Clinical efficacy of ruxolitinib and chemotherapy in a child with Philadelphia chromosome-like acute lymphoblastic leukemia with *GOLGA5-JAK2* fusion and induction failure

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SUPPLEMENTAL METHODS

Chemotherapy backbone regimen

The patient received multi-agent induction chemotherapy on the Children's Oncology Group high-risk B-ALL phase 3 clinical trial AALL1131 and post-induction therapy as per AALL1131 with addition of ruxolitinib 40 mg/m2/dose orally twice daily (PO BID). The specific chemotherapy medications and dosing are listed below.

Induction: vincristine intravenously (IV) 1.5 mg/m^2/dose on days 1, 8, 15 and 22, prednisone orally PO 30 mg/m^2/dose BID on days 1-28, daunorubicin IV 25 mg/m^2/dose on days 1, 8, 15 and 22, peg-asparaginase IV 2500 International units/m^2/dose on day 4, intrathecal (IT) cytarabine 70 mg on day 1, and IT methotrexate 15 mg on days 8 and 29.

Consolidation: cyclophosphamide IV 1000 mg/m2/dose on days 1 and 29, cytarabine subcutaneously (SC) 75 mg/m^2/dose on days 1-4, 8-11, 29-32, and 36-39, 6-mercaptopurine PO 60 mg/m^2/dose daily on days 1-14 and 29-42, vincristine IV 1.5 mg/m^2/dose on days 15, 22, 43 and 50, peg-asparaginase IV 2500 International units/m^2/dose on day 15 and 43, and IT methotrexate 15 mg on days 1, 8, 15 and 22.

Interim Maintenance I: methotrexate IV 5000 mg/m^2 on days 1, 15, 29, and 43 with leucovorin 15 mg/m^2/dose PO every 6 hours until methotrexate clearance, vincristine IV 1.5 mg/m^2/dose on days 1, 15, 29, and 43, 6-mercaptopurine PO 25 mg/m^2/dose daily on days 1-56, and IT methotrexate 15 mg on days 1 and 29.

Subsequent chemotherapy with cyclophosphamide IV 440 mg/m^2/dose and etoposide IV 100 mg/m^2/dose daily days 1-5 was also administered. Lymphodepleting chemotherapy with fludarabine IV 30 mg/m^2/dose daily days 1-4 and cyclophosphamide IV 500 mg/m^2/dose daily days 1 and 2 was administered prior to CD19-redirected chimeric antigen receptor T cell immunotherapy.

Molecular monitoring of GOLGA5-JAK2 fusion

Reverse-transcriptase polymerase chain reaction (RT-PCR), nested PCR, and real-time quantitative (q) PCR assays were used for the confirmation/detection of the *GOLGA5-JAK2* fusion gene. In brief, three forward primers located at exon 8 (F3), exon 9 (F2), and exon 10 (F1) of *GOLGA5* and 3 reverse primers located at exon 19 (R1), exon 20 (R2), and exon 22 (R3) of *JAK2* were designed (Supplemental Table 1). One ug of total RNA was used for reverse-transcription, and the F3/R3 primer pair was used with 2 uL of cDNA to run the first PCR cycle. For the second PCR cycle, primers F1/R1 and F2/R2 were used with 1 uL of the first cycle PCR product. The products of the second PCR cycles were separated via 1.2% agarose gels and visualized with ethidium bromide. PCR products with visible bands were then sequenced on an ABI 3730XL sequencer using standard laboratory procedures. All PCR reagents were from Qiagen.

For reverse transcription, 1 ug of RNA was mixed with 4uL iScript RT supermix and RNase-free water for a total reaction volume of 20 uL. The reaction was incubated at 25 °C for 5 minutes, 42 °C for 60 minutes, and 85 °C for 5 minutes.

For nested PCR, 2 uL of reverse transcription product was mixed with 12.5 uL Qiagen fast cycling mix, 1 uL 10 uM primer mix (F3 + R3), and water for a total reaction volume of 25 uL. The mixture was incubated at 95˚C for 2.5 minutes, 20 cycles of 30 seconds at 95˚C, 30 seconds at 56˚C and 1 minute at 72˚C, then 5 minutes at 72˚C (first round PCR). Subsequently, 1 uL of the first round PCR product was mixed with 12.5 uL Qiagen fast cycling mix, 1 uL 10 uM primer mix (F1 + R1 or F2 + R2), and water for a total reaction volume of 25 uL. The mixture was incubated at 95˚C for 2.5 minutes, 35 cycles of 30 seconds at 95˚C, 30 seconds at 58˚C and 1 minute at 72˚C, then 5 minutes at 72˚C (second round PCR).

For real-time PCR, the reverse transcription product of 1 ug of RNA was diluted 10 times. Two uL of this cDNA template was mixed with 10 uL iTaq Universal SYBR

Green Supermix, 1 uL 10uM primer mix (forward and reverse), and water for a total reaction volume of 20 uL. The reaction was carried out at 95˚C for 2 minutes, then 45 cycles of 15 seconds at 95˚C and 30 seconds at 60˚C. A melting curve step was added at the end of the reactions.

SUPPLEMENTAL TABLES

Supplemental Table 1. Clinical single nucleotide polymorphism (SNP) microarray analysis of diagnostic bone marrow specimen.

Chromosome	Copy Number	Abnormality/Genes
5q33.3	1	Loss/ bkpt in EBF1
6p25.2p25.1	1	Loss
Multiple discontinuous deletions within 6q15q23.2		
6q15q16.1	1	Loss
6q16.1	1	Loss
6q16.2q16.3	1	Loss
6q16.3q21	1	Loss
6q21q22.31	1	Loss
6q22.31	1	Loss
6q22.31	1	Loss/ NKAIN2
6q22.33	1	Loss/ PTPRK
6q23.1	1	Loss
6q23.2	1	Loss
6q23.2	1	Loss
6q27	3	Gain/ MLLT4
7p14.1	0	Loss/ TCRG
7p12.2	1	Loss/ IKZF1
7q34	0	Loss/ TCRBV
9p24.1	3	Gain/ JAK2
chromothripsis of 9p13p22		
9p22.2	1	Loss
9p21.3	1	Loss/ CDKN2A, CDKN2B
9p21.3	1	Loss
9p21.2	1	Loss
9p21.1	1	Loss
9p21.1	1	Loss
9p21.1	1	Loss
9p13.3p13.1	1	Loss/ PAX5
9q21.33	1	Loss/ NTRK2
9q22.2	1	Loss
9q22.31q31.1	1	Loss
9q31.1	1	Loss
9q33.1	1	Loss
14q11.2	0	Loss/ TCRA
14q32.12	3	Gain/ LGMN/GOLGA5
14q32.33	0	Loss
14q32.33	0	Loss
22q11.22	1	Loss/ VPREB1

Supplemental Table 2. Primer sequences used for real-time qPCR analyses.

RT-PCR primer	Sequence	Amplicon size (base pairs)
GOLGA5E10-JAK2E19 F1	CATCCAGAAACAGACCATGC	293
GOLGA5E10-JAK2E19 R1	TACCCTTGCCAAGTTGCTGT	
GOLGA5E10-JAK2E19 F2	CAAAGAACACATTGCAAAGCA	580
GOLGA5E10-JAK2E19 R2	TCATGCTGTAGGGATTTCAGG	
GOLGA5E10-JAK2E19 F3	AGAACTGGAGCGACTGAAGC	847
GOLGA5E10-JAK2E19 R3	TGCCAGATCCCTGTGGATA	
GOLGA5E10-JAK2E19 RT-qPCR F1	AGATGAACTCCGCCTCTGGAAGTAG	172
GOLGA5E10-JAK2E19 RT-qPCR R1	TCAAACTGTGTAGGATCCCGGTCTT	
HPRT1_F (control gene)	GAGGAGTCCTGTTGATGTTGCCAG	173
HPRT1_R (control gene)	GGCTGGCCTATAGGCTCATAGTGC	

E = exon, F = forward primer, R = reverse primer