen were one TP53 mutation (G245S) and 1 FOXO3 mutation (L382fs)
- The three tumors with 592-gene panel carried tumor mutational load of 4, 6 and 8 mutations/megabase.

Result

Figure 2: Comparison of selected biomarker features of DSRCT (N=35) tumors and ES (N=88)tumors investigated. Shown are biomarkers with incidences that are more than 50% different between the two tumor types. Connective lines indicate statistical significance by Fisher-Exact test ($p<0.05$).



- Significantly higher overexpressson of AR in DSRCT (59% vs. 3%, p = 1.7E10) and TUBB3 (56% vs. 29%, p = 0.03) were seen
- Similar to DSRCT, PD-L1 expression on tumor cells is absent in ES; PD-1 expression on TIL was seen in 32% (6 of 19) ES tumors.
- Tumor mutational load was calculate in 11 ES tumors: mean TML was 5 mutations/megabase (range 3-8).

Conclusions

- We investigated 35 tumors of the extremely rare and highly aggressive tumors of DSRCT for molecular alterations.
- We identified high expression of topoisomerase expressions including TOP2A and TOP1, high expression of androgen receptor expression as well as low expression of PD-L1 expression. This supports the use of TOPO2 inhibitors including anthracyclines and etoposide in DSRCT treatment.
- Induction neoadjuvant HD Alkylator and Anthracycline based chemotherapy followed by Maximal resection and consolidation with HIPEC, IMRT leads to better outcomes and is supported by biomarker data
- Our molecular results on PD-L1 expression and tumor mutational load don't support the use of immune checkpoint blockage in DSRCT, however the low patient number precludes a conclusion.
- Small molecule TKI's have shown dismal results so far and have not been represented in trials including Pazopanib approval in the PALETTE trial [9], Eribulin[10] and Trabectedin[11]
- PDGFRa inhibitor (Olartumumab) which is known to be activated in DSRCT was approved in Soft tissue sarcoma (STS) however in the study DSRCT was not represented [12]
- With Significantly higher AR and TUBB3 expression in DSRCT compared to ES tumors suggest androgen-targeted therapy as an interesting option for DSRCT while taxanes may be more effective in Ewing's sarcoma.

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