Preclinical evaluation of new therapeutics

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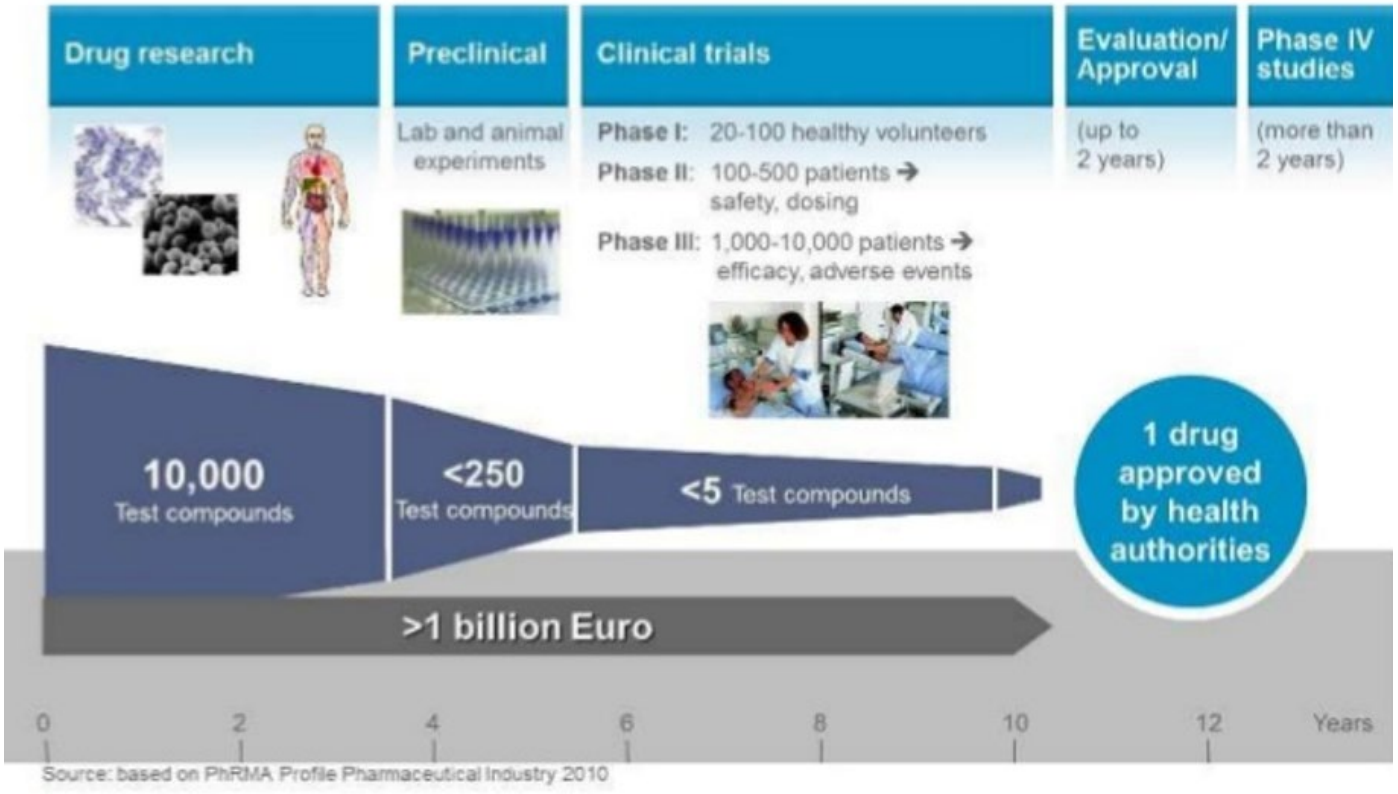
(biologist/biomedical sciences)

ITCC education meeting

Oct 15th 2018, Utrecht

Drug Development process

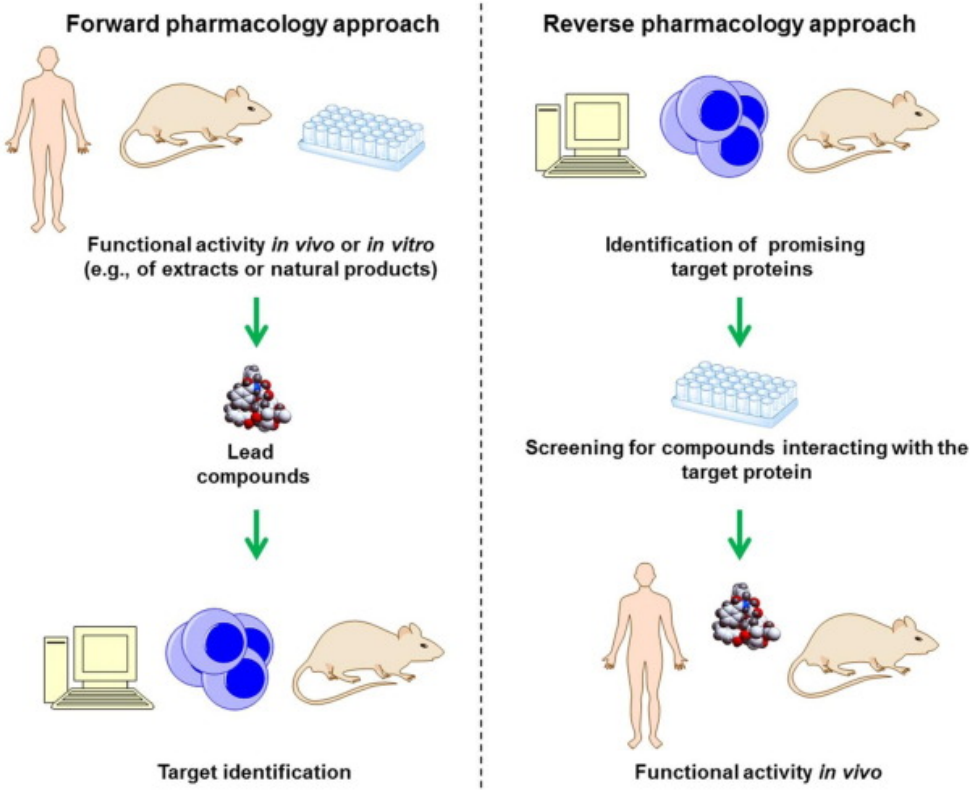


This talk

This presentation: drug discovery

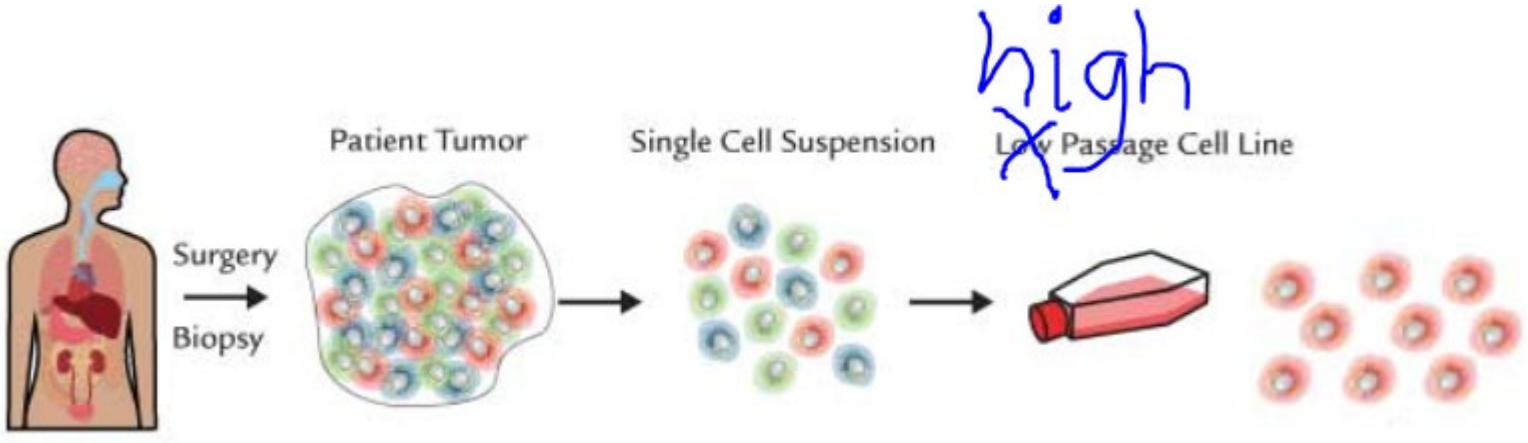
1. Principles of test models and drug testing
2. Principles of target discovery

} 3. Preclinical evaluation of new therapeutics



Atanasov AG et al. Biotechnol Adv. 2015

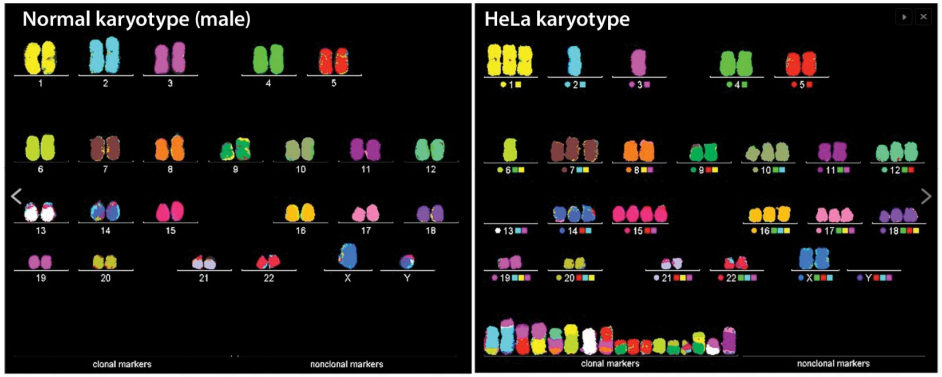
Preclinical test models: in vitro – cell lines



HeLa cell line
cervical carcinoma, 1951

Henrietta Lacks

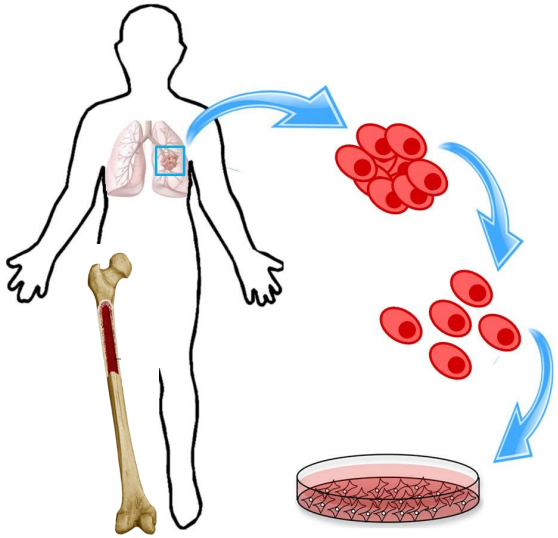
>60,000 papers
in medical science field



The karyotype of a HeLa cell is very different from the karyotype of a normal human, with extra copies of some chromosomes and missing copies of others. credit: Duesberg lab, UC Berkeley *Item 3 of 3*

Caveats:
Genetic drifting
Culture artefacts

Preclinical test models: ex vivo – patients' cells (leukemia)



Nolte, Signals 2014

Pro:

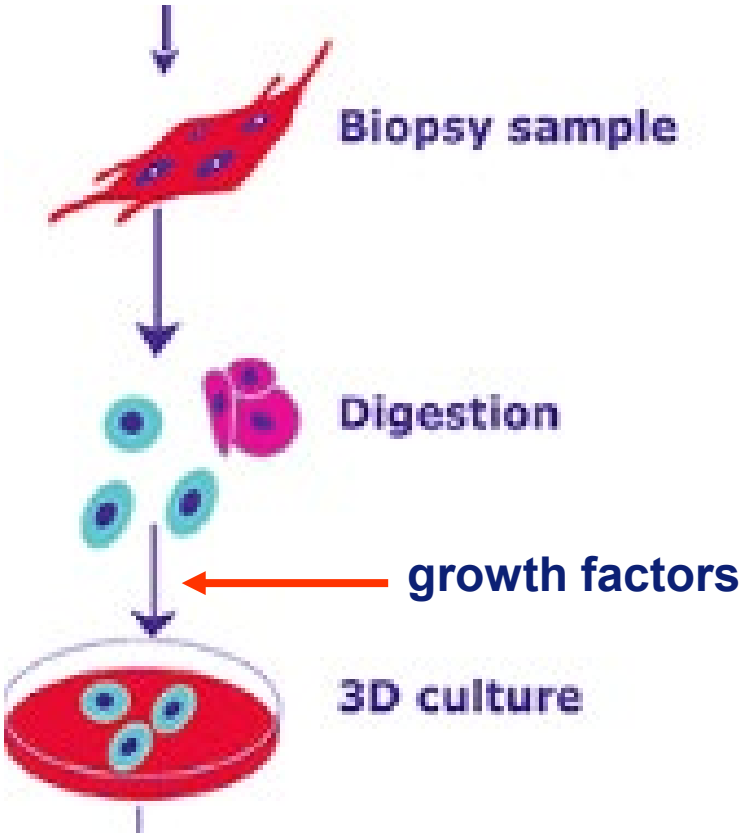
- Closest to patient
- Genetic context intact
- Tumor heterogeneity intact

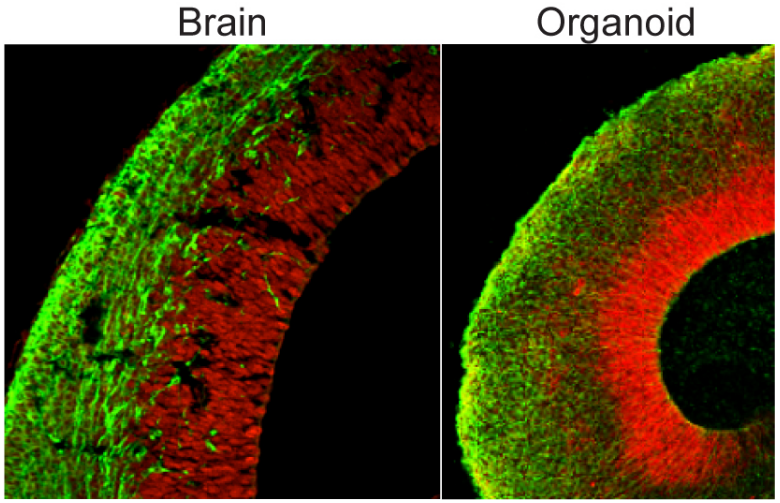
Limitations:

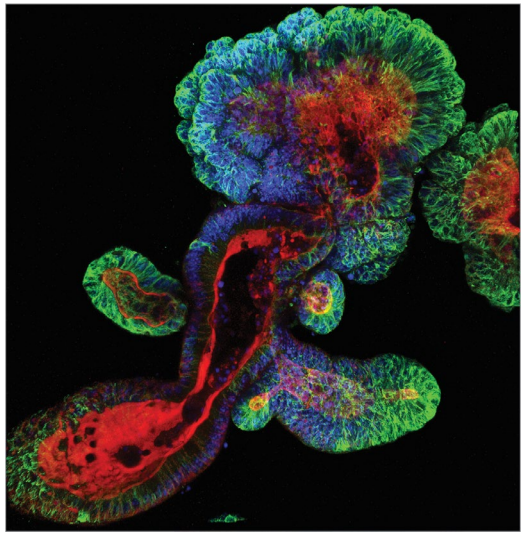
- Limited availability
- Difficult to obtain, to isolate and to preserve
- Short survival time ex vivo (days)
- Disconnected from tumor microenvironment

BUT..

Preclinical test models: ex vivo – tumor organoids (solid and brain tumors)

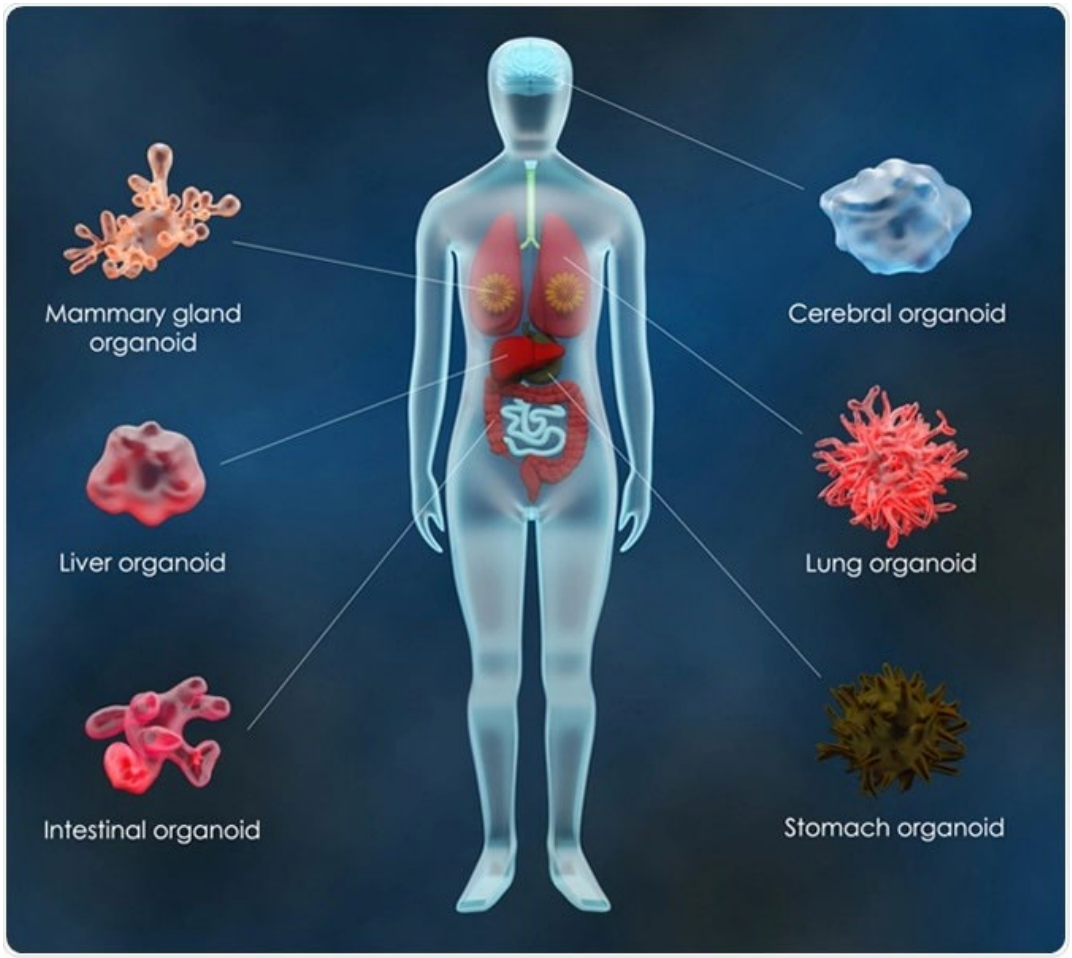






intestinal organoid

Preclinical test models: ex vivo – tumor organoids (solid and brain tumors)



Pro:

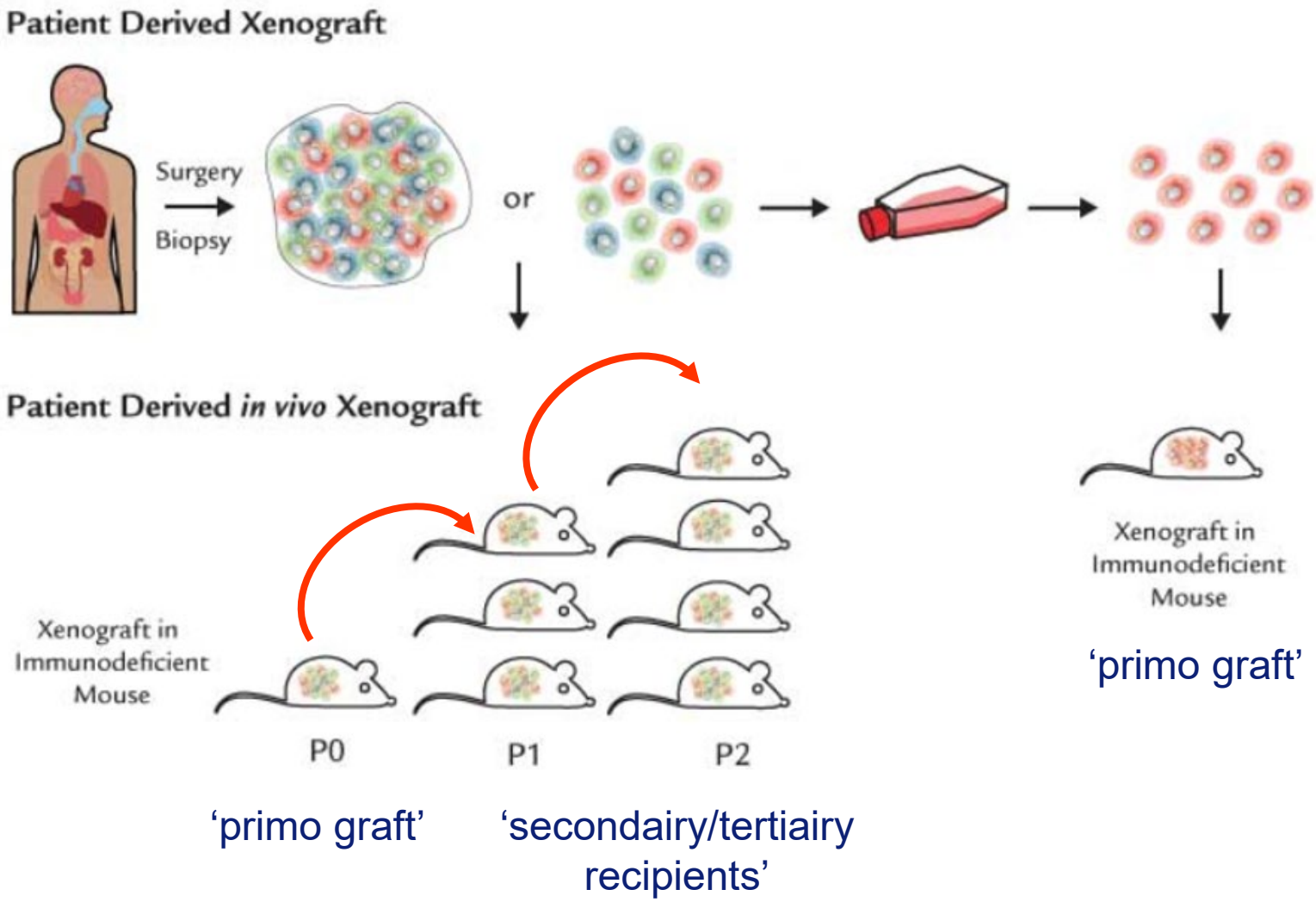
- Closest to patient
- Genetic context intact
- Tumor heterogeneity often intact
- Prolonged culture possible

Limitations:

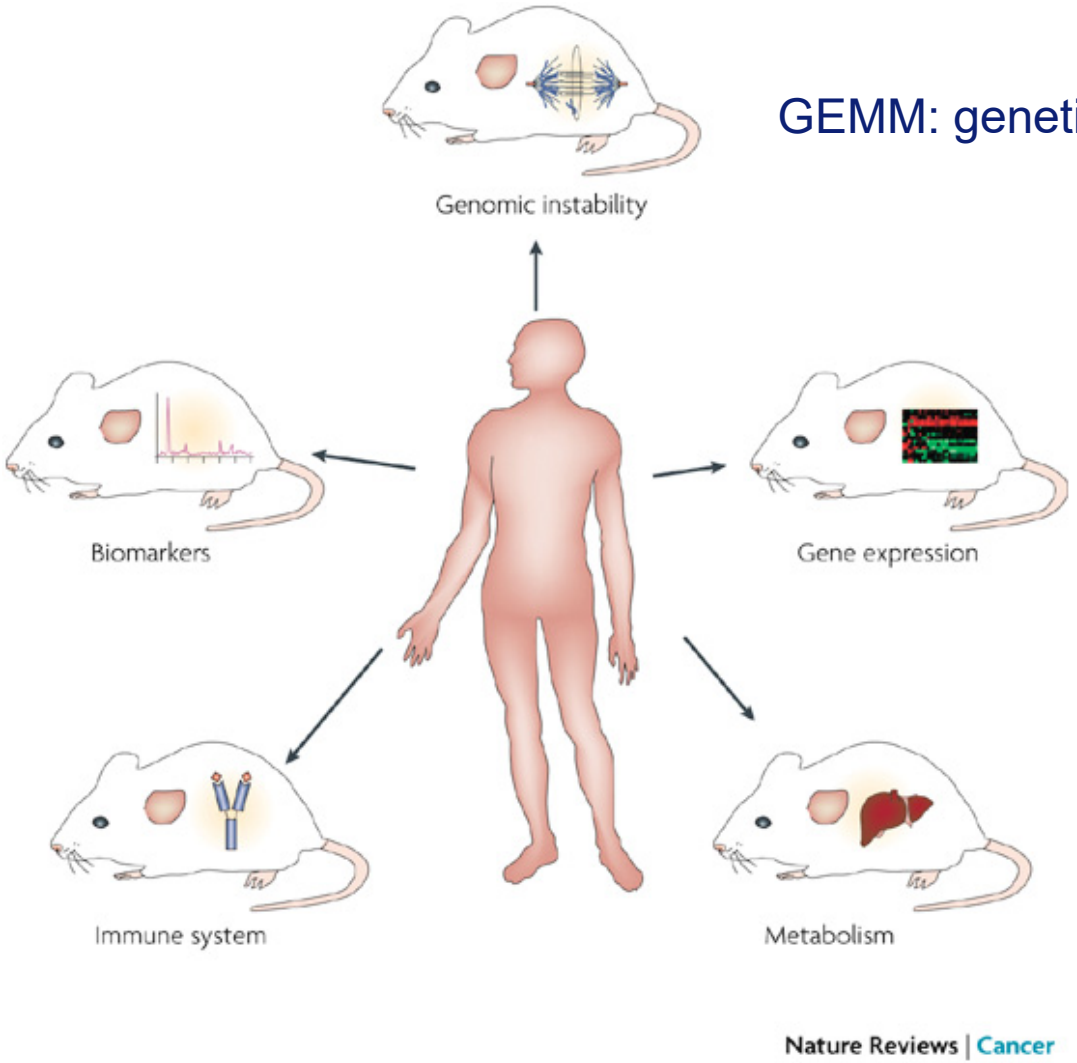
- Success rate varies between 30 and 80%
- Depending on cocktail of external growth factors
- Disconnected from tumor microenvironment

Preclinical test models: in vivo - mouse

In vivo is not synonymous to patients



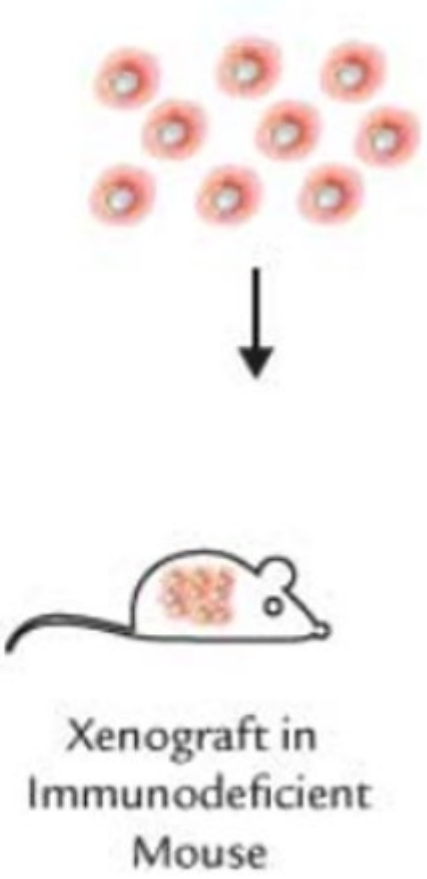
Preclinical test models: in vivo - mouse



GEMM: genetically engineered mouse model

Frese & Tuveson, Nat Rev Cancer 2007

Preclinical test models: in vivo - mouse



Pro:

- More holistic view of cancer
- Real-time monitoring of drug effect possible (response biomarkers)

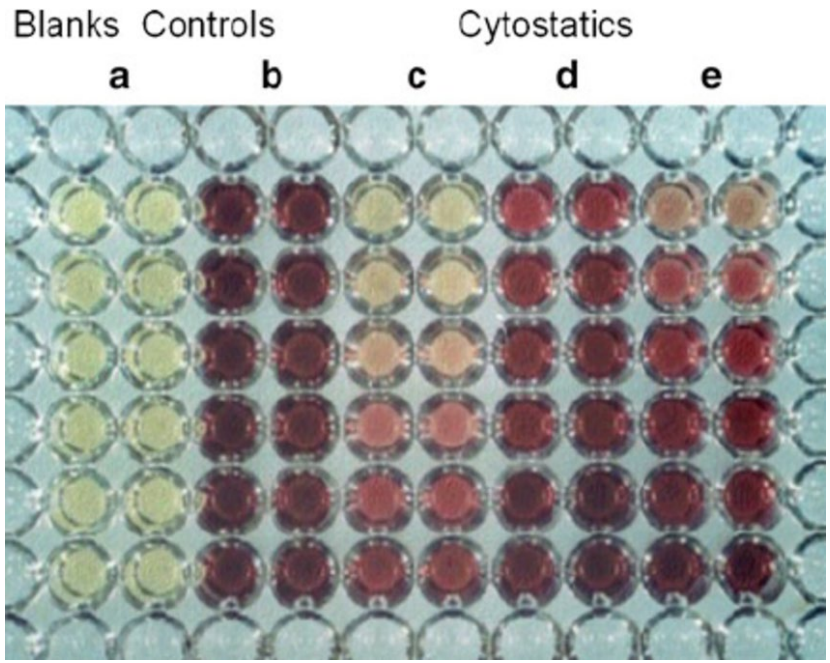
Limitations (PDX):

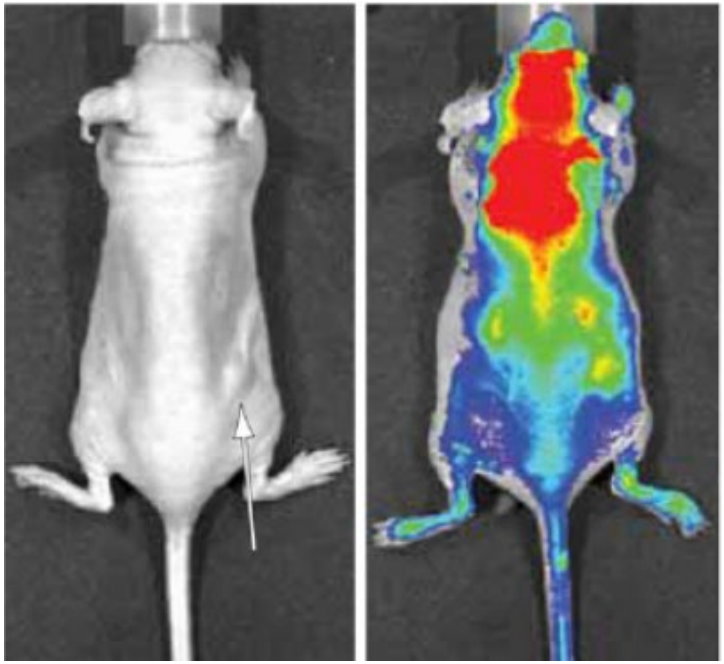
- Irradiation/immunocompromised mice needed to facilitate engraftment:
  - Complement activation/ADCC levels low
  - Immune modulatory agents hard to study
- Often biased to high risk tumors (which engraft)
- PK/PD and microenvironment mouse differs from humans

Principles of drug testing; of mice and man

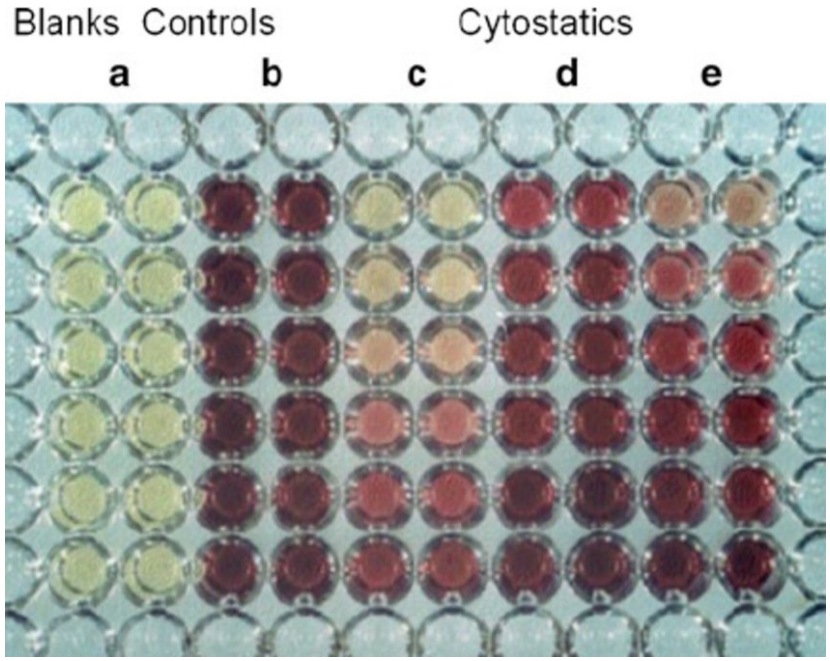
Assay systems to be discussed:

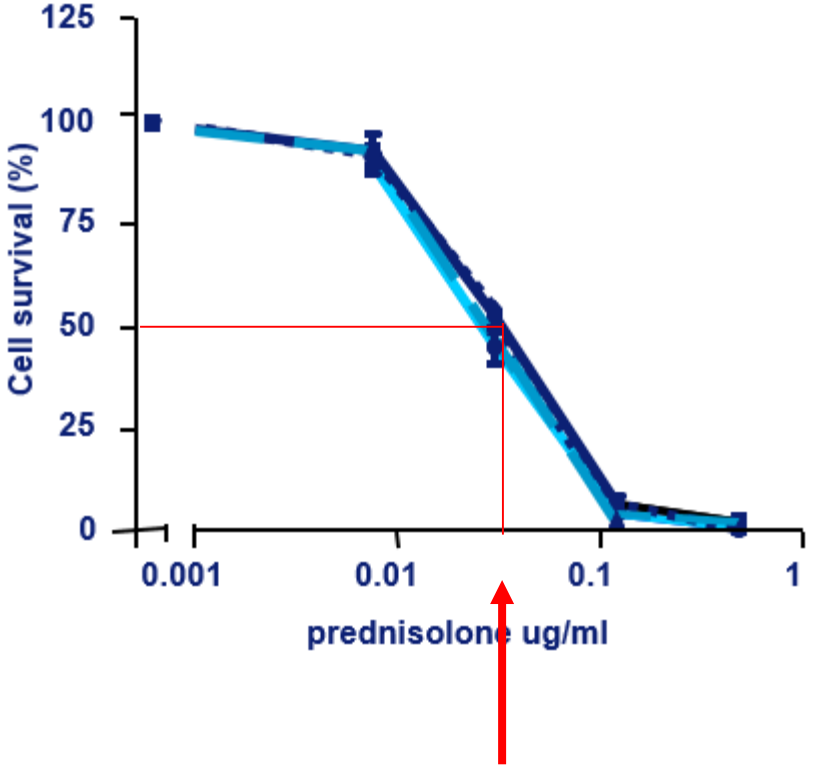
1. Short term culture assays
2. Mouse models





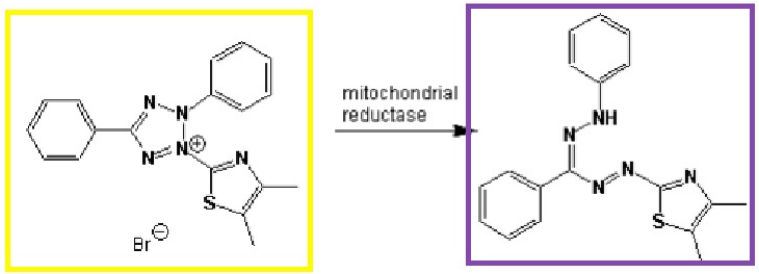
In vitro and ex vivo evaluation





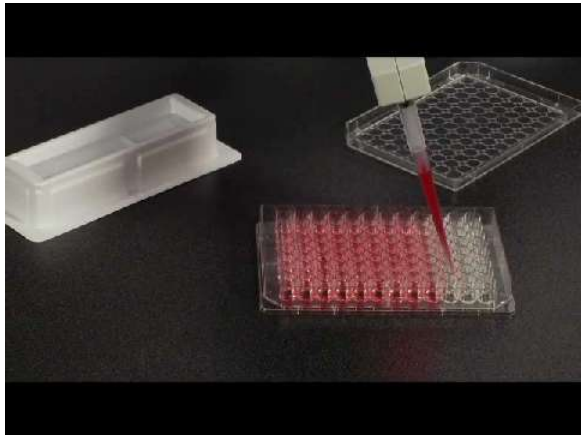
GI50/LC50 value

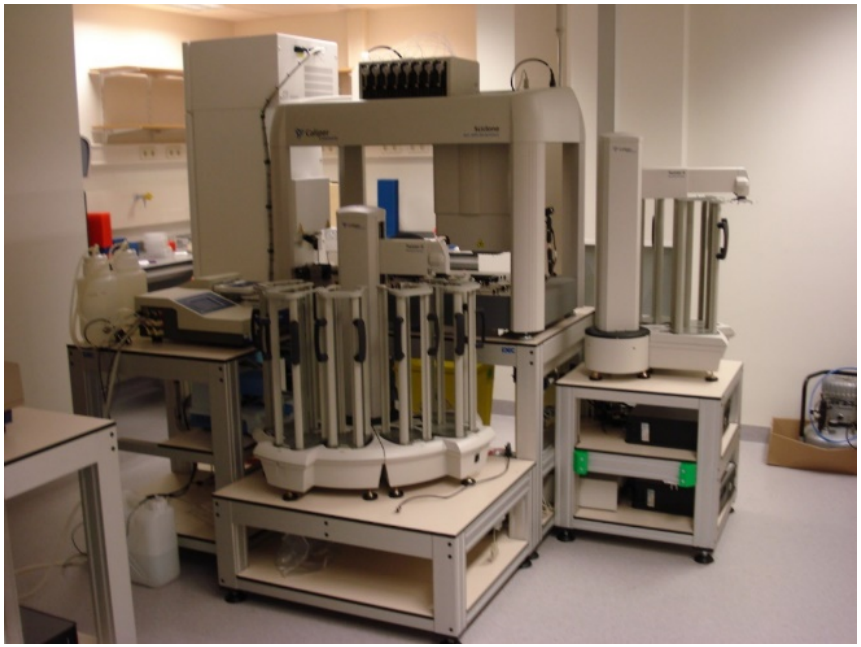
In mitochondria :



Viable cells only

preclinical drug evaluation: drug libraries





Input:

- cell lines
- primary patients' cells
- organoids

Dedicated library of new agents relevant to childhood cancer

Tested compounds in the ITCC cell line panel and primary cells (leukemia)

Compound	Target
(+)-JQ-1	BRD4(1); RD4(2)
ABT-199	BCL-2
ABT263	BCL-X_L; BCL-2; BCL-W
AMG 337	MET
ANA-12	TrkB
AT7519	CDK1; CDK2; CDK4; CDK6; CDK9
AZD6738	ATR
AZD8055	mTORC1; mTORC2
Binimetinib	MEK1; MEK2
C-DIM12	Nurr1
Cobimetinib	MEK1
Crizotinib	c-Met; ALK
CYC065	CDK9
GDC-0575	CHK1
GDC-0623	MEK1
GDC-0994	ERK1; ERK2

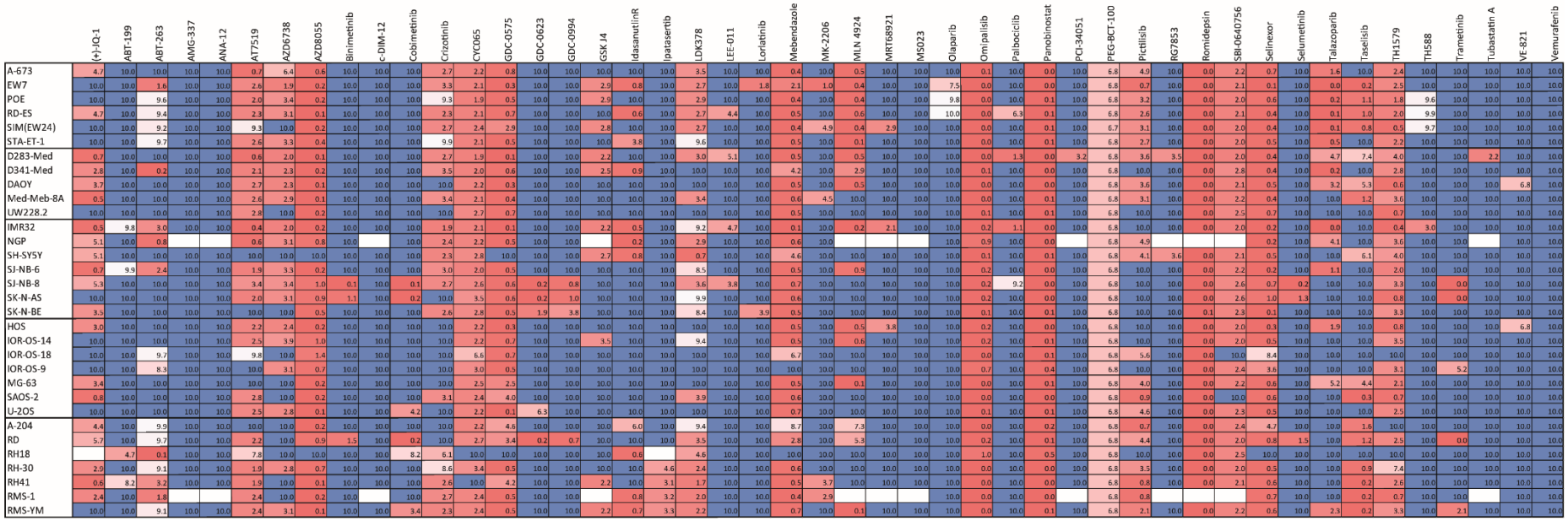
Compound	Target
GSK J4	JMJD3; UTX
Ipatasertib	AKT1; AKT2; AKT3
LDK378	ALK
LEE-011	CDK4; CDK6
Lorlatinib	ALK; ROS1
Mebendazole	
MK-2206	AKT1; AKT2; AKT3
MLN 4924	Nedd8-activating enzyme
MRT68921	ULK1; ULK2
MS023	PRMT1; PRMT3; PRMT4; PMRT6; PMRT8
Olaparib	PARP1; PARP2
Omipalisib	PI3Kα; PI3Kβ; PI3Kδ; PI3Kγ; mTORC; mTORC2
Palbociclib	CDK4; CDK6
Panobinostat	HDAC
PCI-34051	HDAC8
PEG-BCT-100	Arginine

Compound	Target
Pictilisib	PI3Kα; PI3Kδ
RG7388	p53-MDM2
RG7853	ALK
Romidepsin	HDAC1; HDAC2
SBI-0640756	eIF4G1
Selinexor	XPO1; CRM1
Selumetinib	MEK1; p-ERK1; p-ERK2
Talazoparib	PARP1; PARP2
Taselisib	PI3Kα-mutant
TH1579	MTH1
TH588	MTH1
Trametinib	MEK1; MEK2
Tubastatin A	HDAC6
VE-821	ATR
Vemurafenib	BRAFV600E

Courtesy of: Jan Molenaar's group

Prinses MÁXIMA centrum voor kinderoncologie

Successful testing of >140 small molecule drugs



blue: resistant
red: sensitive

Courtesy of: Jan Molenaar's group

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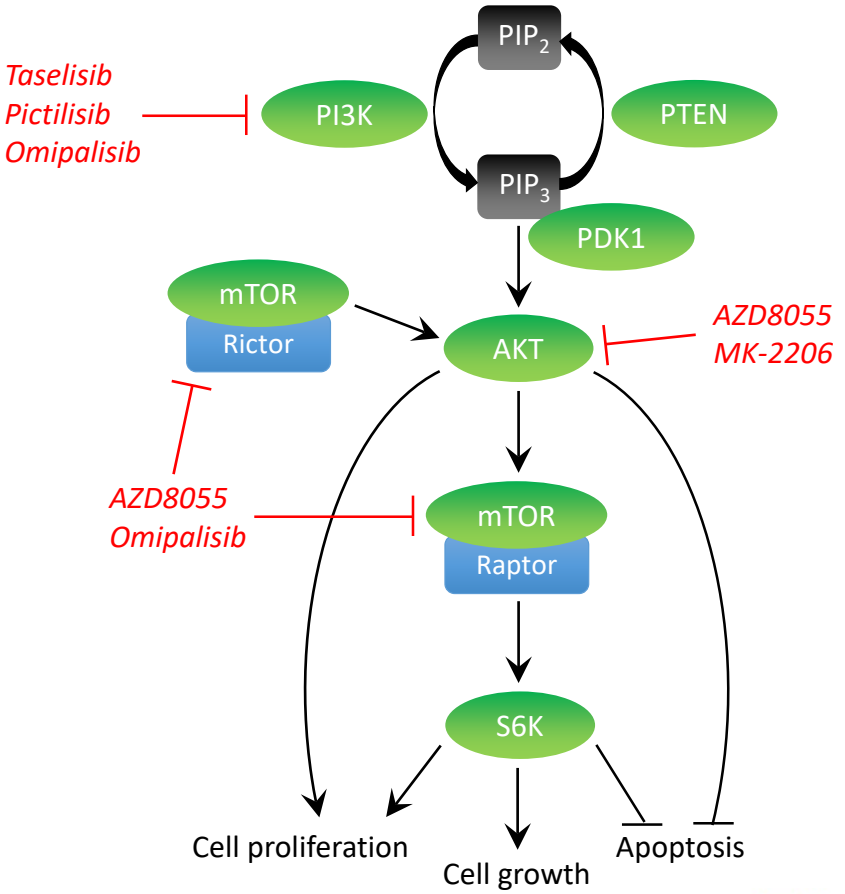
Differential efficacy of inhibitors of the PI3K/AKT/mTOR (survival) pathway

		Taselisib (PI3K α-mutant)	Pictilisib (PI3K)	AZD8055 (mTOR)	Omipalisib (PI3K/mTOR)	Ipatasertib (AKT)	MK-2206 (AKT)
Ewing sarcoma	A-673	10.00	4.88	0.60	0.14	10.00	10.00
	EW7	0.25	0.70	0.17	0.01	10.00	1.05
	POE	1.15	3.23	0.16	0.03	10.00	10.00
	RD-ES	0.95	2.64	0.12	0.02	10.00	10.00
	SIM(EW24)	0.83	3.09	0.15	0.03	10.00	4.92
	STA-ET-1	10.00	2.71	0.44	0.03	10.00	10.00
Medullo-blastoma	D283-Med	7.38	3.57	0.11	0.04	10.00	10.00
	D341-Med	10.00	10.00	0.16	0.13	10.00	10.00
	DAOY	5.31	3.64	0.14	0.03	10.00	10.00
	Med-Meb-8A	1.21	3.12	0.12	0.03	10.00	4.50
	UW228.2	10.00	10.00	0.15	0.14	10.00	10.00
Neuroblastoma	IMR32	10.00	10.00	0.18	0.16	10.00	10.00
	NGP	10.00	4.91	0.84	0.85	10.00	10.00
	SH-SY5Y	6.07	4.10	10.00	0.05	10.00	10.00
	SJ-NB-6	10.00	10.00	0.19	0.17	10.00	10.00
	SJ-NB-8	10.00	10.00	1.00	0.21	10.00	10.00
	SK-N-AS	10.00	10.00	0.89	0.21	10.00	10.00
	SK-N-BE	10.00	10.00	0.51	0.14	10.00	10.00
Osteosarcoma	HOS	10.00	10.00	0.18	0.17	10.00	10.00
	IOR-OS-14	10.00	10.00	1.02	0.19	10.00	10.00
	IOR-OS-18	10.00	5.56	1.37	0.04	10.00	10.00
	IOR-OS-9	10.00	10.00	0.68	0.66	10.00	10.00
	MG-63	4.45	4.03	0.15	0.03	10.00	10.00
	SAOS-2	0.32	0.85	0.19	0.03	10.00	10.00
	U-2OS	10.00	4.56	0.14	0.13	10.00	10.00
Rhabdomyosarcoma	A-204	1.62	0.75	0.20	0.03	10.00	10.00
	RD	1.16	4.38	0.87	0.18	10.00	10.00
	RH18	10.00	10.00	10.00	1.04	10.00	10.00
	RH-30	0.89	3.51	0.68	0.04	4.60	10.00
	RH41	0.15	0.82	0.14	0.01	3.06	3.69
	RMS-1	0.19	0.79	0.15	0.02	3.16	2.86
	RMS-YM	0.16	2.10	0.12	0.02	3.32	10.00
Acute lymphoid leukemia	697	10.00	3.72	0.14	0.03	10.00	10.00
	Call2	0.57	2.65	0.65	0.02	10.00	3.43
	Call4	0.36	1.78	0.56	0.02	10.00	2.45
	Kasumi2	0.12	0.57	0.13	0.02	10.00	0.61
	RCH-ACV	0.03	0.16	0.12	0.00	3.67	0.52
	SupB15	10.00	10.00	0.12	0.10	10.00	10.00



Courtesy of: Jan Molenaar's group

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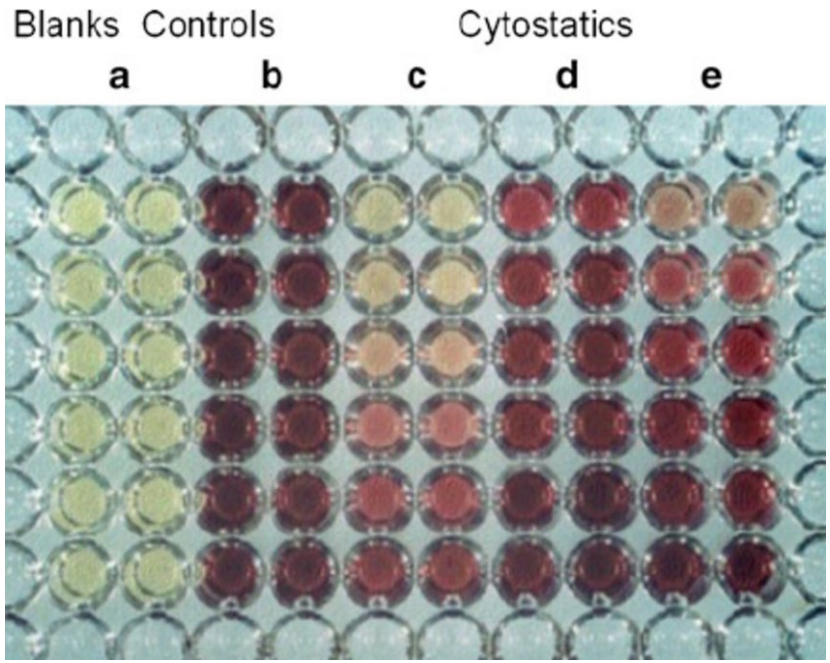
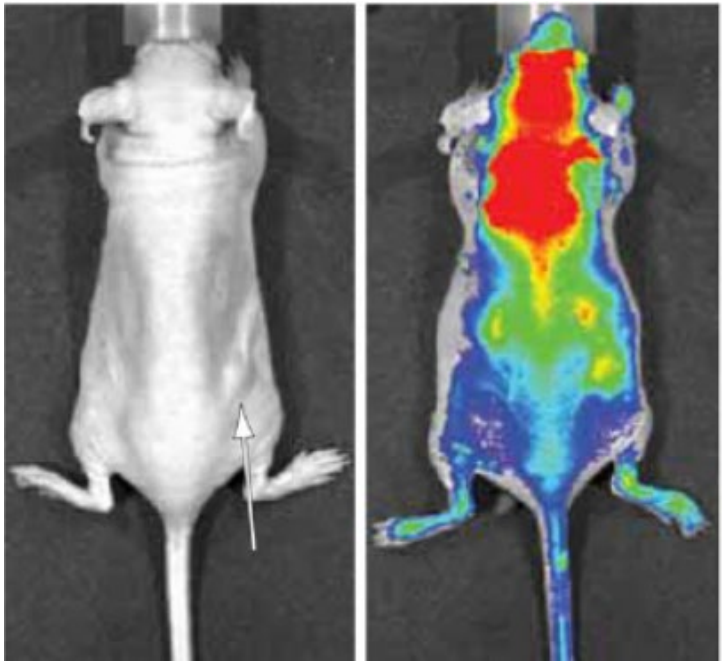
Of mice and man: preclinical evaluation of drugs

Assay systems to be discussed:

1. Short term culture assays
2. Mouse models





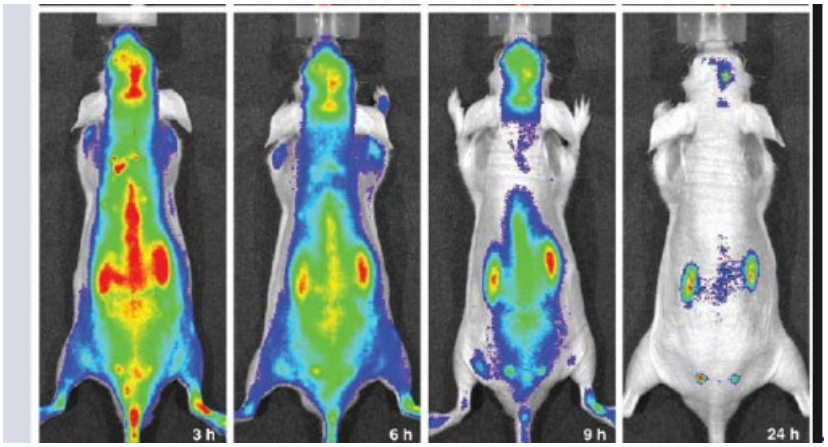
In vivo drug monitoring

Luciferase positive cells

Xenograft in Immunodeficient Mouse

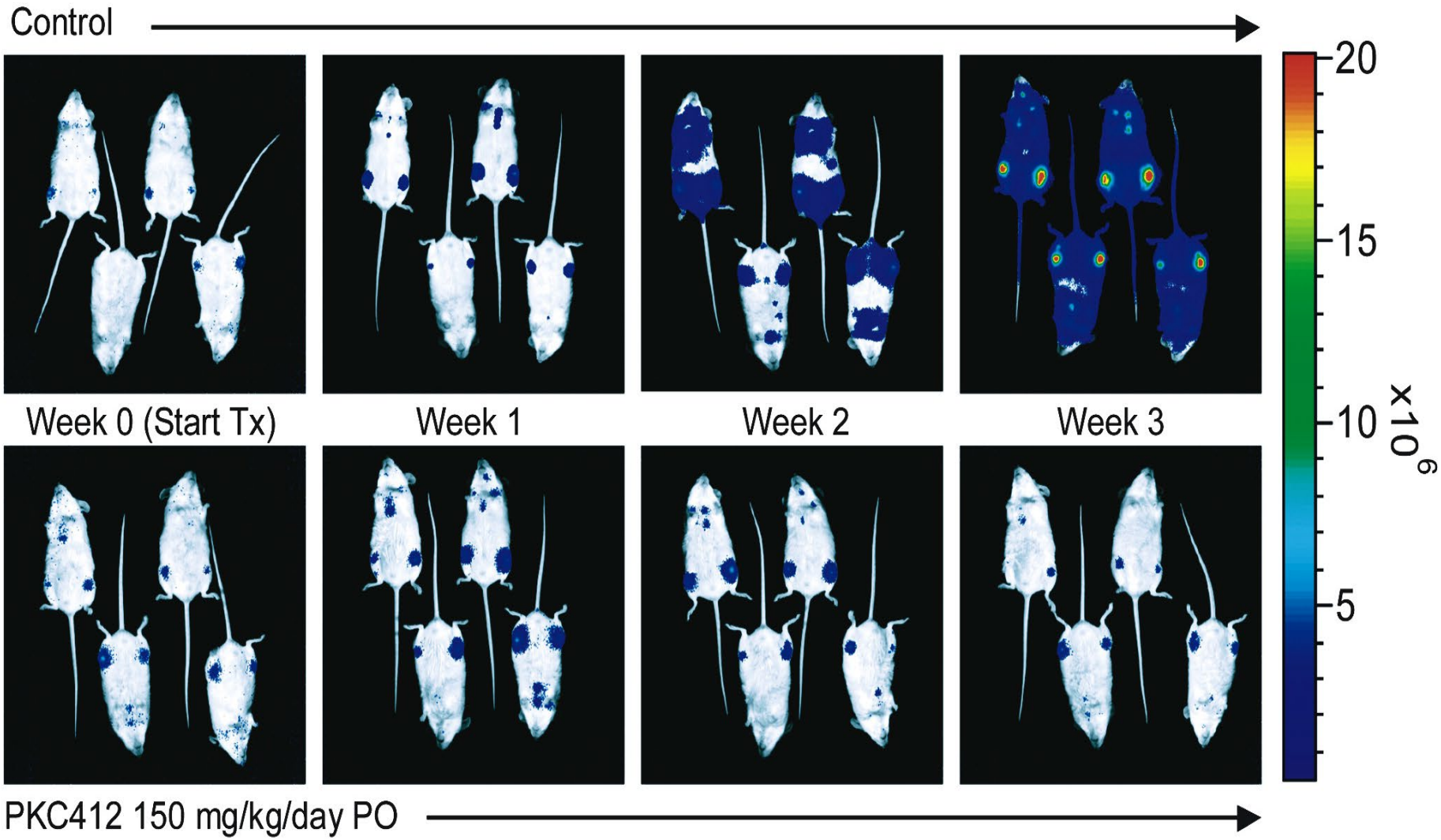
Drug or placebo

tumor reduction



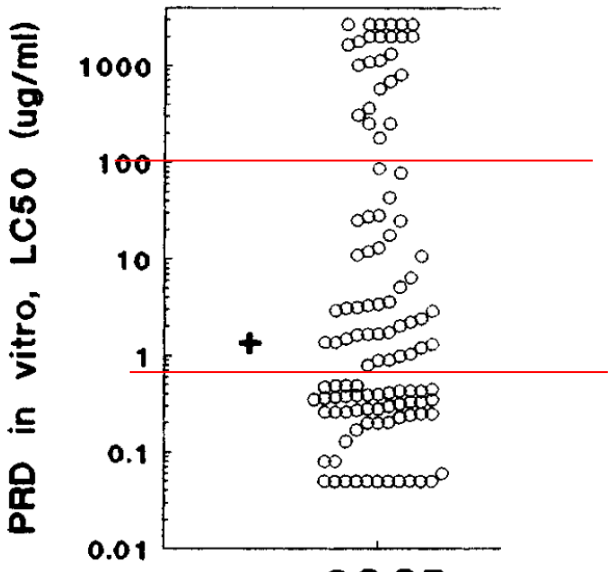
IVIS system

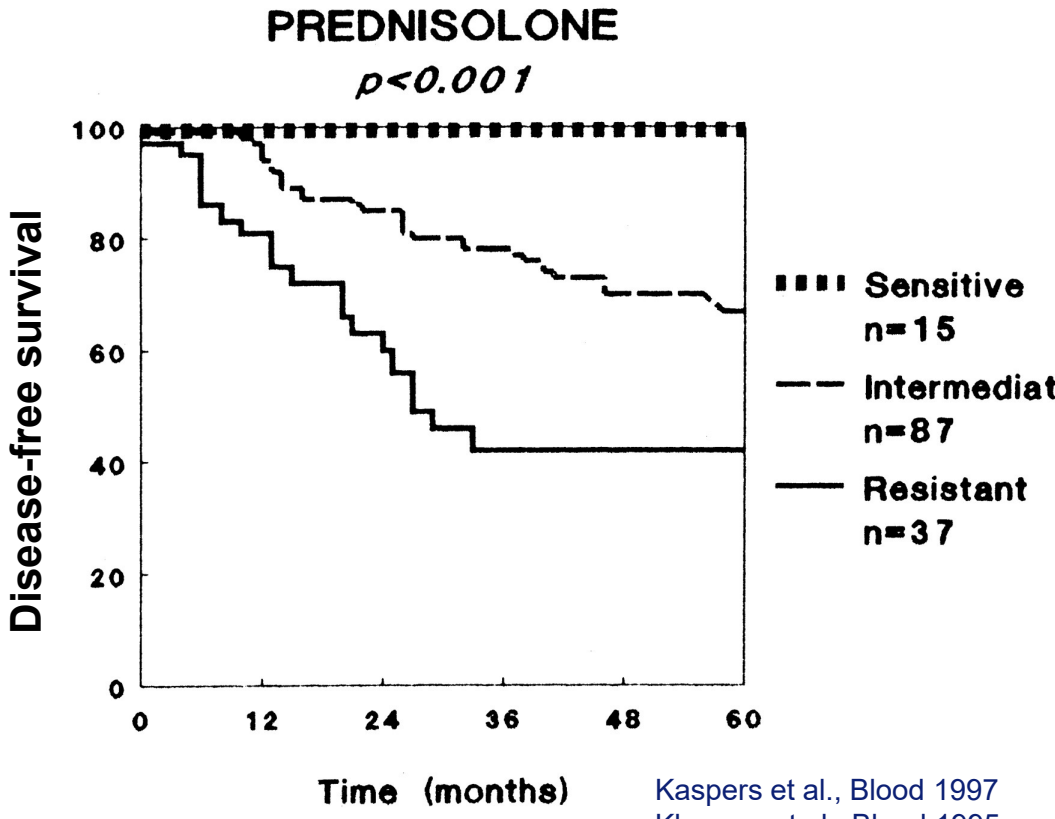
Inhibition outgrowth of MLL^{pos} leukemia



Armstrong et al, Cancer Cell 2003

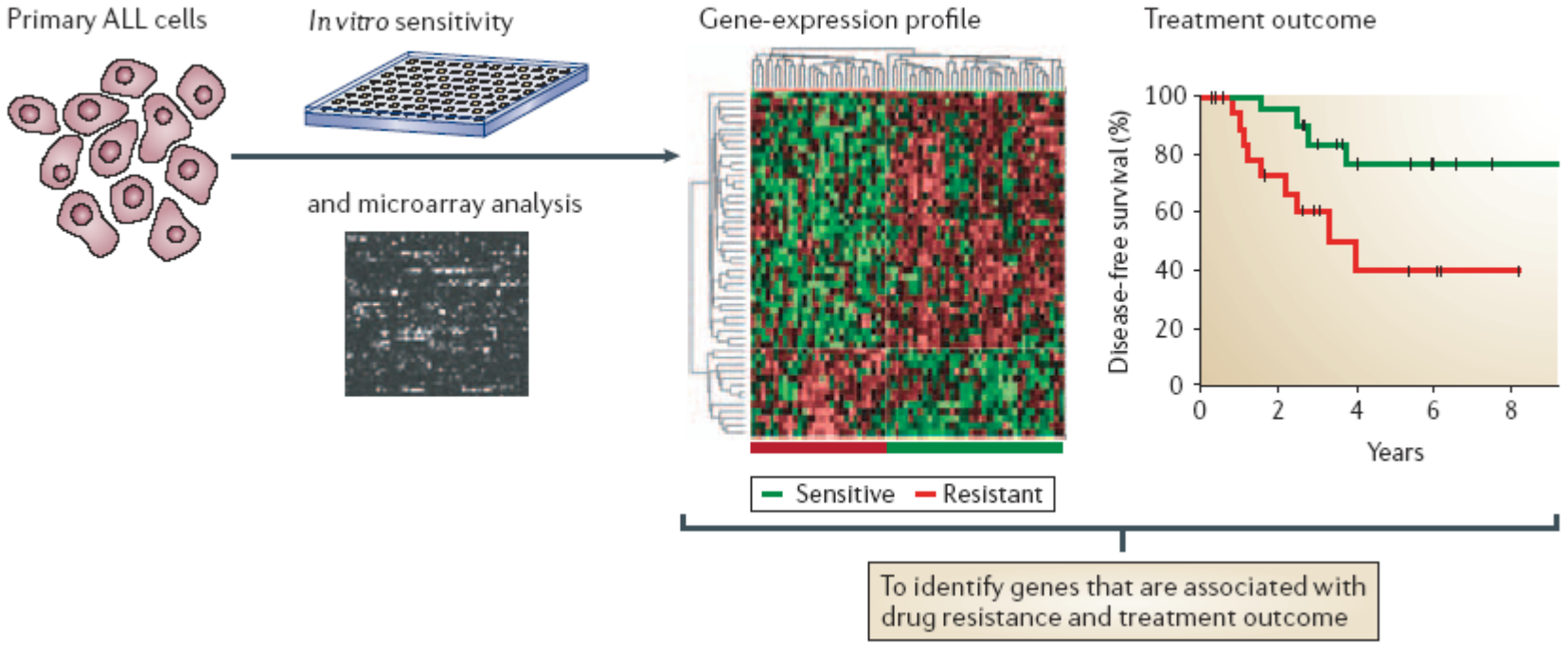
Ex vivo drug cytotoxicity data and target discovery



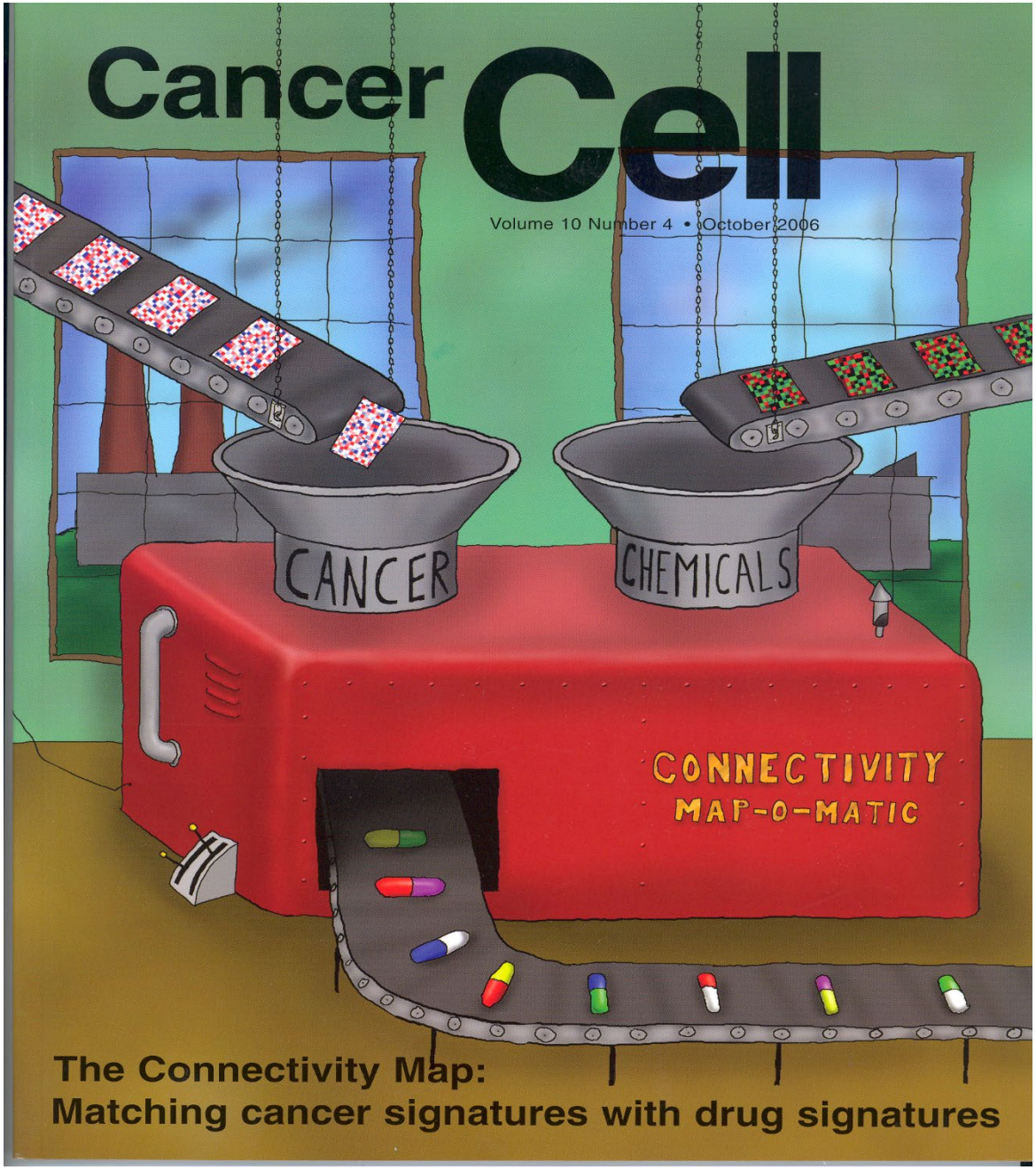


Kaspers et al., Blood 1997
Klumper et al., Blood 1995

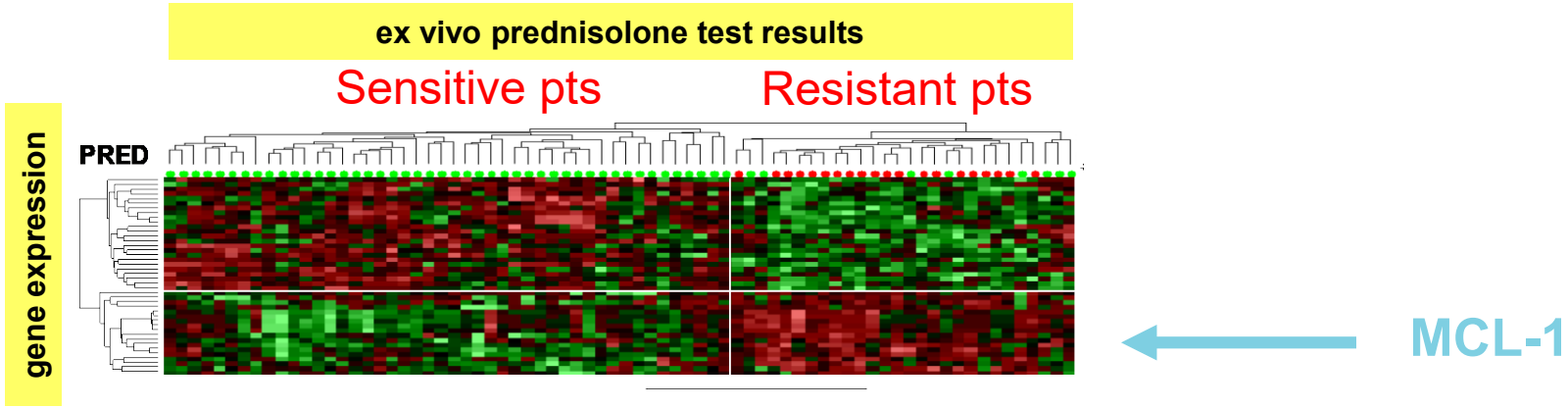
Ex vivo drug cytotoxicity data used for target discovery



Cheok & Evans, Nat Rev Cancer 2006


Cancer Cell
Volume 10 Number 4 • October 2006
CANCER
CHEMICALS
CONNECTIVITY
MAP-O-MATIC
The Connectivity Map:
Matching cancer signatures with drug signatures

Prednisone resistance and Myeloid Cell Leukemia 1 (*MCL-1*) in B-lineage ALL



Holleman *et al*., New Engl J Med 2004

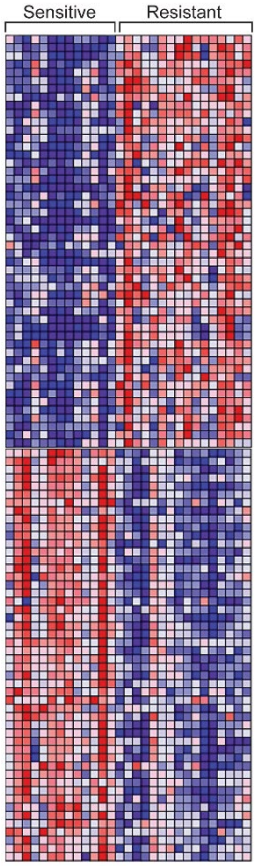
MCL-1:

- **Anti-apoptotic member of the Bcl-2 family**
- **Elevated expression induces resistance to drugs**
- **Role in mitochondrial function**

Targeting MCL1 and glucocorticoid resistance in ALL

Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance

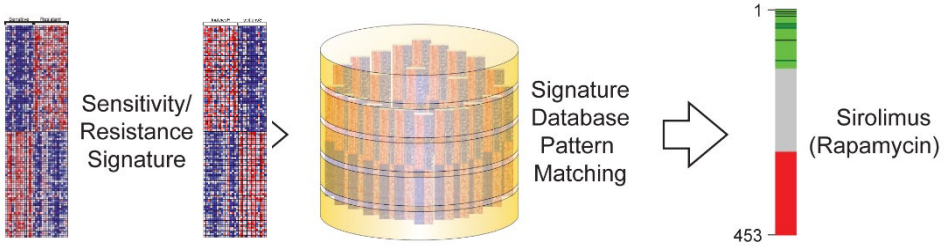
Guo Wei,[1,3] David Twomey,[2,3] Justin Lamb,[3] Krysta Schlis,[1,2] Jyoti Agarwal,[2] Ronald W. Stam,[4] Joseph T. Opferman,[5] Stephen E. Sallan,[1,2] Monique L. den Boer,[4] Rob Pieters,[4] Todd R. Golub,[1,2,3,6] and Scott A. Armstrong[1,2,*]



Connectivity mapping

164 compounds

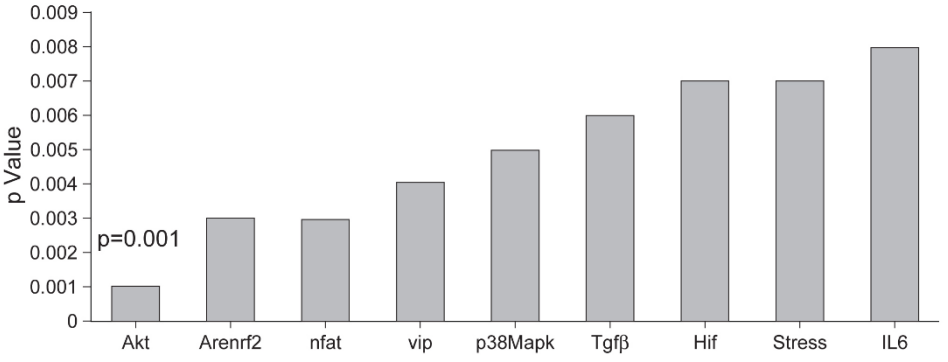
library screen



→ **Rapamycin**

Gene set enrichment analysis

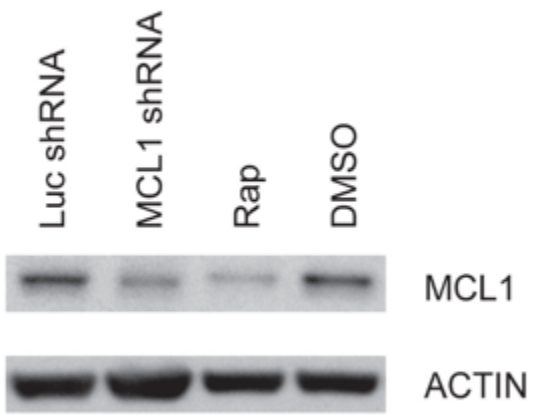
163 pathways

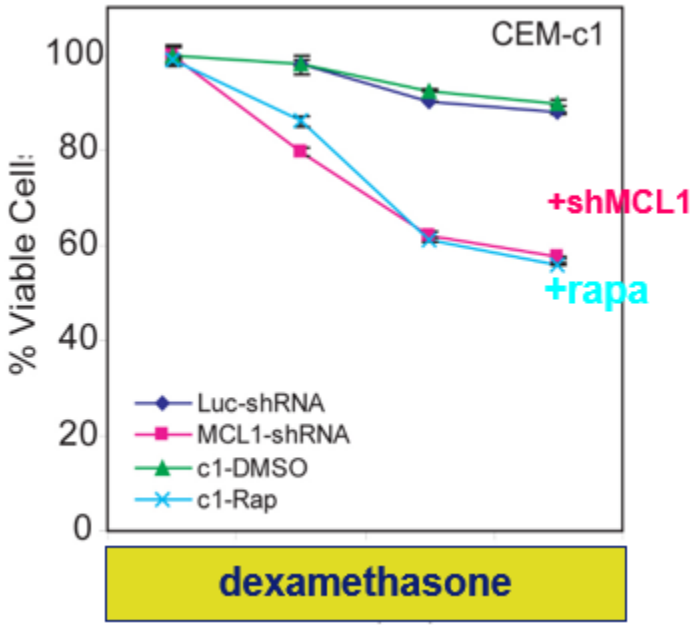


→ **Akt/mTOR-pathway most affected**

Wei *et al.*, Cancer Cell 2006

Silencing MCL-1 sensitizes to dexamethasone

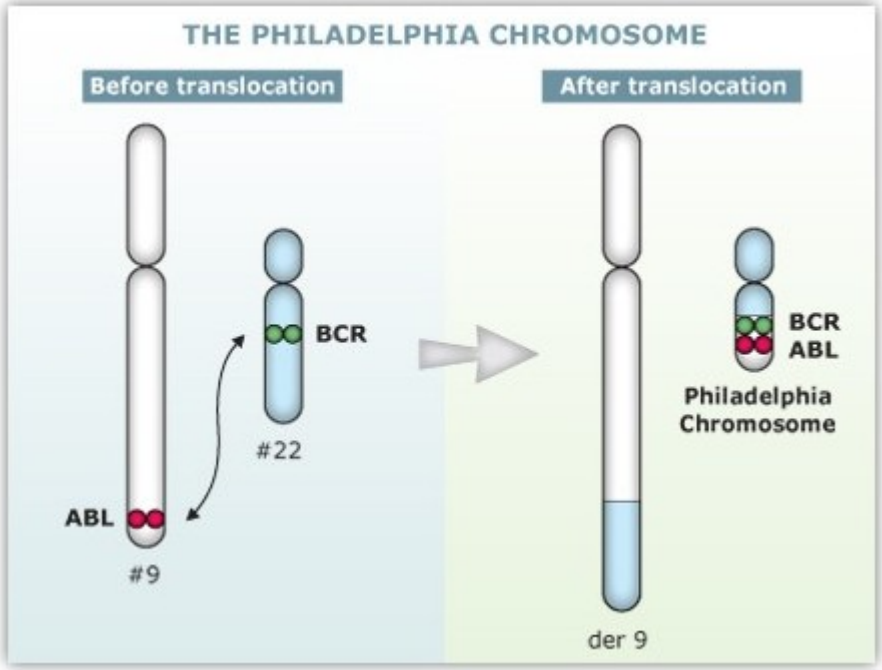


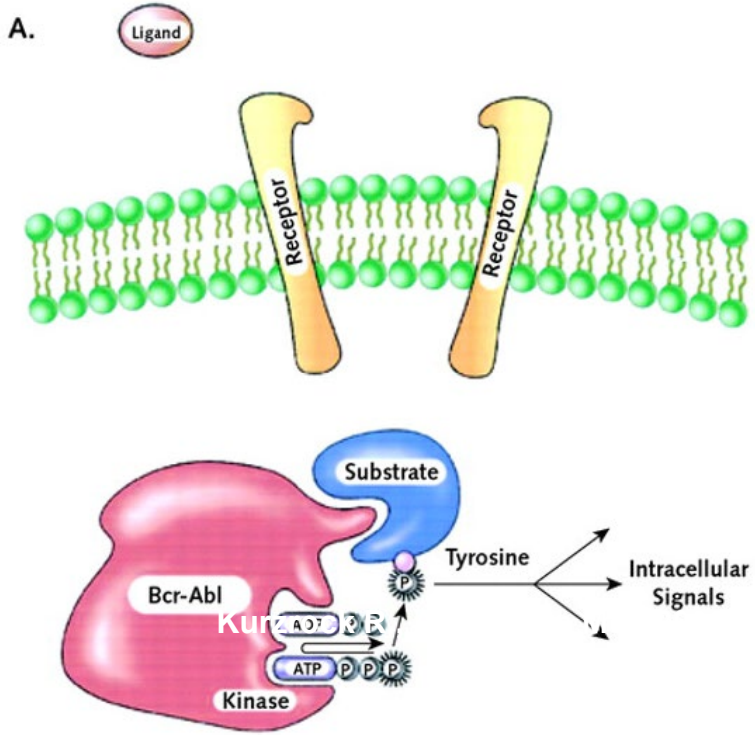


➔ Akt/mTOR inhibition a way to sensitize to glucocorticoids in ALL

Wei *et al*., Cancer Cell 2006

Target discovery: BCRABL1 - Philadelphia Chromosome t(9;22)

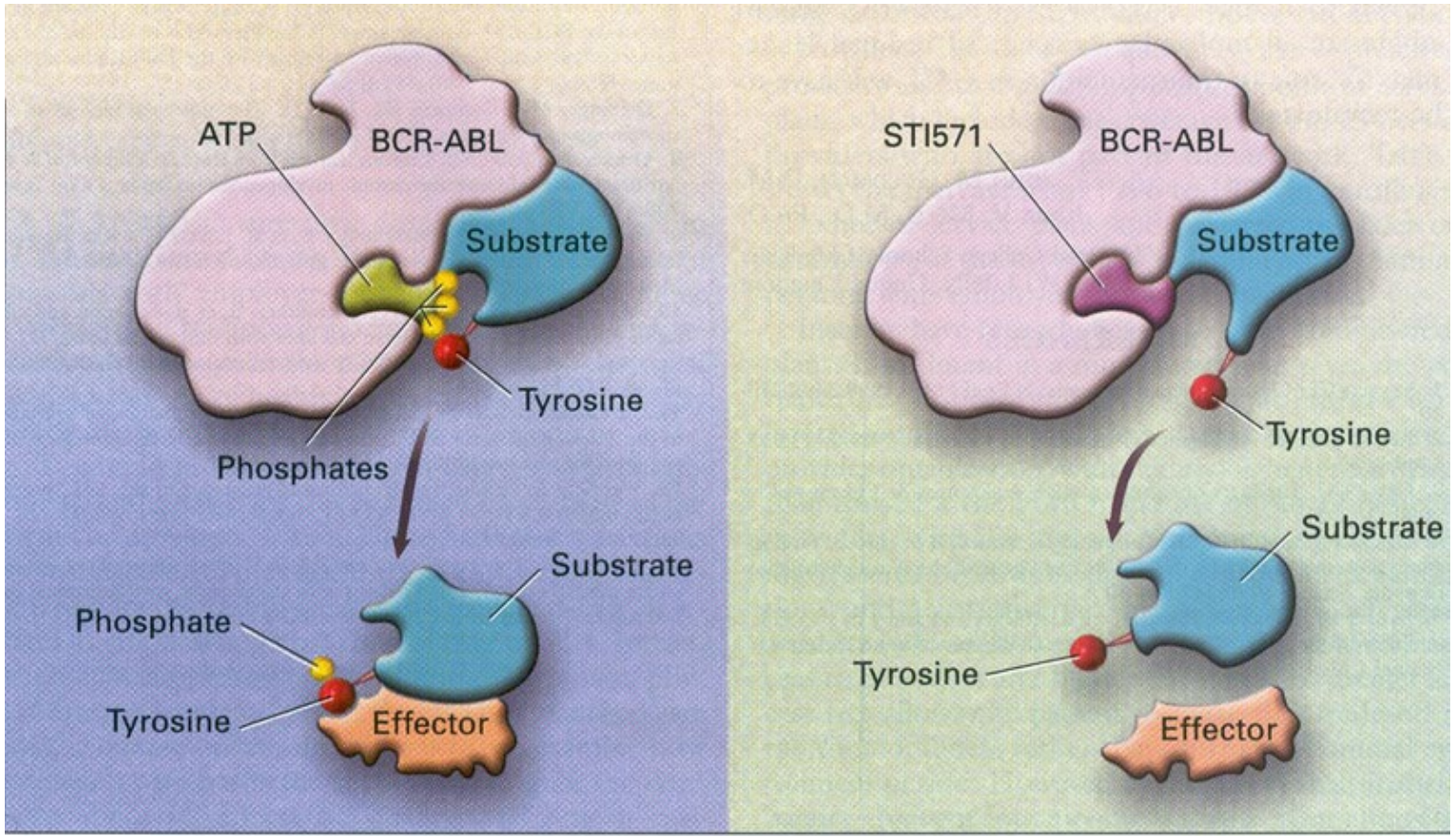




CML and ALL

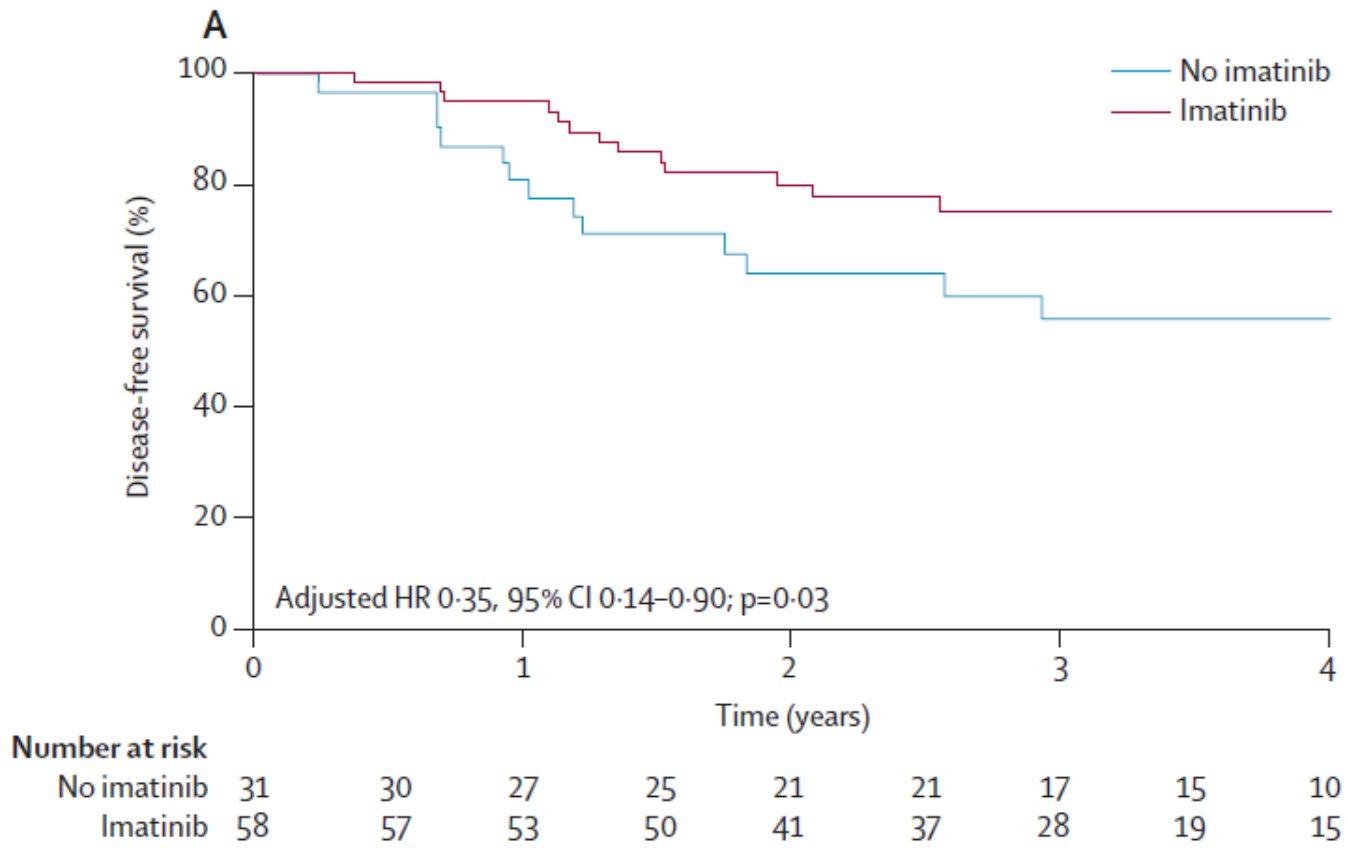
Targeting BCRABL1

Imatinib (STI571), dasatinib et al.



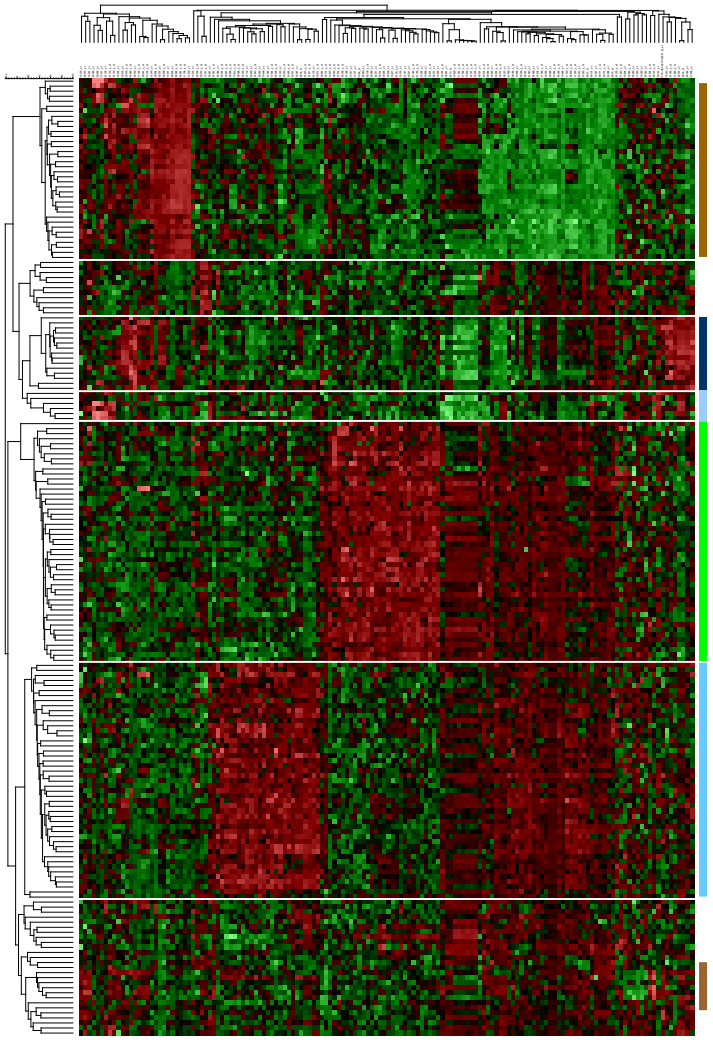
Goldman JM, Melo JV. *N Engl J Med*. 344:1084-1086.

Improved outcome with Imatinib



Biondi et al, Lancet Oncol 2012

Identification of BCR-ABL1-like subgroup by gene expression profiling



T-ALL

E2A-rearranged

MLL-rearranged

TEL-AML1

Hyperdiploid

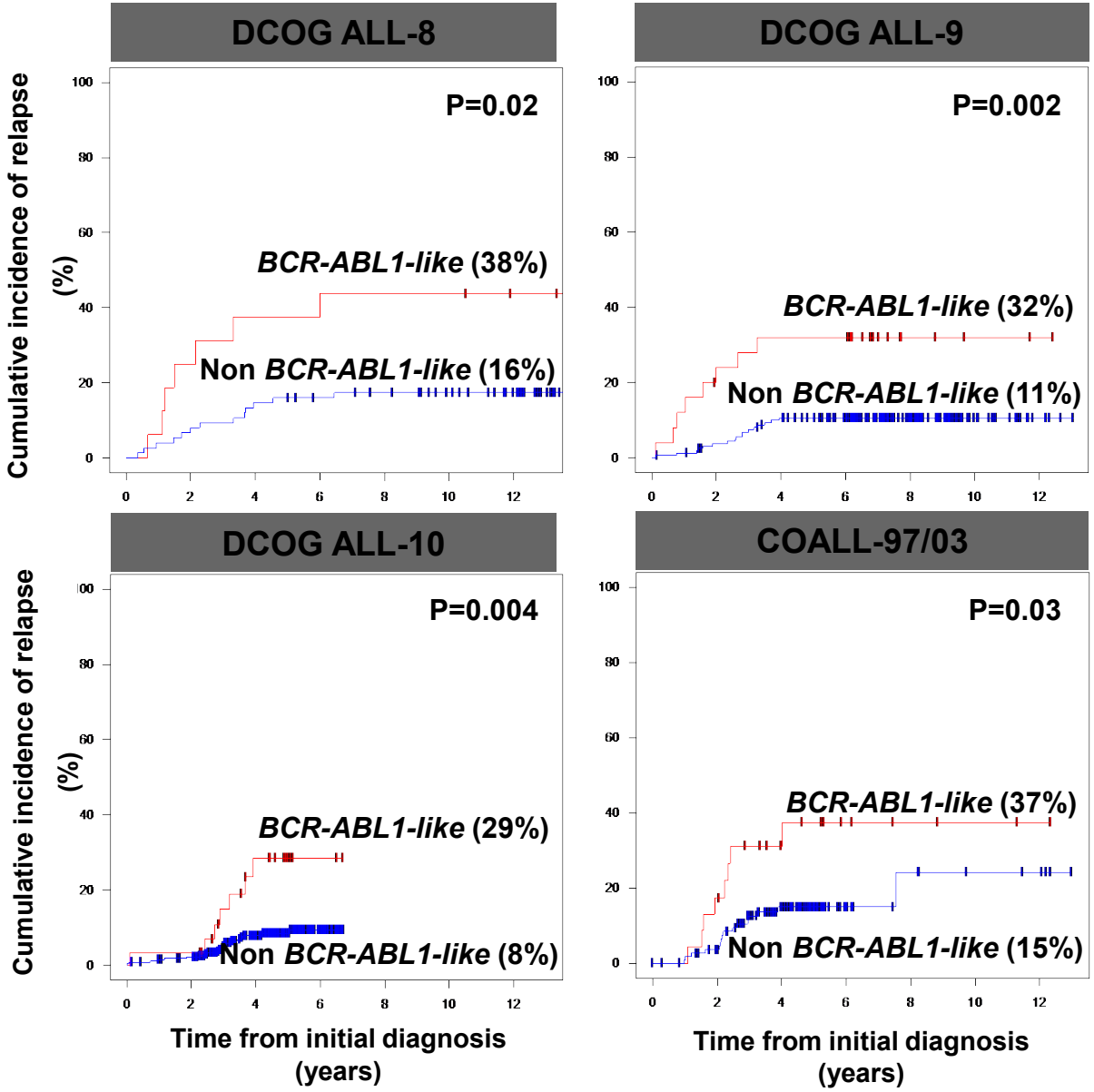
BCR-ABL1

5 real BCR-ABL1

30 BCR-ABL1 *like*

Den Boer et al. Lancet Oncology 2009

Validation of *BCR-ABL1*-like ALL: 4 independent cohorts



BA-like in children:

- 15% of BCP-ALL
- 50% of B-other group
- 70% MRD-medium risk
- independent prognostic factor

Van der Veer et al, Blood 2013

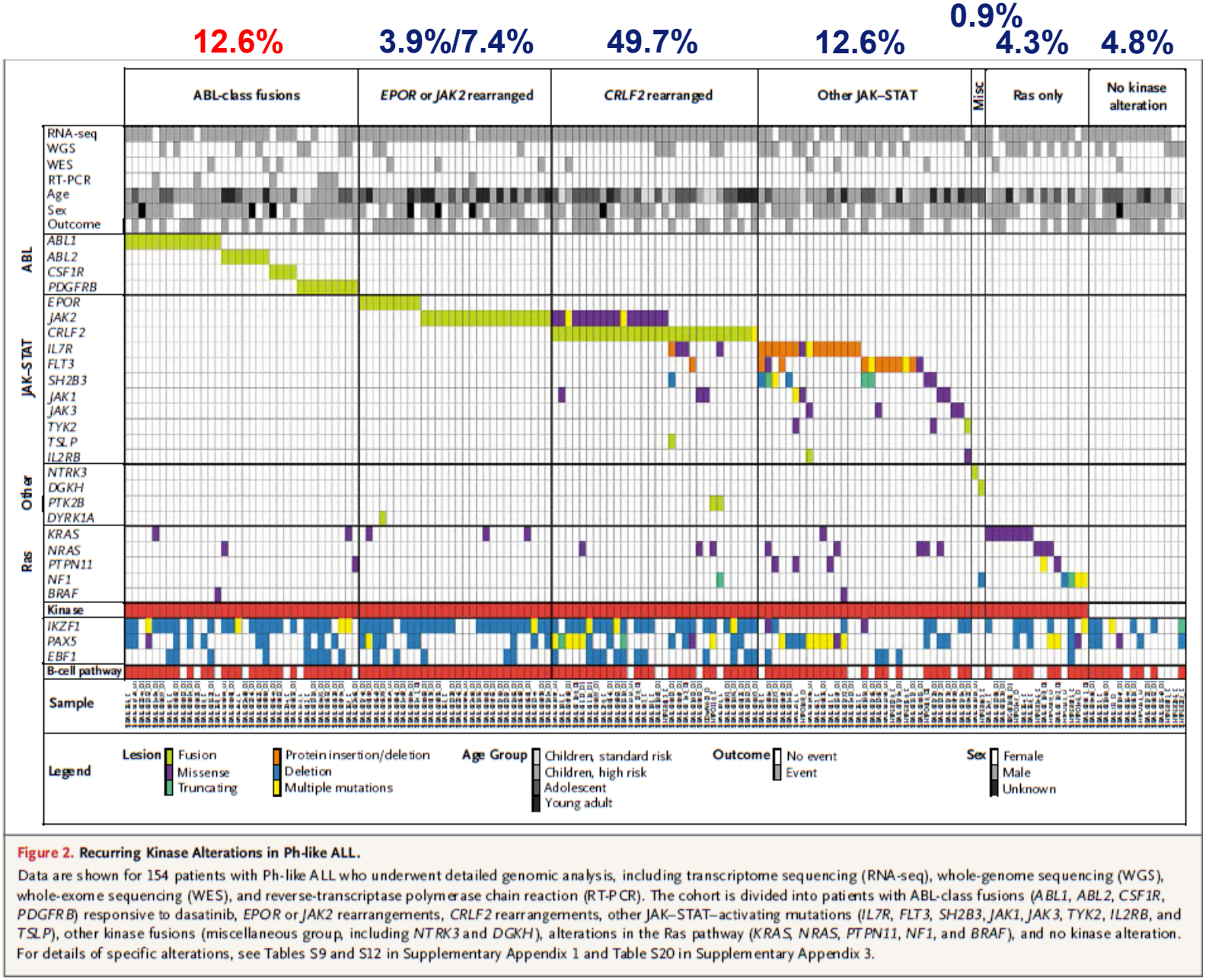


Figure 2. Recurring Kinase Alterations in Ph-like ALL.

Data are shown for 154 patients with Ph-like ALL who underwent detailed genomic analysis, including transcriptome sequencing (RNA-seq), whole-genome sequencing (WGS), whole-exome sequencing (WES), and reverse-transcriptase polymerase chain reaction (RT-PCR). The cohort is divided into patients with ABL-class fusions (*ABL1*, *ABL2*, *CSF1R*, *PDGFRB*) responsive to dasatinib, *EPOR* or *JAK2* rearrangements, *CRLF2* rearrangements, other JAK–STAT–activating mutations (*IL7R*, *FLT3*, *SH2B3*, *JAK1*, *JAK3*, *TYK2*, *IL2RB*, and *TSLP*), other kinase fusions (miscellaneous group, including *NTRK3* and *DGKH*), alterations in the Ras pathway (*KRAS*, *NRAS*, *PTPN11*, *NF1*, and *BRAF*), and no kinase alteration. For details of specific alterations, see Tables S9 and S12 in Supplementary Appendix 1 and Table S20 in Supplementary Appendix 3.

Roberts et al, New Engl J Med 2014

RNAseq/WGS of *BCR-ABL1*-like ALL

Table 1. Kinase Fusions Identified in Ph-like Acute Lymphoblastic Leukemia.

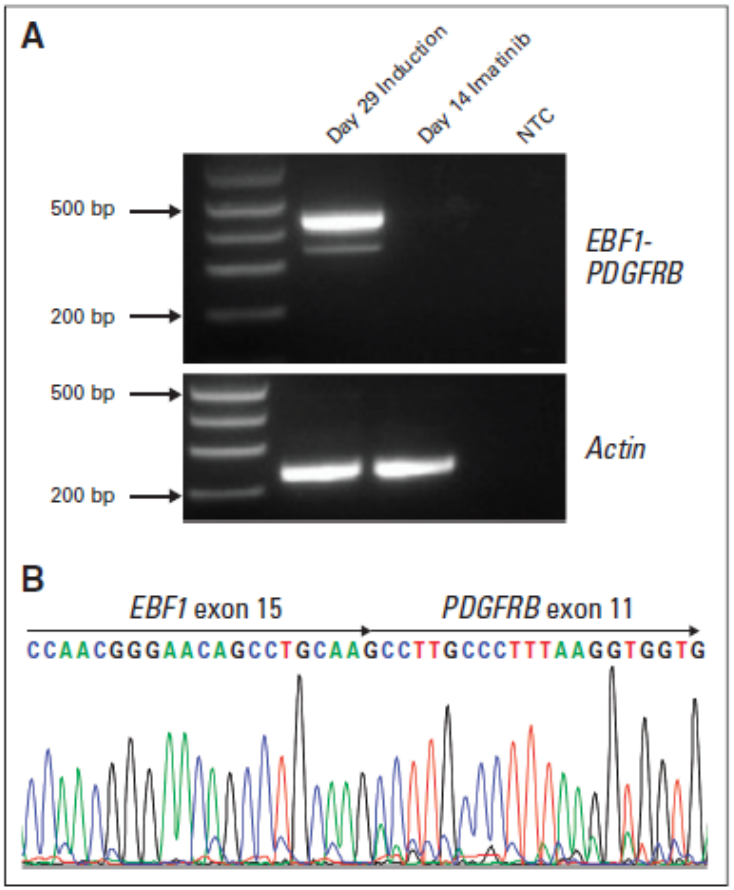
Kinase Gene	Tyrosine Kinase Inhibitor	Fusion Partners	Patients	5′ Genes
		number		
ABL1	Dasatinib	6	14	*ETV6*,[11] *NUP214*,[11] *RCSD1*,[11] *RANBP2*,[11] *SNX2*,[19] *ZMIZ1*[20]
ABL2	Dasatinib	3	7	*PAG1*,* *RCSD1*,* *ZC3HAV1**
CSF1R	Dasatinib	1	4	*SSBP2**
PDGFRB	Dasatinib	4	11	*EBF1*,[11-13] *SSBP2*,* *TNIP1*,* *ZEB2**
CRLF2	JAK2 inhibitor	2	30	*IGH*,[21] *P2RY8*[22]
JAK2	JAK2 inhibitor	10	19	*ATF7IP*,* *BCR*,[11] *EBF1*,* *ETV6*,[23] *PAX5*,[11] *PPFIBP1*,* *SSBP2*,[24] *STRN3*,[11] *TERF2*,* *TPR**
EPOR	JAK2 inhibitor	2	9	*IGH*,[11] *IGK**
DGKH	Unknown	1	1	*ZFAND3**
IL2RB	JAK1 inhibitor, JAK3 inhibitor, or both	1	1	*MYH9**
NTRK3	Crizotinib	1	1	*ETV6*[25-27]†
PTK2B	FAK inhibitor	2	1	*KDM6A*,* *STAG2**
TSLP	JAK2 inhibitor	1	1	*IQGAP2**
TYK2	TYK2 inhibitor	1	1	*MYB**

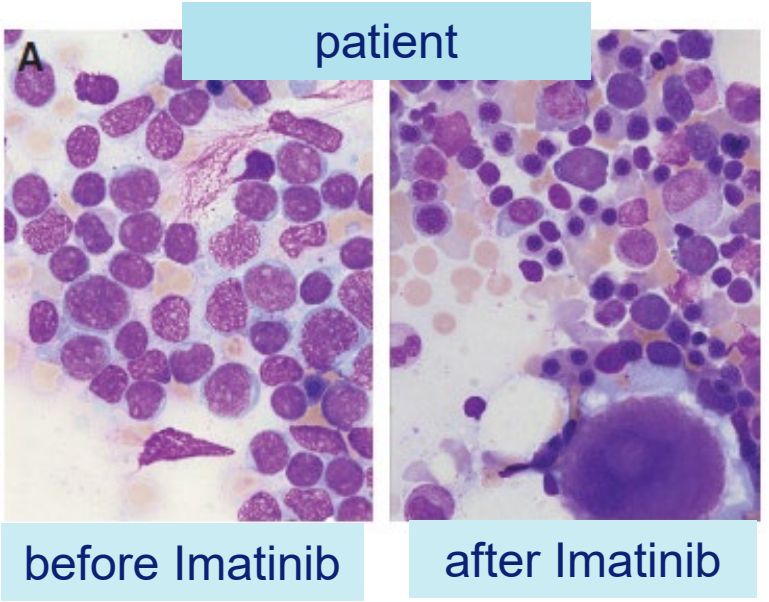
ABL-class fusion genes

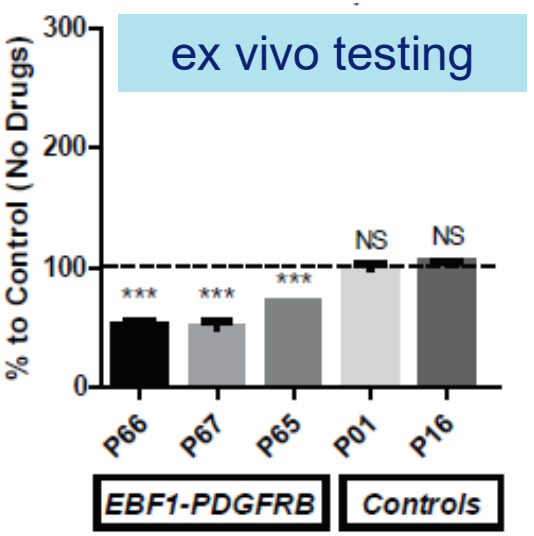
13 kinase/cytokine receptor genes, ~30 fusion partners
+ more emerging (IGH-DUX4, TCF3-ZNF384-fusions)

Roberts et al, N Engl J Med 2014; Yasuda et al, Nat Gen 2016; Lilljebjorn et al, Nat Comm 2016

Imatinib induces CR in a child with refractory *EBF1-PDGFRB*$^{+ve}$ ALL



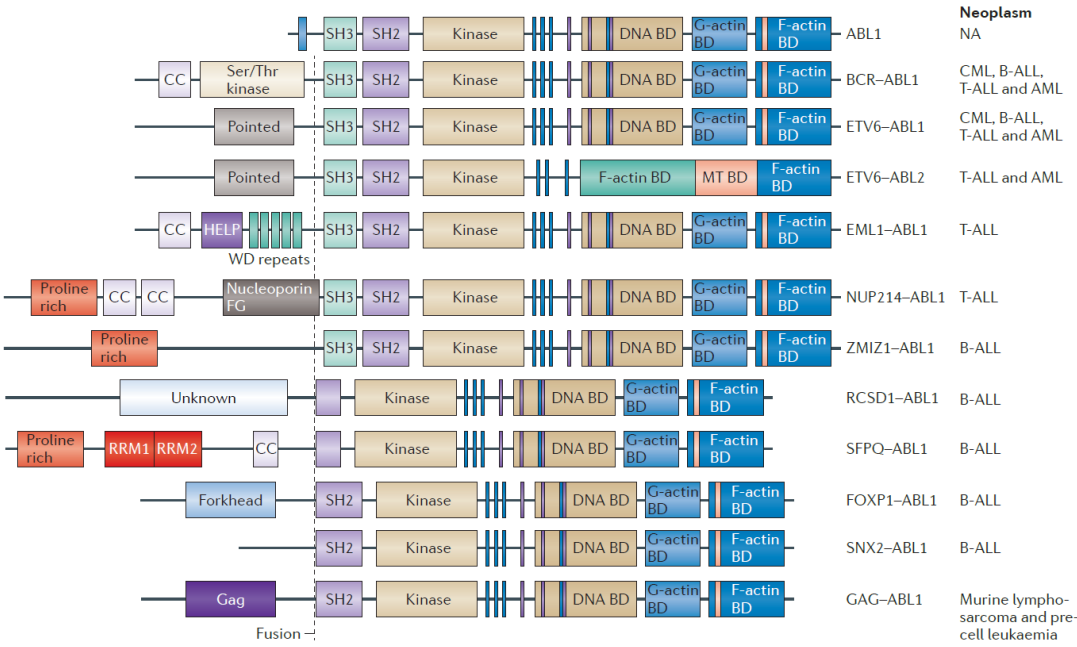




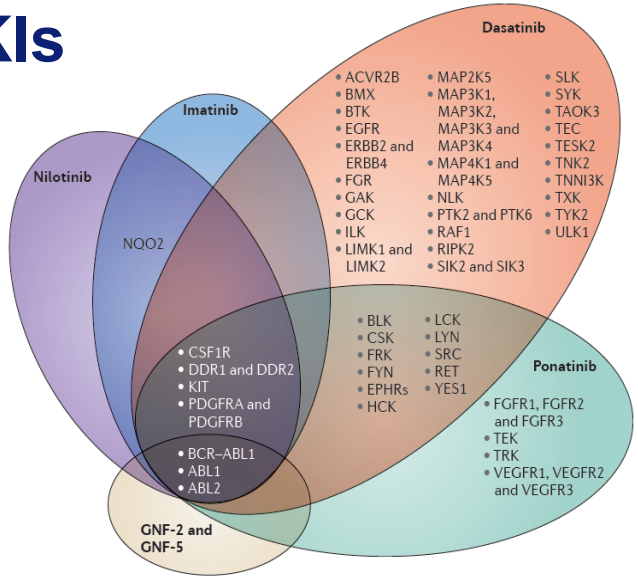
Weston et al, J Clin Oncol 2013; Lengline et al, Haematologica 2013
Roberts et al, NEJM 2014; Boer et al, Oncotarget 2017

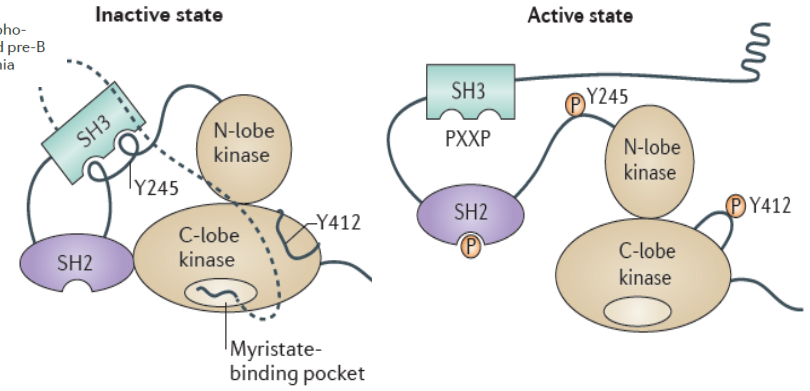
Caveat 1:

Different breakpoints in ABL genes, not all may be equally sensitive to TKIs



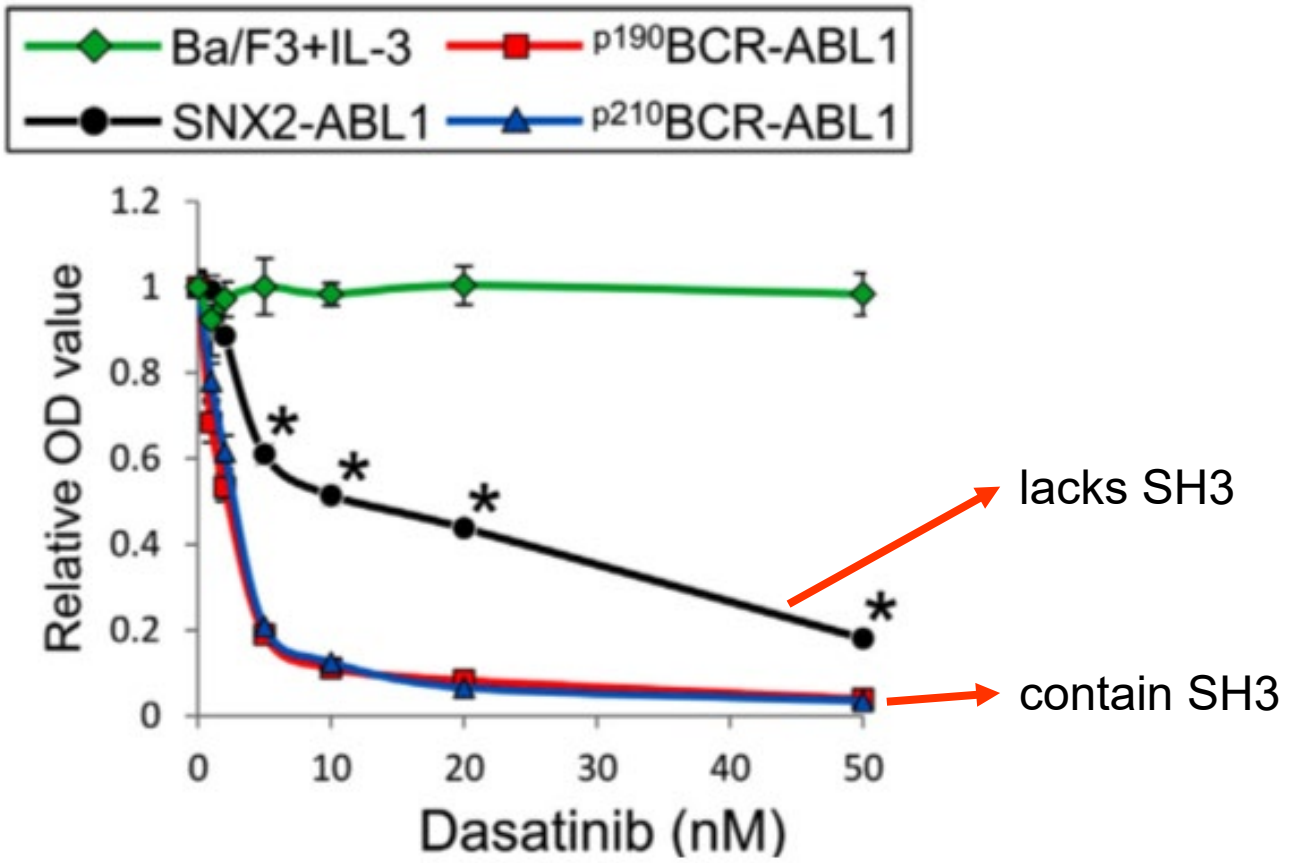
some lack SH3 domain





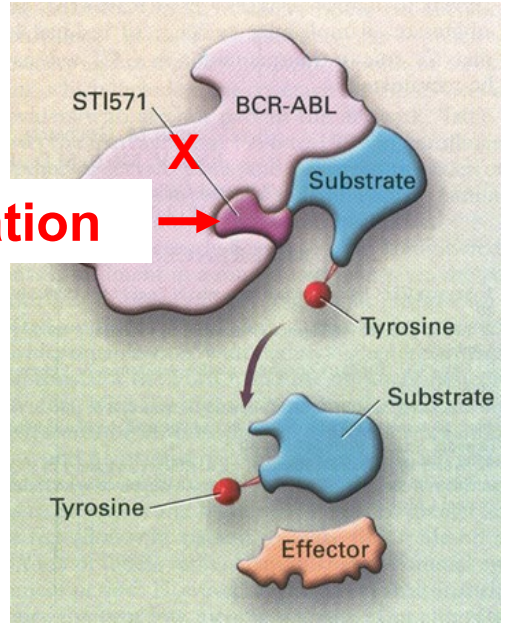
Greuber et al. Nat Rev Cancer 2013

ABL fusion genes not equally sensitive to TKIs (dasatinib)



Tomita et al, Leukemia Research 2014

Caveat 2: inactivating mutations

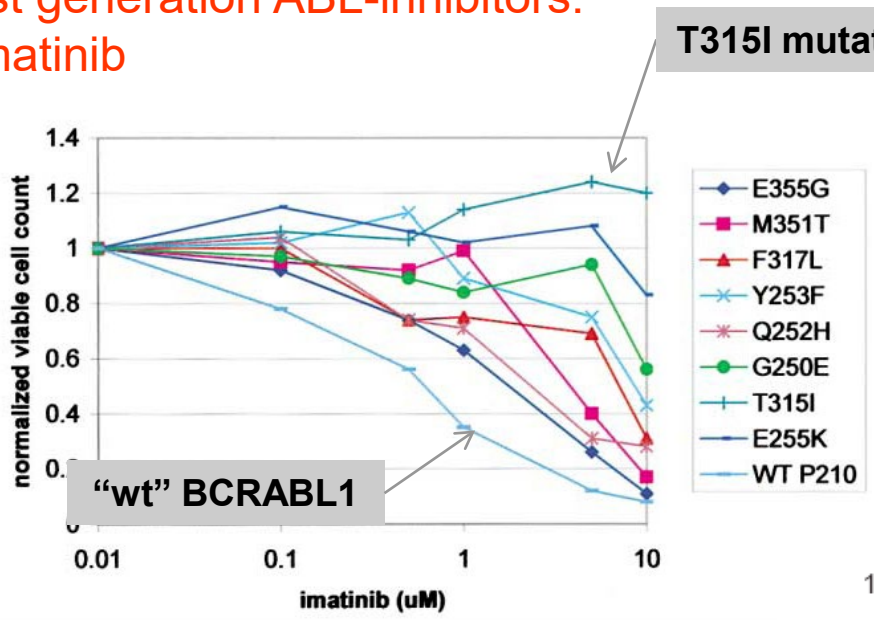


T315I-mutation

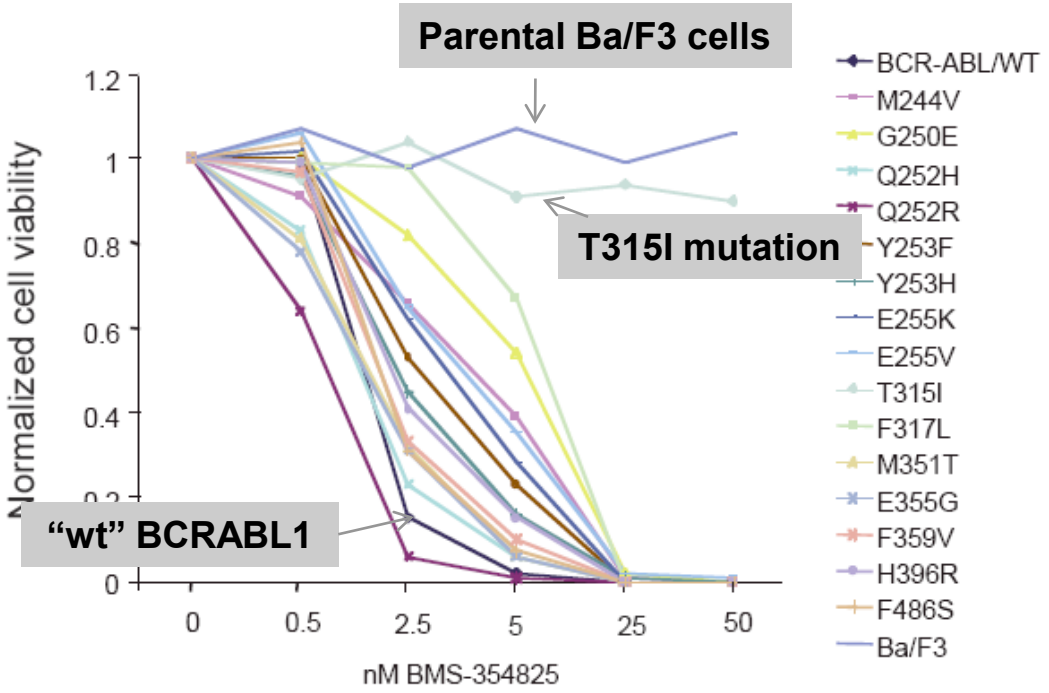
Branford et al, Blood, 2002

‘Imatinib-resistance’ mutations

1st generation ABL-inhibitors:
Imatinib



2nd generation ABL-inhibitors:
Dasatinib



Preclinical and early clinical evidence TKIs for ABL class

	Sensitive to imatinib/dasatinib				
ABL class fusion	**Transforming** IL3/IL7 independent growth in Ba/F3 or IK6 Arf-/-	***In vitro*** growth inhibition in Ba/F3 or IK6 Arf -/-	***Ex vivo*** growth inhibition of patients' cells	**Mouse** growth inhibition in patient-derived xenograft	**Patient** response in early clinical trials or case reports*
ETV6-ABL1	Yes	Yes	Yes	Yes	Yes ; Transient
FOXP1-ABL1					Inconclusive: short FU
SNX2-ABL1	Yes	No, but sensitive to nilotinib			Transient ; Poor/partial
NUP214-ABL1	Yes	Yes	Yes	Yes	Inconclusive: short FU ; Transient
RANBP2-ABL1	Yes	Yes	Yes	Yes	Yes
RCSD1-ABL1	Yes	Yes			Yes ; Partial; short FU ; Transient
ZMIZ1-ABL1	Yes	Yes			Yes
RCSD1-ABL2	Yes	Yes	Yes	Yes	Yes
PAG1-ABL2	Yes	Yes	Yes	Yes	
ZC3HAV1-ABL2					Inconclusive: short FU
EBF1-PDGFRB	Yes	Yes			Yes (6 cases) ; Partial
ETV6-PDGFRB	Yes	Yes			
ZEB2-PDGFRB			Yes		
ATF7IP-PDGFRB	Yes	Yes	Yes		Yes
SSBP2-CSF1R	Yes	Yes	Yes		
MEF2D-CSF1R		Yes			

Roberts et al. 2014; Roberts et al. 2012; Carroll et al. 1997; Yeung et al. 2015; Zuna et al. 2010; Malone et al. 2010; Zaliova et al. 2016; Tomita et al. 2014; Ernst et al. 2011; Masuzawa et al. 2014; De Keersmaecker et al. 2008; Duployez et al. 2016; Perwein et al. 2016; Inokuchi et al. 2011; Mustjoki et al. 2009; Lengline et al. 2013; Weston et al. 2013; Schwab et al. 2016; Ishibashi et al. 2016; Kobayashi et al. 2015; Tasian et al. 2016

This presentation: drug discovery

1. Principles of drug testing
2. Principles of target discovery

} 3. Preclinical evaluation of new therapeutics

Summary:

- Preclinical test models: in vitro – ex vivo – in vivo
- Drug discovery: often forward library screens, combined with gene expression profiling (and/or mutational spectrum)
- Target discovery = functional driven research

Caveats:

- type of cells used for drug discovery (often non-representative cell lines, lack of tumor heterogeneity, genetic context)
- mouse models: often outgrowth inhibition instead of tumor reduction
- intrinsic and/or acquired mutations (T315I in ABL)
- (immune regulation/microenvironment)

‘’B-cell precursor ALL’’ research group

princess máxima center
pediatric oncology

Biomarker/drugable target discovery:

- Femke Hormann, MSc
- Naomi Michels, MD

Leukemic niche:

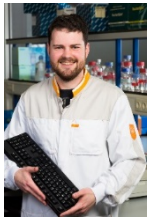
- Femke Meijers-Stalpers, BSc
- Myrthe Vermeeren, BSc
- Mandy Smeets, MSc
- Ilse Dingjan, PhD
- Iris van de Sandt, MSc

Mouse models:

- Cesca van de Ven, PhD
- Aurelie Boeree, BSc

Clinical Genomics:

- Judith Boer, PhD
- Alex Hoogkamer, BSc



Early clinical trial studies:

- Marjolein Bakker, BSc

E: m.l.denboer@prinsesmaximacentrum.nl

Design a drug workshop (figuratively)

Overall aim: Actively think about the drug development process

Objectives:

Design your most optimal drug target

Design your most optimal targeted drug