Molecular profiling of 267 pediatric cancers to identify potential clinically relevant targets

Todd Maney, PhD[1] *, Sherri Z. Millis, PhD[1] *, Josiah Winkler[1], Leonard Sender, MD[2]
*co 1st authorship; [1]Caris Life Sciences, Phoenix, AZ, [2]UC Irvine Medical Center, Orange, CA, Hyundai Cancer Institute, CHOC Children's and UC Irvine CHAO Comprehensive Cancer Center

Abstract #10037

Background: Even though only 1% of cancers occur in children, cancer is the leading cause of death in children. Survival rates depend on the type of cancer, the majority of which arise from the central nervous system, bone, or neuroblasts.
Methods: 267 cases referred to Caris Life Sciences were tested per physician request, including sequencing (Sanger, next generation [NGS]), protein expression (immunohistochemistry [IHC]), gene amplification (CISH or FISH), and/or MGMT methylation. Diagnoses were collected from referring physicians at intake; for this analysis, cases were initially grouped into carcinomas (CA), n=40, sarcomas (SA), n=117, neuroendocrine (NET), n=12, germ line (GL), n=11, or central nervous system (CNS), n=37. Within those groups the specific diagnoses were further delineated. Metastatic pediatric cases were submitted for molecular profiling at Caris Life Sciences between 2008 and 2013. Testing was performed on formalin-fixed, paraffin-embedded tumor samples (fresh samples were not needed) and included a combination of immunohistochemistry (IHC), in situ hybridization (ISH) performed by either fluorescent or chromogenic methods, and Sanger or next-generation sequencing (NGS).

Demographics:

% Female	49
% Male	51
Median Age	12
Age Range	0-17.9
Metastatic?	267 (100%)

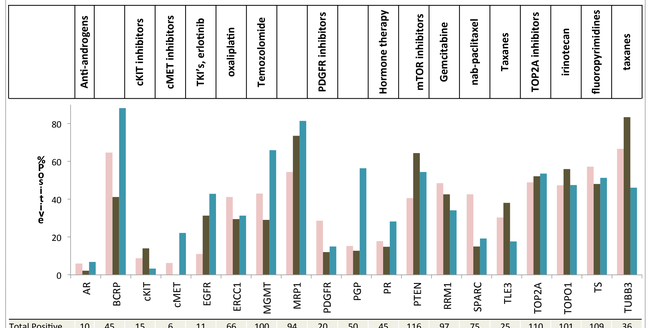
Type of Cancer	# of Patients	% of Patients Profiled
Sarcomas, NET, pNET	131	49%
Central Nervous System	68	25%
Carcinomas	51	19%
Germline	11	4%
Other	6	2%

Carcinomas	# of Patients
Renal, Wilm's	13
Hepatoblastoma, Hepatocellular	10
Adrenal cortical carcinoma	7
CUP	6
Mesothelioma	4
Ovarian serous carcinoma	4
Colorectal adenocarcinoma	3
Pancreatoblastoma	1
Gastric adenocarcinoma	1
Pseudopapillary tumor of the pancreas	1
Choroid plexus carcinoma	1

Results: In this pediatric cohort, biomarker alterations included higher AR protein expression in CA and SA, higher ER expression in CA, GL, and NET, and EGFR amplification in all but GL. MGMT loss was highest in CNS, PTEN loss was highest in GL and both were lowest in CA. PGP was expressed at less than 15% in CNS and SA and 68% in CA. No HER2 protein overexpression, amplification, or gene mutations were seen. TP53 mutations were lowest in SA (9%) and varied between 25 and 50% in the others. Of the gene panel tested, CTNNB1 was mutated in 1 patient in CA and SA, while AKT1, CSFIR, and MPL were mutated in 1 patient each in GL. KRAS was mutated at least once in all but CNS. All other mutations (MT) were exclusive to the CNS group, and included PTEN, SMO, VEGF, ERBB4, EGFR, ALK, and APC. These were specific to the astrocytomas, which also had the only MGMT methylation event, except for ALK MT (neuroblastoma) PTEN MT (medulloblastoma), and EGFR MT (ganglioglioma).
Conclusion: The mutations in the CNS group suggest MEK and mTOR pathway involvement. Biomarker profiling to identify therapeutic targets has potential in pediatric patients and warrants further investigation. Comparison to adult onset of these types of cancers may yield different molecular profiles for a subset of these cancers. Because children typically respond well to chemotherapy, targeting specific molecular alterations identified in childhood cancer could prove very effective.

Results, Molecular Profile

Table 1. Specific biomarker protein expression for all cases, by IHC, and broken out by the 3 most frequently seen subtypes, sarcoma (SA), CNS, and carcinoma (CA).



	AR	BCRP	cKIT	cMET	EGFR	ERCC1	MGMT	MRP1	PDGFR	PGP	PR	PTEN	RRM1	SPARC	TLE3	TOP2A	TOPO1	TS	TUBB3
Total Positive	10	45	15	6	11	66	100	94	20	50	45	116	97	75	25	110	101	109	36
Total Cases, **ALL**	213	75	168	86	42	185	229	143	94	197	221	241	224	245	87	207	210	207	56
Total Positive	6	22	7	2	3	40	48	44	13	16	19	49	54	49	11	51	49	61	14
Total **SA Cases**	111	37	89	36	19	94	112	76	45	95	111	118	112	120	37	102	103	108	20
Total Positive	1	7	6	0	5	13	16	25	3	6	7	38	23	9	8	25	28	24	10
Total **CNS Cases**	47	17	43	20	16	44	55	34	25	47	47	59	54	60	21	48	50	50	12
Total Positive	3	15	1	4	3	10	29	22	3	22	13	25	14	9	3	23	19	20	6
Total **CA Cases**	44	17	30	18	7	32	44	27	20	39	46	46	41	47	17	43	40	39	13

Table 2. Specific biomarker amplification or gene alteration, for all cases, and broken out by the 3 most frequently seen subtypes, sarcoma, CNS, and carcinoma.

No mutations were found in the following genes:
ABL1,ATM,CDH1,cKIT,cMET,ERBB2,FBXW7,FGFR1,FGFR2,FLT3,GNA11,GNAQ,GNAS,HNF1A,HRAS,IDH1, IDH2,JAK2,JAK3,MLH1,NOTCH1,NPM1,NRAS,PDGFRA,PIK3CA,PTPN11,RB1,RET,SMAD4,SMARCB1,STK11,VHL.

		Amplification, FISH				Gene Alteration, Sanger or NGS												Methyl-ation
		ALK	cMET	cMYC	EGFR	AKT1	ALK	APC	BRAF	CSF1R	CTNNB1	EGFR	ERBB4	KDR	KRAS	PTEN	SMO	TP53	MGMT
All Cases	Total Positives	3	2	1	13	1	1	1	5	1	3	3	2	1	3	1	1	6	2
N=267	Total Cases	19	54	17	109	31	33	33	82	33	34	42	33	33	86	29	22	33	7
	% Positive	**15.8**	**3.7**	**5.9**	**11.9**	**3.2**	**3.0**	**3.0**	**6.1**	**3.0**	**8.8**	**7.1**	**6.1**	**3.0**	**3.5**	**3.4**	**4.5**	**18.2**	**28.6**
Sarcomas	Total Positives	1	2	1	5	0	0	0	1	0	1	0	0	0	1	0	0	1	0
N=131	Total Cases	8	22	4	43	11	12	12	28	12	12	14	12	12	44	9	5	12	0
	% Positive	**11.1**	**8.0**	**16.7**	**11.5**	**0.0**	**0.0**	**0.0**	**3.0**	**0.0**	**7.1**	**0.0**	**0.0**	**0.0**	**2.1**	**0.0**	**0.0**	**14.3**	
CNS	Total Positives	2	0	0	6	0	1	1	3	0	0	3	2	1	0	1	1	2	2
N=68	Total Cases	7	14	10	32	9	9	9	22	9	9	14	9	9	14	8	6	9	7
	% Positive	**28.6**	**0.0**	**0.0**	**18.8**	**0.0**	**11.1**	**11.1**	**13.6**	**0.0**	**0.0**	**21.4**	**22.2**	**11.1**	**0.0**	**12.5**	**16.7**	**22.2**	**28.6**
Carcinomas	Total Positives	0	0	0	1	0	0	0	0	0	2	0	0	0	1	0	0	1	0
N=51	Total Cases	2	10	1	17	6	6	6	18	6	7	7	6	6	17	6	6	6	0
	% Positive	**0.0**	**0.0**	**0.0**	**5.9**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**28.6**	**0.0**	**0.0**	**0.0**	**5.9**	**0.0**	**0.0**	**16.7**	

Highlighted columns indicate genes for which clinical therapies are available.

Results, Pediatric CNS Patients

Figure 1. Distribution of CNS subtypes. The median age was 9 and the gender distribution was 44% female, 56% male.

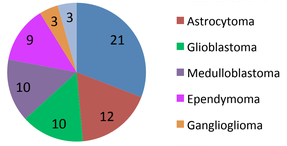
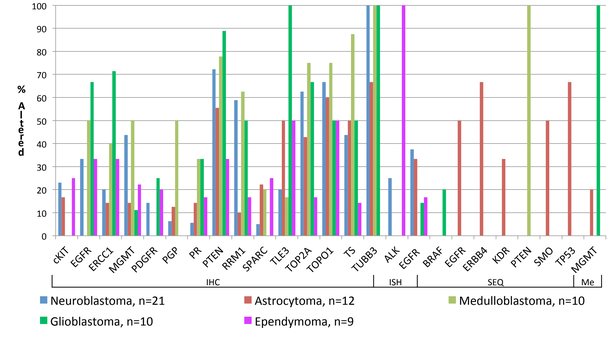


Figure 2. Biomarker alteration frequencies for each subtype. Ganglioglioma and 'other' not shown. A ganglioma case had an EGFR mutation, and one of the 'other' had a BRAF mutation.



Results, Pediatric Sarcoma Patients

Figure 3. Distribution of sarcoma subtypes. The median age was 12 and the gender distribution was 47% female, 53% male.

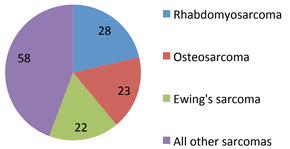


Figure 4. Biomarker alteration frequencies for each subtype.

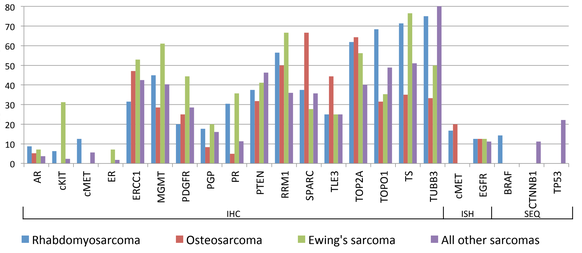


Table 3. Examples of guided therapy recommendations based on multi-platform molecular profiling.

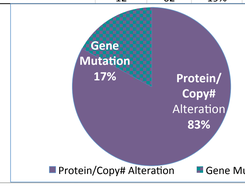
Single Therapy

Biomarker	Technology	Drug or Clinical Trial	Cases w/ Target	Cases Total	Percent Cases	Category
EGFR	IHC, ISH, DNA Seq.	Monoclonal antibodies: cetuximab Small molecule inhibitors: erlotinib, gefitinib, afatinib, and lapatinib	5	16	31.0%	CNS
			3	7	42.9%	Carcinoma
			2	18	11.1%	Sarcoma
SPARC	IHC	nab-paclitaxel	75	245	30%	ALL
MEK	DNA Seq.	AZD6244 selumetinib, trametinib	6	86	7%	ALL
AKT/PIK3CA, PTEN	IHC, ISH, DNA Seq.	Sirolimus, temsirolimus, everolimus, MK-2206	125	241	52%	ALL, PTEN
			1	6	17%	ALL, PIK3CA
PDGFR, cKIT	IHC, ISH, DNA Seq.	Imatinib , sunitinib	18	168	9%	ALL
BRAF	DNA Seq.	vemurafenib, dabrafenib, clinical trials	5	82	6%	ALL
TUBB3	IHC	Vinorelbine, taxanes	7	13	54%	Carcinoma
VEGFR	DNA Seq.	PTC299, cediranib, pazopanib, bevacizumab	1	9	11%	CNS
ERCC1	IHC	cisplatin, carboplatin, oxaliplatin	119	185	64%	ALL
Androgen receptor	IHC	Anti-androgens	10	213	5%	ALL

Combination Therapies

Biomarker	Technology	Drugs/Clinical Trial	Cases w/ Target	Cases Total	Percent Cases	Category
EGFR/MGMT	multiple	erlotinib, gefitinib, afatinib, lapatinib WITH temozolomide, dacarbazine	7	62	11%	CNS
			12	62	19%	All

Figure 5. Comparison of single technology vs. multiple technologies in identifying actionable biomarker changes. Gene mutations were identified in only 17% of cases; the other 83% of cases would have had no actionable recommendations without IHC and ISH testing.



Conclusions

- Molecular profiling using a multi-platform approach provides more actionable results; 83% more cases had actionable recommendations when also evaluated for protein expression and gene copy number.
- The addition of molecular profiling to help guide therapeutic decisions beyond the organ of origin could be of high value in pediatric cancers, as most drugs are not approved for pediatric patients.
- Using a rational approach based on biomarker status to identify clinical trials and off-label options in relapsed pediatric cancer patients may increase survival and may be a useful tool for tumor boards.
- Pediatric clinical trials would be enhanced with the use of biomarker status in inclusion/exclusion criteria, especially for targeted therapies, based on NCCN guidelines for other cancer types.
- PD-1 and PD-L1 protein overexpression was not seen in 25 of 25 neuroblastomas evaluated, indicating that PD-1 and PD-L1-directed immunotherapy may not benefit these patients
- High BCRP, MRP1 and PGP in pediatric carcinomas suggest resistance to chemotherapies.

References

1. Cabanas, et al. Prolonged use of nimotuzumab in children with central nervous system tumors: safety and feasibility. Cancer Biother Radiopharm. 2014 May;29(4):173-8.
2. Russell, et al. Biomarkers of Pediatric Brain Tumors. Front Pediatr. 2013 Mar 21;1:7.