Molecular Profiling of Sarcomas – Comprehensive analysis on 5 cases of Ewings' sarcoma from a tertiary care cancer centre

Vidya H Veldore, Pramod S. Chinder, Ajai Kumar BS | Department of Musculoskeletal Oncology, HCG, Bangalore, India

PROBLEM STATEMENT

- Ewing sarcoma belongs to the group of neoplasms commonly referred to as small, round, blue-cell tumors of child-hood. CD99 positivity by IHC suggests Ewings sarcoma diagnosis in correlation with other clinical and pathologicparam-eters. CD99 is also positive in GIST, NHL and Synovial Sarcoma, hence not a unique marker for Ewings. Hence there is a diagnostic need for unique molecular marker for supporting Ewings Sarcoma diagnosis.

- 80% of the Ewings' Sarcoma recurr within 2 years of diagnosis and the prog-nosis is poor with a 5 year survival of less than 20%. There is no standardized second-line treatment for relapsed or refractory Ewing sarcoma. Therefore developing novel therapy options is the need of time.

INTRODUCTION

Standard Treatment Options for Ewings Sarcoma (ES):

- Multidrug chemotherapy: vincristine, doxorubicin, ifosfamide, and etopo side.
- Radical resection
- Myeloablative therapy? Better than standard chemotherapy
- Allogenic stem cell transplantation compared to autologous ??
- Radiation

Some of the promosing clinical trials on ES in last decade:

- Topotecan+ cyclophosphamide:
  1. Saylors RL et al J Clin Oncol 19 (15): 3463-9, 2001, (Pediatric Oncology Group phase II study) (6/17: 35% responders)
  2. Hunold A et al Pediatr Blood Cancer 47(6): 795-800, 2006. (16/49: 33% responders)

- Temozolomide+Irinotecan: 20 patients Casey DA, Pediatr Blood Cancer 53 (6): 1029-34, 2009. (MSKCC) (25% Complete response, 25% partial response)

- Personalized targeted therapy: IGF1R monoclonal antibody: 10% response in recurrent disease. Temsirolimus (mTOR inhibitor)+IGF1R antibody (Figitumumab): Phase 1 Trial: 2 complete response (11.7%) and 3 partial response (17.6%) Juergens H et al: J Clin Oncol 29 (34): 4534-40, 2011.

Further molecular characterization of the tumors may add value to the response rate by selecting patients who would likely benefit from targeted therapy. We report here our observational findings on genomic profiling of 5 recurrent cases of ES. The choice of technology and the gene panel was focused to arrive at novel targeted therapy options, arriving at new diagnostic, prognostic and predictive markers for the treatment management of the ES disease.

METHODOLOGY

- Tumor genomic DNA isolation and Targeted Re-sequencing of tumors from 5 recurrent cases of Ewings' sarcoma

- Platform and Gene Panel: Ion Torrent Next-Gen-eration Sequencing platform (NGS) (Life Tech-nologies, Carlsbad, CA)

Ion AmpliSeq™ Cancer Panel 1.0

KRAS	BRAF	EGFR	TP53	PIK3CA	CSF1R	JAK2
NRAS	PTPN11	ERBB2	SRC	FGFR3	NPM1	CDKN2A
RET	HNF1A	SMAD4	GNAS	PDGFRA	MPL	ABL1
PTEN	FLT3	STK11	SMARCB1	KIT	MET	NOTCH1
FGFR2	RB1	JAK3	VHL	KDR	SMO	
HRAS	AKT1	ALK	MLH1	FBXW7	ERBB4	
ATM	CDH1	IDH1	CTNNB1	APC	FGFR1	

46 genes ↓ 190 amplicons ↓ 739 mutations

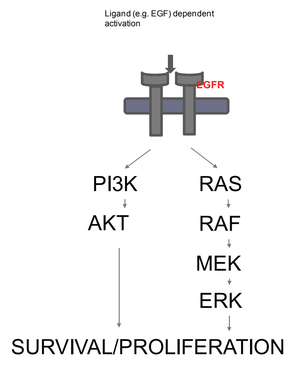
- Median depth of coverage of 1200X with Q20 accuracy. Facilitates detection of rare somatic mutations, insertions and deletions

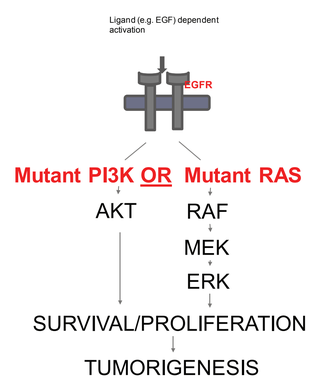
- Sequence Analysis: Torrent Suite Software (TSS), version 2.0

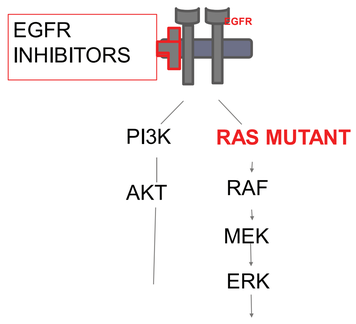
RESULTS

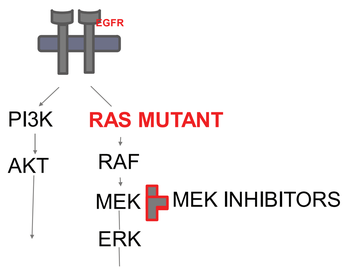
Results / Key Findings : EGFR, FGFR, RET, MET and APC pathways were altered in all the 5 cases with pathogenic mutations

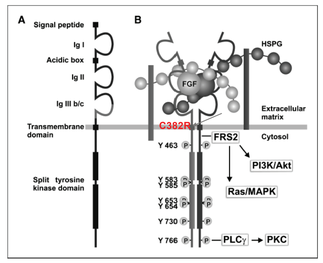
- A homozygous, germline polymorphism in APC gene (COSM19349) which was earlier identified in FAP was a unique observation in all the Ewings' tumors - possible role of genetic predisposition/monoclonality in tumor population
- Somatic mutation C382R (COSM36906) in FGFR2 gene in its transmembrane domain
- Q61R(COSM583;COSM584;COSM582;COSM12725;COSM579) mutation in NRAS
- E545D (COSM27374;COSM765) in PI3KCA
- Mutations in RET, MET and TP53 genes, although at low frequency











TAKE HOME MESSAGE:

- There is a potential role for EGFR and MEK inhibitors for ES management, which requires clinical validation
- There are several new drugs in clinical trials focused on the inhibition of PI3K/AKT, which could be studied further in Ewings sarcoma
- Many FGFR inhibitors are currently being evaluated in clinical trials for several other tumors, considering the presence of recurrent FGFR mutations, there seems to be a great potential for FGFR inhibitors in ES management in the near future.

CONCLUSION / FUTURE DIRECTIONS

Ewings Sarcoma genesis and relapse is a highly complex mechanism. Genome sequencing of tumor from different stages of the disease is very important to understand the biology of recurrence. This pilot study was aimed to identify unique SNP or any specific mutation signature in a panel of genes which marks the identity for Ewings Sarcoma/recurrent ewings sarcoma. These mutation signatures once validated on a larger cohort could become useful as surrogate markers to confirm the diagnosis as well as predictive markers for personalized therapy and management.