LETTER TO THE EDITOR

Crosstalk between β-catenin and WT1 signaling activity in acute myeloid leukemia

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Supplementary Table S1. Clinical characteristics of AML/MDS patient diagnostic/relapse samples used in this study.

Patient no.	Age (at diagnosis)	Sex	WBC count ($x10^9$/L)	Sample type	Secondary disease (Y/N)	Genetic information	Other clinical information
1	76	F	389	LP	N	Normal karyotype, $NPM1^+$, $FLT3^+$	n/a
2	4	M	n/a	BM	n/a	n/a	n/a
3	10	F	n/a	BM	n/a	n/a	Deceased
4	n/a	n/a	>200	PB	Y	n/a	Post-allogeneic transplant. M0/1 (previously diagnosed with M3 10 years previously)
5	n/a	n/a	n/a	n/a	n/a	n/a	n/a
6	17	M	n/a	BM	Y	n/a	Post-BMT for AML following 2 relapses. Deceased
7	6	M	70.7	n/a	Y	n/a	Secondary to Ewings Sarcoma. Myelomonocytic morphology. Deceased.
8	14	F	7.6	BM	N	MLL rearrangement. Karyotype: 46,XX,ins(10;11)(q11.2;q23.1q23.3).ish ins(10;11)?inv(11)(q23.3)(5'MLL+)(q23.1)(3'MLL+)	BMT for high-risk AML. CD33+, MPO+, CD34-, CD117+, TdT+, CD64+, CD11c+, CD15+, CD11b+, NG2+
9	24	M	256	BM	N	46,XY,inv(16)(p13q22)[16].ish inv(16)(p13)(MYH11+,CBFB+)(q22)(CBFB+,MYH11+)[5]	M4, relapsed, deceased.
10	17	M	294	BM	N	46,XY[20]	Alive, complete remission.
11	64	F	13.3	BM	N	Normal karyotype	AML with underlying MDS like changes
12	13	F	91.9	BM	Y		High Risk. Relapsed AML secondary to Rhabdoid tumour. Mixed cellular infiltrate consisting of predominantly monocyte/macrophages (CD14+, CD11c+,

							CD64+, CD34-, CD117-). Deceased
13	4	F	2.6	BM	N	Normal karyotype, $NPM1^{+}$ (exon 12), $FLT3^{-}$	MRD neg. CD13+, CD33+, CD34+, CD117+, MPO+
14	15	F	6.5	BM	N	MLL (KMT2A) rearrangement, t(10;11)(p11-p14,q23), MLL-MLLT10	MRD detected post treatment course 1
15	4	M	3.2	BM	N	t(10;11)(p11.2;q23) KMT2A-MLLT10. Cytogenetically cryptic. KMT2A ex8-MLLT10 ex9 or KMT2A ex9-MLLT10 ex10 fusion detected. $NPM1^{-}FLT3^{-}$	CD13-, CD33+, CD34-, CD117+/-, CD11c+, CD64+, CD14-, NG2+. High risk cytogenetics. BMT. MRD neg post course 1+2.
16	14	F	14.7	PB	N	t(8;21)(q22;q22) RUNX-RUNX1T1. 46,XX,der(8)?del(8)(q11.2q21.3)?dup(8)(q24.3q21.3)?ins(8;21)(q22;q22.12q22.3),der(21)?ins(8;21)(q22;q22.12q22.3)[10]	CD13+, CD33+, CD34+, CD117+, DR+, MPO+, CD19+. MRD negative post treatment course 1, 2 and EOT
17	5	M	1.6	BM	N	n/a	M5. Deceased.
18	14	F	2.1	BM	N	45,XX,-7,add(11)(p11.2)[9]/46,XX[1]	22% Myeloid blasts (CD117+, CD34+/-, CD33+, CD13+, MPO-) + 10% immunophenotypically mature monocytes (CD11c+, CD64+, CD117+) BMT
19	8	F	n/a	BM	N	n/a	M4/5. Deceased.
20	7	F	34.4	BM	Y	MLL rearrangement t(9;11)	M5a morphology. BMT following relapse. Deceased
21	16	F	20.6	BM	N	Normal karyotype 46,XX[20]	CD33+, MPO+, CD34+, CD117+, CD13+, CD14-, CD7+, CD45 weak, CD11c+, TdT-
22	7	M	3.5	BM	N	t(8;21)(q22;q22) RUNX-RUNX1T1 45,X,-Y,t(8;21)(q22;q22)[9]/46,XY[1]	8% myeloid blasts present (CD13+, CD33+, CD34+, CD117+, MPO+)
23	11	M	n/a	BM	N	n/a	BMT for MDS. Deceased.
24	13	F	14.7	BM	N	Karyotype:	Only 2 megakaryocytes

						46,XX,der(8)?del(8)(q11.2q21.3)?dup(8)(q24.3q21.3)?ins(8;21)(q22;q22.12q22.3), der(21)?ins(8;21)(q22;q22.12q22.3)[10] CD13+, CD33+, CD34+, CD117+, DR+, MPO+, CD19+.; AML	seen. Erythropoiesis reduced. Prominent eosinophils and eosinophil precursors - c. 12%. No significant monocytoid population. Densely infiltrated with myeloid blasts. Analysis showed an abnormal female clone with a derivative chromosome 8 from a variant 8;21 rearrangement. This abnormality is consistent with a diagnosis of AML (WHO 2008 subtype: AML with t(8;21)(q22;q22); RUNX1-RUNX1T1) and is reported in association with a favourable prognosis. Alive, complete remission.
25	2	M	2.4	BM	N	t(9;11)(p22;q23), t(11;21)(q23;q8)	n/a
26	4mo	M	5.1	BM	N	t(9;11)	BMT
27	7	F	n/a	BM	N	MPAL, 46XX, del5q, abnormal 21	n/a
28	7mo	F	168.6	BM	N	Karyotype: 47,XX,+21[5]/46,XX[5] nuc ish(CBFA2T3,GLIS2)X3(CBFA2T3 con GLIS2x2)[92/150] + RUNX1.	BMT 24/12/20 due to high-risk genetics. CD33+, CD34+, CD117+, MPO+, DR-, CD13+ Alive, in remission. Received Gemtuzumab Ozogamycin as part of Myechild trial treatment.

29	5	F	29.5	BM	N	Karyotype: 45,X,-X,t(8;21)(q22;q22.1)[8]/46,XX[2]	7% myeloid blasts (CD13+, CD33+, CD34+, CD117+, MPO+) Alive, in remission. Received 2 doses of Gemtuzumab Ozogamycin as part of Myechild trial treatment
30	76	F		BM			D45X Polycythaemia vera. M99503, transformation in to MDS.

BM = Bone marrow
PB = Peripheral blood
LP = Leukapheresis
MRD = Minimal residual disease
BMT = Bone marrow transplant
AML= Acute myeloid leukemia
MDS= Myelodysplastic syndrome
MPAL= Mixed phenotype acute leukemia
MLL = *Mixed-lineage leukemia*
NPM1 = *Nucleophosmin*
FLT3 = *Fms-like tyrosine kinase 3*
RUNX1 = *Runt-related transcription factor 1*
GATA2 = *GATA Binding Protein 2*
WHO = World Health Organisation
n/a = not available

Supplementary Table S2. Enrichment values for β-catenin and WT1 following a TMT-labelled assessment of AML Patient #1 β-catenin interactome by mass spectrometry.

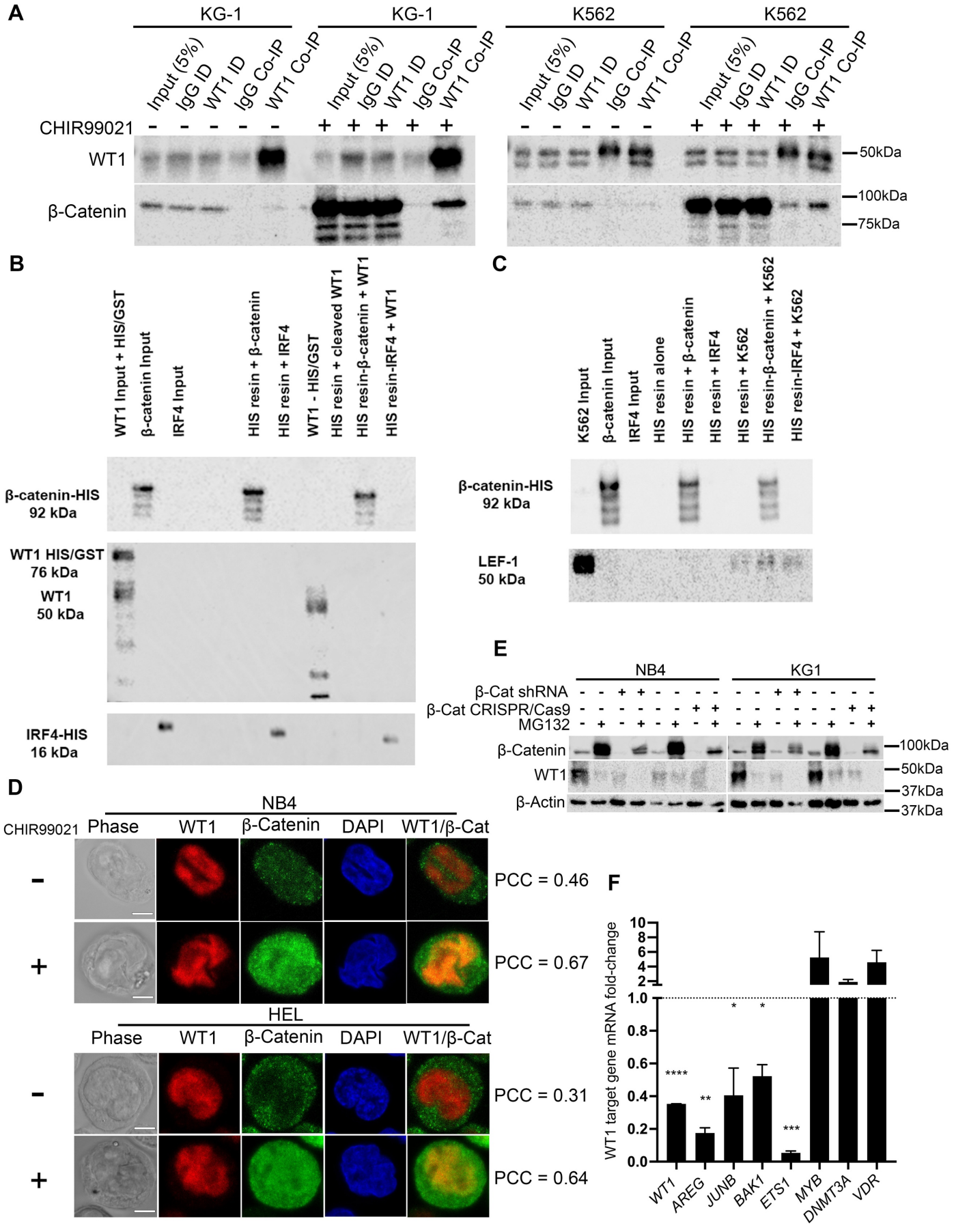
Protein	Score	Coverage	# Proteins	# Unique peptides	# Peptides	PSM	Cyt fold change (vs IgG)	Nuc fold change (vs IgG)
β-Catenin	535.53	64.08	10	33	40	175	8.03	4.159
WT1	2.51	4.49	5	1	1	1	0.889	1.74

Score: Displays the protein score, which is the sum of the scores of the individual peptides.
For Sequest results, the score is the sum of all peptide Xcorr values above the specified score threshold. The score threshold is calculated as follows:
0.8 + peptide_charge × peptide_relevance_factor
where peptide_relevance_factor is an advanced parameter of the SEQUEST or Sequest HT node in the "Protein Scoring Option" category with a default value of 0.4. For each spectrum, only the highest-scoring match is used.
For each spectrum and sequence, the Proteome Discoverer application uses only the highest scored peptide. When it performs a search using dynamic modifications, one spectrum might have multiple matches because of permutations of the modification site.
For Mascot results, the score is:
– Standard score, which is the cumulative protein score based on summing the ion scores of the unique peptides identified for that protein. If a peptide was redundantly identified, only the highest-scoring peptide is used.
–or–
– MudPIT score, which is the sum of the "excess of ions" score over the homology or identity threshold for each spectrum plus the average threshold. For MudPIT scoring, the score for each peptide is not its absolute score but the amount that it is above the threshold. Therefore, peptides with a score below the threshold do not contribute to the score. For each peptide, the threshold is the homology threshold, if it exists; otherwise, it is the identity threshold. By default, the Proteome Discoverer application automatically switches between the standard and the MudPIT score to calculate the protein score in the Mascot node results. It automatically uses the MudPIT score when the number of queries divided by the number of FASTA database entries exceeds 0.001.

Coverage: Displays by default the percentage of the protein sequence covered by identified peptides
Proteins: Displays the number of identified proteins in the protein group of a master protein
Unique Peptides: Displays the number of peptide sequences unique to a protein group
Peptides: Displays the number of distinct peptide sequences in the protein group
PSMs: Displays the total number of identified peptide sequences (peptide spectrum matches) for the protein, including those redundantly identified

The authors wish to acknowledge that the full β-catenin interactome MS data for AML patient #1 is available from bioRxiv link below should researchers desire a full copy:

https://www.biorxiv.org/content/10.1101/2021.11.06.467095v2


A
KG-1
KG-1
K562
K562
Input (5%)
IgG ID
WT1 ID
IgG Co-IP
WT1 Co-IP
CHIR99021
WT1
β-Catenin
50kDa
100kDa
75kDa
B
WT1 Input + HIS/GST
β-catenin Input
IRF4 Input
HIS resin + β-catenin
HIS resin + IRF4
WT1 - HIS/GST
HIS resin + cleaved WT1
HIS resin-β-catenin + WT1
HIS resin-IRF4 + WT1
β-catenin-HIS 92 kDa
WT1 HIS/GST 76 kDa
WT1 50 kDa
IRF4-HIS 16 kDa
C
K562 Input
β-catenin Input
IRF4 Input
HIS resin alone
HIS resin + β-catenin
HIS resin + IRF4
HIS resin + K562
HIS resin-β-catenin + K562
HIS resin-IRF4 + K562
β-catenin-HIS 92 kDa
LEF-1 50 kDa
D
NB4
HEL
CHIR99021
Phase
WT1
β-Catenin
DAPI
WT1/β-Cat
PCC = 0.46
PCC = 0.67
PCC = 0.31
PCC = 0.64
E
NB4
KG1
β-Cat shRNA
β-Cat CRISPR/Cas9
MG132
β-Catenin
WT1
β-Actin
100kDa
50kDa
37kDa
37kDa
F
WT1 target gene mRNA fold-change
WT1
AREG
JUNB
BAK1
ETS1
MYB
DNMT3A
VDR

Supplementary Figure S1. (**A**) Immunoblots showing the level of β-catenin protein present in WT1 Co-IPs derived from KG-1 and K562 under basal (DMSO)or activated (5μM CHIR99021) Wnt signalling conditions. ID= immunodepleted lysate. (**B**) Immunoblot showing purified WT1 (HIS/GST tagged, and non-tagged) abundance in β-catenin-HIS or IRF4-HIS (negative control) resin columns. (**C)** Immunoblot showing level of β-catenin or positive control LEF-1 (derived from whole cell K562 lysates) present in HIS resin β-catenin and IRF4 containing lanes. LEF-1 is a known interactor of β-catenin and confirms recombinant β-catenin protein can still bind established partners. (**D**) CLSM Z-sections showing β-catenin and WT1 subcellular localisation in NB4 and HEL cells +/- 5μM CHIR99021. Phase (gray), WT1 (red), β-catenin (green), DAPI (blue) and merged WT1/β-catenin images are shown alongside Pearsons Correlation Coefficients (PCC; -1= inverse correlation, 0= no correlation, +1= positive correlation) indicating degree of overlap between β-catenin and WT1 signal. White scale bar indicates 5μm. (**E**) Immunoblot showing protein level of β-catenin and WT1 in NB4 and KG-1 cells +/- β-catenin shRNA, +/- β-catenin CRISPR/Cas9 following 16 hours incubation with 1μM proteasome inhibitor MG132. β-Actin detection indicates protein loading. (**F**) Summary graph showing fold change in mRNA expression of genes previously identified as WT1 target genes, in KG-1 cells by qRT-PCR. Fold change is in response to knockdown of WT1 using WT1 shRNA relative to expression in non-targeted shRNA control (dashed line). Expression was normalised to the housekeeping gene β-actin (*ACTB*). All data represents mean ± 1 s.d ($n = 3$). Statistical significance is denoted by **P*<0.05, ***P*<0.005, ****P*<0.0005 and *****P*<0.0001 as deduced by a one-sample t-test.