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Fusion Oncoproteins in Childhood Cancers: Potential Role in Targeted Therapy

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Cancer remains the leading cause of death from disease in children. Historically, in contrast to their adult counterparts, the causes of pediatric malignancies have remained largely unknown, with most pediatric cancers displaying low mutational burdens. Research related to molecular genetics in pediatric cancers is advancing our understanding of potential drivers of tumorigenesis and opening new opportunities for targeted therapies. One such area is fusion oncoproteins, which are a product of chromosomal rearrangements resulting in the fusion of different genes. They have been identified as oncogenic drivers in several sarcomas and leukemias. Continued advancement in the understanding of the biology of fusion oncoproteins will contribute to the discovery and development of new therapies for childhood cancers. Here we review the current scientific knowledge on fusion oncoproteins, focusing on pediatric sarcomas and hematologic cancers, and highlight the challenges and current efforts in developing drugs to target fusion oncoproteins.

ABBREVIATIONS ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; ARMS, alveolar RMS; CML, chronic myeloid leukemia; ERMS, embryonal rhabdomyosarcoma; EWS, Ewing sarcoma; HDAC, histone deacetylase; MLL, mixed-lineage leukemia; MRD, minimal residual disease; RMS, rhabdomyosarcoma; RNAseq, RNA sequencing; RT-PCR, reverse transcriptase–polymerase chain reaction; SS, synovial sarcoma; TKI, tyrosine kinase inhibitor; WHO, World Health Organization

KEYWORDS fusion gene; fusion oncoprotein; leukemia; pediatric; sarcoma

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Introduction

In the United States, an estimated 10,590 new cases of cancer will be diagnosed in children from birth to 14 years of age and approximately 1180 children are expected to die from the disease per year. Despite a declining mortality rate, cancer is the leading cause of death in children >1 years of age.[1,2] The cause of most pediatric cancers is relatively unknown. Inherited genetic abnormalities, exposures to DNA-damaging agents, or exposures to diagnostic or therapeutic radiation account for a small percentage of cancers in children.[3] Further, the types of cancers that commonly occur in children and the molecular alterations in particular cancers generally differ from adults, with pediatric cancers displaying fewer genetic alterations that are distinct from those in the same adult cancers.[3,4] The primary histologic types of pediatric cancer also differ from adults with many pediatric cancers arising from embryonal rather than epithelial cells. With these differences, it is not surprising that the natural history and response to therapy can differ for the same cancer type in pediatrics versus adults.[3] Overall, the contrast between adult and pediatric malignancies represents a complex challenge within oncology research and pediatric drug development.[5]

Pediatric patients are prone to unique toxicities and side effects secondary to their developmental state, posing specific challenges to develop new treatment strategies that limit both short- and long-term adverse events. Specifically, infants, children, adolescents, and young adults still undergoing development can sustain harmful effects from non-specific cellular interference, resulting in a multiplicative effect as damaged developing tissues grow. Therefore, clinical evaluation of treatments must include consideration of age-related metabolic and pharmacologic differences. Leveraging the potential for specificity in cancer treatment can improve cancer prognosis and quality of life by minimizing damage to neighboring cells and tissues.[1,3,5]

To improve efficacy while limiting toxicity, scientists, clinicians, and experts within the field of cancer have focused on identifying molecular targets that affect signaling pathways known to exert growth effects on cancers.[6] Molecular targets are physical molecules, which are usually proteins, that are associated with development of cancer. Drugs can be designed to bind to unique structural parts of these targets to stop cancer cells from functioning or label them for destruction.[7,8] This selectivity helps to protect normal cells and maintain

Table 1. Fusion Oncoproteins in Bone and Soft Tissue Tumors

Reference	Chromosomal Abnormality	Fusion Oncoprotein	Diagnosis
Barr[22]	t(2;13)	PAX3-FOXO1	Alveolar rhabdomyosarcoma
Barr[22]	t(1;13)	PAX7-FOXO1	Alveolar rhabdomyosarcoma
Argani[23]; Ladanyi[24]	t(X;17)	ASPL-TFE3	Alveolar soft-part sarcoma
Argani[23]; Ladanyi[24]	der(17)t(X;17)(p11;q25)	ASPSCR1-TFE3	Alveolar soft-part sarcoma
Oliveira[25]; Sekoranja[26]	t(1;17)	THRAP3(TRAP150)-USP6	Aneurysmal bone cyst
Sekoranja[26]; Oliveira[27]	t(16;17)	CDH11-USP6	Aneurysmal bone cyst
Sekoranja[26]	t(3;17)	CNBP-USP6	Aneurysmal bone cyst
Sekoranja[26]	t(9;17)	OMD-USP6	Aneurysmal bone cyst
Sekoranja[26]	t(17;17)	COL1A1-USP6	Aneurysmal bone cyst
Antonescu[28]	t(12;22)	EWS(EWSR1)-ATF1	Clear cell sarcoma, angiomatoid fibrous histiocytoma
Antonescu[28]	t(2;22)	EWS(EWSR1)-CREB1	Clear cell sarcoma
Waters[29]	t(12;22)	FUS-ATF1	Clear cell sarcoma
La Starza[30]; Gerald[31]	t(11;22)	EWSR1-WT1	Desmoplastic round cell tumor
Hrzenjak[32]	t(7;17)	JAZF1(JJAZ1)-SUZ12	Endometrial stromal sarcoma
Panagopoulos[33]	t(6;7)	JAZF1-PHF1	Endometrial stromal sarcoma
Lee[34]	t(10;17)	YWHAE-FAM22A/B	Endometrial stromal sarcoma
Micci[35]	t(1;6)	MEAF6-PHF1	Endometrial stromal sarcoma
Mendlick[36]; Tanas[37]	t(1;3)	WWTR1-CAMTA1	Epithelioid hemangioendothelioma
Dupain[13]; Zollner[38]; Sankar[39]; Delattre[40]	t(11;22)/t(21;22)	EWSR1-FLI1	Ewing sarcoma
Macarenco[41]	t(17;22)	COL1A1-PDGFB	Giant cell fibroblastoma
Nagasubramanian[42]; De Braekeleer[43]	t(12;15)	ETV6-NTRK3	Infantile fibrosarcoma
Goransson[44]	t(12;16)	FUS-DDIT3	Myxoid/round liposarcoma
Alaggio[45]		VGLL2 rearranged	Sclerosing rhabdomyosarcoma, spindle cell rhabdomyosarcoma
Jin[46]; Panagopoulos[47]	t(5;8)	AHRR-NCOA2	Soft-tissue tumor, angiofibroma
O'Meara[48]	t(17;19)	MLL4-GPS2	Spindle cell sarcoma
Przybyl[49]; McBride[50]; Su[51]; Hayakawa[52]	t(X;18)	SS18-SSX1, SSX2, or SSX4	Synovial sarcoma
Antonescu[53]	t(4;19), t(10;19)	CIC-DUX4	Undifferentiated round cell sarcoma

the overall health and function in patients with cancer. In the last few decades, research has focused heavily on identifying and characterizing tumor molecular profiles that can be used to inform therapy decisions and provide precision medicine to patients.[7-9]

Drugs to treat cancer are generally initially developed in adults. However, the evaluation in children of molecular targeted therapies developed and approved for adults needs careful consideration owing to the differences in the molecular drivers of the cancers between children and adults. Of great promise has been the increasing research and literature related to molecular

genetics in pediatric cancers.[4,9] One molecular alteration seen in pediatric cancers is related to chromosomal rearrangements that lead to the fusion of 2 different genes or a gene fusion.[6,10,11] These fusions can be of genes on the same or on different chromosomes. Although not all fusion genes lead to cancer, those rearrangements that lead to the formation of cancers are referred to as oncogenic fusions. Oncogenic fusions lead to the expression of aberrant proteins, referred to as oncoproteins. These fusion oncoproteins can be a variety of proteins, most typically kinases and transcriptional factors.[12,13]

Fusion genes naturally exist within the human body.[14] However, these fusions can affect cellular functions, including the formation of fusion oncoproteins that can lead to the transformation to cancer cells. These chromosomal rearrangements resulting in fusions can be translocations, inversions, deletions, or insertions.[14] These rearrangements can include in-frame gene fusions that are characteristically found in sarcomas, leukemias, and lymphomas. Fusion oncoproteins are more common in pediatrics because they are early molecular alterations present at a clonal level and are generally observed in cancers with low genetic mutation burden.[13,15,16]

Sarcomas in the pediatric population, such as rhabdomyosarcoma (RMS), Ewing sarcoma (EWS), and synovial sarcoma (SS) are associated with chromosome translocations that generate fusion oncoproteins. These oncoproteins act as transcription factors resulting in a malignant transformation caused by the creation of oncogenic phenotypes (Table 1).[11,14,17-21] Challenges exist in developing drugs that target transcription factors, the products of most oncogenic fusions. In addition, the rarity of these cancers has been an impediment to drug development and has contributed to the limited therapies available for patients.[17,18] Multimodal therapy with intensive chemotherapy, radiation therapy, surgery, and improved supportive care has led to significant improvements in a large portion of patients with localized disease or intermediate-risk disease; however, those with metastatic or recurrent disease continue to fare poorly. Therefore, new therapies are needed for these patients.[54,55]

Point mutations, gene rearrangements, deletions, amplifications, and epigenetic changes induce gene expression and cause genetic and epigenetic alterations in hematopoietic stem cells or progenitors to alter pathways in leukemia.[56] In most leukemias, chromosomal translocations result in fusion genes that produce fusion oncoproteins that affect transcription processes (Table 2).[57] Oncogenic gene fusions are a critical driver of mutations in pediatric leukemias, such as acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).[12,15]

Fusion oncoproteins represent a rapidly growing area in pediatric oncology research and they represent promising targets for therapeutic and prognostic biomarker development.[6,12,13] Potentially, small molecules can be used to inhibit the activity of these fusion oncoproteins by inhibiting their function, blocking their interactions, or triggering their selective degradation.[13,21] This review provides an overview of the current scientific knowledge on fusion oncoproteins in pediatric sarcomas and hematologic cancers, the barriers and challenges that exist in developing drugs to target fusion oncoproteins, and the priorities currently underway to overcome these challenges.

Discovery of Fusion Oncogenes in Cancer Research

The first fusion oncogenes were discovered in leukemia and other hematologic cancers. In the 1980s, a fusion between 3′ of the *ABL1* gene in chromosome 9 and 5′ of the *BCR* gene in chromosome 22 (BCR/ABL) was identified in a subset of patients with leukemia.[13,21,57] In 2001, a tyrosine kinase inhibitor (TKI) targeting *BCR/ABL*, imatinib mesylate, was approved for the treatment of patients with chronic myeloid leukemia (CML) in blast crisis, accelerated phase, or in chronic phase after failure of interferon-alpha therapy.[88] This represented one of the first targeted therapies used for patients with CML. Treatment with imatinib mesylate led to significant improvements in length of remission. The success of this agent showed the promise of targeting oncogenic fusions in the treatment of cancer.[6,13] The progress in sequencing technology has enabled the exploration of additional fusion oncogenes in other cancer types.[21]

Fusion Genes and Fusion Oncoproteins

Fusion genes are caused by chromosomal rearrangements and include translocations, insertions, deletions, and inversions.[13] They are caused by the aberrant rejoining of chromosome breaks that occur from chromothripsis, a genomic catastrophe, or chromoplexy, the joining of chromosomes in loops or loop structures. Rearrangement processes that cause gene fusions are predominantly caused by chromoplexy. These rearrangements, otherwise known as structural variants, drive the formation of fusion genes and are a ubiquitous source of somatic mutations in cancer.[14] Physiological consequences of these changes can differ depending on the fusion gene and sequences affected.[13]

Fusion oncogenes are a key mechanism in carcinogenesis because they can lead to the activation of proto-oncogenes or inactivation of tumor suppressor genes.[6] In cases when one of the fusion partners is an oncogene, several mechanisms can lead to abnormal activation of the oncogene. This includes the oncogene fusing to a stronger promoter, deleting the mRNA regulatory regions, or escaping degradation.[89]

Rearrangements can also generate chimeric oncoproteins and result in a protein that has functional domains derived from both fused genes.[13,21] Most of the chimeric oncoproteins act as transcription factors and cause cell transformation. Fusions genes produced by translocations lose regulatory activity, obtain new

Table 2. Fusion Oncoproteins in Leukemia

Reference	Chromosomal Abnormality	Fusion Oncoprotein	Diagnosis
Liu[58]; Wang[59]	t(4;11)	KMT2A-AFF1	ALL, mixed phenotype acute leukemia
Matsuo[60]; Rahnemoon[61]; De Braekeleer[43]; Wang[59]; Majzner[62]	t(12;21)	ETV6-RUNX1/TEL-AML1	ALL
Wang[59]	t(9;22)	BCR-ABL1	ALL, CML
Carranza[57]; Wang[59]	t(1;19)	TCF3-PBX1	ALL
Wang[59]	t(10;11)	PICALM-MLLT10	ALL
Wang[59]	t(17;19)	TCF3-HLF	ALL
Wang[59]	t(8;14)	TRA-MYC	ALL
Kanayama[63]		PAX5-K220	Ph-like ALL
Meyer[64]; Winters[65]		MLL rearrangement	ALL, AML
Van Vlierberghe[66]	del(9)	SET-NUP214	T-cell ALL, AML
Jaju[67]; Shiba[68]	t(5;11)	NUP98-NSD1	AML
Bhatnagar[69]	t(11;19)	KMT2A-ELL KMT2A-MLLT1	AML, ALL
Roh[70]; Caudell[71]	t(10;11)	CALM-AF10	AML, ALL
Roh[72]	t(8;21)	AML1-ETO	AML
Wang[59]; van der Reijden[73]	inv(16)	CBFB-MYH11	AML
Wang[59]	t(9;11)	KMT2A-MLLT3	AML
Wang[59]	t(3;3)	RPN1-MECOM	AML
Buijs[74]	t(8;13)	ZMYM2-FGFR1	AML
Wang[59]	t(6;9)	DEK-NUP214	AML
Wang[59]	t(3;21)	RUNX1-MECOM	AML
Sotoca[75]	t(16;21)	FUS-ERG	AML
Arber[76]	t(3;5)	NPM-MLF1	AML
Wang[59]	t(3;8)	PVT1-MECOM	AML
Oancea[77]	t(6;9)	DEK-CAN	AML
De Braekeleer[43]	t(3;12)	ETV6-MDS1/EV11	AML
Steinauer[78]	t(8;21)	RUNX1-RUNX1T1	AML
Steinauer[78]	t(16;21)	RUNX1-CBFA2T3	AML
Wang[59]	t(15;17)	PML-RARA	APL
Wang[59]	t(11;17)	ZBTB16-RARA	APL
Wang[59]; Akasaka[79]	t(8;14), t(14;19), t(14;14), t(14;20)	IGH- CEBPD/CEBPA/CEBPE/CEBPB	B-cell precursor ALL
Morrman[80]	t(X;14) or t(Y;14)	IGH-CRLF2	B-cell precursor ALL
Russell[81]	t(6;14)	IGH-ID4	B-cell precursor ALL
Seo[82]	t(4;8)	MYC rearrangement	B-ALL

Table 2. Fusion Oncoproteins in Leukemia (cont'd)

Hu[83]	t(1;19)	E2A-PBX1	B-ALL
Carulli[84]	t(4;11)	MLL rearrangement	B-ALL
De Braekeleer[43]; Strehl[85]	t(9;12)	PAX5-ETV6	B-ALL
Wang[59]	t(2;19)	IGK-BCL3	CLL
Put[86]	t(8;13)	IGH-MYC	CLL
Wang[59]	t(14;19)	IGH-BCL3	CLL
Wang[59]	t(19;22)	BCL3-IGL	CLL
Wang[59]; Acunzo[87]	t(2;11)	CXCR4-MAML2	CLL

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, apromyelocytic leukemia; B-ALL, B-cell acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; Ph-like, Philadelphia chromosome–like

oncogenic functions, and contribute to abnormal gene expression and tumors.[13,89] Translocations that cause fusion oncoproteins have been identified in various tumor types, including hematologic malignancies and soft tissue and bone tumors.[89]

Although fusions oncoproteins typically involve kinases and transcriptional regulators, more advances have been made in the development of therapeutics to target kinases. The most well-known classes of drugs that have provided impressive clinical benefit are those targeting ALK-fusion–positive tumors in non–small cell lung cancer and BCR-ABL in CML.[90] More recently, larotrectinib and entrectinib have been approved for NTRK gene fusions in a tissue agnostic fashion for both adults and children.[91,92]

Transcriptional regulators result in a wide range of phenotypic changes and are characterized by a lack of deep hydrophobic pockets and nuclear localization that have proved more challenging to target with drugs; however, progress has been made.[93] For example, retinoic acid targets the fusion gene transcript RAR-alpha in the childhood leukemia known as acute promyelocytic leukemia. The addition of retinoic acid to arsenic trioxide led to significantly improved outcomes in this population, resulting in high cure rates (>80%) and remission rates (~95%) with retinoic acid in combination with chemotherapy or arsenic trioxide. Prior to the introduction of retinoic acid, the prognosis for acute promyelocytic leukemia was poor with the use of chemotherapy alone.[94] Other treatment approaches that have been evaluated include kinase inhibitors such as those used in BCR-ABL–positive CML, target degradation with retinoic acid in acute promyelocytic leukemia (APL), and other indirect inhibitors such as histone deacetylase (HDAC) inhibitors being evaluated in SS.[13] More research targeted at understanding the molecular vulnerabilities of these fusion proteins will enhance the ability to target them with therapeutic agents.[90]

Pediatric Sarcomas

Pediatric sarcomas are a heterogenous group of mesenchymal tumors that arise from the bone or soft tissue. Sarcomas disproportionately affect children, adolescents, and young adults and have a high mortality rate.[18,95] Although the biology of the various sarcomas differs, the treatment, which includes a combination of conventional chemotherapy, surgery, and radiation, is similar among the sarcoma types. Historically, most pediatric sarcomas have limited prognostic markers for adequate risk stratification to aid in modifying therapy regimens.[17,18,96] Below we review the role of fusion oncoproteins in the most common sarcomas.

Rhabdomyosarcoma. Rhabdomyosarcoma is the most common soft tissue sarcoma of childhood, accounting for 40% of all soft tissue sarcomas.[97-100] Despite the use of intensive multimodal chemotherapy regimens, the 5-year survival rate for patients with metastatic RMS has remained at 30% for decades.[98] Novel agents are lacking.[97,98,101] Thus, characterizing genetic events that underlie RMS is critical to developing more effective diagnostic and prognostic measures, and effective therapeutics.[98]

Rhabdomyosarcoma tumors have 2 major histopathologic variant subtypes: embryonal rhabdomyosarcoma (ERMS) and alveolar RMS (ARMS).[17,99,102] Embryonal RMS is the most prevalent. In addition to the pathologic distinctions, clinical and genetic differences exist between the 2 subtypes.[17,98] The ARMS subtype is generally diagnosed throughout childhood and adolescence, with most cases occurring after the age of 10 years.[17,98] Alveolar RMS fusion-positive patients have significantly poorer survival rates (e.g., the 5-year event-free survival for ERMS is 43% vs ARMS PAX7 at 17% or PAX3 at 8%), have higher frequency of metastasis, and represent an older age distribution.[99,103,104] In contrast, the ERMS subtype is generally diagnosed in young children, and most cases are diagnosed before age 10 years. The ERMS subtype generally occurs as localized tumors in areas such as the head and neck, genitourinary tract,

and retroperitoneum. When localized, the ERMS subtype generally yields a relatively good prognosis (e.g., 5-year event-free survival 43%).[17,97, 99] Patients with ARMS fusion-negative tumors are clinically similar and have similar molecular features to patients with ERMS.[95] Research indicates that no significant difference exists between ARMS fusion-negative and ERMS patients in event-free survival, overall survival, and initial presentation.[103,104] The fusion gene has been associated with a poor prognosis in RMS.[41] Unlike the presence of the *PAX/FOX01* gene as an acceptable prognostic marker for ARMS, no similar marker has been identified for RMS fusion-negative patients. Hingorani et al[95] published the validation of a 5-gene signature to identify different risk groups for fusion-negative patients with RMS, but it is not clear if or how that panel has been incorporated into prospective RMS clinical trials. Lastly, other fusion genes have been identified in RMS, including *MYOD1*-mutant RMS, *VGLL2/NCOA2*-rearranged RMS, and *TCFP2* RMS. These molecular subtypes may have clinical relevance but occur in relatively small subsets of RMS and will require prospective studies to define their role in therapy.

Although RMS tumors have a low mutation rate, alterations such as chromosomal rearrangement, amplification, deletion, and mutations can occur.[98] Most ARMS tumors harbor the t(2;13)(q35;q14) fusion (PAX3/FOX01), or the less common t(1;13)(p36;p14) (PAX7/FOX01) fusion.[98-100,105] The *PAX* fusion gene is thought to be the dominant oncogenic driver and through transcriptional programming can alter a host of downstream targets.[98] The presence of either the *PAX3* or *PAX7* fusion gene is a crucial prognostic indicator of the disease.[17,95,98,101,103,104] Some data suggest that the expression of *PAX3/FOX01* is higher in metastatic tumors and has a slightly inferior outcome than *PAX7/FOX01*.[95,99] Detection of the presence of the fusion gene is not currently part of the World Health Organization (WHO) classification system, although it is used by clinicians to assist with diagnosis.[21,103,104] In addition, the presence of fusion gene status has been proposed for use in eligibility criteria for clinical trials in RMS and is currently being used in an ongoing Children's Oncology Group Study ARST1431 (NCT02567435).[103]

Targeting the fusion has proven to be challenging; however, the role of *PAX3/FOX01* in checkpoint adaptation may provide a potential avenue for intervention.[99] The ability to divide and survive following checkpoint arrest despite unrepairable DNA breaks is known as checkpoint adaptation. To maintain the integrity of the genome, checkpoint controls regulate DNA damage that occurs in cell cycle progression and prevent continued transit through the cell cycle until damage is repaired. If repair occurs, cells can proceed through the cycle and proliferate. If the damage is not able to be repaired, cells will typically die. Cancer cells can alter cell cycle regulation, providing an avenue for unchecked proliferation. Mouse models and human tumor cell lines reveal that *PAX3/FOX01* expression is increased during the G_2/M checkpoint phase of the cell cycle. Under stressful conditions, such as chemotherapy and radiation, *PAX3/FOX01* mediates G_2/M enabling checkpoint adaptation and refractoriness to therapeutic agents.[37] Experimental studies reveal that genetic knockdown of the *PAX3/FOX01* gene improves chemotherapy and radiation sensitivity and reduces tumor re-establishment.

Targeting transcription factors is not an easy task, but efforts to develop drugs are underway. Preclinical data reveal entinostat, a class I HDAC inhibitor, can silence *PAX3/FOX01* mRNA and protein levels. In preclinical studies using orthotopic xenografts, entinostat with chemotherapy suppressed the abundance and activity of *PAX3/FOX01* and growth of ARMS fusion-positive tumors. Entinostat is currently being evaluated in a pediatric phase 1b trial in patients with recurrent or refractory solid tumors (NCT02780804) and in combination with immunotherapy in another trial (NCT03838042).[101] As thoroughly reviewed by Chen and colleagues,[93] investigators are attempting to target multiple pathways involving the fusion proteins relevant to RMS as they work to develop therapeutic approaches to treat children with fusion-positive tumors.

The clinical and pathologic differences that exist between ARMS and ERMS are reflected in and likely driven by the different biologic mechanisms of tumorigenesis found in the 2 RMS subtypes.[97,106] Unlike translocations occurring in ARMS, a wide range of identifiable genetic aberrations have been associated with ERMS; loss of heterozygosity has been seen at 11p15.5 and mutations in *TP53*, *NRAS*, *KRAS*, *HRAS*, *PIK3CA*, *CTNNB1*, and *FGFR4* have been noted.[98] Furthermore, congenital syndromes, such as Beckwith-Wiedemann, Costello, Li-Fraumeni, and neurofibromatosis type-1 (NF1) have been associated with ERMS.[106]

Fusion-negative tumors appear to have a higher mutational burden than fusion-positive tumors.[98,107] Mutations in fusion-negative tumors include those in the tyrosine kinase/RAS/PI3K pathway.[18] In one sequencing study, the RAS pathway is mutated in 45% of tumors of *PAX* gene–negative tumors.[98] The RAS mitogen-activated protein kinase (MAPK) pathway signal transduction has been studied extensively because of its role in oncogenesis.[44] Early preclinical evidence of activity in RMS has been observed with MEK and PI3K inhibitors.[98,108]

Ewing Sarcoma. Ewing sarcoma is the second most common pediatric bone malignancy.[17,109] Ewing sarcoma is a poorly differentiated tumor affecting children and young adults and has a peak incidence at 15 years of age.[17,109] Up to a third of patients with EWS will present with metastatic disease; the most common sites of metastasis are the lung, bone, and bone marrow.[17,38,109] The outcome for patients with metastatic EWS is poor

with an overall survival rate of less than 30%.[38,110]

Ewing sarcomas have high genomic stability.[47] The oncogenic transformation of EWS is caused by one underlying prototypical chromosomal translocation, which is the fusion of the *EWS* gene on chromosome 22q24 with 1 of 5 E-twenty-six (ETS) transcription factor gene family members (*FLI*, *ERG*, *ETV1*, *E1AF*, and *FEV*). Of the EWS/ETS translocations, a reciprocal translocation of t(11;22)(q24;q12) is a cytogenetic hallmark in 85% of patients with EWS.[17,19,105,109] *EWS/FLI* is the most common gene rearrangement in EWS and is used as a molecular diagnostic marker for the disease.[44] The translocation rearrangement results in the fusion between the amino terminus (5′ end) of the *EWS* gene, a member of the TET (TLS/EWS/TAF15) family of RNA-binding proteins, and the carboxy terminus (3′ end) of the *FLI1* gene, a member of the ETS family of transcription factors.[13,38,39] The transcription factor loses the regulatory domains of both proteins when translocated, which leads to an active transcription factor that modulates the expression of more than 500 genes.[19] Because *EWS/FLI* affects the cell in a multitude of different ways, its contribution to tumorigenesis is complex.[109]

The EWS/FLI fusion protein is a potent oncogene that has the ability to transform murine fibroblast cells.[19] In several different studies, inhibition of the endogenous EWS/FLI function or expression demonstrated reduction of oncogenic transformation *in vitro* and *in vivo*.[19] This implies that sustained expression of the fusion oncoprotein is required to maintain the oncogenic phenotype of EWS cells, leading to increased interest in targeting this fusion.[39] The reciprocal translocated chromosome, *FLI/EWS*, is not expressed in EWS tumors.[39]

EWS/FLI has proven to be a challenging drug target likely owing to the complex network described; however, the discovery of therapeutic vulnerabilities in EWS/FLI may potentially lead to drug development. RNA-interference screening has found that *EWS/FLI* fusion is vulnerable to the loss of RNA processing proteins that require splicing to occur at and downstream of the fusion protein.[18] When the splicing of *EWS/FLI* is disrupted, gene expression is altered, and genes required for survival of EWS cells are reversed.[20] Therefore, the disruption of the *EWS/FLI* fusion transcript may lead to a potential strategy for the treatment of EWS.[20] RNA-interference approaches using microarray technology have also resulted in identification of a large number of target genes dysregulated by *EWS/FLI* in EWS.[39] The upregulation of target genes such as *NR0B1*, *NKX2*, and *GLI1* have been identified as critical in the oncogenic process of *EWS/FLI*. Additional target genes that are necessary for sustained tumorigenesis, including cell proliferation, evasion of apoptosis, drug resistance, cell cycle control, evasion of growth inhibition, immortalization, angiogenesis, adhesion, and maintenance of pluripotency, include the following genes: *CCND1*, *IGFBP3*, *GSTM4*, *p21*, *TGFBRII*, *hTERT*, *VEGF*, *CAV*, and *EZH2*, respectively. The large array of target genes indicates that *EWS/FLI* modulates a whole network of downstream effects on genes to achieve oncogenesis.[39]

Other fusions that arise from different translocations have also been identified in rare cases of the disease.[50] Fusions have been identified between the TET family member *TLS*, also called *FUS*, and 2 different ETS family members, *ERG* and *FEV*. *TLS/ERG* and *TLS/FEV* fusion proteins are found in <1% of patients with EWS. They create a fusion that mimics *EWS/FLI*, thus functioning in an analogous manner by binding to the ETS target sites. Large-scale molecular and functional studies are currently ongoing to dissect alternative mechanisms that result in non-*TET/ETS* fusions in Ewing-like tumors. Newfound knowledge will shed additional light on mechanisms that drive EWS, which may potentially translate into new targeted therapies that affect patients with the disease.[39]

In the past decade, investigators have conducted studies to molecularly identify patients with EWS to reduce the number of patients with what has been called *atypical Ewing sarcoma*. In doing so, they have created 3 categories in addition to EWS, including round cell sarcomas with EWSR1 gene fusion with non-ETS family members, CIC-rearranged sarcomas, and BCOR-rearranged sarcomas. Only a few patients have the round cell sarcomas with EWSR1 gene rearrangements that involve non-ETS fusion partners. The *CIC* gene is the human homolog of the Drosophila gene capicua. *DUX4*, the most common fusion partner, is found on the long arm of chromosomes 4q35 or 10q26.3, and its physiological function is unknown. In approximately 5% of cases, the *CIC* gene may fuse with other gene partners (e.g., *FOXO4*, *LEUTX*, *NUTM1*, and *NUTM2A*). CIC sarcomas have a dismal prognosis (5-year overall survival rate ~50%) because most present with lung metastasis at diagnosis. The *BCOR-CCNB3* fusion-positive sarcoma is a relatively rare member of the "Ewing-like" family of tumors and this fusion accounts for 60% of *BCOR* gene alterations. This fusion originates from a paracentric inversion on the X chromosome and splicing of the end of the *BCOR* coding sequence to the *CCNB3* exon 5 splice acceptor site. The resultant fusion protein is composed of full-length BCOR, a transcriptional repressor encoding the Bcl-6 corepressor, and the C-terminus of CCNB3. *In vitro* studies suggest that the BCOR-CCNB3 fusion protein is oncogenic and drives proliferation in this sarcoma. Compared with patients with EWS, patients with BCOR-rearranged sarcoma may have an indolent clinical course.[111]

Synovial Sarcoma. Synovial sarcoma is a high-grade malignant tumor that primarily originates in the lower (62%) and upper (21%) extremities, but may occur elsewhere.[17,49] Despite the term *synovial sarcoma*, the tumor type does not have a biological or pathologic

relationship to the synovium.[49] Synovial sarcoma can occur at any age but is more common in adolescents and young adults. It is the most common sarcoma in adolescents and young adults after RMS, and in total SS accounts for 5% to 10% of soft tissue sarcomas.[17,49-51] Approximately 50% of SS patients develop metastatic disease, which characteristically occurs in the lungs and is fatal despite conventional chemotherapy.[49,51] Synovial sarcoma is commonly divided into monophasic or biphasic. The monophasic type has spindle-shaped mesenchymal cells and biphasic has epithelial in addition to spindle-shaped mesenchymal cells.[17,49,51] Additionally, a rare form of SS is classified as poorly differentiated with ovoid or rounded small cells associated with the worst clinical outcome.[49]

In >90% of cases, SS exhibits a t(X;18)(p11.2;q11.2) reciprocal chromosomal translocation between chromosome X and 18 and is a crucial factor in SS pathogenesis.[17,49,51,52,105] The translocation involves the *SS18* gene, previously known as *SYT*, on chromosome 18 and either *SSX1* or *SSX2* gene on chromosome X. The hallmark fusion oncoprotein, SS18/SSX, has all but 8 amino acids of *SS18* and 78 amino acids of *SSX1*, *SSX2*, or rarely *SSX4*.[17,52-55] The *SS18/SSX1* gene occurs in approximately 66% of cases and is more commonly seen in biphasic SS whereas monophasic SS can contain either the *SSX1* or *SSX2* translocated gene.[17,49] *SS18* is involved in the regulation of transcription, cell motility, and cytoskeletal organization and affects the expression of genes important for embryonic and placental development. The *SSX* genes are expressed in a wide array of cancers, such as melanoma, multiple myeloma, non-Hodgkin lymphoma, neuroblastoma, brain tumors, carcinomas of different origins, and sarcomas.[49]

SS18/SSX functions as an oncoprotein, and studies show continued expression is required for cell survival and is necessary and sufficient to support tumorigenesis.[54] Although there are multiple mechanisms to support tumorigenesis, the expression of SS18/SSX, which is similar to other sarcoma fusion oncoproteins, is thought to contribute to aberrant transcriptional activity and dysregulated gene expression.[49,51] The fusion competes for binding and leads to an altered complex that lacks a tumor suppressor gene that affects cell growth, cell proliferation, the TP53 pathway, and chromatin remodeling mechanisms.[18,49] Evidence also suggests that SS18/SSX pays a role in gene regulation via epigenetic mechanisms and can serve as a biomarker for SS.[52]

SS18/SSX acts on chromatin remodeling by binding with proteins such as mSIN3A, a part of the HDAC complex.[49] Similar to RMS, preclinical models show antitumor activity of HDAC inhibitors in SS.[51] Studies indicate that HDAC can suppress SS tumors by effectively reversing *SS18/SSX* non-mutational gene inactivation. Treatment with an HDAC inhibitor, FK228, revealed that cultured SS cells and xenografts in mice were significantly reduced and tumor weight and density decreased after treatment. Preclinical studies to evaluate mechanisms that disrupt *SS18/SSX* are currently ongoing; however, the involvement and repressor activity of HDAC suggests that it could provide a framework for therapeutic SS targets. Other agents, such as CDK inhibitors (e.g., CDK4/6 inhibitor palbociclib), EZH2 inhibitors, and BRD9 inhibitors that target proteins associated with fusion proteins such as SS18/SSX, were reviewed by Hale et al[112] and Li et al.[113] To gain a thorough understanding of effective targeted therapy, additional research efforts are required to study the interaction of SS18/SSX oncoproteins and the pathways that regulate these processes.[49,51]

Hematologic Cancer

Acute Myeloid Leukemia. Acute myeloid leukemia is a malignant disease that forms immature blood cells in the bone marrow and causes a decrease in red blood cells, platelets, and normal white blood cells. Acute myeloid leukemia causes uncontrollable proliferation of myeloid stem cells or progenitor cells and if left untreated the disease can progress rapidly.[70,72,114] In 40% to 50% of pediatric AML cases, representing a higher proportion than seen in adults, gene rearrangements cause random chromosomal translocations and lead to the expression of fusion proteins.[60,72,114,115] Leukemic transformation occurs because of the alteration of target genes needed for AML development.[114] Acute myeloid leukemia can be divided into subtypes based on chromosomal aberrations or mutations. The 4 most common translocations identified in AML, which have a frequency of 3% to 10%, include *AML1/ETO*, *MLL* fusions, *PML/RARα*, and *CBFβ/MYH11*.[114,116]

The most common fusion oncoprotein that occurs in AML is AML1/ETO.[72] The chimeric translocation t(8;21)(q22;q22) occurs in 10% of AML cases and involves the *AML1 (RUNX1)*, a DNA-binding transcription factor responsible for hematopoietic differentiation, and *ETO*, a protein responsible for transcription repressor activities.[70,72,114,115,117] Together the 2 fused genes harbor the fusion oncoprotein AML1/ETO, which has negative dominant activity and dysregulates the expression of genes that are responsible for myeloid cell development and differentiation. The prognosis for *AML1/ETO*-positive leukemia is poor. However, studies reveal that suppression of *AML1/ETO* via small interfering RNA can result in normal myeloid differentiation of positive leukemic cells, thus highlighting a potential target for AML therapy.[70,72,114,117]

Occurring in 10% of acute leukemias including, AML, ALL, and biphenotypic acute leikemia the mixed-lineage leukemia gene (*MLL*) is implicated in carcinogenesis.[114,118] MLL is a large nuclear protein involved in chromatin regulation that supports the transcription of genes and plays a role in normal development.[118] MLL is found in diverse myeloid and lymphoblastic leukemia subtypes

and is strongly associated with pediatric leukemias.[119,120] In B-cell ALL, the *MLL* gene and translocated *MLL/AF4* fusion occur at a higher incidence in infants than in children older than 1 year (50% and 2%–3%, respectively).[57]

An *MLL* fusion, which can occur in 1 of >50 genes, acts as a potent oncogene and harbors a poor prognosis for patients.[114,119,120] Patients with *MLL* rearrangements have long-term survival rates ≤50%.[118] When *MLL* rearrangements occur, the expression of fusion proteins causes chromatin complexes to dysregulate genes, disrupt normal cellular processes controlling chromatin, and cause leukemia.[114,118] There are 4 translocation genes that have been associated with >75% of cases of *MLL* fusion, namely *AF4*, *AF9*, *AF10*, and *ENL*.[118,120] Studying *MLL* rearrangements has helped gain insight into aberrant gene expression caused by dysregulated chromatin inhibition mechanisms. Clinical trials suggest that these approaches may provide further insight into the disease and lead to new therapeutic developments of small molecule inhibitors targeting epigenetic mechanisms.[118]

In 95% of APLs, a subtype of AML, the t(15;17)(q22;q21) translocation occurs, causing the *PML/RARα* fusion.[114,115] The *PML/RARα* fusion acts as a transcriptional repressor that interferes with differentiation, apoptosis, and self-renewal.[114] Breakpoints and isoform variants have been identified in fusion genes, including *PML/RARα*, which has prognostic implications, thus influencing prognosis and treatment in patients.[115] In 8% of AML cases, the translocation inv(16)(p13;q22) results in the generation of *CBFβ/MYH11*. *CBFβ* causes *AML1* to be stimulated, resulting in *AML1* repressing transcription.[114,115] The high cure rates (>80%) and remission rates (~ 95%) with retinoic acid in APL makes targeting fusion proteins a promising approach. More targeted therapies are being evaluated in early trials as discussed in Lonetti et al.[121]

Detection methods for AML are not standardized.[60] Minimal residual disease (MRD), which refers to residual leukemic cells remaining during or after treatment, is a prognostic marker of increased risk of relapse and shorter survival in patients with AML.[60] However, unlike ALL, the use of MRD is challenging in AML owing to difficulty and lack of standardization in measurement.[122] Few studies have evaluated MRD use in pediatrics because of the small number of patients.[60] However, studies reveal that multiplex RT-PCR can amplify more than 1 type of gene sequence to detect *AML1/ETO*, *PML/RARα*, and *CBFβ/MYH11* fusions, thus revealing a potential effective diagnostic technique for AML.[115]

Acute Lymphoblastic Leukemia. The most common childhood malignancy in children and young adults is ALL, accounting for 18.8% of all cancer cases in this age group.[123] Acute lymphoblastic leukemia accounts for approximately 80% of leukemia cases in childhood. The 2 major subtypes in ALL, determined by immunophenotyping, are T-cell, which represents 10% to 20% of ALL cases, and B-cell, which represents 80% to 90% of ALL cases.[124] Although survival rates of children and adolescents with ALL have improved over time because of supportive care, precise risk stratification, and personalized chemotherapy, 15% to 20% of patients will relapse.[125-128] Relapsed ALL is a common cause of cancer mortality in children in the United States, thus it is important to identify higher-risk children with ALL and develop new therapies.[127] Understanding the genetic heterogeneity of ALL has contributed to early treatment response; however, many children likely to relapse have genetic features that are indistinguishable from children who are cured.[125] Next-generation genome and transcriptome sequencing have helped to gain further insight into leukemogenesis, classify subtypes of leukemia, and identify potential targets for therapy.[126] These advances in technology will help to develop targeted agents for personalized treatment and less toxic treatments for patients with ALL.[126]

A spectrum for genetic abnormalities in ALL exists, which include translocations, deletions, and amplifications.[129] Structural chromosomal rearrangements leading to the expression of oncogenic proteins play a vital role in the malignant transformation of several cancers, including ALL. Fusion transcripts and various somatic mutations are the hallmark of ALL and dictate the pathogenesis and progression of the disease.[15,130] The various genetic abnormalities associated with pediatric ALL have clinical implications on prognosis and drug selection for treatment. In pediatric ALL, common fusion oncogenes include *BCR/ABL*, *ETV6/RUNX1*, and *MLL/AF4*.[74] BCR/ABL1-like ALL, also known as Philadelphia chromosome–like (Ph-like), is similar to *BCR/ABL* but lacks the *BCR/ABL1* fusion.[125,126,131-133] Genetically defined groups within ALL, such as *BCR/ABL1*-like ALL, have poor survival rates.[132]

The chromosomal translocation t(12; 21) (p13; q22) that leads to the fusion of *ETV6/RUNX1*, also known as *TEM/AML1*, is the most prevalent fusion in ALL in children 1 to 4 years of age and is found in 25% of all B-cell ALL cases.[57,61,131] The *ETV6/RUNX1* translocation has a higher incidence in younger children, which is attributed to the prevalence of recurring rearrangements, and decreases with increasing age.[57,130] *ETV6/RUNX1* plays a vital role in the pathogenesis of leukemia B-ALL cases. This fusion occurs in Ph-like ALL and is associated with a high risk of relapse and mortality.[132]

Traditionally, B-cell ALL was divided by cellular appearance and included the following subtypes: acute, acute precursor, and pre–B-cell ALL. The various subtypes within ALL reveal differences in clinical features, laboratory values, and treatment response. However, in 2016, the WHO updated B-cell ALL classifications into several groups based on chromosomal translocations.[134] Chromosome alterations, including rearrangements, deregulate oncogenes or encode proteins and result in the formation of chimeric fusion proteins that are distinct from the non-fused counterparts.[130,132] Fusion proteins in B-cell ALL play a vital role in leukemogenesis by

disturbing hematopoietic development, tumor suppression, kinase signaling, and chromatin remodeling, hence they are important markers for therapeutic targets.[130,132] *In vitro* studies reveal that the *ETV6/RUNX1* fusion is sensitive to TKIs, thus demonstrating a potential utility of TKI in ALL patients.[131]

Discussion

Cancer remains the leading cause of death from disease in children, and toxicity from treatment continues to affect most children cured of cancer.[3] Oncogenic fusions are common in soft tissue sarcomas as well as in leukemia.[12,14] Soft tissue and bone sarcomas are characterized by chromosomal translocations that result in the expression of fusion oncoproteins critical for oncogenesis.[135] Fusion oncoproteins transform cells of known origin and dysregulate normal developmental and regulatory pathways.[136] They are oncogenic drivers of many childhood cancers that are found in primary and relapsed tumors. Translocations such as *PAX/FOXO1* in RMS, *EWS/FLI1* in EWS, and *SS18/SSX* in SS are some of the hallmark fusion oncoproteins in pediatric sarcomas.[135,136] Fusion oncoproteins in leukemia span a wide array of protein classes, which include kinases and transcription factors, thus contributing to the complexity in developing targeted therapeutic agents.[12]

Research has shown that fusion proteins deregulate complexes that control gene expression or chromatin.[12,135] Currently, few drugs target fusion oncoproteins, thus a deeper understanding of protein complexes that drive pediatric cancer is needed.[18,101,114,136] Modern technologies, such as CRISPR-Cas9–associated screening, will help gain an understanding of these deregulated complexes, their functional domains, and interaction with other proteins.[12,136] These technologies can also assess genes associated with tumorigenesis resulting from specific fusion oncoproteins and further progress future drug discovery.[136] Understanding the function of fusion genes, involvement in signaling pathways, and characterization of genomic targets for fusion oncoproteins will help develop new therapeutic options for patients.[6,114]

A better understanding of basic molecular mechanisms to guide therapeutic approaches in childhood cancers is needed.[114] To date there are no systematic assessments of unique fusion oncogenes, thus various research groups continue to screen therapeutic targets and identify fusion-driven childhood cancers and more work is still needed.[135,136] A collaborative, systematic approach to cell line collection, data generation and storage, and analyses is required to gain further insight in pediatric cancers.[136] Novel genomic technologies including next-generation sequencing has helped to understand the landscape of pediatric cancer; however, translating knowledge to the clinic requires biomarkers and model systems that accurately characterize tumor types.[18,114]

Currently, there are more than 30 bioinformatic tools, which differ in sensitivity and selectivity, to detect fusion genes.[6] Whole genome sequencing of germline or tumor samples and targeted RNA sequencing will help usher in a new era of unprecedented analysis that uses precision medicine to analyze the genome for children with high-risk, refractory, or relapsed cancers.[21,137] RNA sequencing (RNAseq) provides genomewide surveillance of fusion genes in a single test, but careful interpretation of sequencing is required owing to the risk of false positives. To improve the sensitivity of RNAseq, targeted RNAseq methods have been used.[21] Nonetheless, this method could lead to improved cancer diagnosis, prediction of treatment resistance, additional prognostic information, and an enhanced understanding of gene biology.[21]

The Cancer Moonshot, otherwise known as Cancer Breakthroughs 2020, was passed by Congress in 2016 with the goal of accelerating cancer research. It aims to make more therapies available to patients and improve the ability to prevent and detect cancer at an early stage.[136] The Blue Ribbon Panel for the National Cancer Moonshot Initiative, a Working Group of the National Cancer Advisory Board, provides scientific expertise from cancer researchers, oncologists, patient advocates, and representatives from the private sector and government agencies on the scientific opportunities that could be accelerated through this initiative. Improving the understanding of fusion oncoproteins in pediatric cancer is one of the goals that have been established to address the Cancer Moonshot research initiatives.[136] The Fusion Oncoproteins in Childhood Cancers Consortium is a collaborative research network that is actively advancing the understanding of fusion oncoproteins in childhood cancers to develop targeted treatments for pediatric patients.[138] The consortium aims at focusing on improving knowledge of pediatric cancers that are at high risk for treatment failure or have no known effective targeted therapies.[138] Areas of research and investigation include developing model systems to examine the function of fusion oncoproteins within genetic models that reflect minority and underserved groups, using functional genomic screening to identify the effects of oncoproteins on molecular pathways, and developing functional assays to test the effects of small molecules on blocking the negative effects of fusion oncoproteins.[139] Additional areas of focus are collecting data for computational models to assist with drug design, and identifying the effects of oncoproteins on gene expression and protein complexes or post-translational modifications that could be targeted to intercept the oncogenic properties.[138]

In addition, the National Cancer Institute made a call for exploratory developmental grant applications to investigate the molecular mechanisms of oncogenic fusion genes for pediatric sarcomas.[139] These grants are aimed at gaining a better understanding of molecular pathways that are activated by chromosomal translocations, the relationship to oncogenesis and tumor progression, and elucidating mechanisms of cancer pathogenesis to

discover novel therapeutics.[140] The research is intended to accelerate and promote the understanding of fusion oncoproteins in pediatric cancer and to promote the development of cancer therapeutics by identifying potential drug targets.[139]

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