Application of NGS-guided Precision Medicine in Ewing Sarcoma

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Overview of Ewing Sarcoma

- Epidemiology:
- Annual Incidence (USA): 3.0 cases per 1 million children and adolescents (<20 years).
- Metastasis at Diagnosis: ~25% present with metastases; common sites: lungs, bones, bone marrow.
- Genetic and Molecular Factors: EWSR1-ETS fusions (e.g., EWSR1-FLI1) in >95%; germline predisposition (e.g., FANCC variants) in ~13%.
- Survival Rates: 5-year survival: 74.5% overall; localized disease 84.7%, metastatic disease 50.4%.
- Poor prognosis for relapse/progression.

Next-Generation Sequencing

- Key Advantages in Oncology:

- Comprehensive genomic profiling in single assay
- Detection of multiple alteration types (SNVs, CNVs, fusions)
- Requires small tissue samples

NGS in Diagnosis and Subclassification

- Enhanced Diagnostic Accuracy:
- Gold standard confirmation through fusion detection
- Distinguishes from other small round blue cell tumors
- Identifies rare fusion variants missed by conventional FISH
- Molecularly distinct entities requiring different management
- Critical for appropriate treatment selection

Molecular Subclassification and Risk Stratification

- Proposed Risk Stratification Model:

Genetic Subgroup	Estimated Frequency	Clinical Implications
Low-Risk	~50%	Fusion-only, may benefit from therapy de-escalation (future)
Intermediate-Risk	~30%	`STAG2` mutant, `CDKN2A` mutant
High-Risk	~20%	`TP53` mutant, `STAG2+TP53` co-mutant

From Genomic Data to Actionable Targets

- Direct Targeting of the Fusion Oncoprotein
- Targeting Germline Predispositions and Associated Pathways

- Immunotherapeutic Avenues and Biomarkers:
  - Checkpoint Inhibitors: ES generally has a low tumor mutational burden (TMB) and a cold immune microenvironment, which may explain the limited efficacy of single-agent PD-1/PD-L1 inhibitors observed to date.

From Genomic Data to Actionable Targets

- Novel Immunotherapeutic Strategies:
  - Combination Approaches: Strategies to inflame the tumor microenvironment, such as combining DNA-damaging agents (e.g., chemotherapy, PARP inhibitors) with immunotherapy, are being explored to convert "cold" tumors into "hot" ones.
  - CAR-T and Bispecific Antibodies: Research is ongoing into cell-based therapies targeting surface antigens such as GD2 or IGF-1R, which may offer a targeted immunologic approach

Liquid Biopsy and Minimal Residual Disease Monitoring

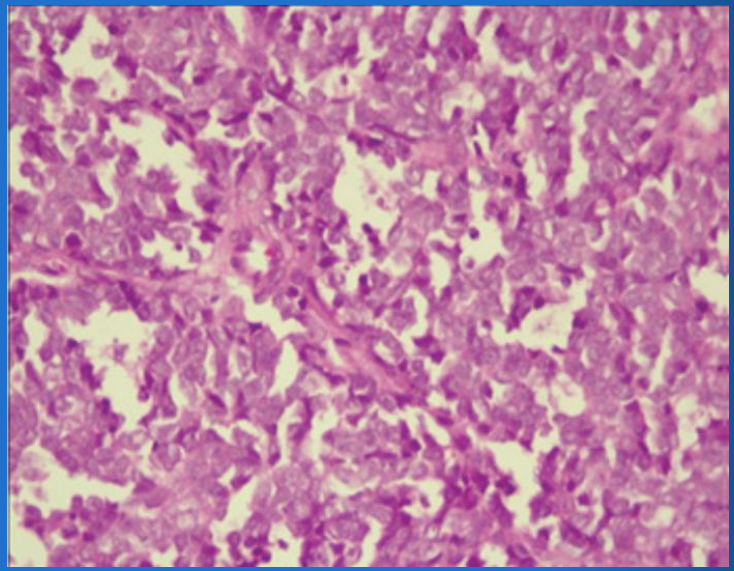
- Circulating Tumor DNA (ctDNA) Analysis:
- Non-invasive approach to monitor tumor dynamics
- Detection of EWSR1-ETS fusions in plasma
- Potential for real-time assessment of treatment response
- Early detection of recurrence before radiographic evidence
- Assessment of molecular response during therapy
- Identification of emerging resistance mutations

Case Presentation

- A 17-year-old male presented with acute lower extremity paraesthesia and gait imbalance.
- His symptoms rapidly progressed over days to include paralysis, bowel and bladder dysfunction.
- MRI revealed a 55 × 43 × 50 mm mass in the right paravertebral musculature at the L3-L4 level.
- Surgical resection via neurosurgical intervention achieved near-total removal of the lesion, which extended into the epidural space.

Case Presentation

- Histopathology sections revealed uniform, undifferentiated cells with scant cytoplasm arranged in islands separated by fibrous strands. Individual cells showed Inconspicuous nucleoli

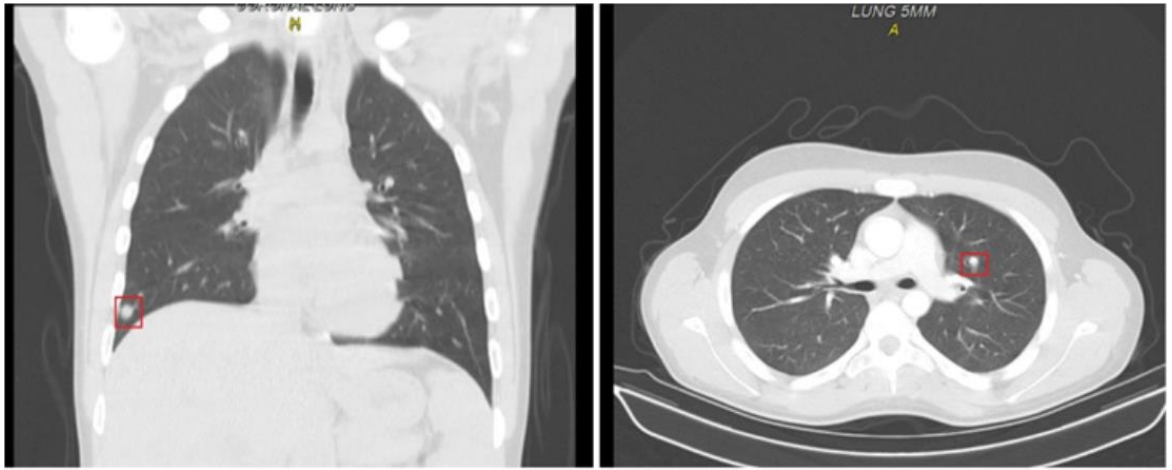


Case Presentation

- IHC markers showed strong membranous CD99 staining and focal positivity for NSE.
- Other markers, such as LC A, CK, S100, Synaptophysin, and Chromogranin, were negative.
- As the next step to confirm the diagnosis of Ewing sarcoma, FISH for t (11;22) was needed, which was not available in our centre.
- Since the patient was a very good Candidate for targeted therapy, an NGS panel that also covered the EWSR1-FLI1 fusion was performed for him.

Case Presentation

One month after surgery, follow-up imaging demonstrated bilateral pulmonary nodules (4 mm - 9 mm) consistent with metastases.



Case Presentation

- Radiotherapy was initiated.
- Upon completion of 25 fractions, systemic chemotherapy commenced according to the Lanzkowsky protocol, which involved alternating cycles of vincristine, Cyclophosphamide, doxorubicin, and Ifosfamide with etoposide.
- After the first cycle, the patient developed vincristine-induced neuropathy, confirmed by EMG/NCV.
- Vincristine was discontinued.

Case Presentation

- To guide further treatment, tumour tissue and blood were sent for comprehensive genomic profiling.

Case Presentation

Mr. Farazmandi Mohammad Navid

M240301422

exacta
ENCYCLOPEDIC TUMOR ANALYSIS

Patient Details

Name	:	Mr. Farazmandi Mohammad Navid
Birth Date	:	06-Jun-2010
Gender	:	Male
Address	:	Iran
Referring Doctor	:	Dr. Ramin Ajami

Specimen Details

Tumor Type	:	Ewing sarcoma
Specimen Type	:	Blood, FFPE Tumor Block
Draw Date	:	12-Mar-2024
Accession Date	:	15-Mar-2024
Report Date	:	04-Apr-2024

Specimen Analysis Summary

Tissue

FFPE Tumor Specimen	:	**50%** Neoplastic Cellularity (1172-I-2 24)
Tumor DNA/RNA	:	511 Genes (SNAs | Indels | CNAs | Fusion Transcripts | TMB)
Sarcoma fusions	:	59 Genes
mRNA	:	20802 Genes
Microsatellite biomarkers	:	BAT-25 | BAT-26 | NR-21 | NR-24 | MONO-27
IHC	:	PD-L1 | AR

Blood

cf Total Nucleic acids	:	52 Genes (SNAs | Indels | CNAs | Fusion Transcripts)
CTC	:	FLI1 | CD45
CTC- ICC	:	mTOR | VEGFR1 | VEGFR2 | VEGFA | EGFR
Chemosensitivity analysis	:	29 Drugs
Pharmacogenetic analysis	:	23 Drugs

07 Clinical Trials	:	Refer to page no. 39 - 40

Case Presentation

Indications	USFDA Approved* / NCCN recommended*	Off Label Therapy*	
TMB - Low 2 Mutations/Mb	⊟ None	⊟ None	
PD-L1-22C3 TPS - <1%	⊟ None	⊟ None	
Microsatellite Status - Stable (MS-S)	--	--	
VEGFR1/FLT1 and VEGFR2/KDR ICC Positive **PDGFRB** Overexpression (+2.34 FC)	☑ Regorafenib	☑ Pazopanib ☑ Axitinib ☑ Ponatinib	☑ Sunitinib ☑ Sorafenib ☑ Tivozanib
VEGFR1/FLT1 and VEGFR2/KDR ICC Positive	☑ Cabozantinib	☑ Lenvatinib	☑ Fruquintinib
PDGFRB Overexpression (+2.34 FC)	⊟ None	☑ Dasatinib	
EIF4EBP2(EBP2) Overexpression (+2.13 FC)	⊟ None	☑ Everolimus	☑ Temsirolimus
VEGFR2/KDR ICC Positive	⊟ None	☑ Vandetanib	☑ Ramucirumab
AR IHC Negative	⊟ None	☒ Enzalutamide ☒ Nilutamide ☒ Darolutamide ☒ Flutamide	☒ Bicalutamide ☒ Apalutamide ☒ Leuprolide ☒ Abiraterone

☑ SOC Drugs with Benefit ☑ Off Label Drugs with Benefit ☒ Drugs without Clinical Benefit / with Potential Resistance

TMB: Tumor mutation burden; TPS: Tumor Proportion Score; ICC: Immunocytochemistry; FC: Fold change; IHC: Immunohistochemistry; CTC: Circulating Tumor Cells; SOC: Standard of Care; NCCN: National Comprehensive Cancer Network - Bone Cancer, Ewing sarcoma.

Case Presentation

Chemosensitivity Analysis : % Cell Death (CD) ± Molecular biomarker

USFDA Approved / NCCN recommended	
Drugs	**Result**
☑ Doxorubicin	70% CD; TOP2A (+2.34 FC)
☑ Cyclophosphamide	65% CD
☑ Etoposide	61% CD; TOP2A (+2.34 FC)
☑ Ifosfamide	57% CD
☑ Temozolomide	52% CD
☑ Gemcitabine	32% CD
☑ Carboplatin	29% CD
☑ Vincristine	26% CD; TUBB overexpression
☒ Dactinomycin	<25% CD
☒ Docetaxel	<25% CD
☒ Irinotecan	<25% CD
☒ Topotecan	<25% CD

Off Label Therapy	
Drugs	**Result**
☑ Bleomycin	69% CD
☑ Mitoxantrone	63% CD
☑ Cisplatin	61% CD
☑ Paclitaxel	50% CD; TUBB overexpression
☑ Pemetrexed	49% CD
☑ 5-Fluorouracil/Capecitabine	42% CD
☑ Cabazitaxel	41% CD; TUBB overexpression
☑ Oxaliplatin	41% CD
☑ Epirubicin	39% CD; TOP2A (+2.34 FC)
☑ Mitomycin	34% CD
☑ Eribulin	31% CD; TUBB overexpression
☑ Dacarbazine	28% CD
☒ Melphalan	<25% CD
☒ Methotrexate	<25% CD
☒ Trabectedin	<25% CD
☒ Vinblastine	<25% CD
☒ Vinorelbine	<25% CD

Case Presentation

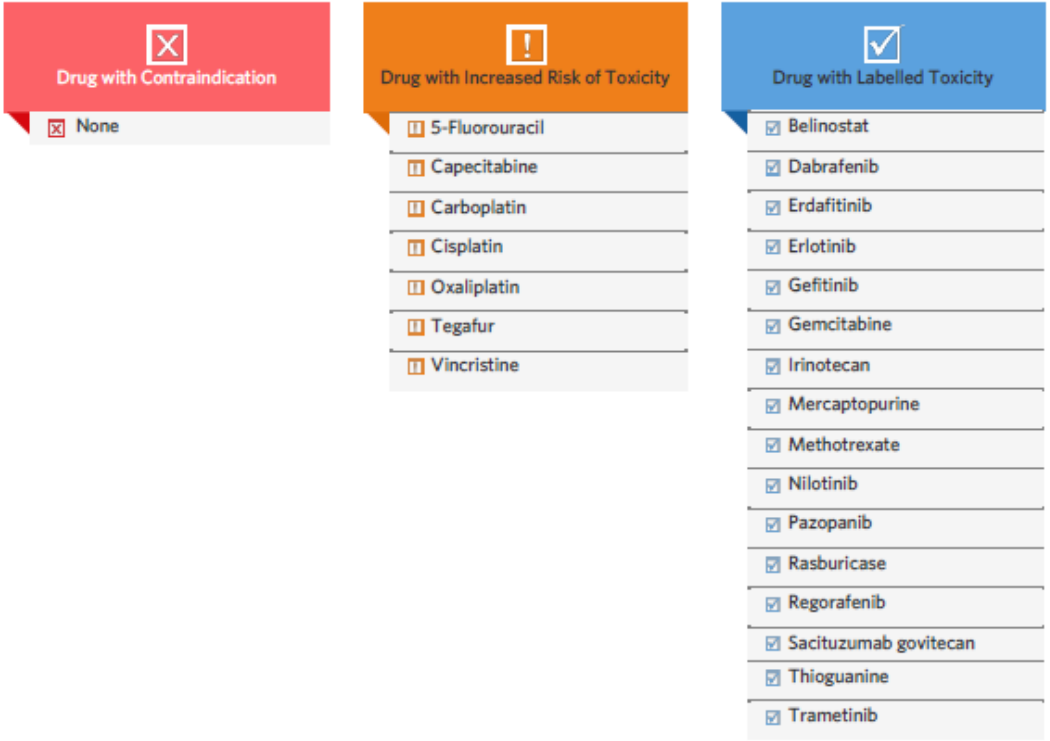
Additional Report Highlights

Indications for Non-Oncology Drugs

Drug	Indication
☑ Atorvastatin	MAPK pathway activation - MAPK3 (+2.05 FC), MAPK14 (+2.40 FC), MAP3K10 (+2.65 FC) overexpression
☑ Celecoxib	MAPK pathway activation - MAPK3 (+2.05 FC), MAPK14 (+2.40 FC), MAP3K10 (+2.65 FC) overexpression; WNT pathway activation - WNT5A (+5.04 FC) overexpression
☑ Quercetin	WNT pathway activation - WNT5A (+5.04 FC) overexpression
☑ Curcumin	BIRC5 (+3.72 FC) overexpression

☑ Drugs with Benefit

Case Presentation



Case Presentation

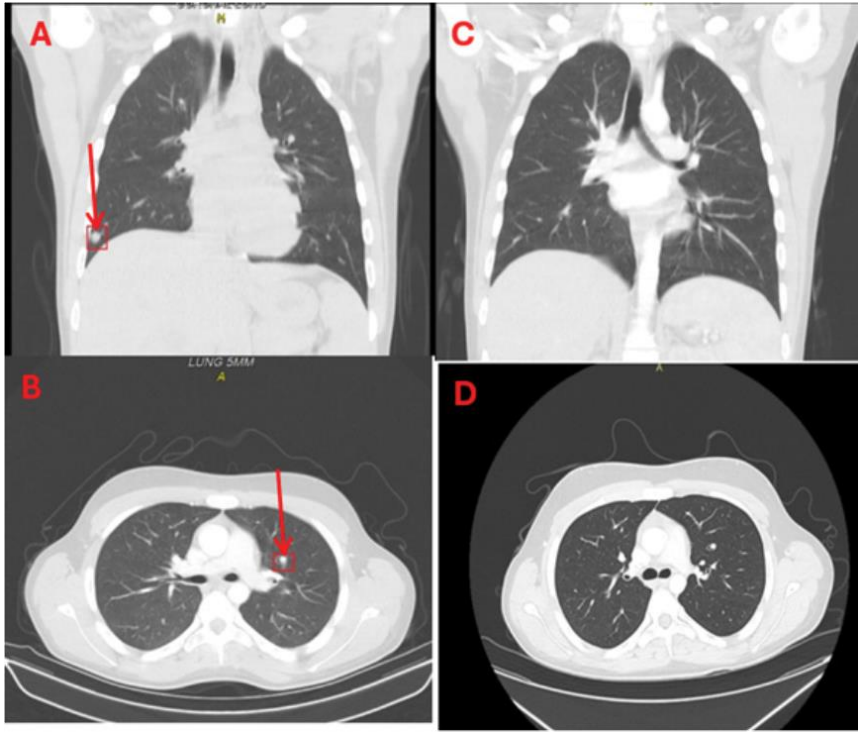
- Immunocytochemistry of circulating tumour cells showed positive expression of VEGFR1 and VEGFR2, suggesting potential benefit from anti-angiogenic therapy.
- Based on the molecular findings and treatment tolerability, a tailored combination regimen was developed, consisting of the following agents:
- 1. Sorafenib 200 mg daily
- 2. Atorvastatin 60 mg daily
- 3. Celecoxib 100 mg twice daily
- 4. Curcumin daily
- 5. Monthly Zoledronic Acid 4 mg IV
- These agents were administered in combination with ongoing chemotherapy.
- Due to neuropathy caused by vincristine, that drug was omitted from his protocol.

Outcomes

- After seven treatment cycles, re-evaluation included spiral chest CT, spinal MRI, and whole-body scan.
- Neurologically, the patient demonstrated partial motor recovery in the lower extremities, accompanied by improved ambulation and independence in daily activities.

Case Presentation

- Compared to the last CT scan, the previous pulmonary nodules have been resolved at the present time, indicating a complete response to the treatment.



Discussion

- This case illustrates how precision medicine can be effectively applied, even in resource-constrained settings where treatment options are often limited.
- In this patient’s case, the path to a tailored regimen began with a thorough molecular characterization of the tumour.
- Low TMB and negative PD-L1 expression ruled out the use of immunotherapy.
- However, the detection of VEGFR1/2 positivity created an opportunity for targeted anti-angiogenic therapy.

"What distinguished this case was not just the use of targeted agents, but the deliberate and evidence-informed combination of repurposed drugs, chosen not only for their individual merit, but also for their synergistic potential

Discussion

• **Sorafenib**: An anti-angiogenic agent, was selected for its inhibitory effects on VEGFR1 and VEGFR2.

- Its role in disrupting tumour vasculature may also improve chemotherapy delivery and overcome resistance.

• **Celecoxib** and **Curcumin**: Both targeting the COX-2 pathway, were combined to modulate tumour-promoting inflammation.

- Preclinical data suggest their synergy enhances apoptosis and reduces tumour progression.

• **Atorvastatin**: Typically used for dyslipidaemia, was included for its anti-proliferative effects and ability to potentiate antiangiogenic therapy.

• **Zoledronic Acid**: While providing skeletal support, may also exhibit direct anti-tumour effects, particularly valuable in cases with bone involvement.

Clinical Case Example

American Journal of Clinical Case Reports

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Case Report

A Successful Application of NGS-guided Precision Medicine in Ewing Sarcoma in Iran: A Case Report

Shahdad Farokhmanesh[1], Omid Reza Zekavat[2], Mohammadreza Bordbar[3], Golsa Shekarkhar[3], Mahdi Shahriari[2], Hadi Mottaghipisheh[2] and Ramin Ajami[4*]*

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Abstract

Background: Ewing sarcoma is a rare, aggressive malignancy of bone and soft tissue, primarily affecting adolescents and young adults. Even with access to standard chemotherapy and radiotherapy, patients with metastatic or recurrent Ewing sarcoma, particularly in lower-resource settings, continue to experience limited survival outcomes.

Case Presentation: We report the first known use of Next Generation Sequencing (NGS) testing to guide individualised treatment for Ewing sarcoma in Iran. A 17-year-old male presented with a paraspinal tumour and rapid neurological decline. Following surgical resection and histopathologic confirmation of Ewing sarcoma, he received standard chemoradiotherapy. Genomic profiling, including Tumour Mutational Burden (TMB) and circulating tumour cell immunocytochemistry, was conducted to identify precision therapeutic options. The patient's TMB was low (2 mutations/Mb), pMMR and MSI-low, ruling out immunotherapy candidacy. However, VEGFR1/2 expression supported the inclusion of Sorafenib. A customised regimen combining oncology and repurposed non-oncology agents was initiated. After seven treatment cycles, imaging revealed resolution of lung metastases and a reduction in spinal involvement,

Challenges and Limitations

- Technical Considerations:
- Tissue quality and quantity requirements (FFPE limitations)
- Tumor heterogeneity and clonal evolution
- Analytical validation and standardization needs
- Accessibility and Cost:
- Bioinformatics expertise requirements
- Economic barriers in resource-limited settings
- Reimbursement issues
- Disparities in global availability

Conclusions and Clinical Implications

- NGS has revolutionized Ewing sarcoma management:
- Provides definitive diagnostic confirmation
- Identifies rare fusion variants and complementary mutations
- Guides targeted therapy selection and clinical trial enrollment

Thanks..

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