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Case Report

EVI1 Disruption Post Neuroblastoma Treatment: A Case Analysis of Treatment-Associated Acute Myeloid Leukemia in a Pediatric Patient

Xin Zhang

Department of Pediatric Hematology and Oncology, Xinhua Hospital Affilliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

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Second malignant neoplasms · Neuroblastoma · Mutation · Leukemia · Child

Abstract
In recent years, there has been an increasing focus on understanding the long-term consequences of pediatric cancer treatments, particularly the emergence of secondary malignant neoplasms (SMNs). Here, we present a case study highlighting the aftermath of treatment, where a pediatric patient, initially treated for neuroblastoma, developed treatment-related acute myeloid leukemia (tAML) 6 years later. Our investigation emphasizes the crucial role of *EVI1* disruption in accelerating the progression of secondary tumors. This case underscores the significant risk of SMNs following pediatric cancer therapy. By analyzing genetic anomalies, we identified variations in the *PTPN11* and *KMT2C* genes, suggesting a complex interplay between genetic susceptibility and chemotherapy-induced mutagenesis in tAML development. Furthermore, our exploration of the involvement of topoisomerase II inhibitors in tAML provides insights into potential future therapeutic approaches. Reporting this case is vital for deepening our understanding of the mechanisms driving SMNs after pediatric cancer treatments. Through a comprehensive analysis of genetic anomalies and treatment variables, we can offer more precise clinical diagnoses and treatment strategies. This approach holds the potential to reduce the occurrence of secondary tumors and improve the long-term prognosis for pediatric patients.

Correspondence to:
Xin Zhang, zxcongufo@163.com

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Introduction

While the adverse effects of cancer treatment, such as nausea, alopecia, and myelosuppression, are well-known, the emergence of late-term toxicities in long-term cancer survivors poses a more intricate challenge in detection and management. Among these complexities, the emergence of secondary malignant neoplasms (SMNs) stands as a formidable complication. SMNs denote novel malignancies that arise autonomously from the primary ailment, distinct from metastatic tumors [1, 2]. In the cohort of individuals diagnosed with primary malignant neoplasms, SMNs constitute a prominent contributor to non-relapse-related late-stage mortality, accounting for approximately 50% of non-relapse-related deaths in cancer survivors at the 5-year mark [3]. The most robust correlation between chemotherapy and the incidence of SMNs is observed in cases of treatment-related acute myeloid leukemia (tAML). According to the World Health Organization (WHO), tAML is characterized as a belated consequence of cytotoxic chemotherapy employed for either neoplastic or non-neoplastic conditions. Almost universally, alkylating agents and topoisomerase II inhibitors administered in this therapeutic context bear a substantial, dose-dependent susceptibility to tAML development, elevating the risk by over tenfold. Antecedent accounts underscore that patients afflicted by tAML typically display a more unfavorable prognosis in comparison to their de novo counterparts with primary AML [4]. Hence, comprehending the genetic predisposition and treatment-associated risk facets linked to the incidence of tAML assumes paramount importance in the surveillance and prompt intervention for affected patients [5]. In this narrative, we present an exceptional case of tAML characterized by heightened *EVI1* expression and −7/del(7q), alongside an elucidation of pertinent literature.

Case Report

A 10-year-old male, previously diagnosed with advanced neuroblastoma, ceased treatment for 6 years. He was admitted with 2 weeks of fatigue. In January 2014, he sought medical help for poor appetite, vomiting, and a palpable mass in his left abdomen. Tests revealed an unusually large and distorted left kidney, with a solid mass causing kidney swelling. Detailed imaging revealed a widespread mass infiltrating his left kidney, pancreas, and left lumbar muscle, along with enlarged lymph nodes in the right iliac region. The mass also affected his abdominal aorta and inferior vena cava, and there were similar growths in his posterior mediastinum. A bone scan confirmed widespread skeletal metastases, but brain MRI and chest CT were normal. A bone marrow biopsy confirmed the diagnosis of stage IV neuroblastoma with neural infiltration and *MYCN* amplification. He began high-risk chemotherapy as per the CCCG-NB-2009 protocol (Fig. 1; Table 1). Post the fourth cycle, a CT angiography showed partial regression of the tumor, although it still affected his left kidney, pancreas, and left lumbar muscle. After six cycles, bone marrow stem cells were collected, revealing an improved situation with the renal mass. In July 2014, he underwent surgery elsewhere, removing the tumor and his left kidney. Pathology revealed residual neuroblastoma in the remaining kidney, with some degeneration, calcification, and necrosis. He then received four more cycles of chemotherapy, followed by radiotherapy. A PET-CT scan in January 2015 showed no signs of relapse or metastasis. He concluded treatment in October 2015. In July 2021, after 6 years, he experienced 2 weeks of fatigue. Follow-up revealed a blood panel displaying hemoglobin of 67 g/L and a white blood cell count of 52.66×10^9/L, with an immature cell ratio of 63%. As shown in Figure 2, accounting for approximately 63.4% of nucleated cells in the bone marrow represents abnormal cells. The expression of relevant antigens is shown in Table 2. Genetic tests showed mutations in *PTPN11* (NM_002834:exon3:

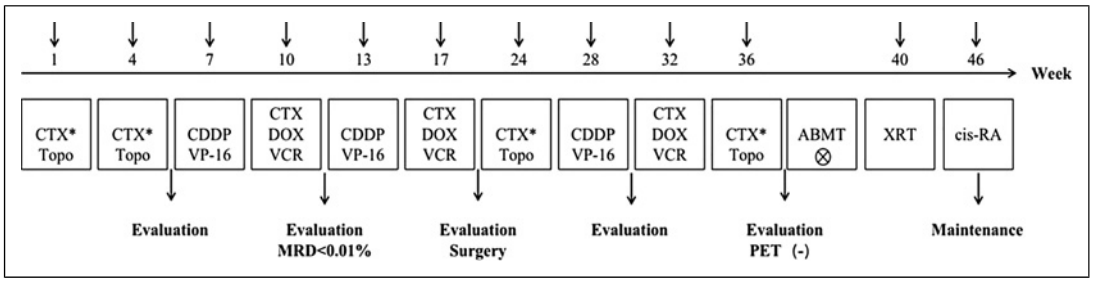

Fig. 1. Treatment schedule. XRT: external beam radiotherapy; 13-cis-RA: cis-retinoic acid.

c.215C>T:p.A72V; mutation frequency: 54.4%) and *KMT2C* (NM_170606:exon42:c.9565C>T: p.Q3189X). Qualitative screening revealed *EVI1* positivity, and analysis showed chromosome 7 deletion in most cells (Fig. 3). Genome-wide sequencing confirmed mutations associated with AML. After the diagnosis of tAML, the patient received prompt hydration and initiated oral allopurinol therapy, along with supportive treatment using hydroxyurea. Simultaneously, chemotherapy (DNR+Arac+Vp16) and lumbar puncture were administered. In December 2021, the patient continued treatment at an external hospital. Unfortunately, due to the inability to undergo a bone marrow transplantation, the patient passed away. The CARE Checklist has been completed by the authors for this case report, attached as in online supplementary Figure 1 (for all online suppl. material, see https://doi.org/10.1159/000533571).

Discussion

This case presents a high-risk neuroblastoma patient who, after 6 years of varied treatments, developed tAML, confirmed through abnormal blood routine findings. The overall SMNs incidence in neuroblastoma is around 0.87%, increasing due to enhanced survival rates and therapies [6]. The heightened risk of SMNs in relapsed or refractory cases is attributed to genetic predisposition, intensified chemotherapy, and radiation. This risk extends even to low-risk pediatric patients predominantly treated with surgery, underscoring the significant role of genetic susceptibility [7].

Hematopoietic SMNs typically appear within 5 years post-initial treatment with a brief latency. Non-hematopoietic SMNs tend to emerge between 5 and 15 years posttreatment, linked to specific agents like etoposide [8]. Topoisomerases, pivotal enzymes facilitating DNA processes, prominently feature topoisomerase II in DNA replication and cell division. Topoisomerase II inhibitors, functioning as chemotherapeutic agents, disrupt enzyme activity, perturb DNA structures, and induce cellular apoptosis, impacting cancerous and normal cells, thus contributing to tAML. The extended latency challenges tAML detection, emphasizing vigilance [9, 10]. Vigilant monitoring and follow-ups are crucial. In this case, the patient displayed fatigue and anomalous blood exams during follow-up, urging early intervention. Developing agents targeting topoisomerase II for safety is vital.

Mutations in *PTPN11* and *KMT2C* genes are detected in this case. *PTPN11* mutation impact treatment responses, elevating tAML risk [11]. These mutations may interact with other genetic abnormalities, hastening AML progression. When combined with *KMT2C* mutation, the tAML risk escalates further. *KMT2C* deletion affects stem cell cycle, elevating tAML incidence. *KMT2C* mutation occurs in 4.3% of AML patients, associated with shorter survival. This case harbors a *Q3189X* mutation in the *KMT2C* gene [12]. Chromosome 7 aberrations, including deletions and monosomy, associate with adverse AML prognosis.

Table 1. CCCG-NB-2009 protocol

Agents	Dose	Route	Time
CTX*+Topo			
Cyclophosphamide*	400 mg/m^2 (<12 kg, 13.3 mg/kg)	IV	D1-5
Topotecan	1.2 mg/m^2	IV	D1-5
Irinotecan	120 mg/m^2		D1-3
CDDP+VP-16			
Cisplatin	50 mg/m^2 (<12 kg, 1.67 mg/kg)	IV	D1-4
Etoposide	200 mg/m^2 (<12 kg, 6.67 mg/kg)	IV	D1-3
CTX+DOX+VCR			
Cyclophosphamide	1,800 mg/m^2 (<12 kg, 60 mg/kg)	IV	D1-2
Mesna	420 mg/m^2 (0, 4, and 8 h after CTX injection)	Push	D1-2
Doxorubicin	25 mg/m^2 (<12 kg, 0.83 mg/kg)	IV	D1-3
Liposomal doxorubicin	20 mg/m^2		D1-3
Vincristine	0.017 mg/kg (<12 months)	Push	D1-3
	0.67 mg/m^2 (<12 months and 12 kg)		D1-3
	0.022 mg/kg (>12 months, below 12 kg)		D1-3
XRT	23.4 Gy/13Fx		
13-cis-retinoic acid (13-cis-RA)	5.33 mg/kg/d (>12 kg, 120–160/m^2)		D1-14

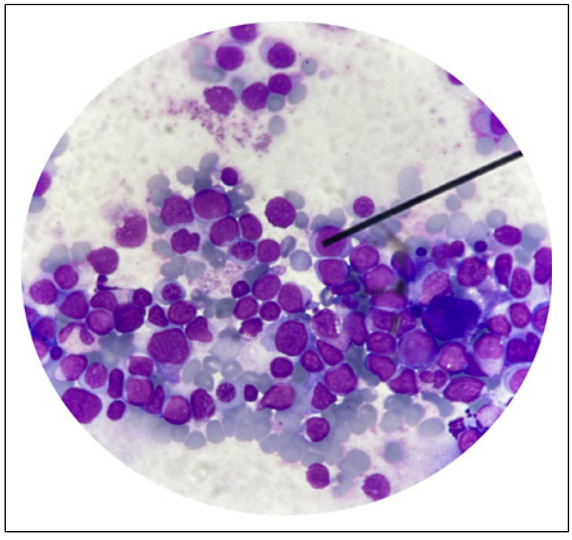

Fig. 2. Bone marrow smear (Giemsa staining ×1,000).

tAML characterized by −7/del(7q) usually follows alkylating agent exposure and has a poor prognosis (median survival of 8 months) [13]. *KMT2C*, an anti-oncogene, is frequently deleted in −7/del7q anomalies, even haploinsufficiency accelerating AML. Elevated *EVI1* expression in AML often links to chromosomal damage, considered a marker of poor prognosis. *EVI1* encodes a transcription factor essential for hematopoiesis. It is well-established that prevalent mutations in inv(3)/*t*(3; 3) chromosomal rearrangement-induced AML encompass monosomy 7 and genes associated with the *RUNX1*, *IKZF1*, and *RAS* signaling pathways (*NRAS, KRAS, PTPN11,* and *NF1*) [14, 15]. SMNs development may

Table 2. Flow immunophenotyping results

B-lineage		T-lineage		Myelocyte		Stem progenitor cell markers and others	
CD	%	CD	%	CD	%	CD	%
CD19 (APC)	0	CD7	83.3	MPO	22.9	CD34	98.8
cCD79a	0	cCD3	0	CD33	53.2	HLA-DR	99.4
cCD22	0	TCR αβ	0	CD13	97.9	CD9	0
CD10	0	TCR γδ	0	CD11b	0	CD123	72.7
CD20	0	CD3	0	CD64	0	CD66c	0
CD22	0	CD4	7.2	CD36	0	CD56	0
smIgM	0	CD8	0	CD14	0	CD117	64.6
cu	0	CD1a	0	CD15	0	CD38	68.1
TDT	0	CD2	0	CD71	0	CLL1	79.5
		CD5	0	CD61	0		

Approximately, 63.4% of abnormal myeloid cells were found in the bone marrow.

cCD79a, cCD22, cCD3, and MPO are cytoplasmic stains, which are specific markers for B-ALL, T-ALL, and AML. CD19 and CD7 are sensitive markers for B-ALL and T-ALL, respectively.

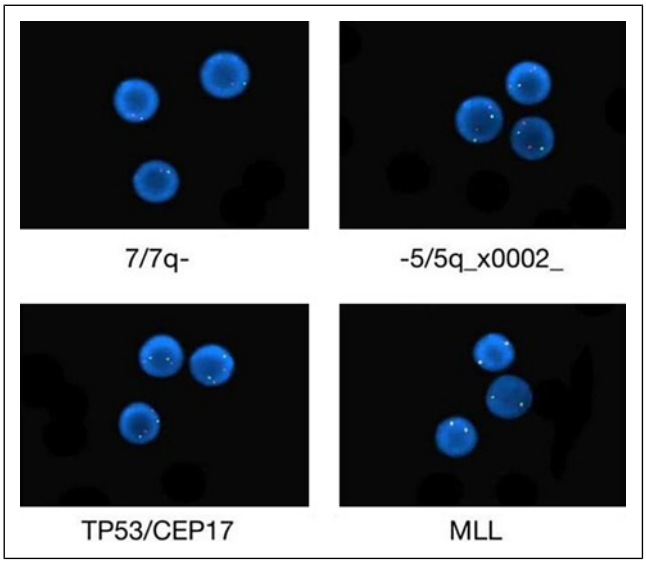

Fig. 3. Results of molecular fluorescence in situ hybridization (FISH) analysis using specific DNA probes.

arise randomly or due to genetic susceptibility or mutagenic effects of initial cancer treatments. Chemotherapy's impact on SMN development varies significantly, underlining genetic mutations' role in this process.

Conclusion

High-risk AML demands immediate treatment to avert rapid mortality. The connection between topoisomerase II toxicity and leukemia seems inherent to the drug's mechanism. Whether tAML differs in features and prognosis from other AML types remains uncertain, yet topoisomerase II inhibitors are linked to tAML. This study presents a unique case of *EVI1*-

disrupted tAML, yielding essential insights into tAML's progression. Emphasizing tAML's importance enhances precise monitoring and prompt treatment. Future research on topoisomerase II's link to leukemia and improved therapies holds paramount importance for better outcomes in high-risk AML pediatric patients.

Statement of Ethics

Written informed consent was obtained from the patient's MOTHER for publication of the details of their medical case and any accompanying images. This study protocol was reviewed and approved by Ethics Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Approval No. XHEC-D-2023-122).

Conflict of Interest Statement

The authors declare no conflict of interest.

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Author Contributions

X.Z. collected the information and wrote the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

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