Differential drug sensitivity patterns in myelodysplastic syndrome patients are recapitulated by *ex vivo* drug response profiling

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568 / 11
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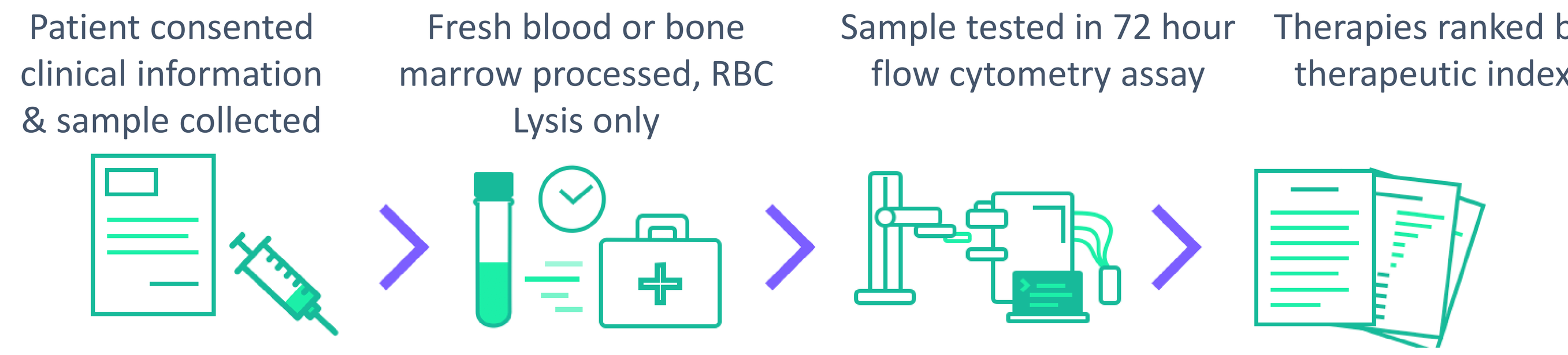
ABSTRACT

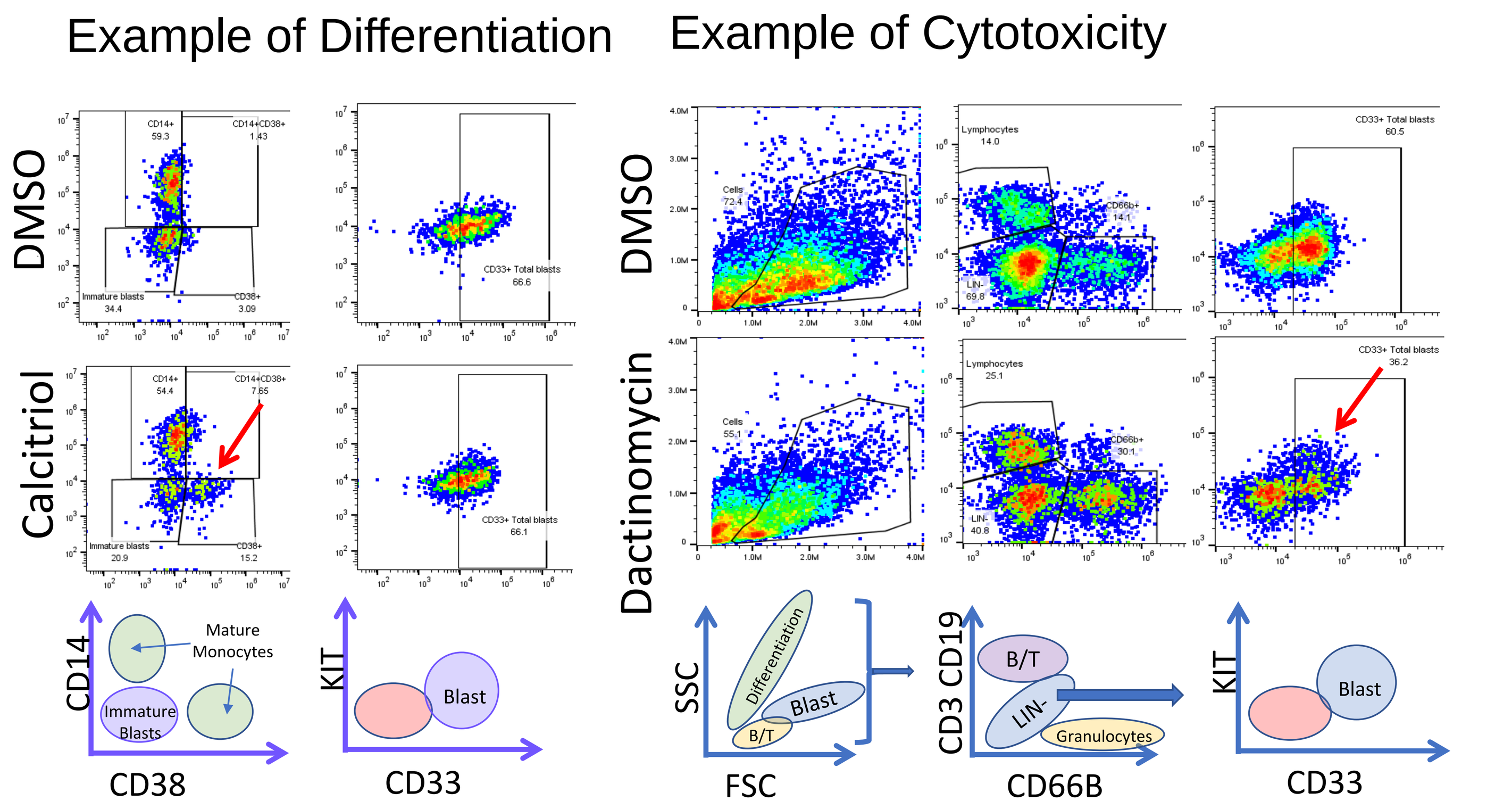
Myelodysplastic syndromes (MDS) are a collection of clonal diseases of dysfunctional hematopoietic stem cells, characterized by ineffective hematopoiesis, cytopenias, and dysplasia. Limited conventional treatment options exist for these patients, with hypomethylating agents remaining the standard of care for higher-risk MDS patients. Drug sensitivity and resistance testing on myelodysplastic syndromes (MDS) samples should provide important functional information to guide actionable target and biomarker discovery. We provide proof-of-concept data by profiling the effects of 55 common oncology drugs on 27 myelodysplastic (MDS) samples from both treatment-naïve and refractory cases.

METHODS



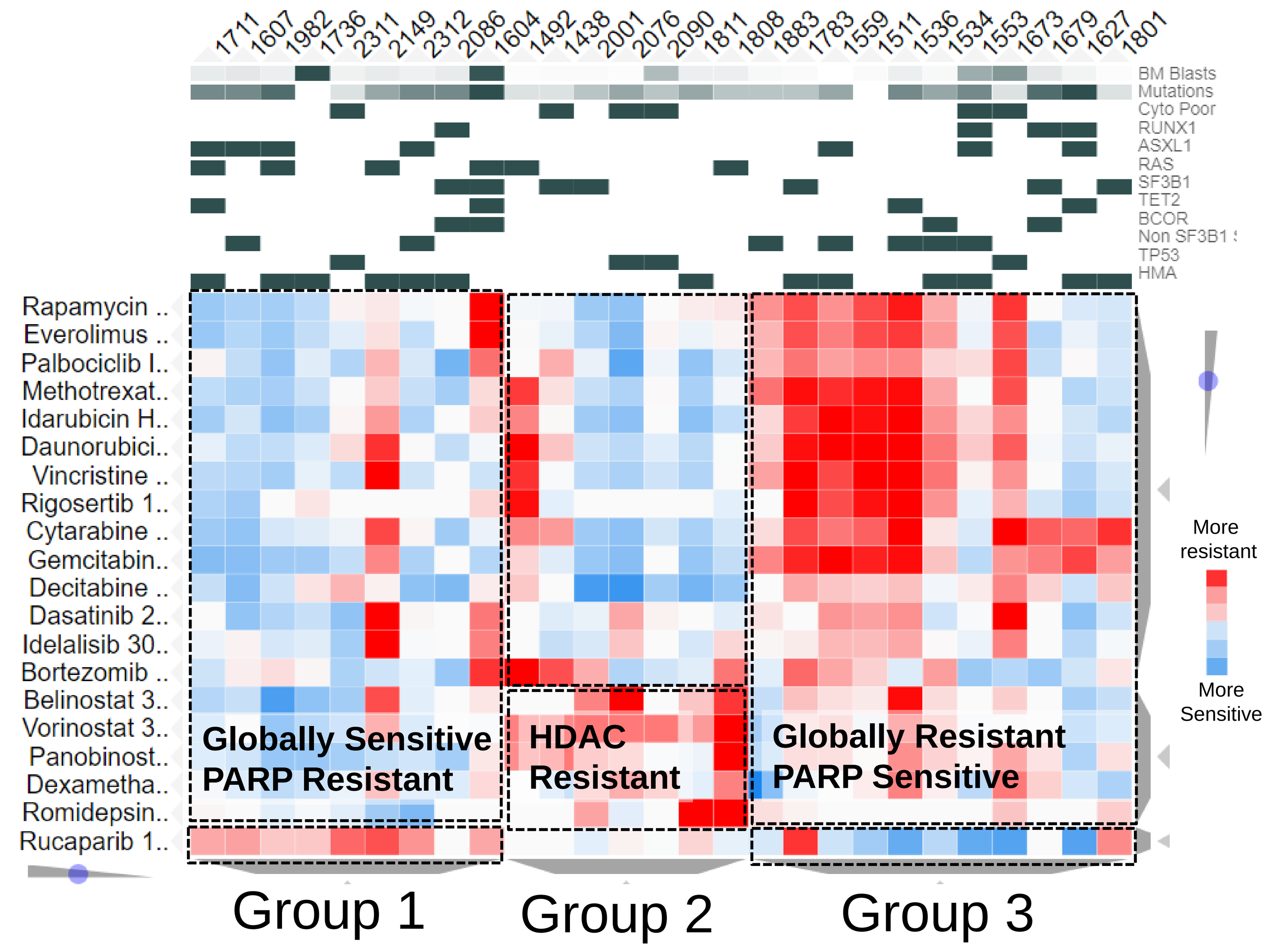
Blood or bone marrow samples were red blood cell lysed upon arrival, counted and resuspended at the appropriate concentration in proprietary serum free media with cytokines. The samples were then plated in 384 well microtiter plates and treated with drugs in triplicate (for each staining panel) using an Echo acoustic dispenser. 72 hours post-drugging the samples were stained with appropriate antibodies (panel details below) and read out on an Intellicyt iQue Plus flow cytometer.

CHARACTERIZATION OF RESPONSES



Live cells were gated using FSC/SSC and DAPI exclusion, and then further defined by cell surface marker expression. Absolute counts for each population are provided, as well as counts normalized to a vehicle-only (DMSO) control. Representative samples, provided as a PDF, demonstrated the gating strategy for quantified populations, as well as a qualitative view of differentiation (compared to DMSO control).

ex vivo DRUG SENSITIVITY SCREEN


1711 1607 1982 1736 2311 2149 2312 2086 1604 1492 1438 2001 2076 2090 1811 1808 1883 1783 1559 1511 1536 1534 1553 1673 1679 1627 1801
BM Blasts
Mutations
Cyto Poor
RUNX1
ASXL1
RAS
SF3B1
TET2
BCOR
TP53
HMA
Rapamycin
Everolimus
Palbociclib
Methotrexat..
Idarubicin
Daunorubici..
Vincristine
Rigosertib
Cytarabine
Gemcitabin..
Decitabine
Dasatinib
Idelalisib
Bortezomib
Belinostat
Vorinostat
Panobinost..
Dexametha..
Romidepsin..
Rucaparib
Globally Sensitive
PARP Resistant
HDAC Resistant
Globally Resistant
PARP Sensitive
More resistant
More Sensitive
Group 1
Group 2
Group 3

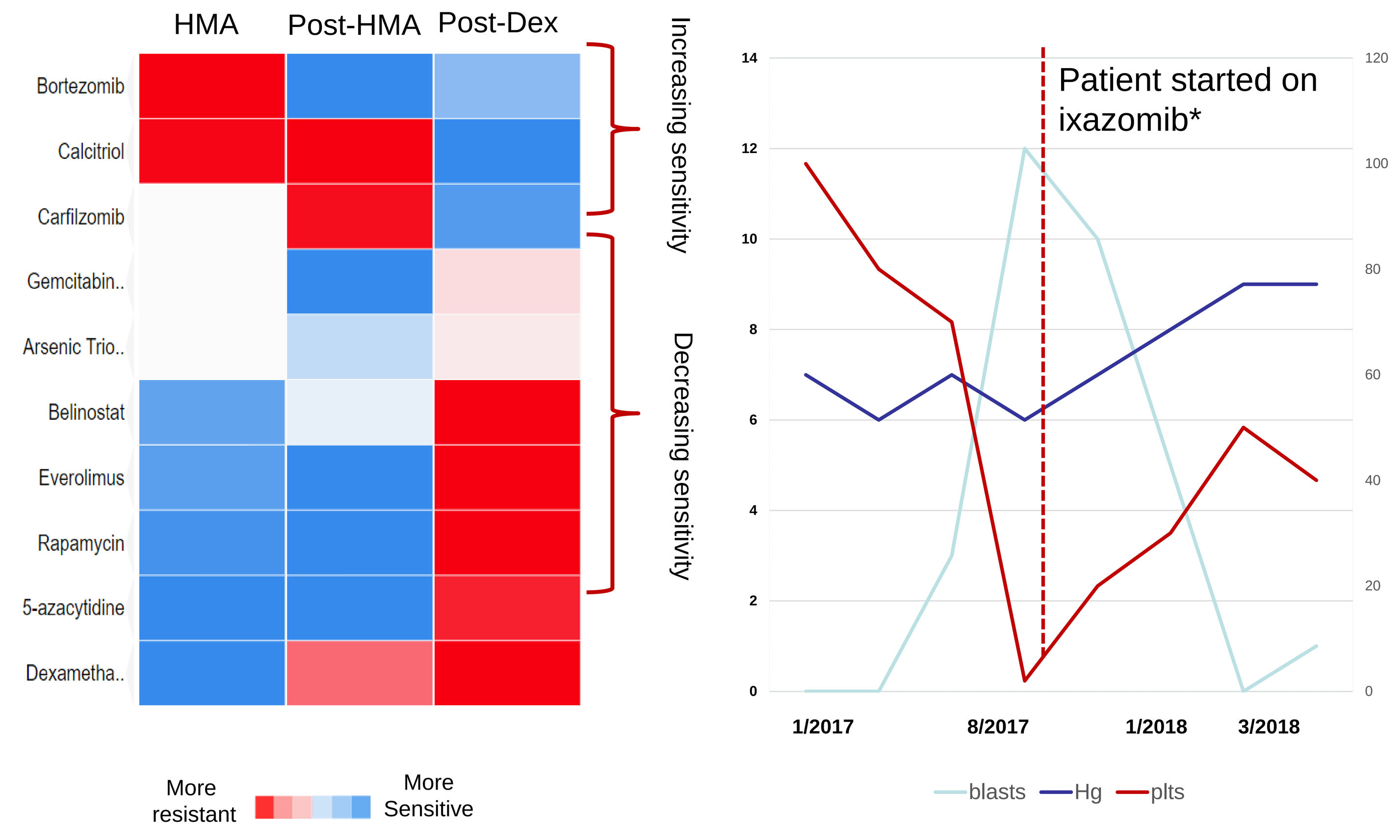
Ex vivo drug sensitivity screen. The heatmap of samples were clustered with the complete linkage method using Euclidean distance measures using top 30% of compounds with the highest variance.

PATIENTS (n=27)

Patient ID	Age	Sex	Sample	Diagnosis	Prior HMA	BM Blasts(%)	Cytopenias	Cytogenetics
1438	90	F	BM	RARS	Naïve	2	A	46,XX,t(2;4)(p13;q25)?c,del(7)(q22q32)[7]/46,XX,t(2;4)(p13;q25)?c[14]
1492	66	M	BM	CMML-0	Naïve	1	T	46,XY[22]
1511	86	M	BM	MDS (del 5q)	Naïve	2	A, T	46,XY,del(5)?(q15q35)[cp3]/46,XY[9]
1534	70	M	BM	MDS	Yes	3	A, T	46,XY[20]
1536	71	M	PB	CMML-1	Naïve	8	T	46,XY[20]
1553	86	M	BM	sAML	Yes	42	A, T, N	45,XY,-7[19]
1559	84	M	PB	CMML-1	Yes	0	A	46,XY[21]
1604	87	M	BM	sAML	Naïve	87	A, T, N	47,XY,+4[11]/46,XY[10]
1607	61	M	BM	MDS-EB2	Naïve	12	N	46,XY
1627	80	M	BM	MDS-EB1	Yes	5	A, T, N	46,XY
1673	64	M	BM	sAML	Naïve	53	A, T, N	45~46,XY,add(2)(q35),add(4)(q21),?der(5)t(5;17)(q13q21),-7,-9,?del(1 2)(p12p13),+13, add(15)(p11.2),-17,+r,+1~2mar[cp20]/46,XY[1]
1679	63	M	BM	MDS-EB2	Naïve	12	N	46,XY,del(12)(p12p13)[18]/
1711	79	M	PB?	MDS/MPN	Yes	10	A, T	47,XY,+8[20]
1736	79	M	BM	sAML	Yes	90	A	46,XY,t(11;19)(q23;p13.1)[?11]
1783	81	M	BM	RARS	Yes	2	A	47,XY,+8[11]
1801	83	M	PB	MDS-EB2	Yes	8	A,T	46, XY, t(11;21)[9]
1808	73	M	BM	RARS	Naïve	1	A	46,XY[24]
1811	77	M	PB	MDS/MPN	Yes	8	A,T	46, del(20)
1883	83	M	BM	MDS-MDS	Naïve	3	A,N	47,XY,+8[4]/46
1982	77	F	BM	MDS/MPN	Yes	8	A,T	46,XX[19]
2086	62	M	BM	MDS-EB2	Yes	10	A,N	46,XY,del(12)(p12p13)[
2090	47	M	BM	sAML	Naïve	34	A, T, N	45,XY,?t(1;6)(q42;q27),del(3)(p14),del(5)?t(5;14)(q15;q11.2),-6,-14,add(18)(q21),?der(22)t(6;22)(q13;q13.3),+mar1[cp13]/
2076	75	M	BM	MDS	Naïve	1	A, T, N	74<3n>,XYY,+Y,+Y,-5,-7,+8,+13,-18,+19,+22,+mar1x2[cp10]/46
2311	67	M	BM	MDS-EB1	Naïve	6	A, T, N	43 X, - Y, t(3;21), der(5;17), der(11), -14
2001	78	F	BM	RARS	Naïve	2	A	47,XX,+8[18]
2312	83	M	PB	CMML-1	Yes	7	A, T	46,XY
2149	78	M	BM	MDS/MPN	Yes	10	A, T, N	47,XY,+8[19]/46,XY

BM (bone marrow), PB = peripheral blood, RARS = refractory anemia with ring sideroblasts, EB = excess blasts, sAML = secondary acute myeloid leukemia, HMA = hypomethylating agent, A= anemia, T thrombocytopenia, N = neutropenia

ex vivo SENSITIVITIES OVER TIME


HMA
Post-HMA
Post-Dex
Bortezomib
Calcitriol
Carfilzomib
Gemcitabin..
Arsenic Trio..
Belinostat
Everolimus
Rapamycin
5-azacytidine
Dexametha..
Increasing sensitivity
Decreasing sensitivity
More resistant
More Sensitive
Patient started on ixazomib*
1/2017
8/2017
1/2018
3/2018
blasts
Hg
plts

*Ixazomib was used instead of bortezomib, given observed sensitivity to bortezomib and ease of oral administration. After 6 weeks of therapy with ixazomib / dexamethasone patient achieved a partial response and has remained transfusion independent for the past 3 months.

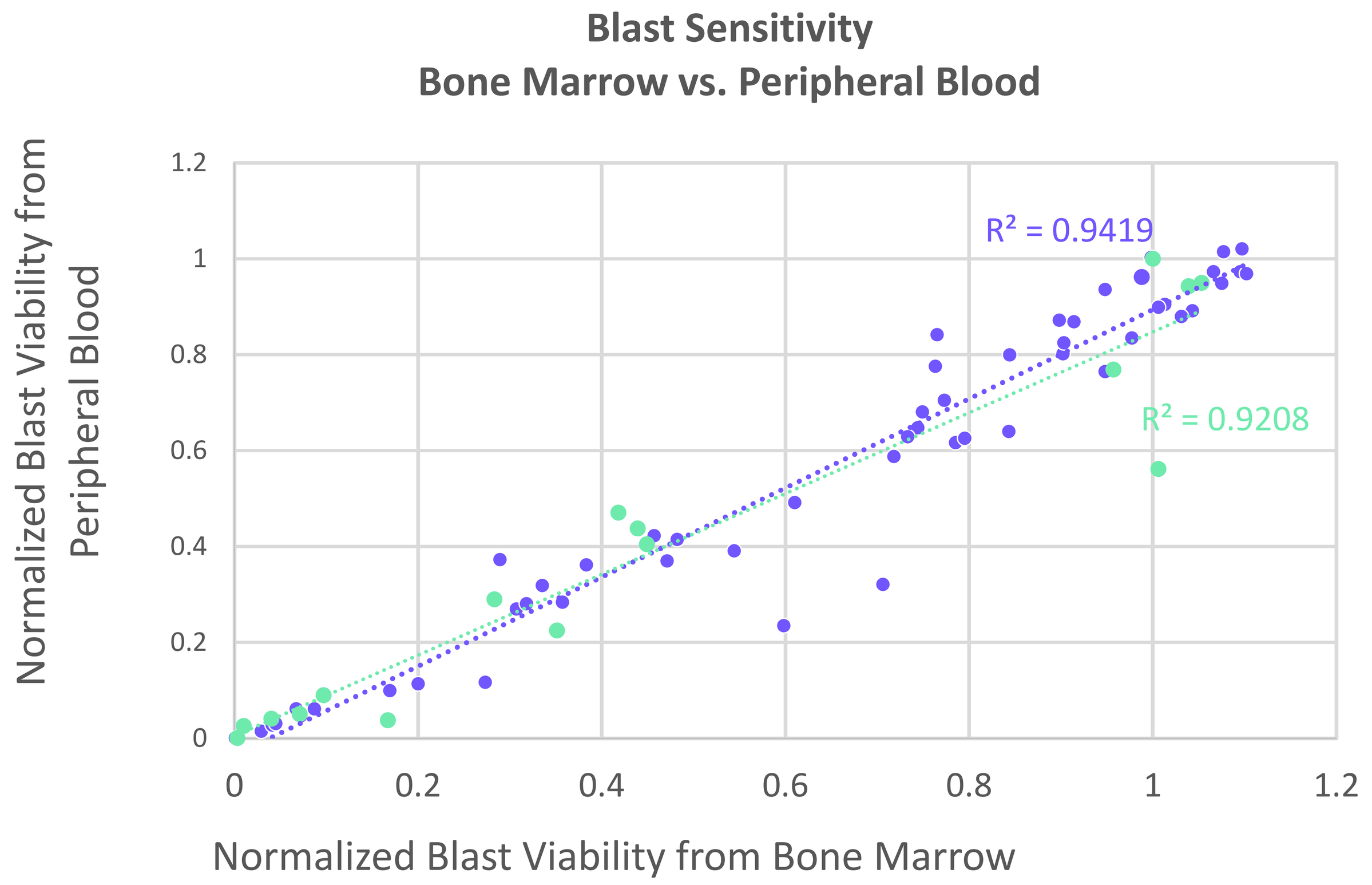
HMA = hypomethylating agent therapy, Dex = dexamethasone, Hg = hemoglobin, plts = platelets

DIFFERENTIAL RESPONSES BY GROUP

	Group 1	Group 2	Group 3	p-value
Average # of Mutations	3.44	2.00	2.82	0.244
Clinical Prior HMA Therapy	67%	14%	55%	0.105
Clinical HMA Response %	37%	66%	0%	**0.03**
Vorinostat (SD away from average)	-0.15	0.66	0.00	**2.13E-06**
Panobinostat (SD away from average)	-0.32	0.40	0.11	**0.001**
Decitabine (SD away from average)	-0.11	-0.27	0.10	**0.002**
Rucparib (SD away from average)	0.25	0.00	-0.25	**7.25E-05**

Groups 1-3 defined as per above. One-way ANOVA used to calculate p-values

PB vs BM



High correlation between PB and BM blast sensitivity. PB = peripheral blood. BM = bone marrow.

CONCLUSIONS

- Demonstrate utility of *ex vivo* approach to analysis of primary MDS PB and BMBx specimens
- Identify 3 distinct subgroups of MDS characterized by differential ex-vivo responses to HDAC, PARP, and HMA agents
- Demonstrate temporal evolution of *ex vivo* drug sensitivity which correlates with clinical responses
- Clinical feasibility study to guide personalized therapy in HMA R/R MDS ongoing

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