The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

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

Asciminib Is Effective Against *ABL1* Gene Fusions in Acute Lymphoblastic Leukemia but Only When the *ABL1* SH3 Domain Is Present

Laura N Eadie, BSc, PhD [1,2], Caitlin E Schutz, BSc(hons) [2], Elyse C Page, BSc(hons), PhD [1,2], Tace S Conlin, BSc [2,1], Susan L Heatley, PhD [2,1], Elias Lagonik, BSc(hons) [2,1], Jacqueline A Rehn, BEd, MBiotech [1,2], David T Yeung, MBBS,PhDBSc,FRACP,FRCPA [3,4,5,6], Timothy P Hughes, MDMBBS,FRACP,FRCPA [1,7,8,4], Deborah L White, PhD [4,1,2]

[1] Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, Australia
[2] Precision Cancer Medicine Theme, South Australian Health and Medical Research Institute, Adelaide, Australia
[3] Blood Cancer, Precision Medicine Theme, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia
[4] Australasian Leukaemia and Lymphoma Group (ALLG), Melbourne, Australia
[5] Haematology, Central Adelaide Local Health Network (CALHN), Adelaide, Australia
[6] Adelaide Medical School, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, Australia
[7] Haematology Department, Royal Adelaide Hospital, Adelaide, Australia
[8] South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia

Background

Ph-like acute lymphoblastic leukemia (ALL) is high risk and comprised of many kinase activating gene fusions including those involving the *ABL1* gene (*ABL1*-rearranged, *ABL1*r). *ABL1*r ALL is associated with poor relapse-free and overall survival and incidence increases with age. Tyrosine kinase inhibitors (TKIs) such as dasatinib effectively target *BCR::ABL1*+ ALL and are in clinical trials for *ABL1*r ALL. However, resistance to TKIs is often observed, and novel treatments are required. The allosteric inhibitor asciminib binds the myristate pocket of Bcr-Abl kinase and is currently in Phase III trials for treatment of *BCR::ABL1*+leukemias. We assessed asciminib efficacy in *ABL1*r ALL.

Methods

Ba/F3 cells were transduced with four *ABL1* fusions observed in ALL and asciminib efficacy evaluated in AnnexinV/7-AAD cell death assays (LD $_{50}$). Specific regions of the *ABL1* fusion partner were deleted by site-directed mutagenesis (SDM). STAT5/CRKL phosphorylation and hCD45 levels were investigated by flow cytometry. Cells from three *NUP214::ABL1* ALL patients were injected into NSG mice to establish patient-derived xenograft (PDX) models. Once mice reached 5% hCD45+ cells in peripheral blood (PB), treatment was commenced: vehicle control, dasatinib, asciminib. At experimental endpoint (>50% hCD45+ cells in PB) mice were humanely killed and organs harvested for analysis.

Results

Transduced Ba/F3 cells demonstrated varying sensitivity to asciminib (Table 1). While asciminib was not efficacious against Ba/F3 *RCSD1::ABL1* and *SNX2::ABL1* cells, Ba/F3 *RANBP2::ABL1* (Heatley *et al* 2021)and *NUP214::ABL1* cells demonstrated LD $_{50}$ concentrations within the clinically achievable range (3 μM, p<0.0001; 0.8 μM, p=0.0238 compared with control, respectively). Following asciminib treatment, Ba/F3 *NUP214::ABL1* cells demonstrated reduced phosphorylation of STAT5 (mean fluorescence intensity [MFI]=1820 to 790, 57% reduction) and CRKL (MFI=2069 to 951, 54% reduction), confirming asciminib inhibits kinase signalling. No phosphorylation of STAT5 or CRKL was observed in control cells (MFI=290 and 406, STAT5 and CRKL, respectively). Additionally, *ex vivo* assessment of *ETV6::ABL1* patient blasts demonstrated reduced phosphorylation of STAT5 (MFI=3395 to 1788, 47% reduction) and CRKL (MFI=5039 to 3000, 40% reduction) following asciminib treatment. Interrogation of the different *ABL1* fusion breakpoints (Figure 1) revealed fusions demonstrating asciminib sensitivity retained exons 2 or 3 of *ABL1* (corresponding to complete/partial SH3 and complete SH2 domains). Conversely, gene fusions lacking exons 1-3 (truncating the SH3 domain and retaining a partial SH2 domain) were resistant to asciminib suggesting a critical region in the SH3 and/or SH2 domain/s required for asciminib efficacy. To further investigate this we systematically deleted

regions of *ABL1* exon 3 using the *NUP214::ABL1* construct as the model system and generated transduced Ba/F3 cells expressing different SDM regions. Cell death assays indicated the SH3 domain is critical for asciminib efficacy (Table 1). Asciminib also demonstrated efficacy in pre-clinical mouse modelsof *NUP214::ABL1* ALL (where *ABL1* exon 3 and partial SH3 domain is retained). Asciminib treatment significantly increased survival outcomes compared with control mice (84 vs 46d, p=0.01) by a time comparable to that of dasatinib treatment (93 vs 46d, p=0.0046). Results were confirmed in two additional *NUP214::ABL1* PDX models demonstrating asciminib's potential to treat *NUP214::ABL1* patients. Asciminib treatment reduced spleen and liver weights to that of healthy mice and resolved the leukemic immunophenotype of PB and bone marrow cells.

Conclusion

*ABL1*r ALL is associated with high rates of treatment failure and relapse. For the first time, we demonstrate the efficacy of asciminib in this high risk disease. Our data support addition of asciminib to treatment regimens of patients with *NUP214::ABL1* ALL, the most common ABL class gene fusion affecting up to 6% of B-ALL patients and up to 10% of T-ALL patients. We also identify a critical region of *ABL1* required for asciminib efficacy in *ABL1*r ALL. We propose interrogation of *ABL1*r patient breakpoints to determine inclusion/exclusion of the *ABL1* SH3 domain as a surrogate test to be used in conjunction with *in vitro* kinase sensitivity assays for predicting whether a patient is likely to respond to asciminib treatment.

Table 1: LD$_{50}$ values from cell death assays using asciminib to target different *ABL1* fusion genes identified in ALL. Note the steady state C$_{max}$ of asciminib in patients treated with 200 mg BID is 15.2 μM

	LD$_{50}$ (μM)	Efficacy	SH3 domain	SH2 domain
Vector control	22.1	N	N/A	N/A
RCSD1::ABL1	34	N	N	partial (33 aa)
SNX2::ABL1	42	N	N	partial (33 aa)
RANBP2::ABL1	3	Y	complete (54 aa)	complete (94 aa)
NUP214::ABL1	0.8	Y	partial (33 aa)	complete (94 aa)
NUP214::ABL1 SDM1	9.7	reduced	partial (8 aa)	complete (94 aa)
NUP214::ABL1 SDM2	0.6	Y	partial (33 aa)	partial (69 aa)

aa=amino acids present in the fusion
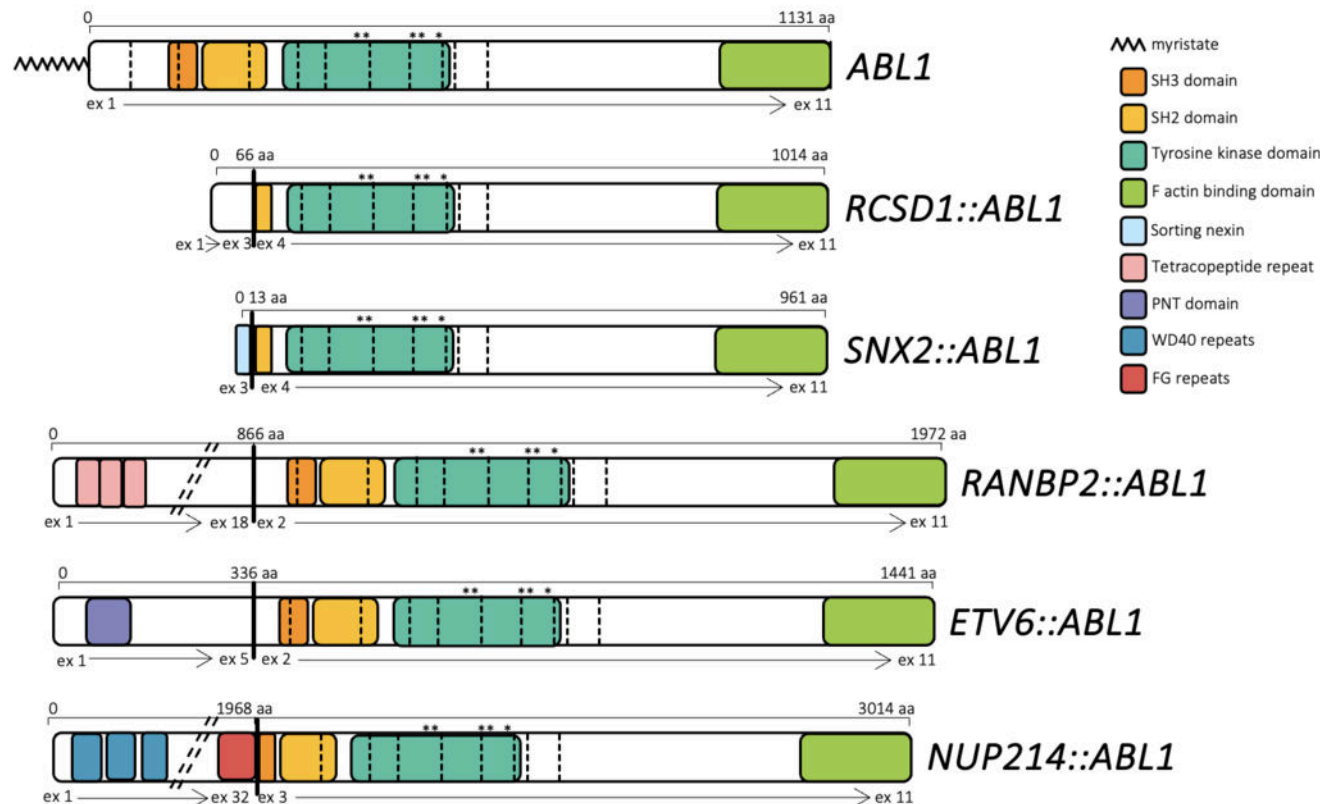
Figure 1. Schematics depicting the functional domains incorporated in each *ABL1* fusion gene. In all fusion genes, the *ABL1* tyrosine kinase domain is retained and the autoinhibitory N-terminus myristate moiety is truncated. The residues required for formation of the myristate binding pocket are retained in all fusions as indicated by (*), however, the regions of the SH3 and SH2 domains included in the fusions are variable. Breakpoints are denoted by vertical bold black lines and exons by vertical dashed lines. Diagonal dashed lines indicate where regions of the some fusion partner genes have been omitted for simplicity.

Figure 1