Sentosa® SQ Leukemia Panel: Development of targeted Next Generation Sequencing IVD test for detecting mutations in Leukemia patients

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Introduction

- The *Sentosa* SQ Leukemia Panel is an NGS panel designed for detection of diagnostic, prognostic, and predictive biologic markers associated with the major types of leukemia.
- The panel is designed to detect ~4,500 mutations in 49 key cancer genes associated with leukemia.
- Mutation detection sensitivity of the panel is 10% at 500x coverage using peripheral whole blood and bone marrow as specimen types, respectively.

Design: Target gene list with target exons

Gene	# Amplicons	# Targeted Exons
ABL1	5	4,5,6,7
ASXL1	13	5,6,12,13
ATM	45	5,7,8-11,13-15,17-21,23,25,26,28,29,32,34-39 ,41-45,48-50,52-56,58-60,62,63
BCOR	10	4,7,9,12,15
BRAF	1	15
CALR	1	9
CBL	4	5,8,9,12
CBLB	1	10
CEBPA	4	1
CREBBP	15	2,8,15,16,18,20,23,24,26,27,30,31
CSF3R	1	17
DDX3X	2	10, 12
DNMT3A	13	4,10,11,12,13,14,15,16,17,18,19,20,22,23
ETV6	4	3,5,6,7
EZH2	16	3,4,5,6,7,8,9,12,14,15,16,17,18,19,20
FBXW7	9	2,3,5,8,9,10,11,12
FLT3	9	5,11,13,14,15,17,18,20
GATA1	3	2, 3
GATA2	2	4, 5
HRAS	2	2, 3
IDH1	2	3, 4
IDH2	1	4
IKZF1	4	3,4,5,8
IL7R	2	5,6
JAK1	2	14, 15

Gene	# Amplicons	# Targeted Exons
JAK2	7	12,13,14,16,20,25
JAK3	6	5,11,15,18,19,21
KDM6A	3	12,16,26
KIT	10	2,3,8,9,11,12,14,17,18,20
KRAS	4	2,3,4
MLL	3	3,7,27
MPL	3	4,6,10
NOTCH1	8	6,13,26,27,31,34
NPM1	1	12
NRAS	2	2, 3
PAX5	3	2,3,5
PTEN	3	5,7
PTPN11	3	3,12,13
RUNX1	6	3,4,5,6,7,8
SF3B1	6	13,14,15,16,18
SRSF2	1	1
STAT3	2	20,21
SUZ12	1	5
TET2	31	3,5,6,7,8,9,10,11
TP53	9	2,3,4,5,6,7,8,9,11
U2AF1	2	2,6
WT1	6	6,7,8,9,10
ZMYM3	3	2,15,21
ZRSR2	2	2, 8
System control	1	NA

Results and discussion

- In a first "proof of concept" approach, randomly-selected frozen PBMC (n=18) and peripheral blood (n=9) leukemia samples were included for mutation detection, followed by Sanger sequencing of all detected variants on selected samples (Table 1 and 2).
- Another 8 samples of known leukemia variants were tested using the panel, which showed 100% concordance with previously detected mutations. Additional variations were detected using the *Sentosa* SQ Leukemia Panel (Table 3).
- Diagnostics accuracy of mutation detection in both whole blood and bone marrow samples was tested in matched samples collected from the same patient at the same disease stage and showed 100% concordance for samples with >20% blast count (Table 4).

Table 1: Variant detection in Leukemia PBMC samples (n=18)

Patient ID	Status	Blast Count	Gene	CDS Mutation	AA Mutation	In dbSNP	Var Freq
ALL135L	Diagnosis	blood - 70%; BM - 80%	CREBBP	c.3250delA	p.I1084fs*15	No	0.019
CML064	Diagnosis	No	CREBBP	c.3250delA	p.I1084fs*15	No	0.021
CLL430L	Diagnosis	70% lymphocytes in BM	IL7R	c.731C>T	p.T244I	Yes	0.497
AML272L	Diagnosis	blood - 79%; BM - 80%	IL7R	c.731C>T	p.T244I	Yes	0.503
ALL137L	Diagnosis	blood - 1%; BM - 59%	JAK3	c.452C>G	p.P151R	Yes	0.425
CLL423L	Diagnosis	80% lymphocytes in BM	IL7R	c.731C>T	p.T244I	Yes	0.520
			SF3B1	c.2223G>T	p.K741N	No	0.084
			SF3B1	c.2223G>C	p.K741N	No	0.355
			DNMT3A	c.1711_1711delG	p.A571fs*80	No	0.012
CML063L	Diagnosis	N.A.	JAK2	c.1849G>T	p.V617F	No	0.182
			IL7R	c.731C>T	p.T244I	Yes	0.504
AML277L	Diagnosis	blood - 74% ; BM - 93.4%	IL7R	c.731C>T	p.T244I	Yes	0.535
			KRAS	c.436G>A	p.A146T	No	0.018
ALL139L	Diagnosis	blood - 3%	CALR	c.1113A>T	p.E371D	No	0.027
ALL136L	Diagnosis	blood - 90%; BM - 93%	CALR	c.1113A>T	p.E371D	No	0.062
			WT1	c.1181G>A	p.R394Q	No	0.464
CLL427L	Progression	N/A	DNMT3A	c.2206C>T	p.R736C	No	0.080
CLL422L	Diagnosis	80% lymphocytes in BM	CALR	c.1113A>T	p.E371D	No	0.041
AML281L	Diagnosis	blood - 77%; BM - 98%	NPM1	c.863_864insTCTG	p.W288fs*12	No	0.412
			DNMT3A	c.1711_1711delG	p.A571fs*80	Yes	0.018
			SRSF2	c.284C>T	p.P95L	Yes	0.465
			IDH2	c.419G>A	p.R140Q	No	0.480
			CBL	c.1249C>T	p.P417S	No	0.426
AML279L	Diagnosis	blood - 10%; BM - 22%	EZH2	c.2035G>A	p.V679M	No	0.422
			IL7R	c.731C>T	p.T244I	Yes	0.984
			PTPN11	c.181G>T	p.D61Y	No	0.023
			NRAS	c.182A>G	p.Q61R	No	0.383
AML280L	Diagnosis	blood - 84%; BM - 90%	JAK2	c.1849G>T	p.V617F	No	0.808
			IL7R	c.731C>T	p.T244I	Yes	0.981
			DNMT3A	c.1711_1711delG	p.A571fs*80	No	0.016
			SRSF2	c.284C>T	p.P95L	No	0.406
CML066L	Diagnosis	N.A.	IL7R	c.731C>T	p.T244I	Yes	0.510
			DNMT3A	c.1711_1711delG	p.A571fs*80	No	0.018
CLL429	N.A.	N.A.	NPM1	c.863_864insTCTG	p.W288fs*12	No	0.421
			IDH1	c.394C>A	p.R132S	No	0.425
			DNMT3A	c.1711_1711delG	p.A571fs*80	No	0.022
			SRSF2	c.284C>G	p.P95R	No	0.368
ALL140L	Diagnosis	BM - 28%	IL7R	c.731C>T	p.T244I	Yes	0.990
			ATM	c.1402_1403delAA	p.K468fs*18	Yes	0.322

Table 2: Variant detection in Leukemia whole blood samples (n=9)

Patient ID	Status	Blast Count	Gene	CDS Mutation	AA Mutation	dbSNP	Var Freq
ALL142_II*	Resistant to Therapy	blood - 1%; BM - 29%	JAK2	c.1711G>A	p.G571S	Yes	0.488
			IL7R	c.731C>T	p.T244I	Yes	0.500
			JAK3	c.452C>G	p.P151R	Yes	0.485
			CREBBP	c.3250delA	p.I1084fs*15		0.011
AML295WB*	Diagnosis	blood - 14%; BM - 48.4%	NPM1	c. 863_864insTCTG	p.W288fs*12	No	0.397
			IL7R	c.731C>T	p.T244I	Yes	0.491
			DNMT3A	c.2644C>T	p.R882C	No	0.443
ALL145WB*	Diagnosis	blood - 30%; BM - 89%	FLT3	c.1793_1794ins24	p.Y597_E598insDYVDFREY	No	0.215
			IL7R	c.731C>T	p.T244I	Yes	0.490
AML293 blood*	Relapse	BM - 5%	ASXL1	c.2957A>G	p.N986S	No	0.493
			IL7R	c.731C>T	p.T244I	Yes	0.464
			DNMT3A	c.2578T>C	p.W860R	No	0.055
			JAK3	c.452C>G	p.P151R	Yes	0.458
ALL144 blood	Relapse	blood - 10%; BM - 8%	IL7R	c.731C>T	p.T244I	Yes	0.480
CLL431 blood*	Resistant to therapy	BM - 50% lymphocytes	JAK3	c.452C>G	p.P151R	Yes	0.434
			STAT3	c.1940A>T	p.N647I	No	0.019
			STAT3	c. 1968_1969insTAT	p.G656_Y657insY	No	0.020
ALL137II_WB	Relapse	BM - 5%	DNMT3A	c.1711_1711delG	p.A571fs*80	No	0.017
			JAK3	c.452C>G	p.P151R	Yes	0.452
CLL436WB	Relapse	BM - 80% lymphocytes	RUNX1	c.167T>C	p.L56S	No	0.472
			ASXL1	c.3115C>T	p.Q1039*	No	0.031
			ASXL1	c.2338C>T	p.Q780*	No	0.039
			DNMT3A	c.1711_1711delG	p.A571fs*80	No	0.022
CLL424II_WB	Relapse	BM - 60% lymphocytes	IL7R	c.731C>T	p.T244I	Yes	0.506
			SF3B1	c.1997A>G	p.K666R	No	0.091
			DNMT3A	c.1711_1711delG	p.A571fs*80	No	0.020
			CEBPA	c.454delG	p.A152fs*8	No	0.020

*Sanger sequencing performed

Table 3: Variant detection concordance (n=8)

Sample ID	Expected Mutation	Gene	CDS Mutation	AA Mutation	Var Freq
1	NPM1	NPM1	c.863_864insTCTG	p.W288fs*12	0.155
		KRAS	c.436G>A	p.A146T	0.111
		IL7R	c.731C>T	p.T244I	0.516
		FLT3	c.1669G>A	p.V557I	0.494
2	SNP in IDH1 c.315C>T	JAK2	c.3323A>G	p.N1108S	0.531
		IL7R	c.731C>T	p.T244I	0.535
		DNMT3A	c.1711_1711delG	p.A571fs*80	0.013
		IDH1	c.315C>T	p.G105G	0.500
3	JAK2 V617F	JAK2	c.1849G>T	p.V617F	0.565
		IL7R	c.731C>T	p.T244I	0.542
4	MPL p.W515L	TET2	c.1648C>T	p.R550*	0.188
		MPL	c.1544G>T	p.W515L	0.313
5	R172K in IDH2	IL7R	c.731C>T	p.T244I	0.498
		IDH2	c.515G>A	p.R172K	0.483
		DNMT3A	c.2206C>T	p.R736C	0.470
		DNMT3A	c.1711_1711delG	p.A571fs*80	0.014
		DNMT3A	c.1792C>T	p.R598*	0.345
		ASXL1	c.1888_1910del23	p.E635fs*15	0.159
6	IDH2 R172K+FLT3 c.2504A>C	TP53	c.544T>A	p.C182S	0.010
		NRAS	c.179G>A	p.G60E	0.017
		IDH2	c.515G>A	p.R172K	0.492
		FLT3	c.2504A>C	p.D835A	0.096
		DNMT3A	c.2578T>C	p.W860R	0.461
		DNMT3A	c.2245C>T	p.R749C	0.486
		ASXL1	c.3824C>G	p.S1275*	0.020
		TP53	c.545G>A	p.C182Y	0.038
7	NPM1+FLT3 Duplication in exon 12-13	SRSF2	c.284C>A	p.P95H	0.561
		NPM1	c.863_864insTCTG	p.W288fs*12	0.420
8	CALR c.1099_1150del	IDH2	c.419G>A	p.R140Q	0.487
		SF3B1	c.1998G>C	p.K666N	0.019
		RUNX1	c.167T>C	p.L56S	0.298
		IL7R	c.731C>T	p.T244I	0.501
		IDH2	c.419G>A	p.R140Q	0.029
		CALR	c.1137G>C	p.E379D	0.196
		CALR	c. 1092-1143del	p. L367fs*52	0.237

Table 4: Mutation detection in blood-bone marrow paired samples (n=7)

Patient ID	Tissue Type	Status	Blast count	Gene	AA Mutation	In dbSNP	Var Freq
AML279 WB	WB	Diagnosis	blood - 10%	EZH2	p.V679M	No	0.4216
				NRAS	p.Q61R	No	0.3831
				IL7R	p.T244I	Yes	0.9837
AML279BM	BM		blood - 22%	EZH2	p.V679M	No	0.4319
				NRAS	p.Q61R	No	0.3657
				IL7R	p.T244I	Yes	0.9966
AML277WB	WB	Diagnosis	blood - 74%	IL7R	p.T244I	Yes	0.535
AML277BM	BM		BM - 93.4%	IL7R	p.T244I	Yes	0.5038
ALL136WB	WB	Diagnosis	blood - 90%	WT1	p.R394Q	No	0.464
				CALR	p.E371D	No	0.062
ALL136BM	BM		BM - 93%	WT1	p.R394Q	No	0.371
				CALR	p.E371D	No	0.067
ALL140WB	WB	Diagnosis	N.A.	IL7R	p.T244I	Yes	0.9901
				ATM	p.K468fs*18	No	0.3216
ALL140BM	BM		28%	IL7R	p.T244I	Yes	0.9904
				ATM	p.K468fs*18	No	0.247
ALL145WB	WB	Diagnosis	30%	IL7R	p.T244I	Yes	0.4902
				ASXL1	p.N986S	No	0.4927
ALL145BM	BM		89%	IL7R	p.T244I	Yes	0.4978
				ASXL1	p.N986S	No	0.5015
CLL430WB	WB	Diagnosis	N.A.	IL7R	p.T244I	Yes	0.4967
CLL430BM	BM		70%	IL7R	p.T244I	Yes	0.5087
AML293WB	WB	Relapse	N.A.	DNMT3A	p.W860R	No	0.055
				JAK3	p.P151R	Yes	0.458
				IL7R	p.T244I	Yes	0.464
AML293BM	BM		5%	DNMT3A	p.W860R	No	0.065
				JAK3	p.P151R	Yes	0.415
				IL7R	p.T244I	Yes	0.514
				PAX5	*p.V26G*	No	0.156

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