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POSTER ABSTRACTS

618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

A *D e Novo* Supernumerary Ring Chromosome 1 Causes B-Cell Acute Lymphoblastic Leukemia in Monozygotic Twins Due to Independent and Partially Convergent Evolutionary Trajectories

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Introduction

B-cell acute lymphoblastic leukemia (B-ALL) is the most common pediatric cancer. In recent years, our understanding of B-ALL biology has benefited from genomic and transcriptomic analyses that identify driver alterations in 98% of patients. In addition, prior studies of monozygotic twins, who both developed B-ALL, have provided insight into the developmental timing and natural history of the disease, as well as the identification of novel mechanisms of leukemogenesis.

Herein we study a case of monozygotic twins, who developed B-ALL **(Table 1).** We report the characterization of a *de novo* supernumerary ring chromosome 1 (SRC1) as an initiator event that followed independent but partially convergent clonal trajectories in each twin. Thus, SRC1 resulted in genomic rearrangements of *MEF2D* in both twins but targeted distinct fusion gene partners (*BCL9* versus *ARNT*). To our knowledge, this study represents the first description of *MEF2D-ARNT* as an event involved in B-ALL, and the association of SRC1 Syndrome with leukemia.

Methods

We performed integrative whole genome sequencing (WGS) and RNAseq analysis on B-ALL samples at diagnosis and compared this to WGS data from minimal residual disease negative bone marrow aspirates. Coverage levels for tumors and matched normals were: Twin A=104 & 59; Twin B=81 & 53. Tumor purity estimations were 0.80 in Twin A and 0.31 in Twin B. For RNAseq, expression profile efficiencies were 61% and 36% respectively. Comprehensive variant calling was performed using the Isabl platform (Medina et al, 2020) to map all putative somatic and germline events. ALLCatchR (Beder et. al, 2022) was used for molecular B-ALL subtype allocation in 26 samples from our pediatric cohort of patients including the twins (age range 1-23), and MutationTimeR (Gerstung et al, 2020) to calculate the relative timing of copy number gains. All research studies were approved by NYU and MSKCC Institutional Review Boards.

Results

The tumor mutational burdens for each leukemia sample were 0.86 for Twin A and 0.47 mutations/Mb for Twin B, and they showed independent alterations in oncogenic genes **(Table 1)**. The only B-ALL driver that both twins had partially in common was an intrachromosomal translocation resulting in *MEF2D* fusions, but each with a distinct partner gene (*BCL9* versus *ARNT*) which was confirmed by RNAseq (supporting reads: Twin A=48, Twin B=107) and PCR assays **(Fig. 1)**. Each sample demonstrated enhanced transcription levels of *MEF2D* compared to our reference B-ALL cohort (transcripts per million: Twin A=48.0, Twin B=77.4, median=27.7, sd=19.9), and were transcriptionally classified as MEF2D-subtype by the ALLCatchR classifier. These results functionally supported the role of *MEF2D-ARNT* as a new oncogenic event.

Analysis of germline variants showed a tandem duplication flanking the pericentromeric region of chromosome 1 in both twins (1:120,163,074-157,714,717) where *MEF2D BCL9*and *ARNT* are localized, that had been previously identified as SRC1 in

only one of them by constitutional genotyping **(Table 1)**. Cell fractions in normal and tumor tissues were: Twin A=14.8% & 50%, Twin B=8.6% & 26.5%. PCR interrogation of parental germline DNA failed to identify SRC1, suggesting this to be a *de novo* event in the affected twins **(Fig. 1).**

We compared the relative timepoint in which the SRC1 took place in each sample using MutationalTimeR, but we were only able to obtain results in Twin A due to a higher mutational burden (number of variants in SRC1: Twin A=44, Twin B=13). As expected, the tandem duplication that formed the SRC1 was identified as an early mutational event (confidence interval of relative time of appearance: 0-0.4).

Conclusions

Our results identify SRC1 as the common initiating genomic event leading to *MEF2D* fusion driven B-ALL in both twins. These findings support a model in which a tandem duplication around a pericentromeric area was an initiating event which caused the scission and circularization of DNA without the loss of genetic material. This led to a *de novo* acquisition of a SRC1, that after independent but partially convergent clonal trajectories gave rise to 2 distinct oncogenic *MEF2D* fusion driven leukemias in each twin . Moreover, *ARNT* is a novel partner gene to *MEF2D* which has not been previously described.

This is the first report of B-ALL association with SRC1 Syndrome, which emphasises the utility of the study of monozygotic twins to identify novel alterations underlying B-ALL.

Disclosures Gundem: *Isabl Inc.:* Consultancy. **Levine:** *Isabl Inc.:* Current Employment, Current holder of *stock options* in a privately-held company, Patents & Royalties. **Medina Martinez:** *Isabl Inc.:* Current Employment, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees. **Kung:** *Isabl Inc.:* Current Employment, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees. **Shukla:** *Syndax Pharmaceuticals:* Honoraria. **Papaemmanuil:** *Isabl Inc.:* Current equity holder in private company, Current holder of *stock options* in a privately-held company, Other: CEO, Patents & Royalties: Whole genome cancer analysis; *TenSixteen Bio:* Current equity holder in private company.

Table 1: Clinical and genomic features of both twins.

Clinical feature	Twin A	Twin B
Age at diagnosis	11 years	15 years
WBC	7.3k/μL	18.5k/μL
Phenotype	CD45 dim to negative (mostly), CD19 +, CD20 minimal+, CD10 +, CD34 partial +, CD38 +, CD22 +, CD56 dim+, HLA-DR+, TDT +	CD45 dim +, CD19+, CD20 mostly +, CD10+, CD34-, CD38+, CD22+, CD5 partial dim +, CD9+, CD58+, HLA-DR+, TdT partial +
CNS	CNS1 (1 WBC, 42 RBC)	CNS3 (10 WBC, 208 RBC)
Cytogenetics	47,XX,+r[7]/47,XX,add(12)(p11.2),+mar[7]/46,XX[6] FISH: *E2A* rearrangement in 85% nuclei and 1 copy of EVT6 in 98% cells	46,XX,del(6)(q13q23),dic(7;9)(p11.2;p11.2),+mar[8]/46,XX[13] FISH: Deletion of *CDKN2A* (9p21) in 29% of the nuclei
Constitutional genotype at diagnosis		*De novo* chromosomal mosaicism for a supernumerary small ring chromosome that originated from chromosome 1 (SRC1)
WGS/RNAseq somatic alterations	*SETDB1* (p.L1543R), *ARID1A* (p.Q1835fs*46) *SMARCA4* (p.R978fs*1) ***MEF2D/BCL9* fusion** MEF2D RNA subtype	*GNB1* (p.I80N) ***MEF2D/ARNT* fusion** MEF2D RNA subtype
WGS/RNAseq germline alterations	Tandem-duplication (chr1:120,163,074-157,714,717)	Tandem-duplication (chr1:120,163,074-157,714,717)

Figure 1: PCR-gel validation of *MEF2D* translocations and SRC1.

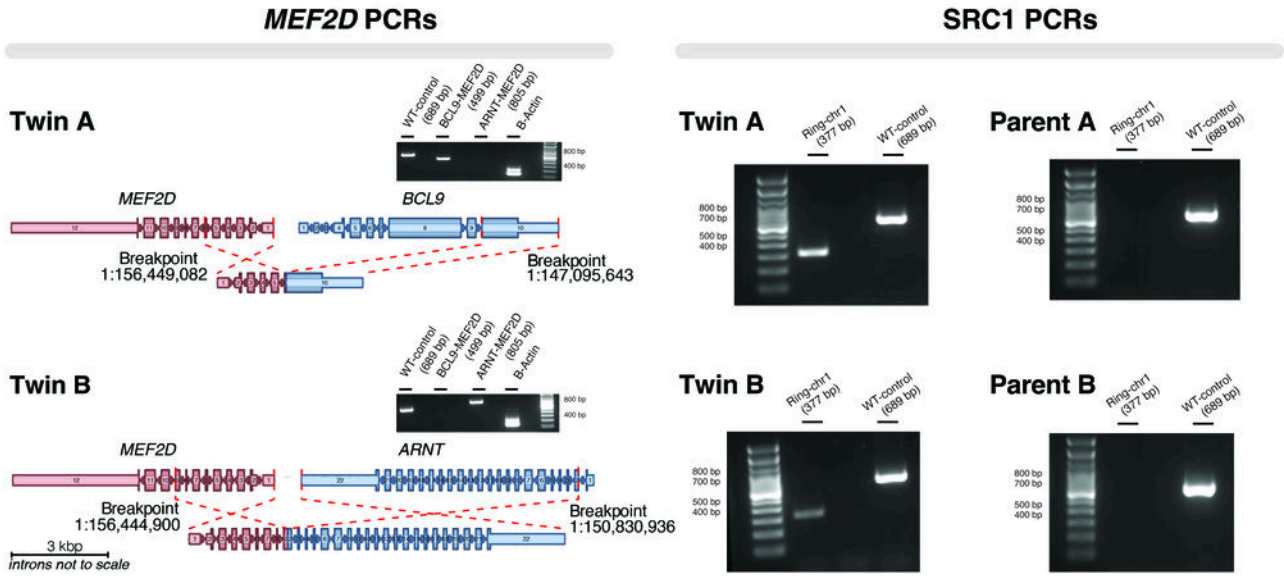

Figure 1

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