The use of the AML xenograft model to translate leukemia stem cell (LSC) biology to the clinic

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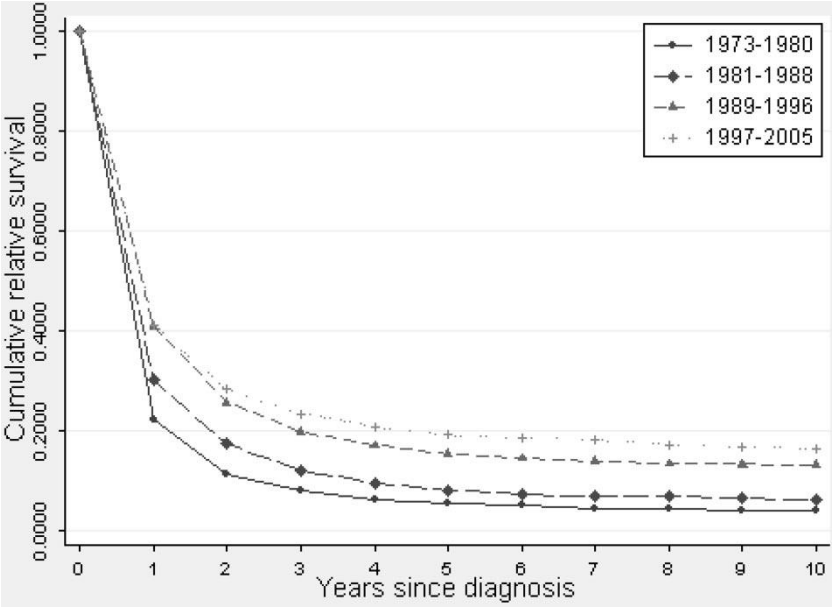
MaRS

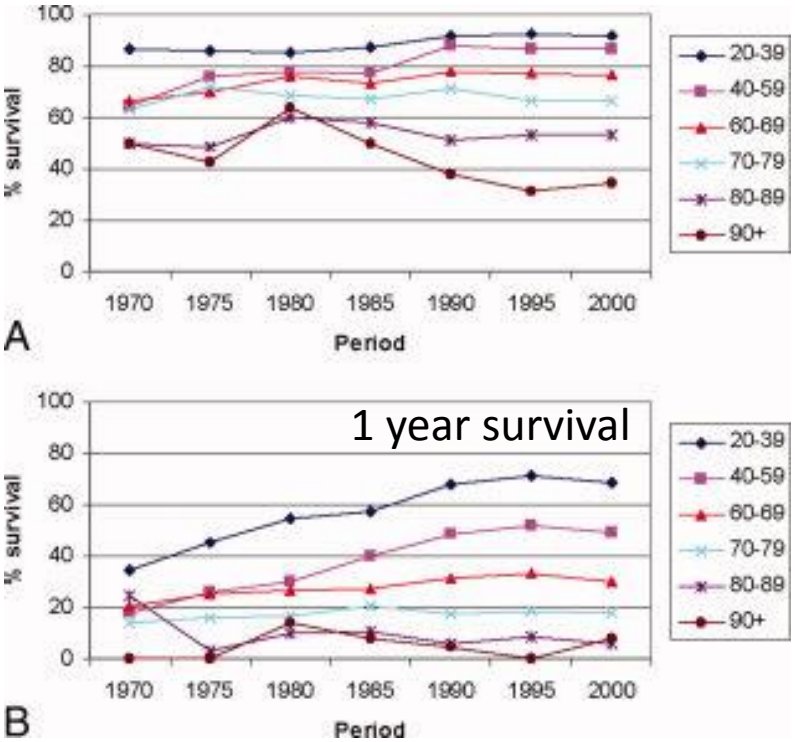
Patient survival in AML is poor

Cumulative relative survival in AML



Derolf et al, *Blood* 2009

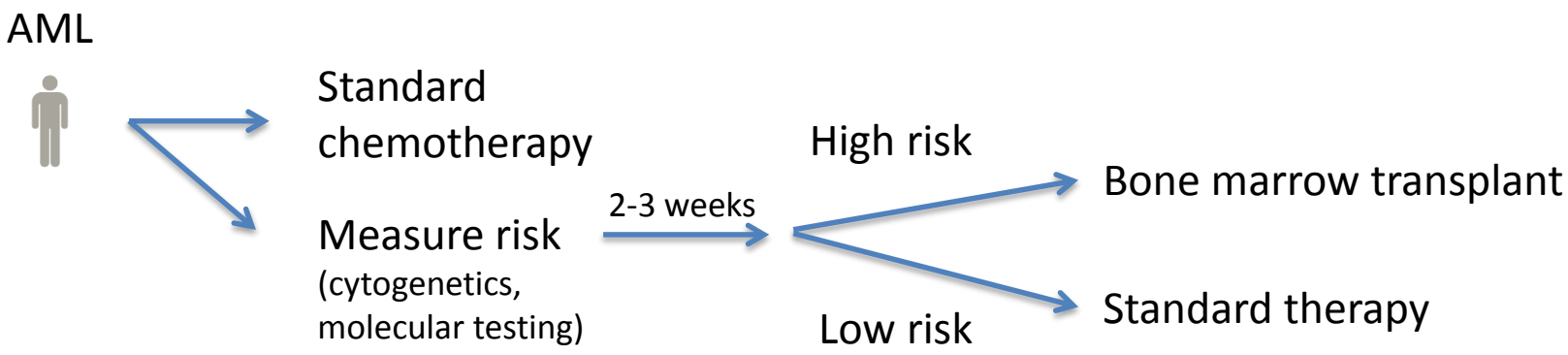
30 day survival



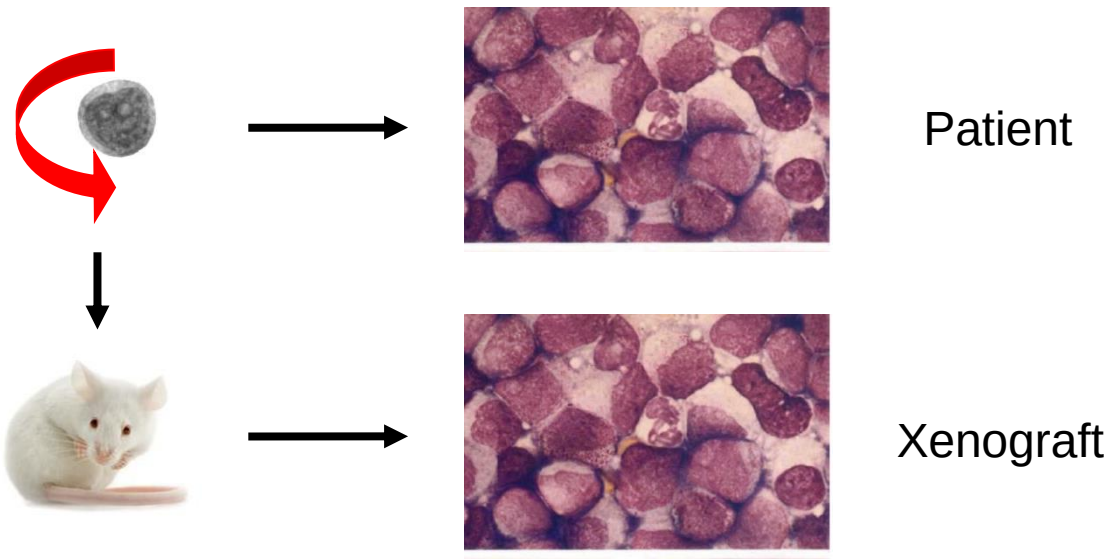
Alibhai et al, *Cancer* 2009

- survival is poor
- disease is complex (both inter- and intra-patient)
- basic treatment has not advanced for decades

A new treatment strategy for AML patients based on risk



Are LSCs assayed by xenotransplantation clinically relevant?



If LSCs are biologically important,

then patient outcome should be linked to LSC properties

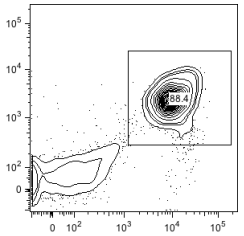
Heterogeneous engraftment ability of LSCs from AML patients

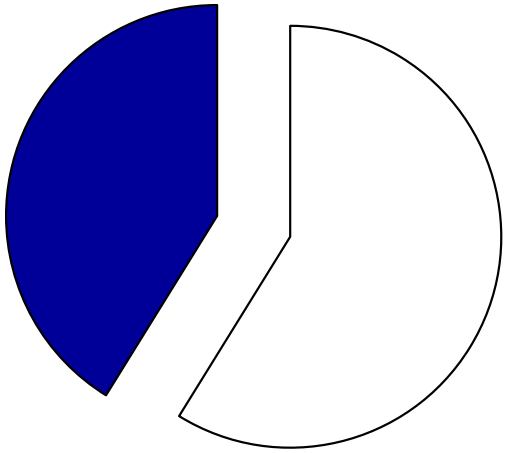
272
Diagnostic
AML

Intrafemoral
Injection

5×10^6 cells

8-10 w





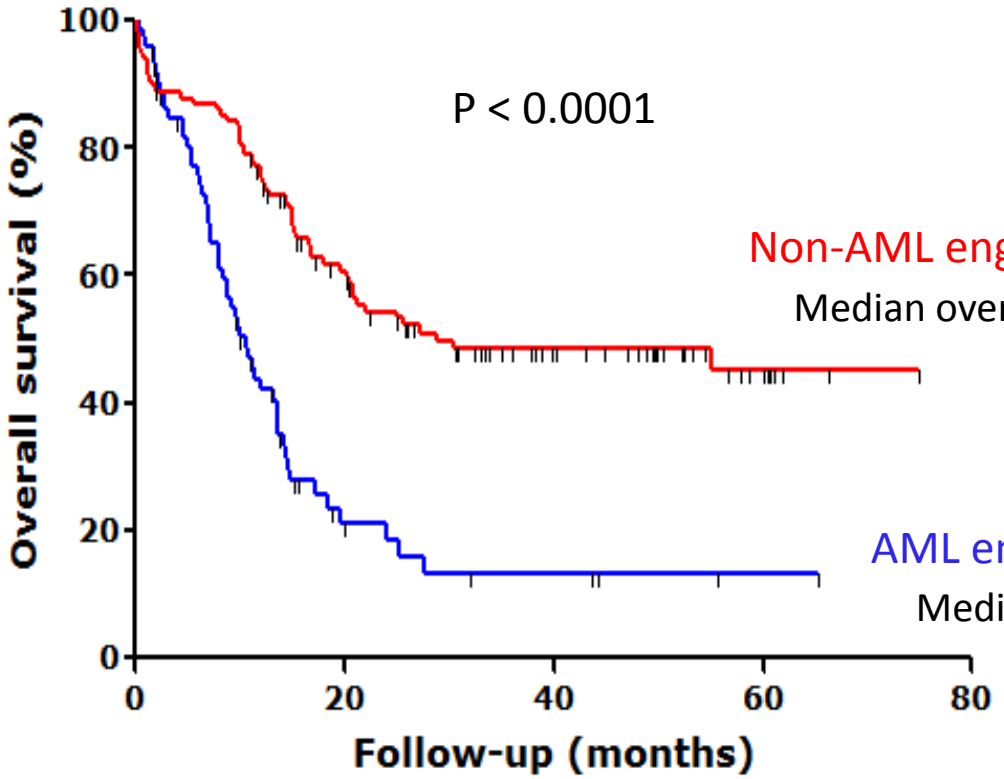
AML graft
112 (41%)

Non-AML graft
160 (59%)

Amanda Mitchell, James Kennedy

AML engraftment in xenograft assay is associated with poorer survival following induction chemotherapy

Survival post-induction chemotherapy



P < 0.0001

Non-AML engrafters (*n=114*)
Median overall survival **28.7** mos

AML engrafters (*n=73*)
Median overall survival **10.6** mos

Also Pearce, Bonnet et al, *Blood* 2006

James Kennedy, Amanda Mitchell

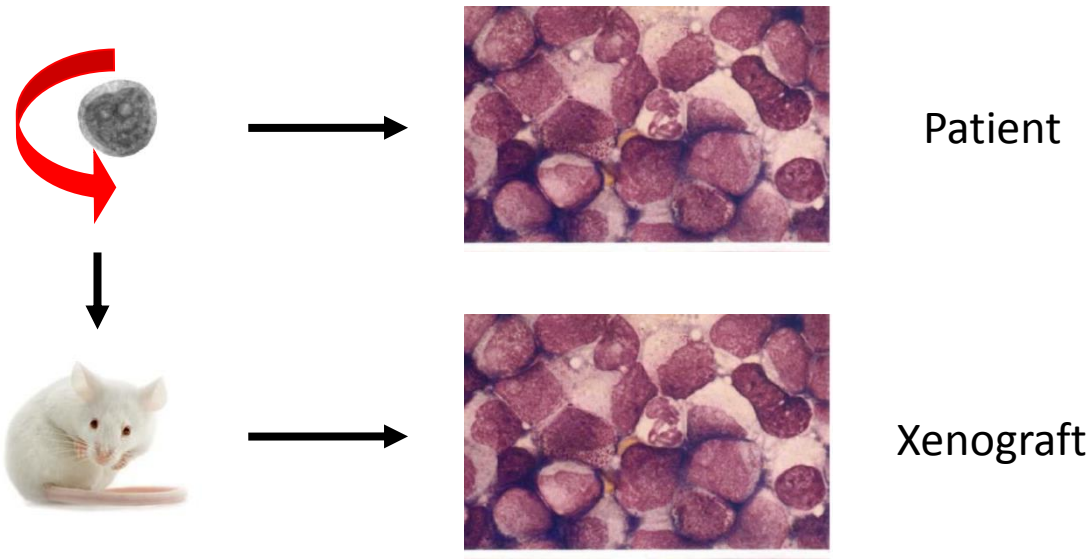
AML engraftment in xenograft assay is associated with poorer survival following induction chemotherapy

Multivariate analysis

I. Overall survival							
Entire induction cohort (n = 179)				***Normal Karyotype AML (n = 89)***			
Parameter	**HR**	**95% CI**	**P-value**	**Parameter**	**HR**	**95% CI**	**P-value**
Adverse cytogenetics†	3.89	(2.32-6.51)	P< 0.0001	AML engraftment	4.15	(2.21-7.80)	P< 0.0001
AML engraftment	2.51	(1.66-3.81)	P< 0.0001	WBC count	1.005	(1.0004-1.009)	P=0.032
WBC count	1.005	(1.002-1.007)	P= 0.001	*NPM1c* mutation	0.52	(0.27-0.97)	P=0.041
Secondary AML	1.74	(1.03-2.94)	P=0.040	Secondary AML	2.50	(1.02-6.15)	P=0.045
Age	1.014	(0.998-1.029)	NS*	*FLT3*-ITD	1.41	(0.76-2.62)	NS
Favorable cytogenetics†	0.93	(0.49-1.75)	NS	Age	1.005	(0.98-1.03)	NS

James Kennedy

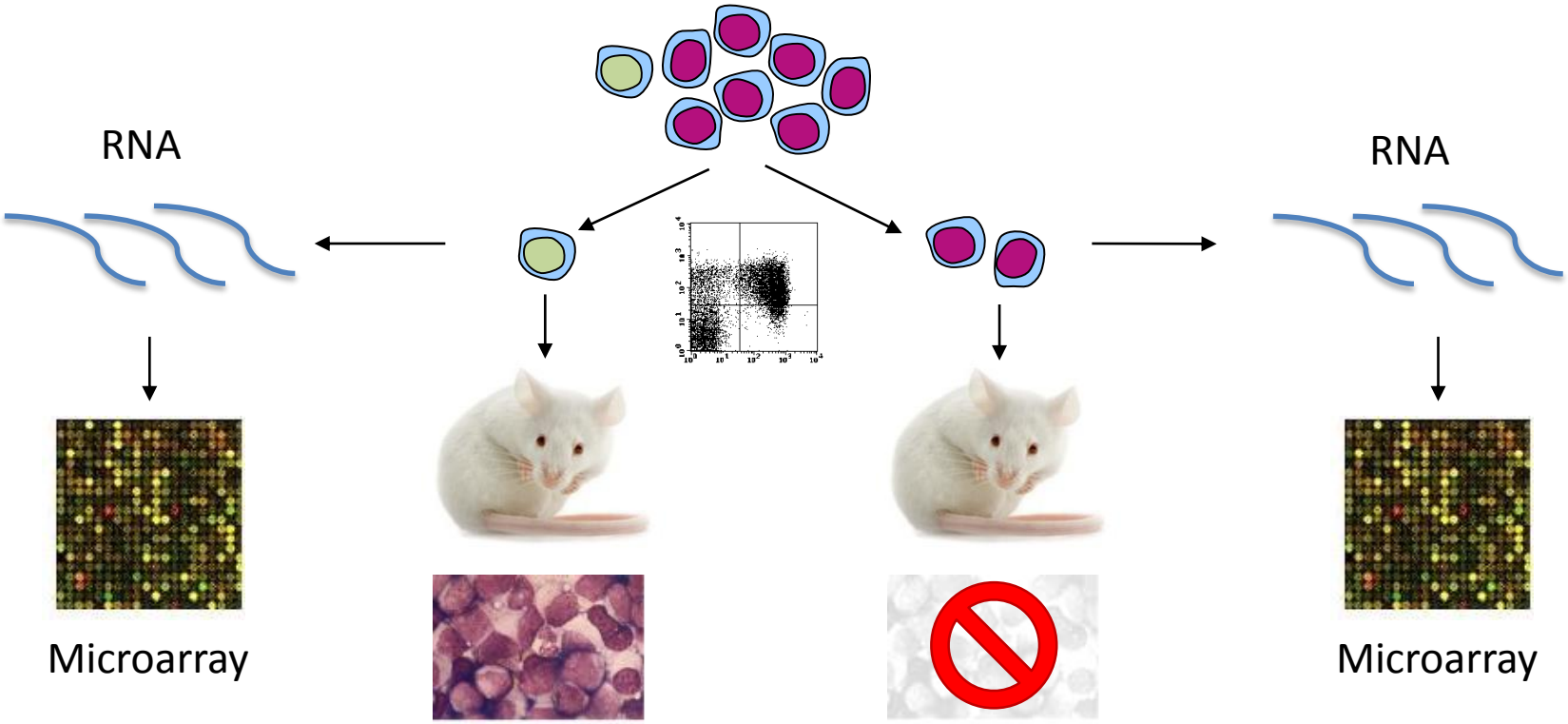
LSC properties are linked to outcome



LSCs are linked to therapy resistance and relapse

Analysis of LSC-specific molecular programs may identify prognostic biomarkers

An LSC gene expression profile generated from functionally validated cell populations



LSC-specific gene expression signature

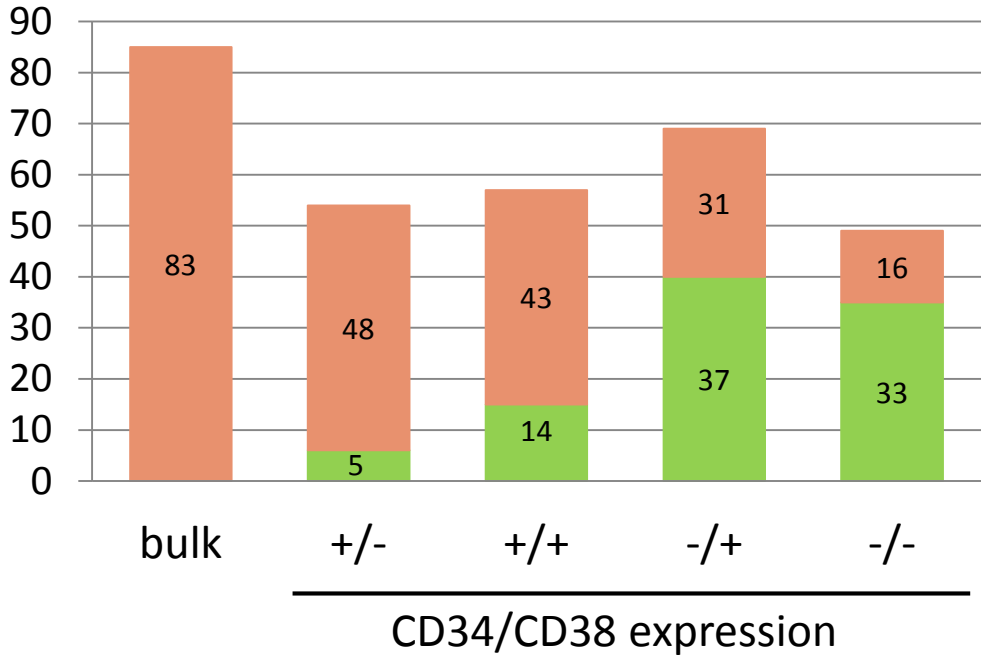
LSCs are found in multiple sorted fractions

78 AML patients

n=83

LSC+ 138

LSC– 89



	+/–	+/+	–/+	–/–
512	N	N	N	N
590	Y	Y	Y	N
596	Y	Y	N	N
598	Y	Y	N	Y
618	N	N	N	N
636	Y	Y	N	N
646	Y	Y	N	N
698	Y	N	N	N
713	Y	Y	Y	Y
740	N	N	Y	N
840	Y	N	N	N
858	Y	N	N	N
874	Y	Y	Y	Y
9515	norm	N	N	N
9578	N	N	N	N
9601	N	N	N	N
9677	norm	N	Y	N
9700	Y	Y	N	N
80008	Y	Y	Y	N
80179	Y	Y	N	N
80185	N	N	N	N
80280	N	Y	Y	Y
80281	Y	Y	Y	N
80291	Y	Y	N	N
80408	Y	Y	Y	N
80488	?	?	Y	Y
80527	Y	N	N	N
80534	Y	Y	N	N
80561	Y	Y	N	Y
90077	Y	Y	N	N
90087	Y	Y	N	N
90135	Y	N	N	N
90147	Y	N	N	N
90156	N	N	N	N
90181	Y	Y	Y	Y
90184	Y	Y	Y	Y
90185	Y	N	Y	Y
90190	Y	N	Y	N
90191	Y	N	N	N
90239	Y	Y	Y	Y
90240	Y	Y	Y	Y
90295	Y	N	N	N
90365	Y	Y	Y	N
90392	Y	N	Y	N
90394	Y	Y	N	N

	+/–	+/+	–/+	–/–
90409	Y	Y	N	N
90428	Y	Y	Y	Y
90429	Y	N	N	N
90444	Y	N	N	Y
90454	Y	Y	N	N
90476	Y	Y	N	N
90481	Y	N	N	N
90521	Y	Y	N	Y
90543	N?	Y	N	N
90582	N	Y	N	N
90590	Y	Y	Y	Y
90668	Y	N	-	N
90686	Y	N	N	N
90716	Y	N	N	N
100006	Y	Y	N	N
100016	Y	Y	Y	N
100091	N	Y	Y	N
100176	Y	Y	N	N
100183	Y	Y	N	Y
100255	Y	Y	Y	Y
100274	Y	Y	Y	Y
100327	Y	Y	Y	Y
100348	Y	Y	N	N
100454	Y	Y	Y	Y
100474	N	N	Y	Y
100490	Y	N	N	N
100507	norm	Y	N	N
100565	Y	Y	N	N
100685	Y	N	N	N
100719	Y	Y	N	N
100770	N	N	Y	N
100818	Y	Y	N	N
100874	Y	Y	N	N
100885	Y	Y	N	N
110002	Y	N	N	N
110080	Y	Y	Y	Y
110102	Y	Y	N	Y
110120	Y	Y	Y	N
110180	Y	Y	N	N
110260	norm	N	N	Y
110283	N	Y	Y	N
110438	?	N	Y	Y
110484	Y	Y	N	N
110500	Y	Y	N	Y
33xxxx1	Y	Y	Y	Y

Amanda Mitchell

An LSC gene expression profile generated from functionally validated cell populations

1676 differentially expressed genes

$p < 0.01$

FC > 2

Top 104 genes

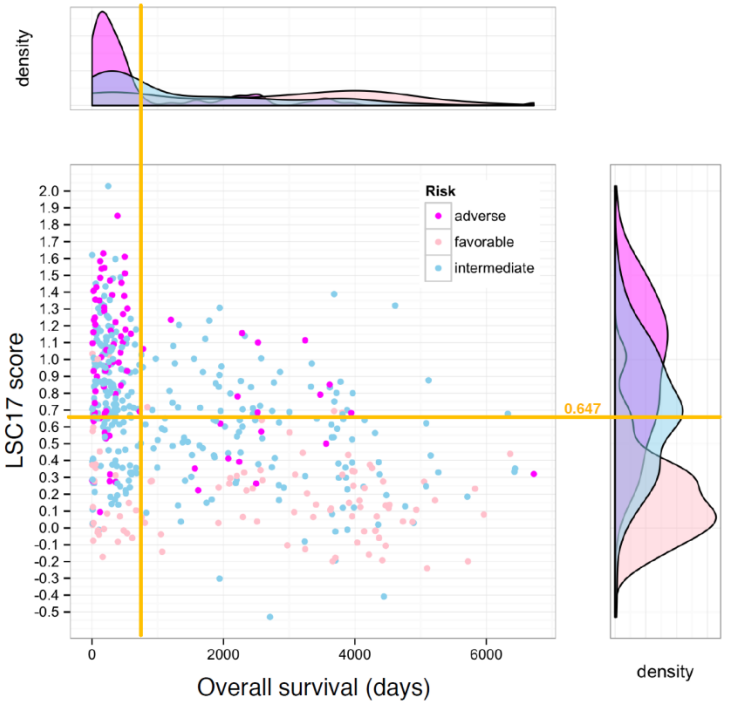
48 higher in LSC+

Statistical regression (43)

17 gene LSC signature

Adult AML

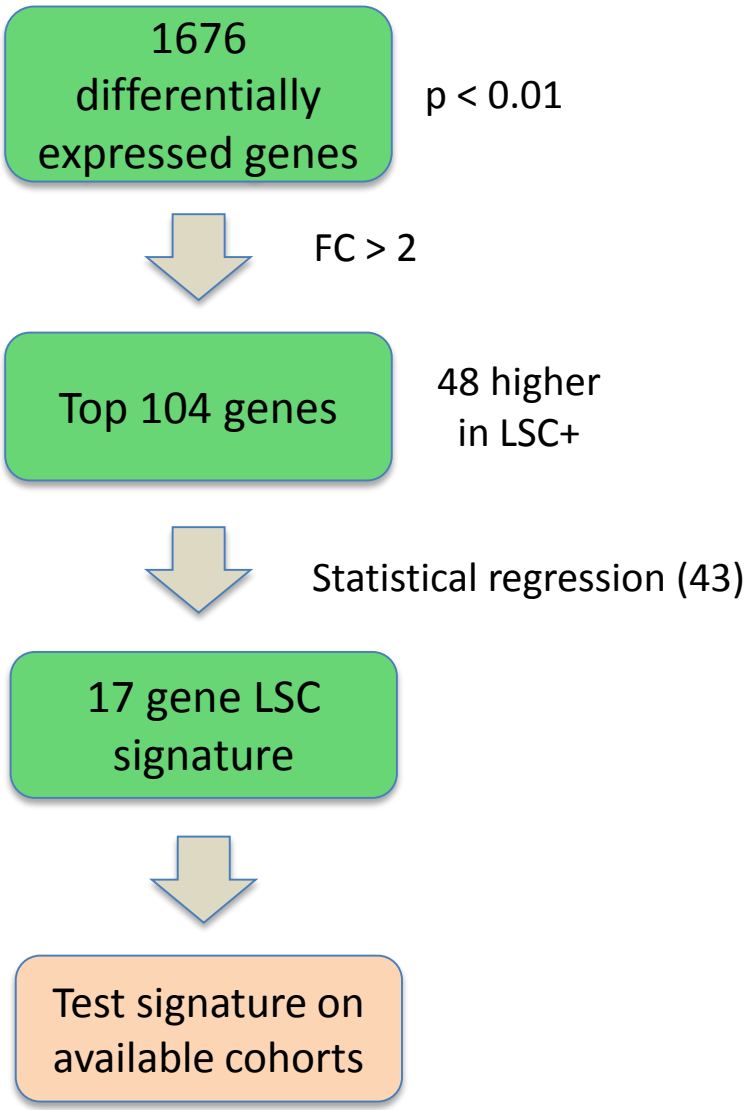
Cohort	Source	#samples
Training	Valk (GSE6891) Affymetrix U133Plus2.0	495



Median LSC17 score splits the intermediate cytogenetic risk group

Stan Ng

An LSC gene expression profile generated from functionally validated cell populations



Adult AML

Cohort	Source	#samples
Training	Valk (GSE6891) Affymetrix U133Plus2.0	495
Testing #1	TCGA (GSE10358) Affymetrix U133Plus2.0	183
Testing #2	Metzeler (GSE12417) Affymetrix U133A/B	156
Testing #3	Metzeler (GSE12417) Affymetrix U133Plus2.0	70
Testing #4	Ulm (GSE15434) LMR Affymetrix U133Plus2.0	70

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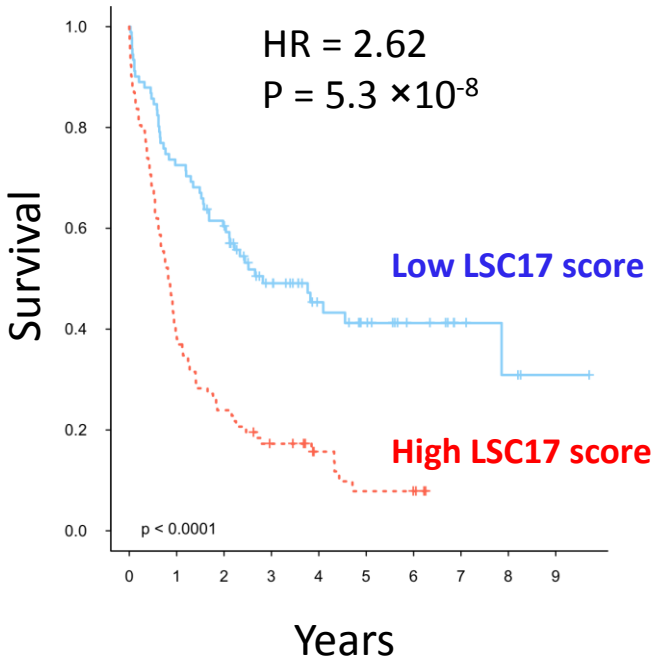
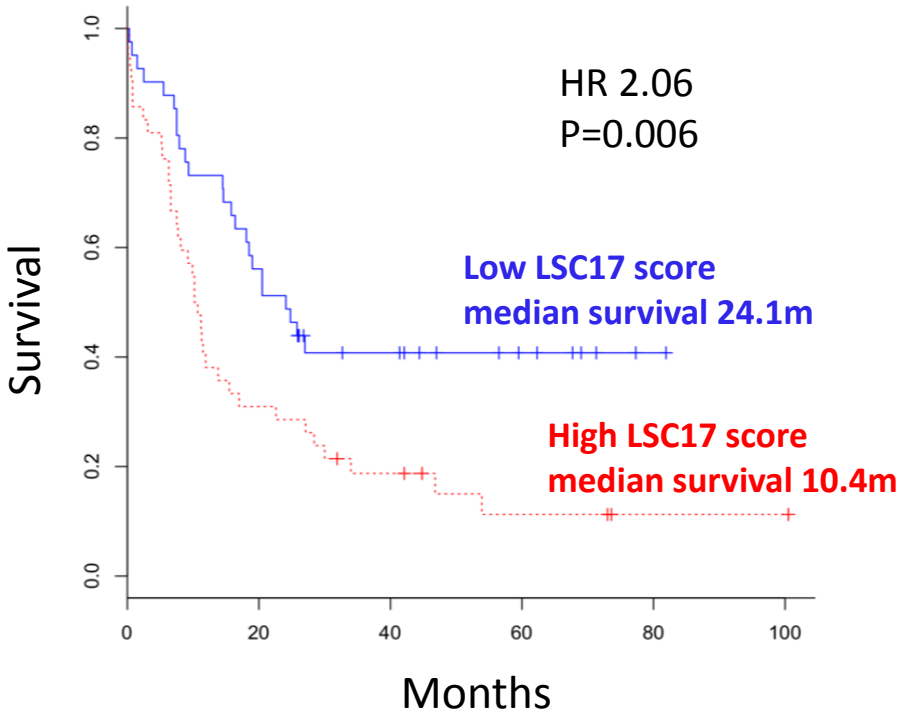
LSC17 score is prognostic in multiple independent AML cohorts

Affy array data

TCGA AML (n=183)



TCGA CN-AML (n=83)



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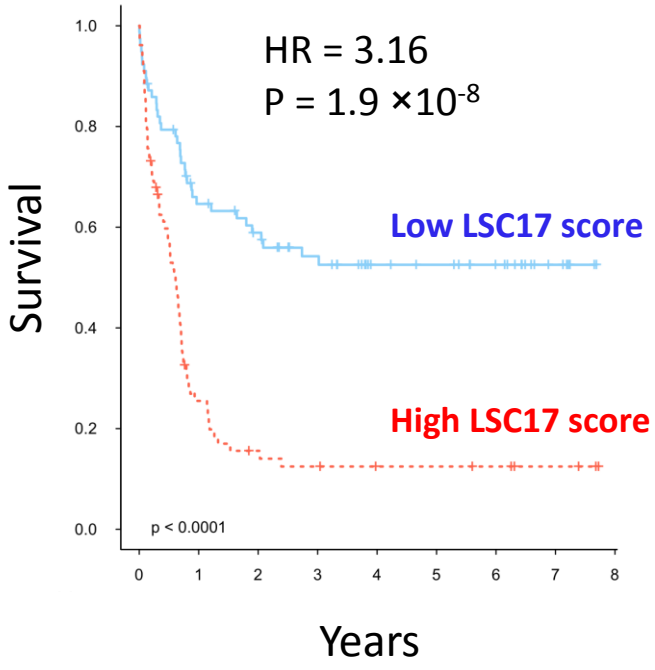
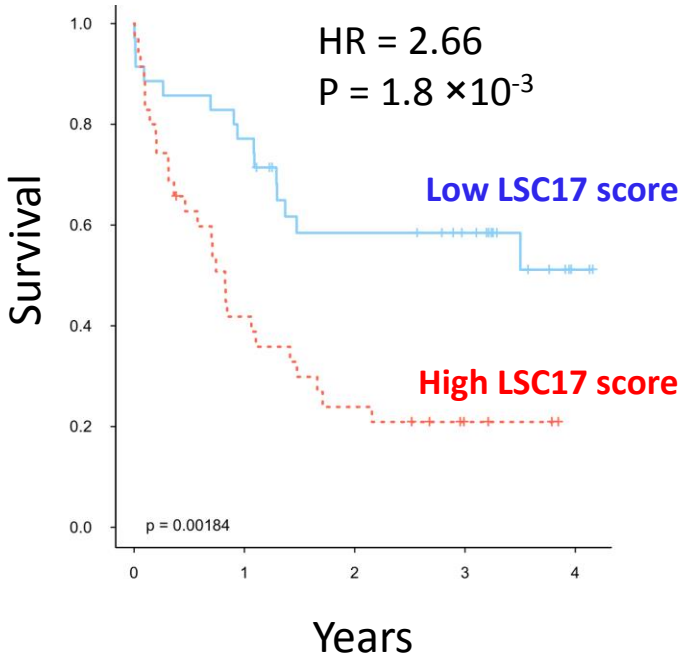
LSC17 score is prognostic in multiple independent AML cohorts

Affy array data

GSE12417 CN-AML #1 (n=156)



GSE12417 CN-AML #2 (n=70)



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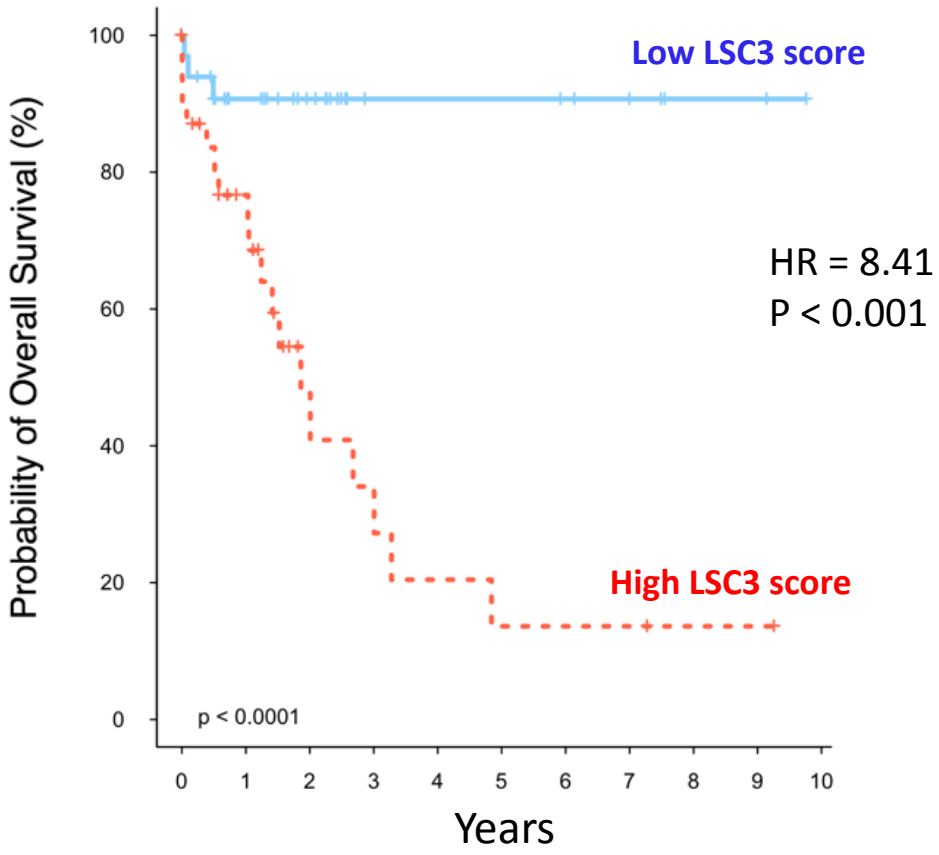
LSC3 score is prognostic in low molecular risk patients

LSC17 score re-weighted on LMR patients in GSE6891 training cohort (n=44)→ LSC3 score

*NPM1*mut
FLT3-ITD neg

GSE15434 CN-LMR (n=70)

Test cohort (Ulm)



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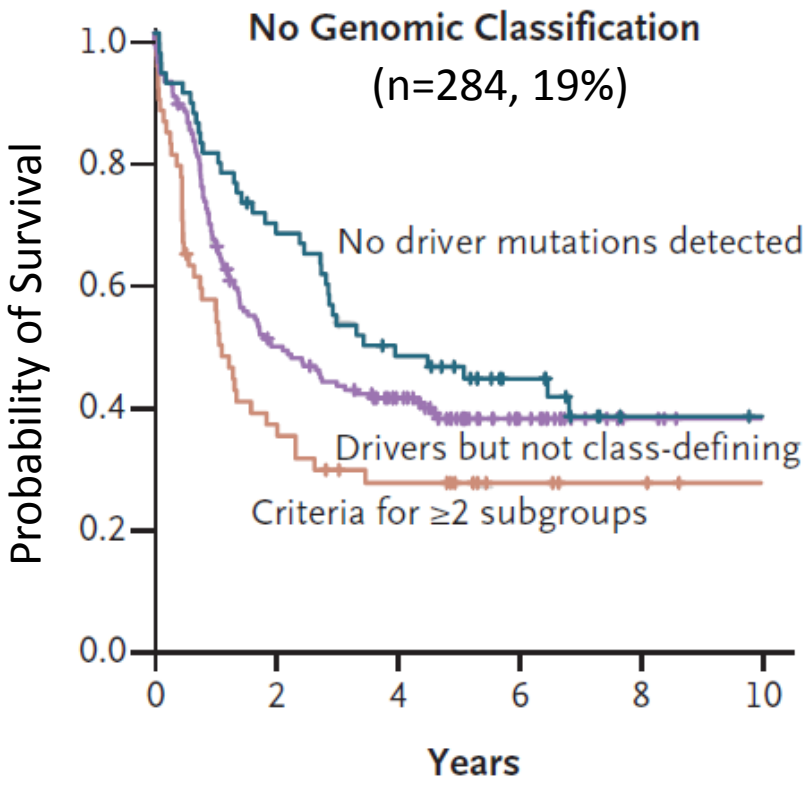
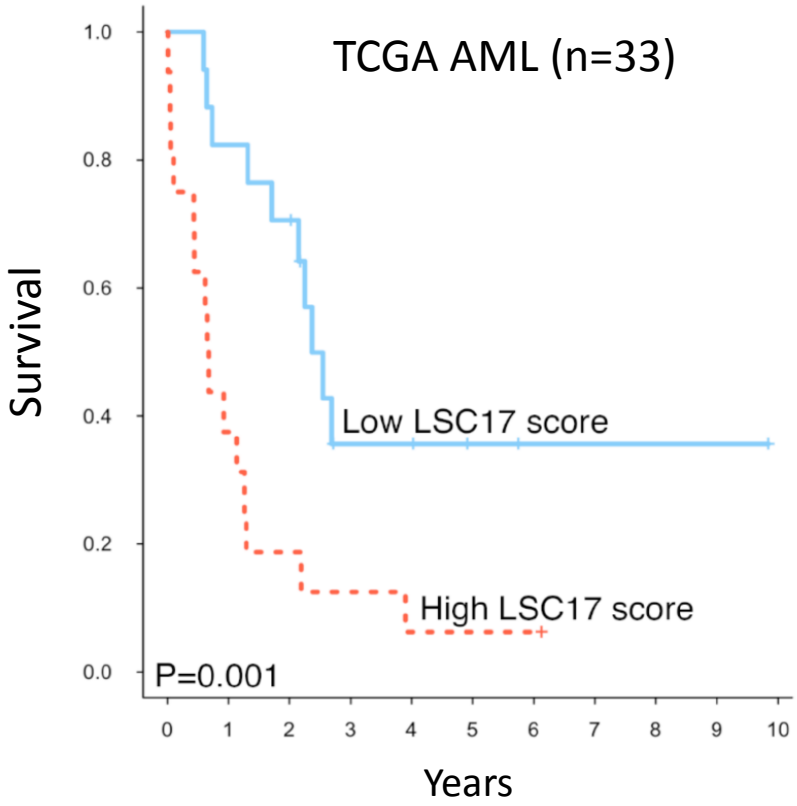
The LSC17 score refines genomic classification schemes

The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 JUNE 9, 2016 VOL. 374 NO. 23

Genomic Classification and Prognosis in Acute Myeloid Leukemia

Papaemmanuil et al.





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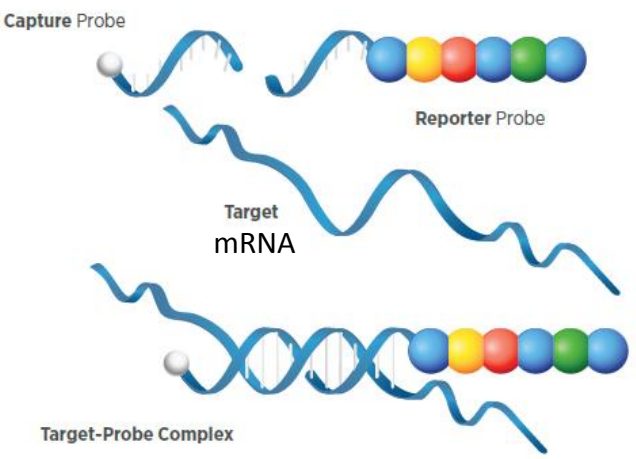
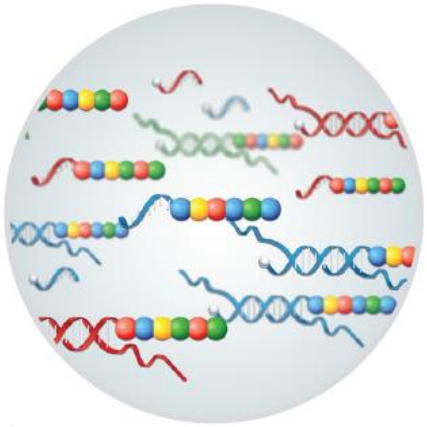
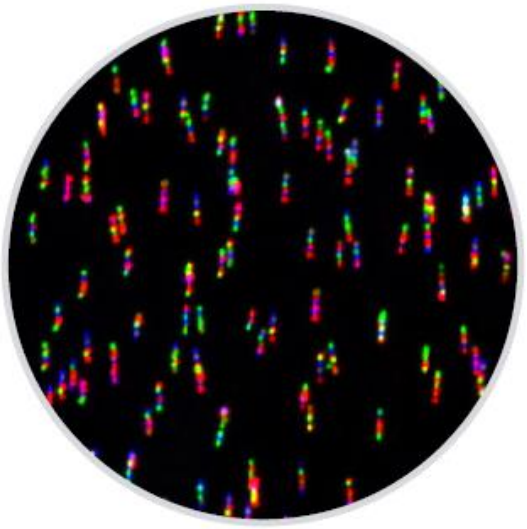
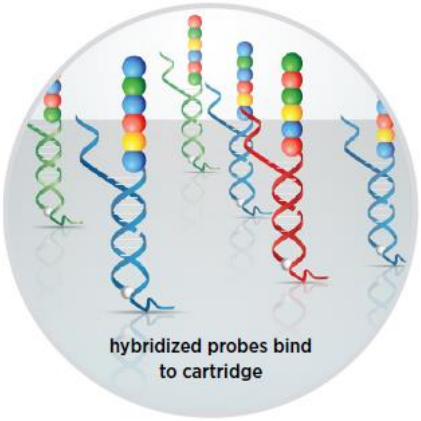
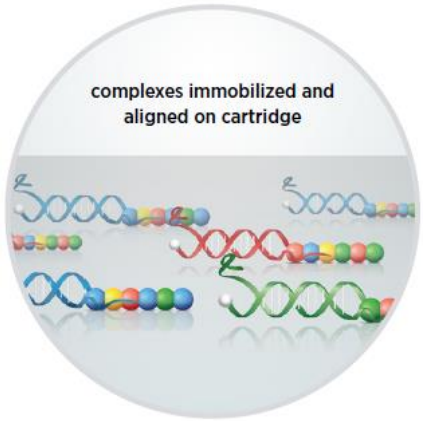
NanoString nCounter® Analysis System











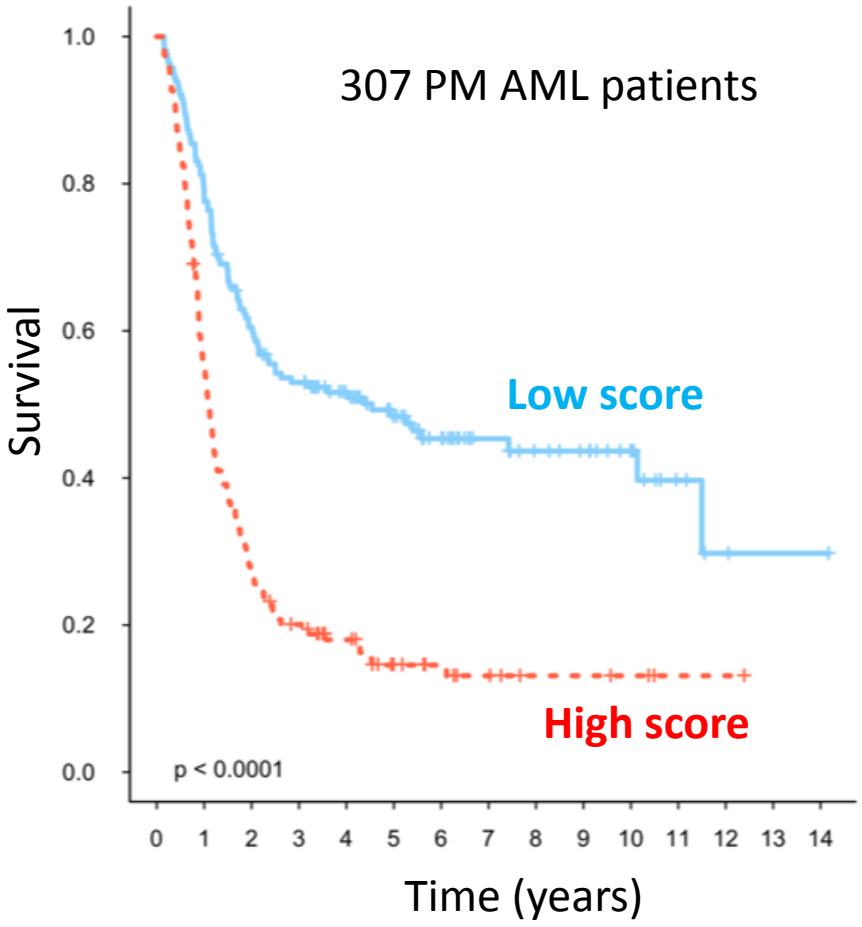
Sensitive
Reproducible
Cost-effective

Barcode	Counts	Identity
	3	XLSA
	2	FOX5
	1	INSULIN

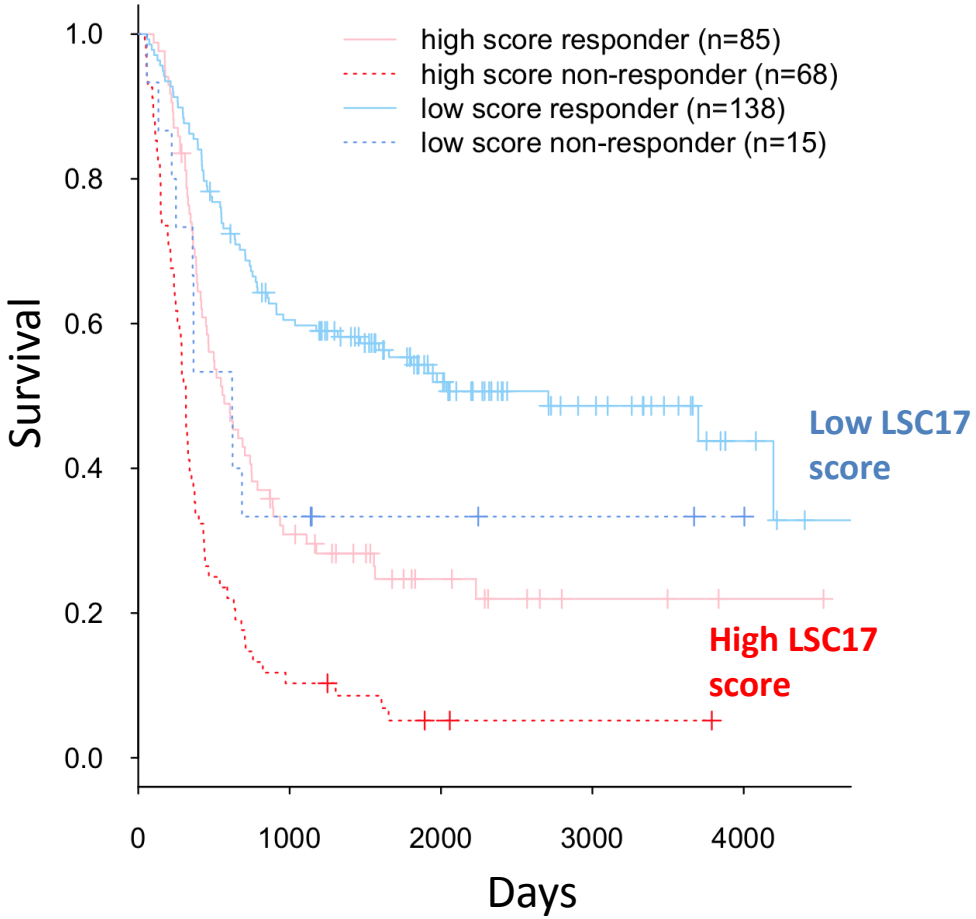
Barcodes are counted and tabulated for each target molecule.

LSC17 score predicts survival and therapy response in AML

NanoString data



High score patients have worse survival regardless of whether or not remission was achieved

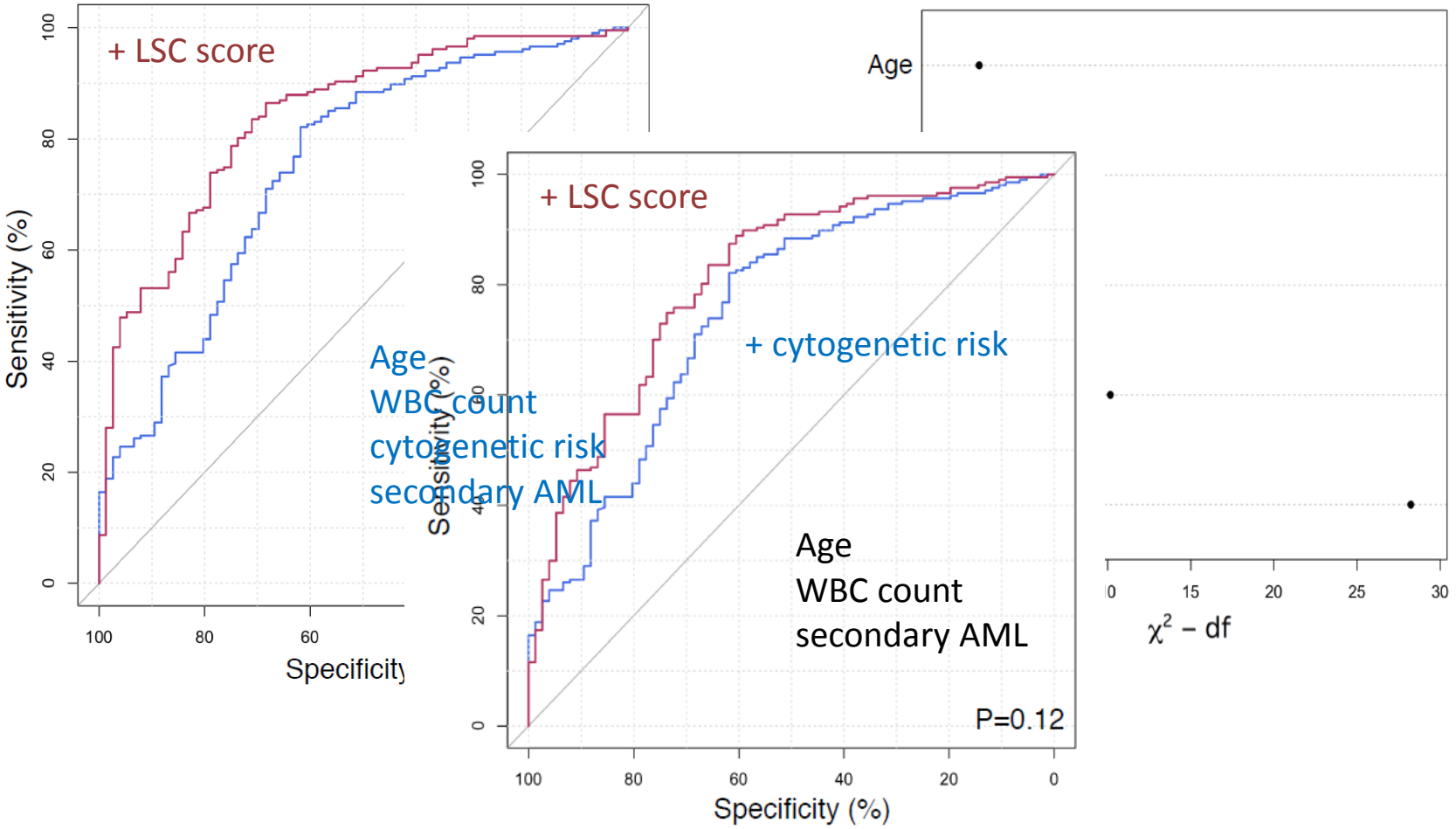


Better upfront therapies are needed for high score patients

Stan Ng, Amanda Mitchell et al, *Nature* 2016;540:433

LSC17 score predicts therapy resistance

Therapy resistance is a major barrier to cure



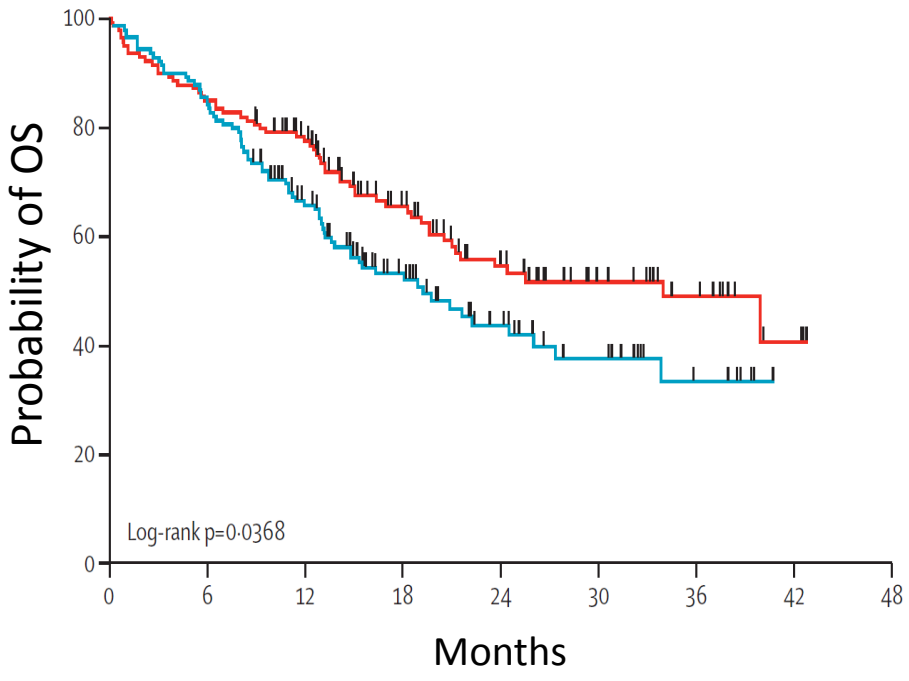
Stan Ng

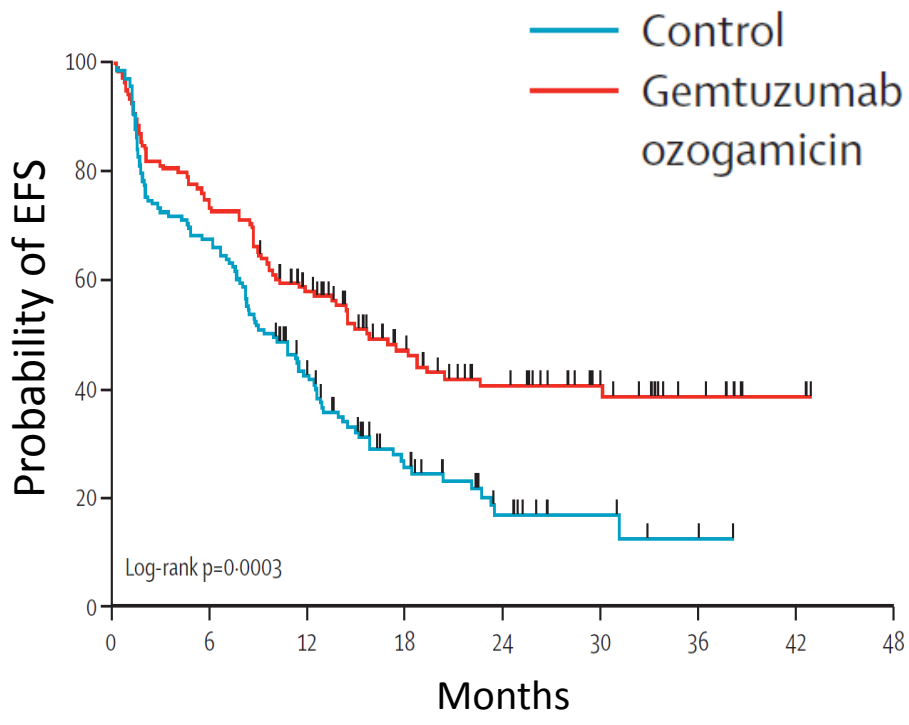
The LSC17 score predicts response to gemtuzumab ozogamicin (GO)

Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study

Lancet 2012; 379: 1508–16

Sylvie Castaigne, Cécile Pautas, Christine Terré, Emmanuel Raffoux, Dominique Bordessoule, Jean-Noel Bastie, Ollivier Legrand, Xavier Thomas, Pascal Turlure, Oumedaly Reman, Thierry de Revel, Lauris Gastaud, Noémie de Gunzburg, Nathalie Contentin, Estelle Henry, Jean-Pierre Marolleau, Ahmad Aljijakli, Philippe Rousselot, Pierre Fenaux, Claude Preudhomme, Sylvie Chevret, Hervé Dombret, for the Acute Leukemia French Association

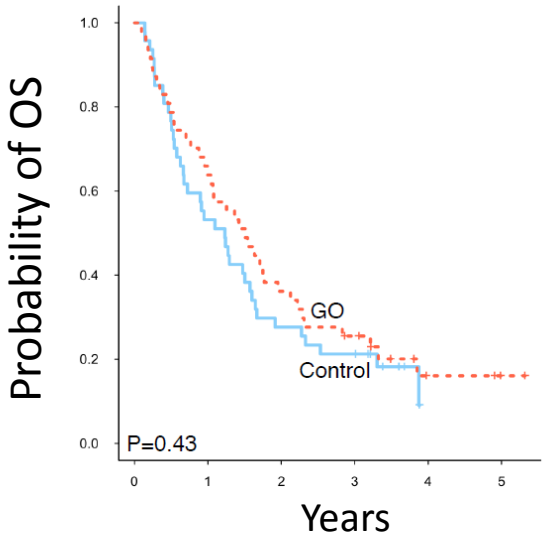


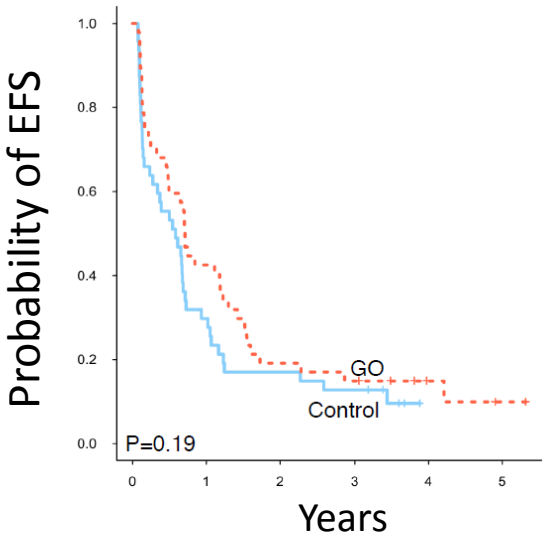


The LSC17 score predicts response to gemtuzumab ozogamicin

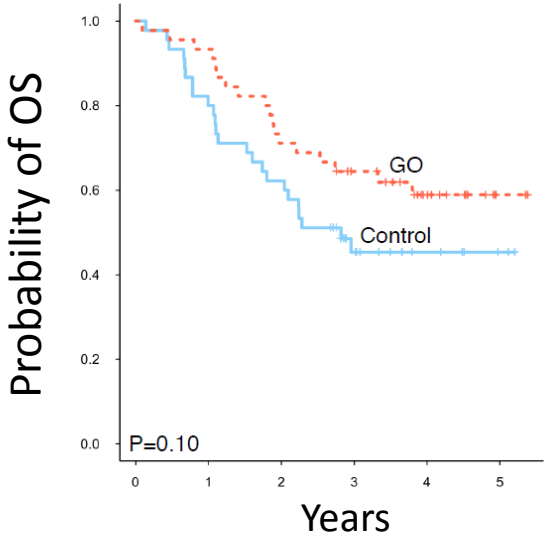
Patients surviving more than 30 days
ALFA-0701 trial

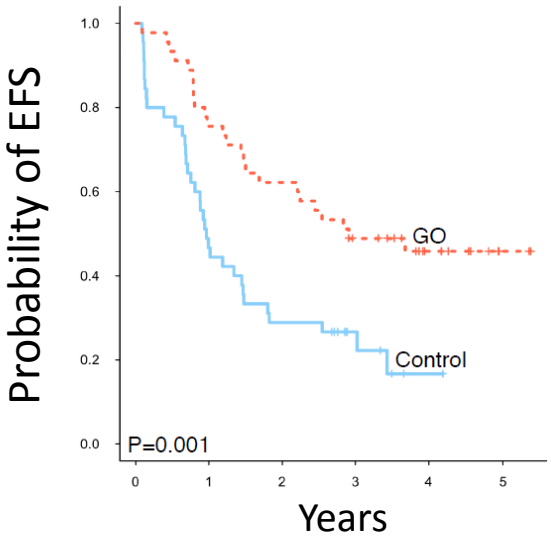
High LSC17 score
n=94





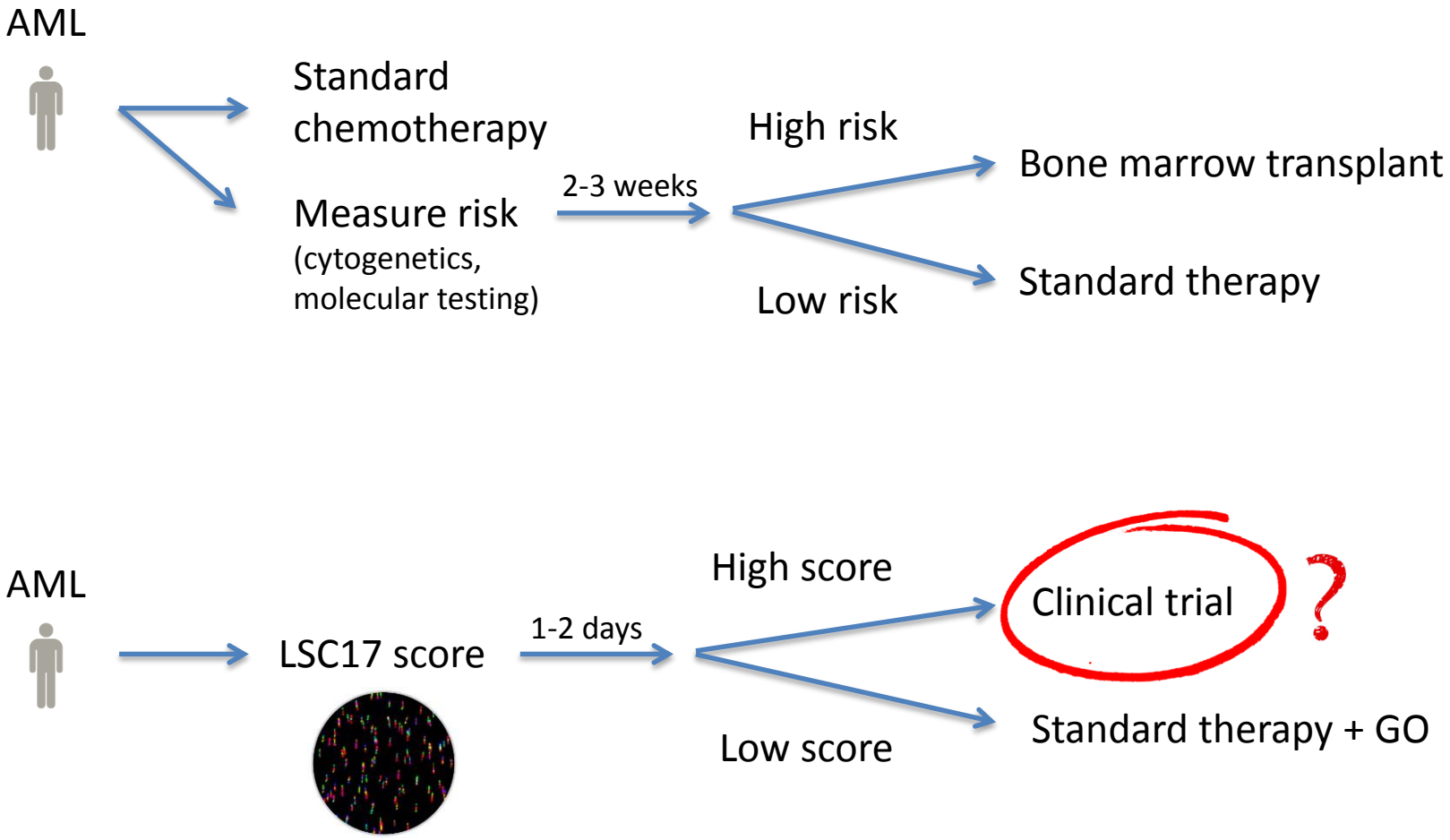
Low LSC17 score
n=90





Stan Ng

A new treatment strategy for AML patients based on risk

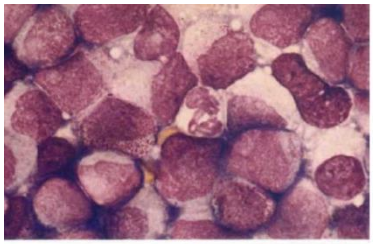


A better strategy for anti-cancer drug development

Cancer cell lines

Drug selected based on ability to kill cancer cell lines

LEUKEMIA STEM CELLS



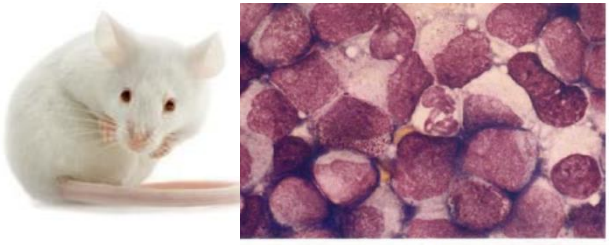
LEUKEMIA

A better strategy for anti-cancer drug development

Cancer cell lines

Dru s ect d based on ability
kill ca cer cell lines

Transplant



LEUKEMIA
STEM CELLS

LEUKEMIA

Test drugs for ability to reduce leukemia in mice: Capture heterogeneous responses

AML cells
from patients

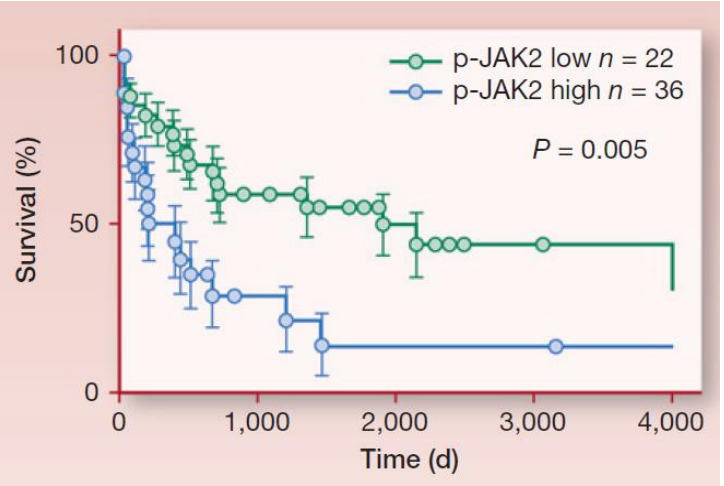
JAK2 inhibition in AML

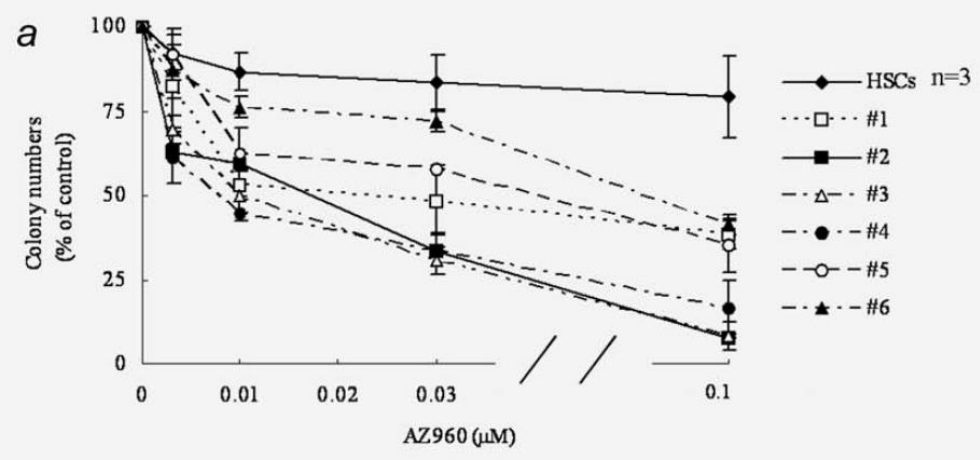
Int. J. Cancer: 129, 2512–2521 (2011)

Expression of p-JAK2 predicts clinical outcome and is a potential molecular target of acute myelogenous leukemia

Takayuki Ikezoe[1], Shinsuke Kojima[1], Mutsuo Furihata[2], Jing Yang[1], Chie Nishioka[1,3], Asako Takeuchi[1], Mayuka Isaka[1], H. Phillip Koeffler[4,5] and Akihito Yokoyama[1]

de novo AML





AZ960 (JAK2 inhibitor) inhibits colony formation by CD34+ AML cells

Heterogeneous response to a JAK2 inhibitor in AML

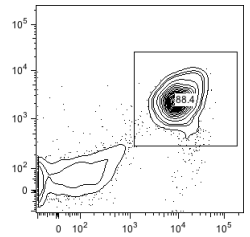
Primary AML samples

Intrafemoral transplantation

2-3 wks

FED or VEH

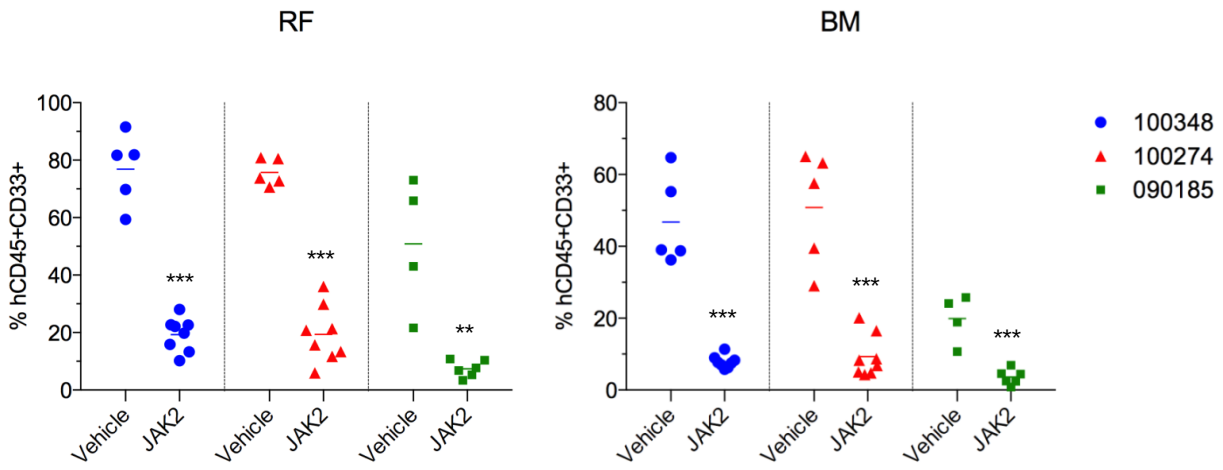
oral gavage × 14d



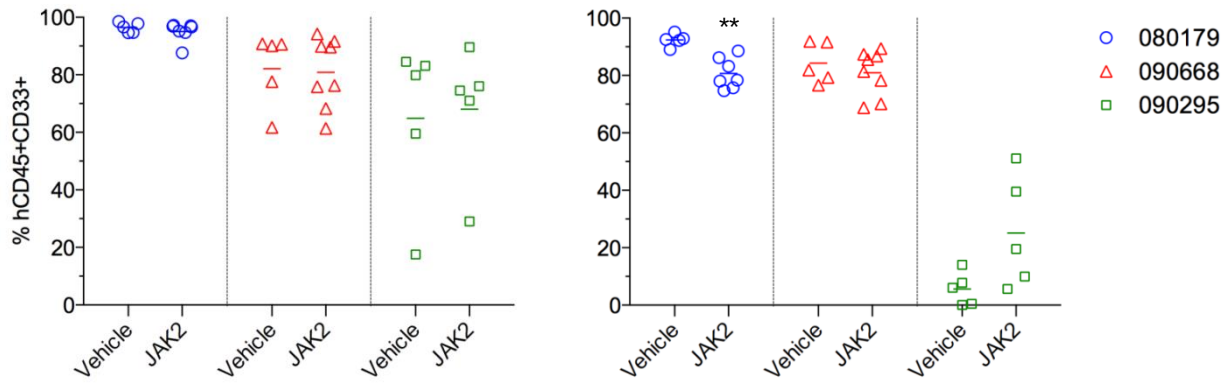
Check engraftment

Secondary transplantation

Responders



Non-Responders



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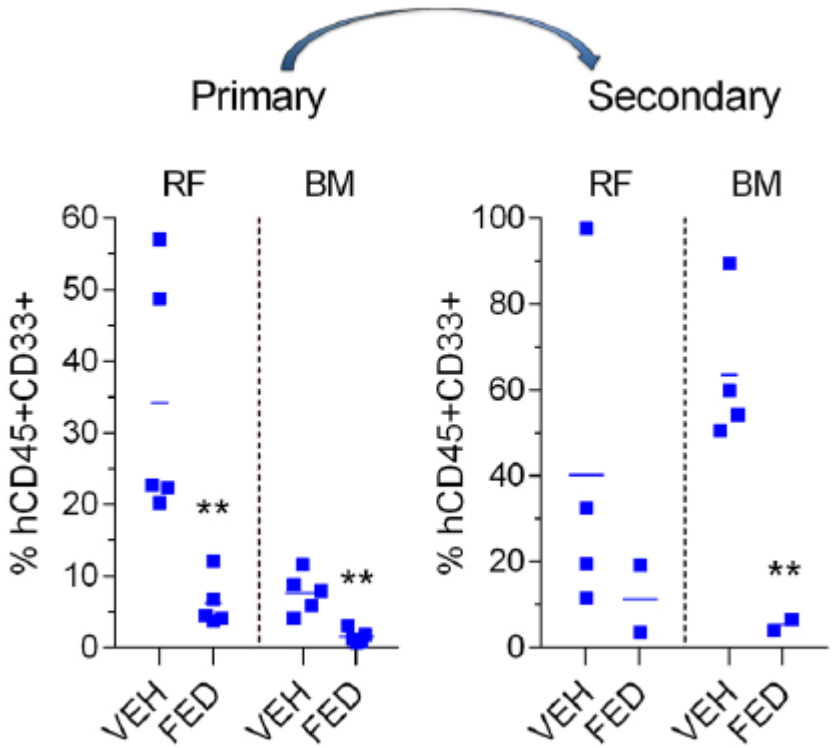
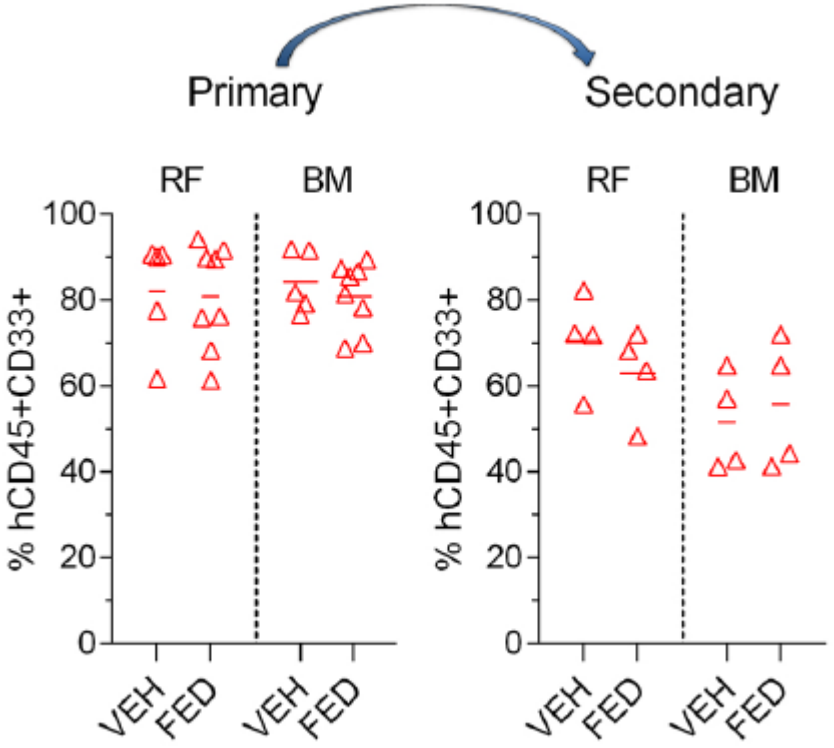
Heterogeneous response to a JAK2 inhibitor in AML





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Heterogeneous response to a JAK2 inhibitor in AML

Primary AML samples

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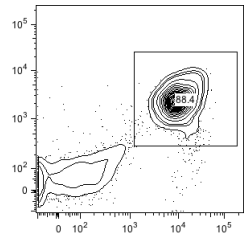

Check engraftment

Secondary transplantation

Phosphoflow cytometry

34 patient samples

"Model" Clinical Trial

Heterogeneous response

17/34 Responders (50%)

5/34 Partial Responders (15%)

12/34 Non-Responders (35%)

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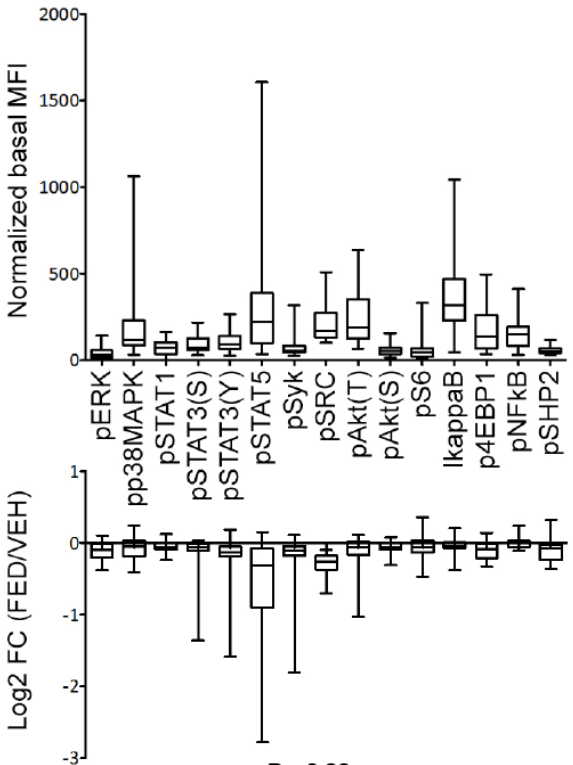
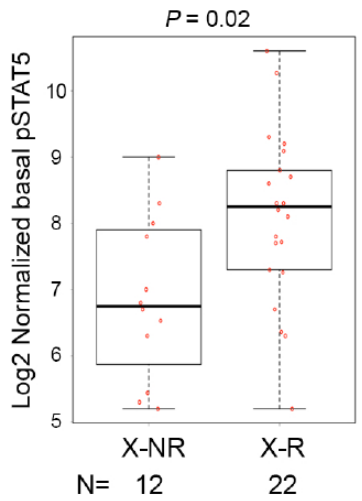
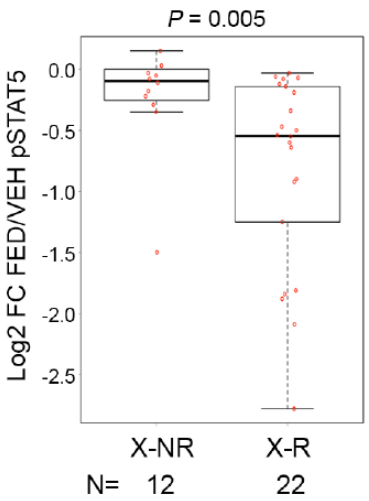
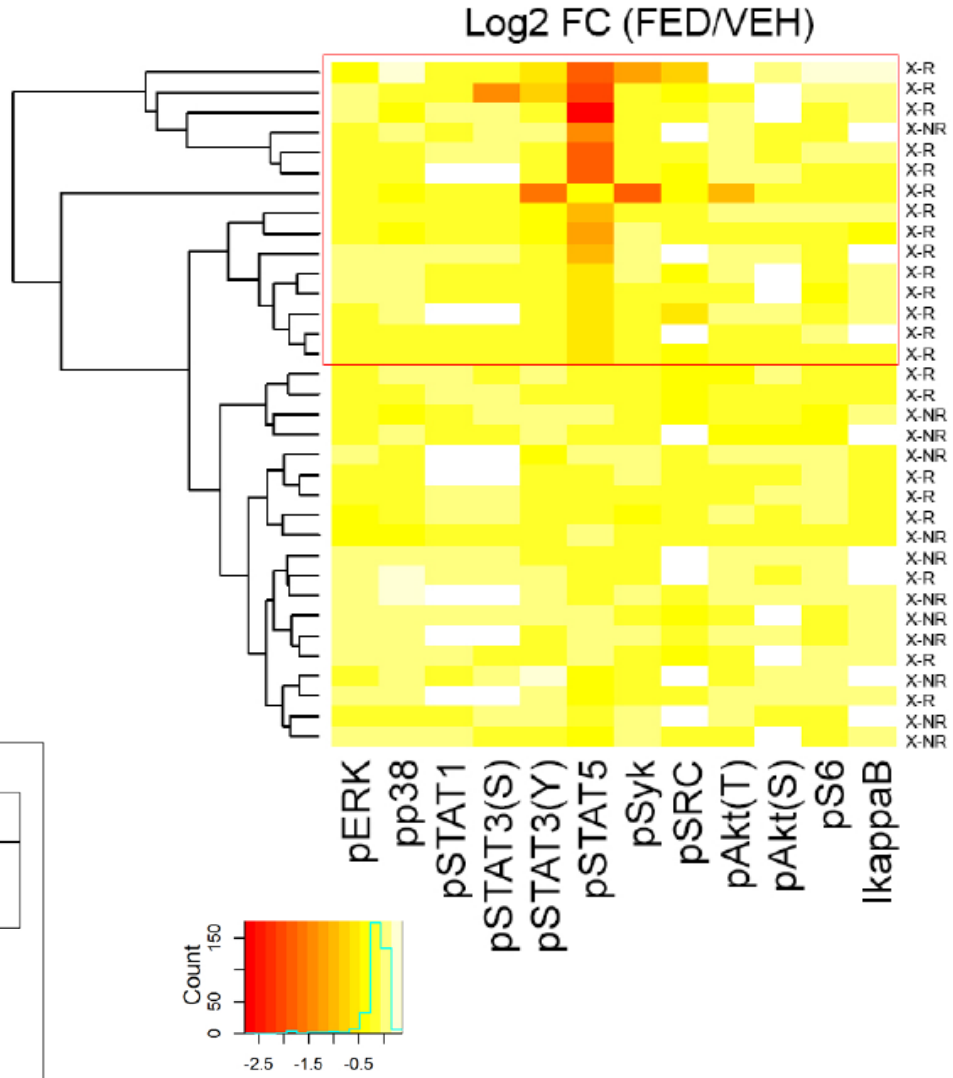
FED-sensitive pSTAT5 provides a biomarker of *in vivo* response to FED









Julie Yuan, Cindy Guidos

FED-sensitive pSTAT5 provides a biomarker of *in vivo* response to FED

In vivo drug response

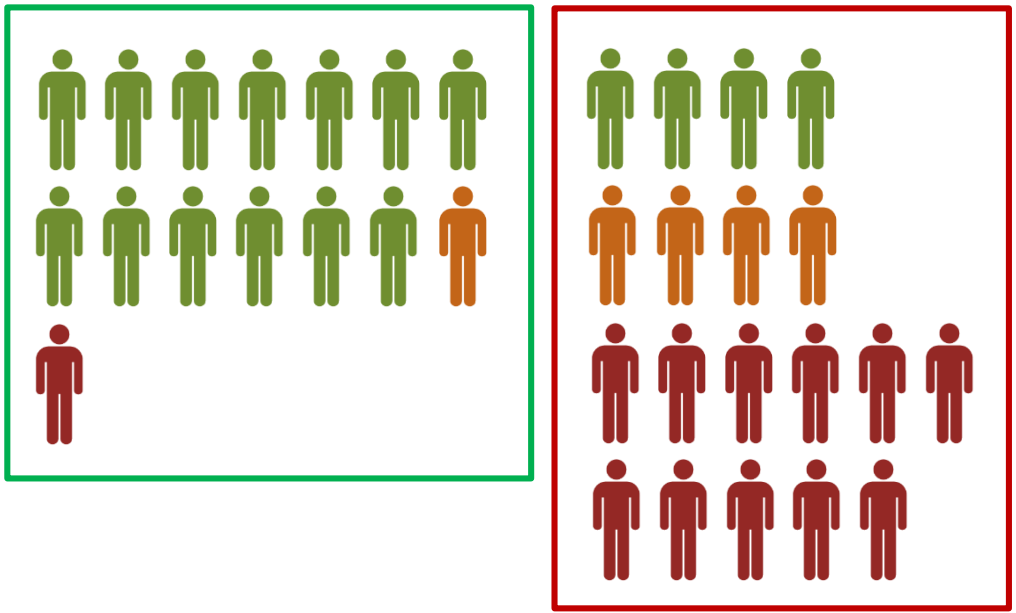
17/34 Responders (50%)

5/34 Partial Responders (15%)

12/34 Non-Responders (35%)

PF Responders

PF Non-Responders



PF Response:
Log2 FC ≥ 0.4

Sensitivity 64%

Specificity 92%

Positive predictive value 93%

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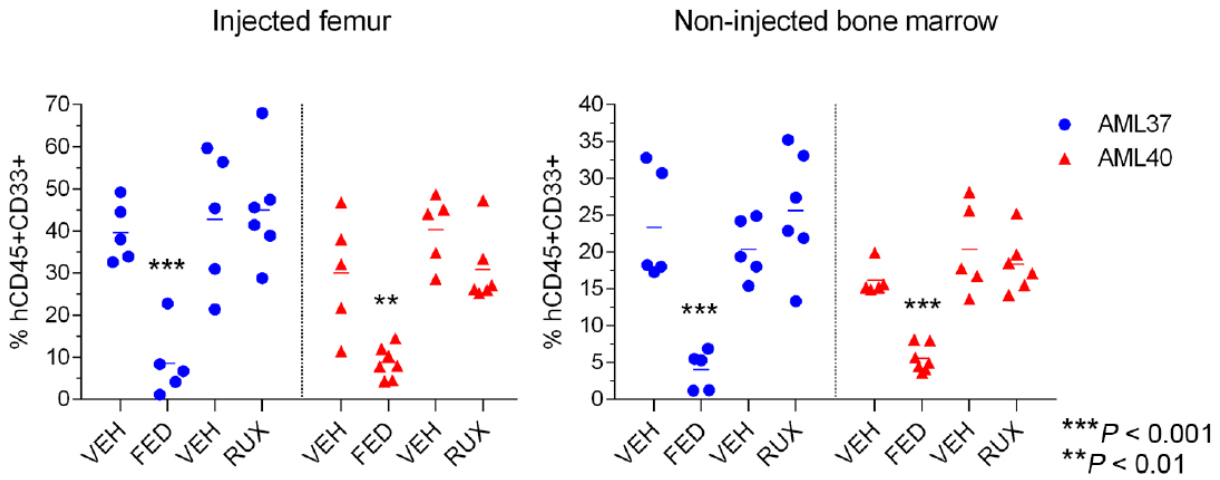
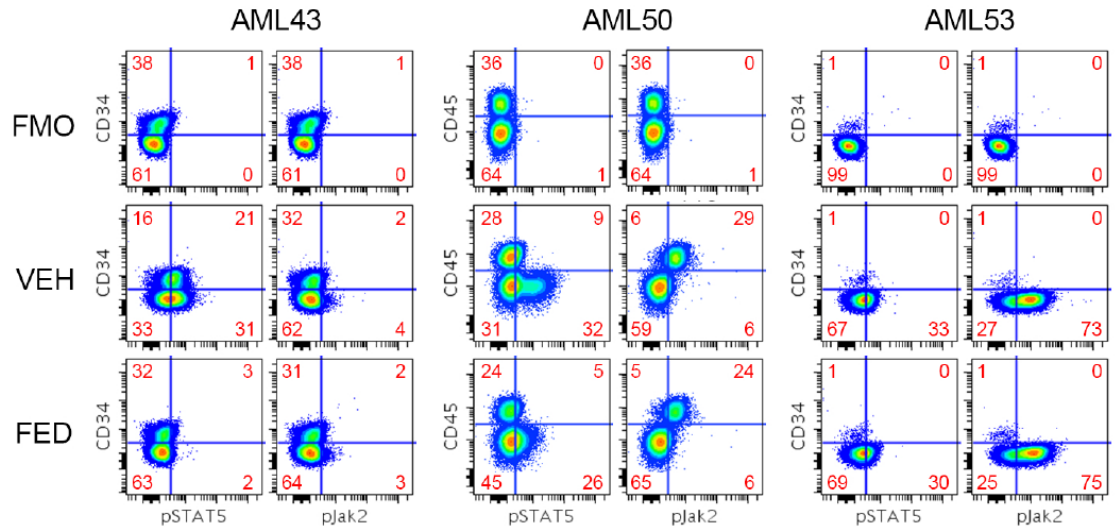
Discordant responses to FED and RUX – a non-JAK2-dependent mechanism



Sample ID	*FLT3-ITD*	In vivo response		Log2FC drug/veh pSTAT5	
		FED	RUX	FED	RUX
AML48	+	PR	NR	-1.22	-0.2
AML37	+	R	NR	-1.52	-0.48
AML38	+	R	PR	-0.52	-0.07
AML40	+	R	NR	-0.76	0.15
AML2	+	R	NR	-2.09	ND
AML12	+	PR	NR	-3.03	-0.44
AML43	+	R	NR	-1.6	-0.21
AML1	-	R	NR	-0.35	-0.01
AML36	-	R	NR	-0.03	0.04
AML7	-	R	PR	-0.56	-0.64



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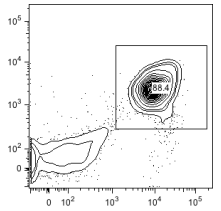
A better strategy for anti-cancer drug development

Large number of AML patient samples

transplantation

Drug vs. control



Determine drug response

Biomarker studies: correlate with drug response

Biomarker for treatment stratification

"Model" Clinical Trial

Validation cohort

Test drug and biomarker

Clinical Trial

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The Future is in Our Genes.

California Institute for Regenerative Medicine

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