BIOLOGIE DE LA LEUCEMIE MYELOIDE CHRONIQUE

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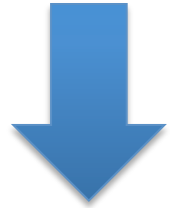


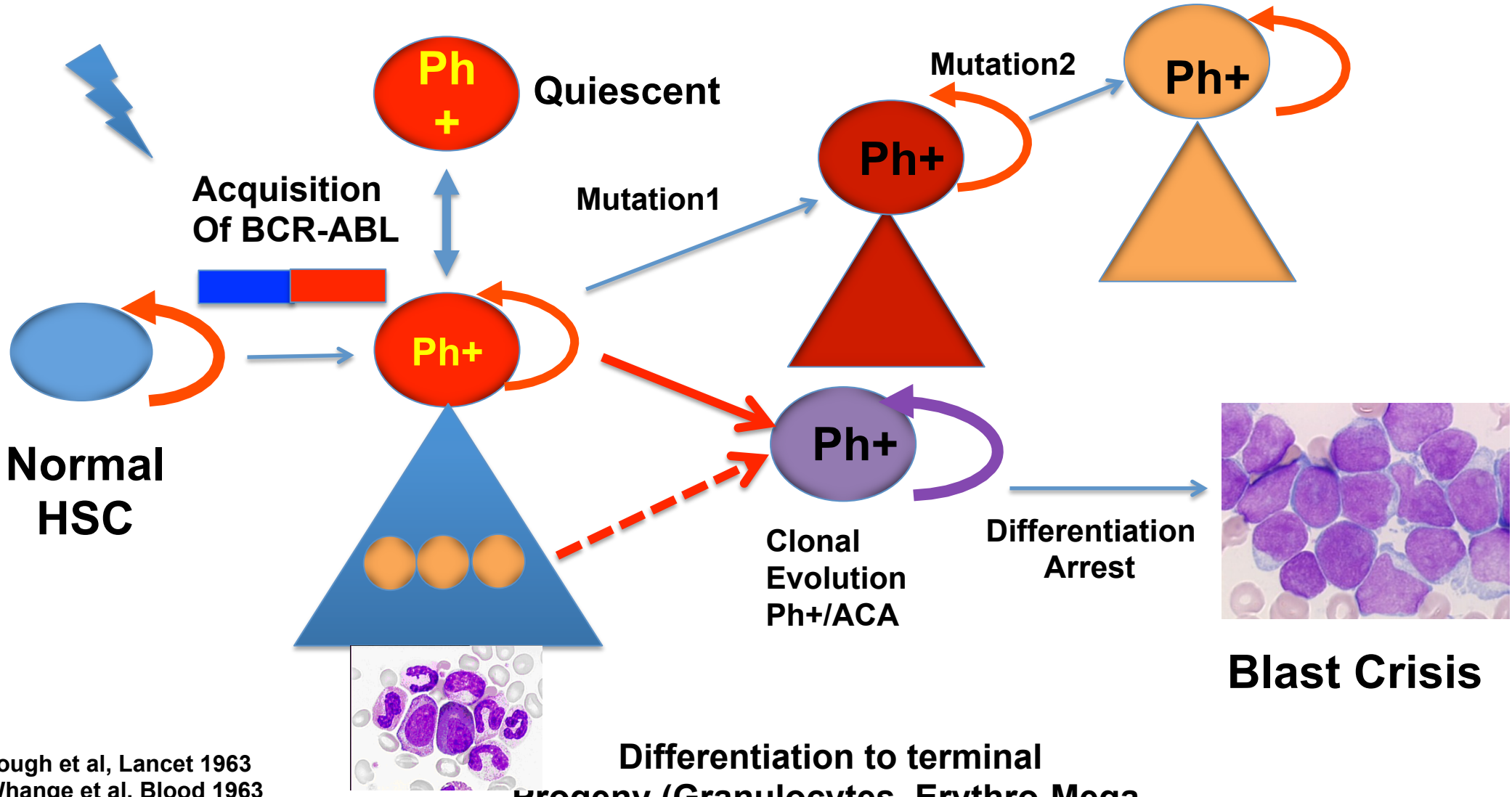
Rappel: Biologie des cellules souches de LMC

Resistance & Instabilité Génétique

Persistance

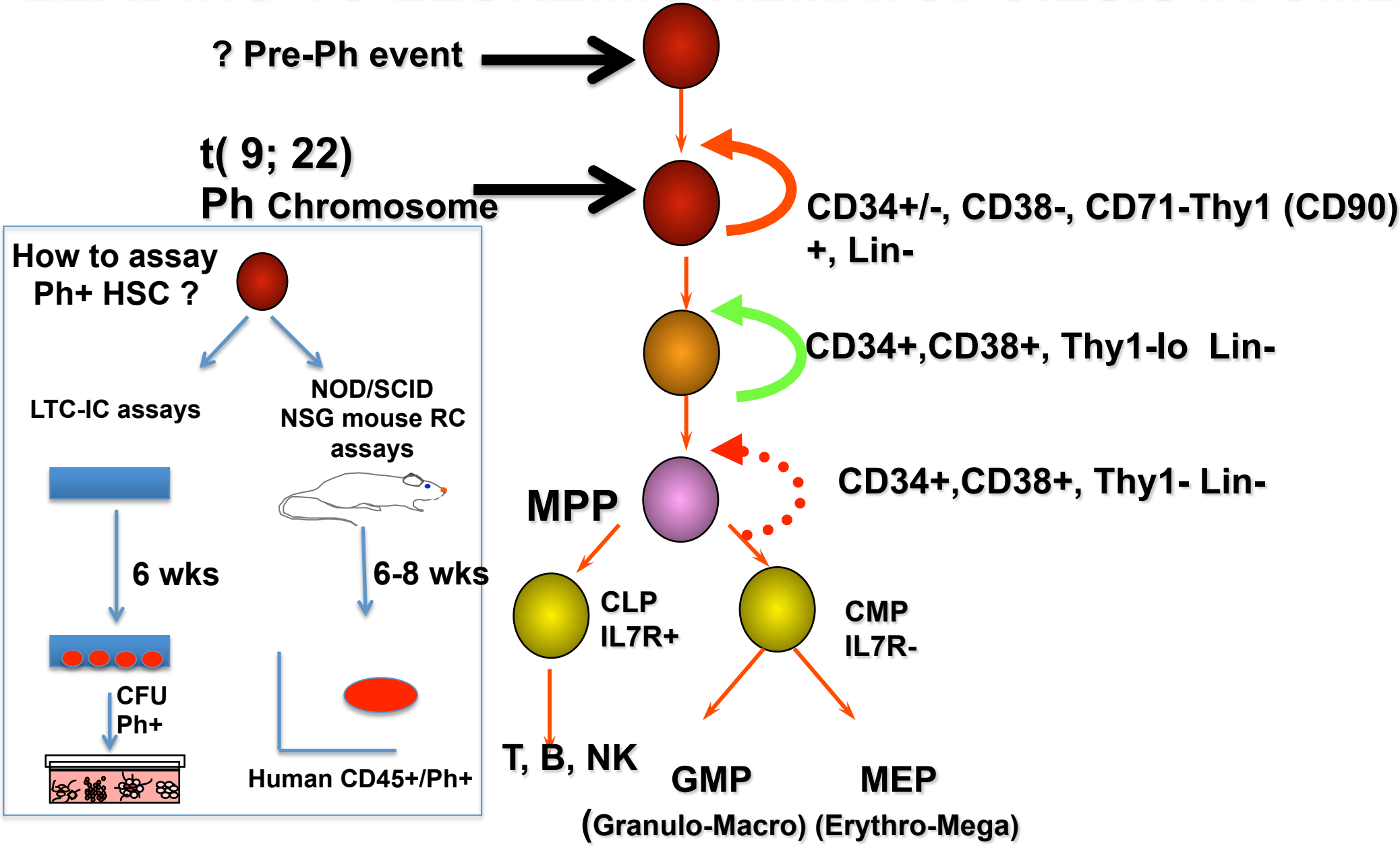
Nouvelles stratégies de ciblage

CML: A CANCER STEM CELL MODEL WITH CLONAL EVOLUTION

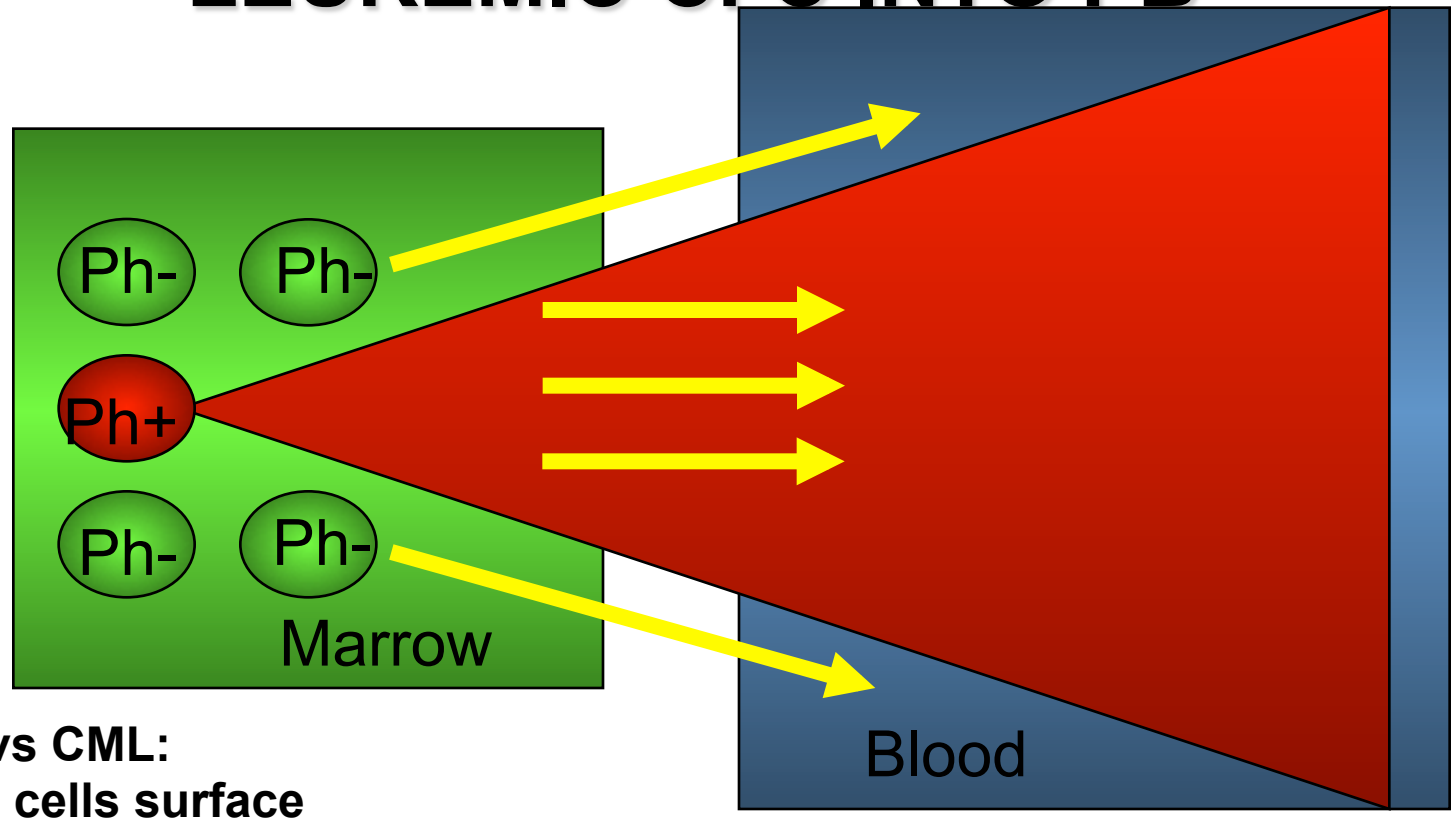


Tough et al, Lancet 1963
Whange et al, Blood 1963
Fialkow et al PNAS 1967

INITIAL CLONAL EVENT(S) LEADING TO LEUKEMIC HEMATOPOIESIS IN CML



STEM CELL PROLIFERATION IN CML: SPONTANEOUS MOBILIZATION OF LEUKEMIC CFC INTO PB



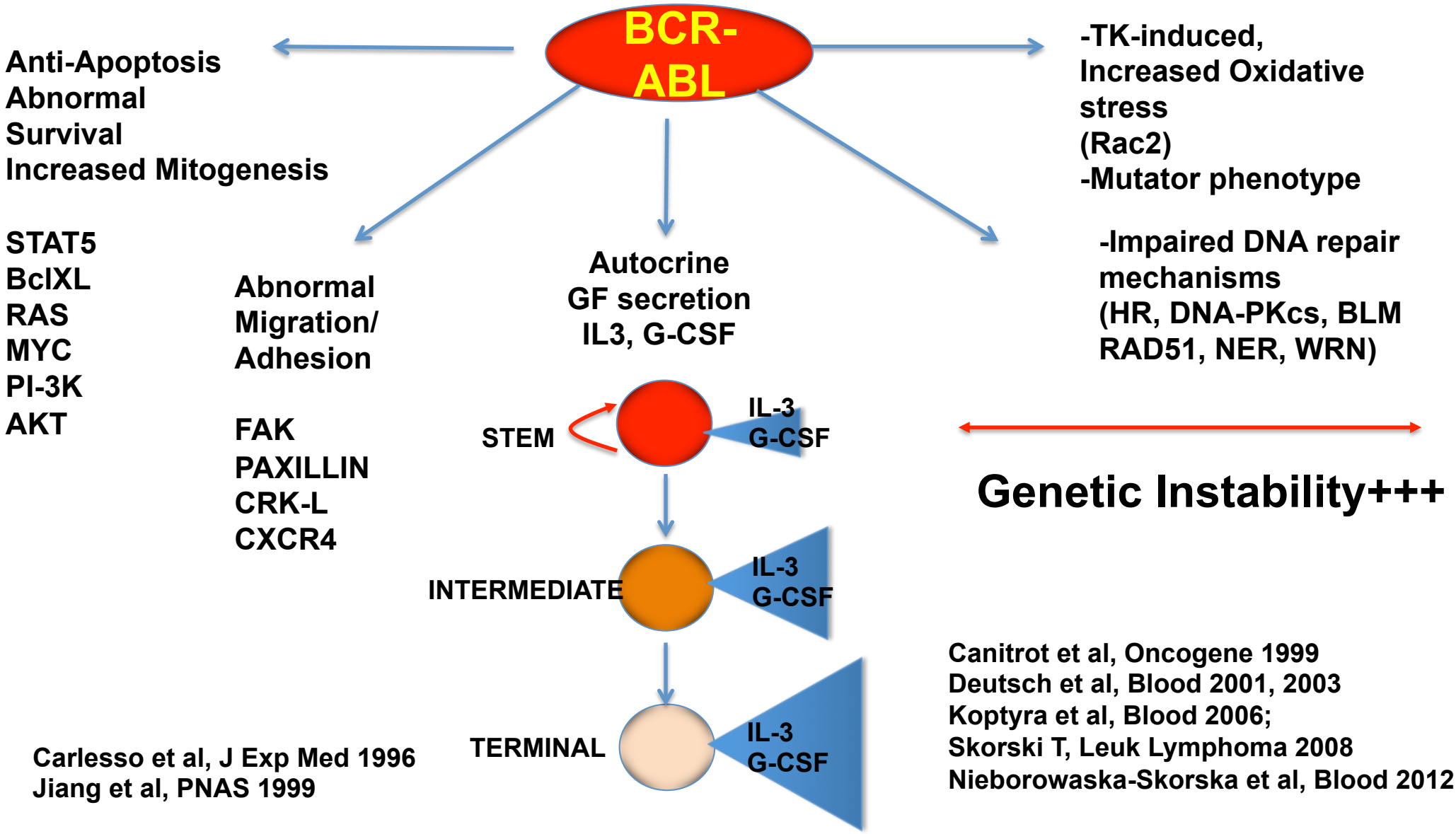
Normal vs CML:
Identical cells surface Markers

Differentiation programs:
Not perturbed

PB CFU-C at diagnosis = 1000- 10000 fold Increase as compared to normal

Hypersensitivity to growth factors++

CML STEM CELLS: CELL-AUTONOMOUS ABNORMALITIES

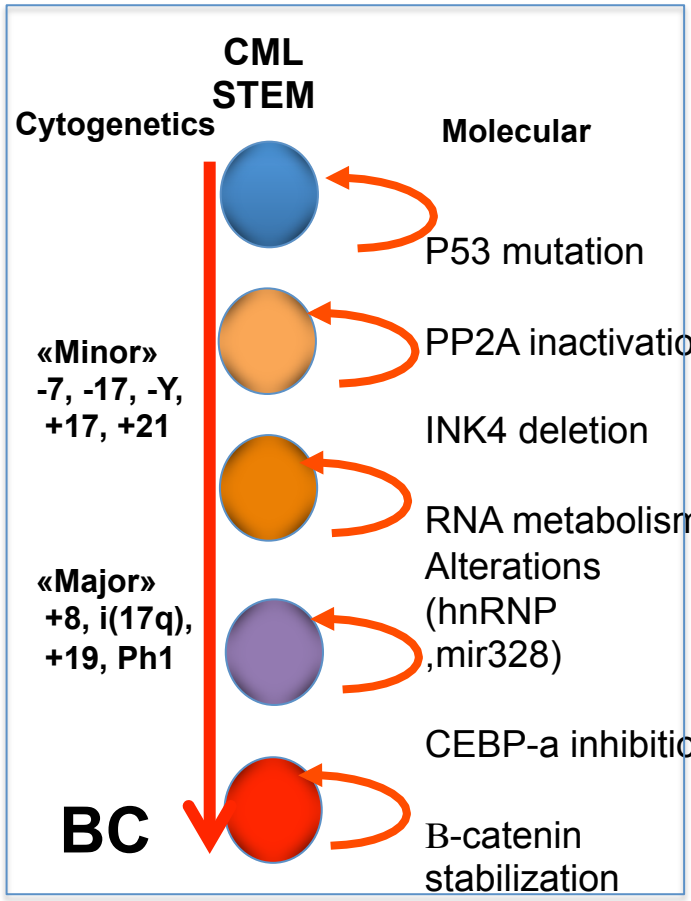


Carlesso et al, J Exp Med 1996
Jiang et al, PNAS 1999

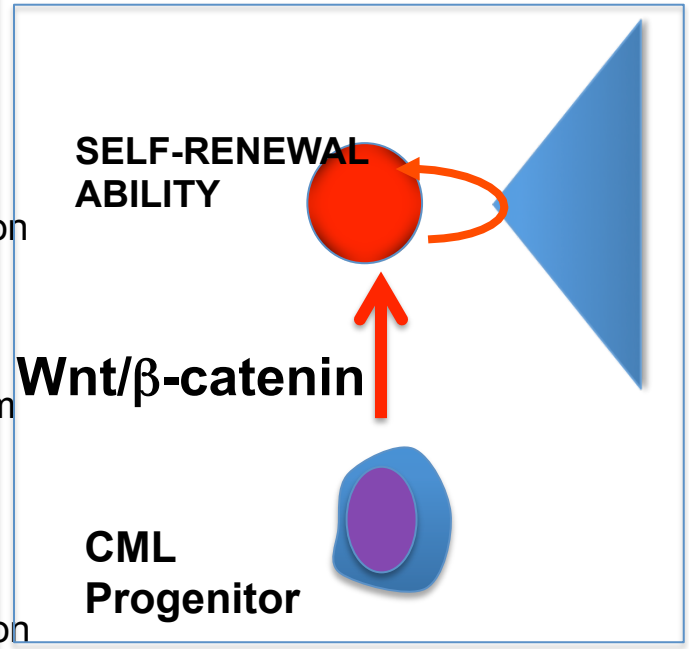
Canitrot et al, Oncogene 1999
Deutsch et al, Blood 2001, 2003
Koptyra et al, Blood 2006;
Skorski T, Leuk Lymphoma 2008
Nieborowaska-Skorska et al, Blood 2012

CML STEM CELLS: ORIGIN OF BLAST CRISIS

Acquisition Of Additional Genetic Abnormalities

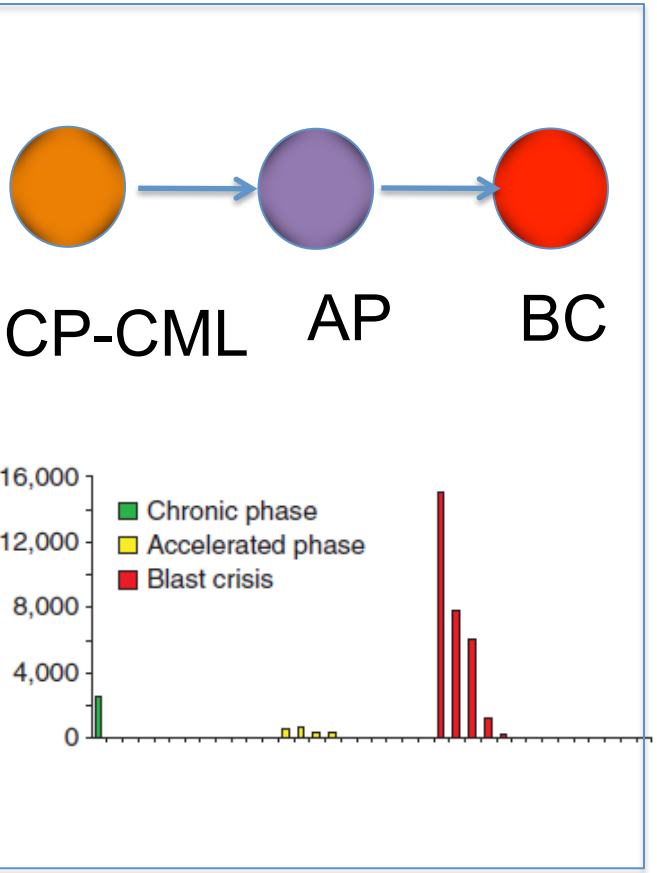


Acquisition Of a Stem Cell Phenotype by a Progenitor

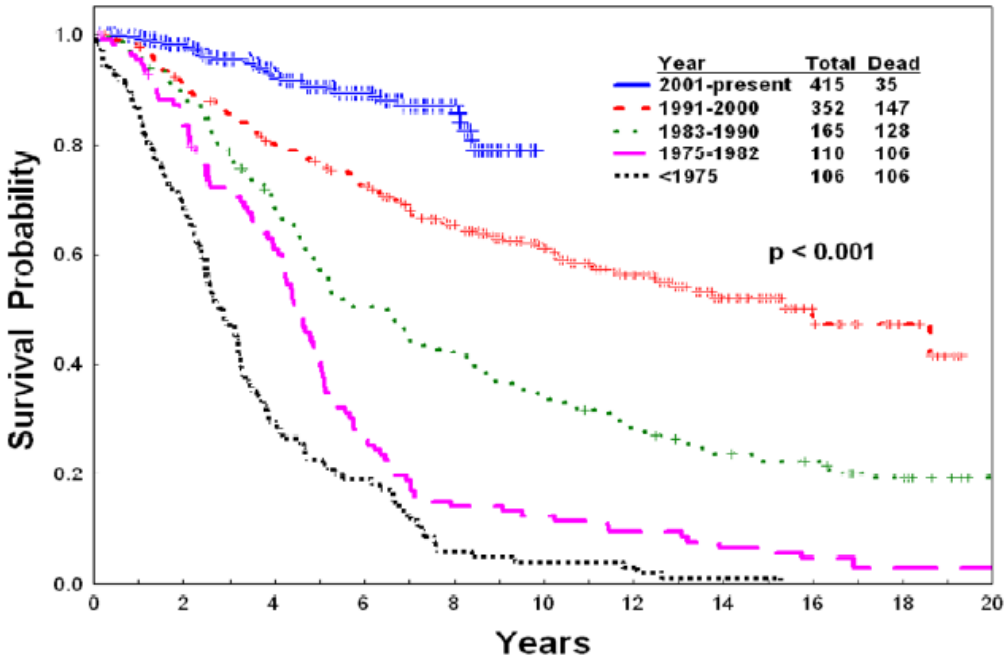


Calabretta & Perrotti 2004, Blood
Jamieson et al, N Engl J Med 2004
Perrotti & Neviani Clin Cancer Res 2007
Wisvanathan et al Nat Genetics 2009
Eiring et al, Cell 2010

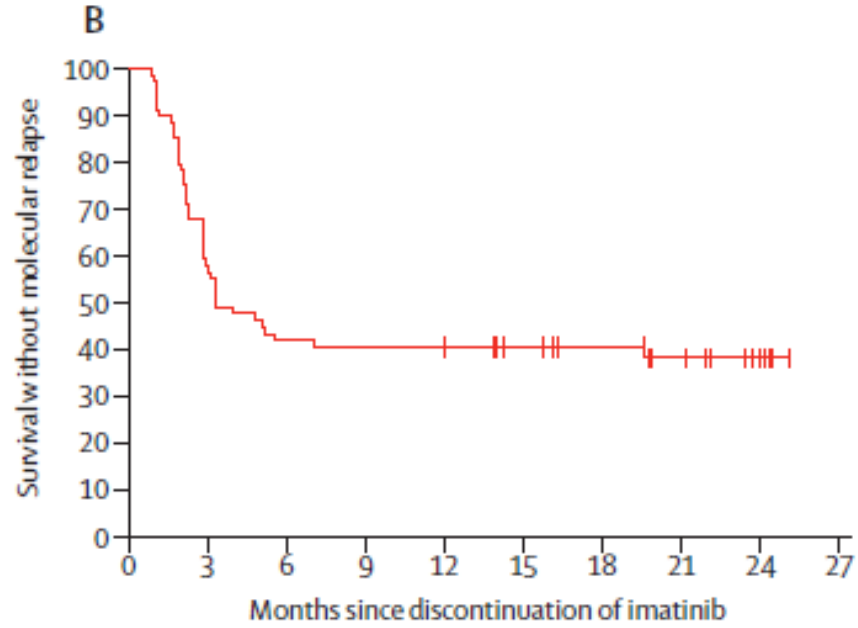
Expression Of Pluripotency Genes: Let7 Regulator Lin28



CML STEM CELLS IN THE ERA OF TARGETED THERAPIES



Kantarjian et al, Blood 2012

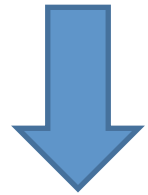


Mahon et al, Lancet Oncol 2010

WHY DO PATIENTS IN COMPLETE MOLECULAR RESPONSE RELAPSE UPON IM DISCONTINUATION?

CML: WHAT REMAINS TO BE DONE ?

-Very efficient first line therapies
-Very efficient second line therapies
- Unprecedented survival (90 % 5 yrs, 80% 10 years)

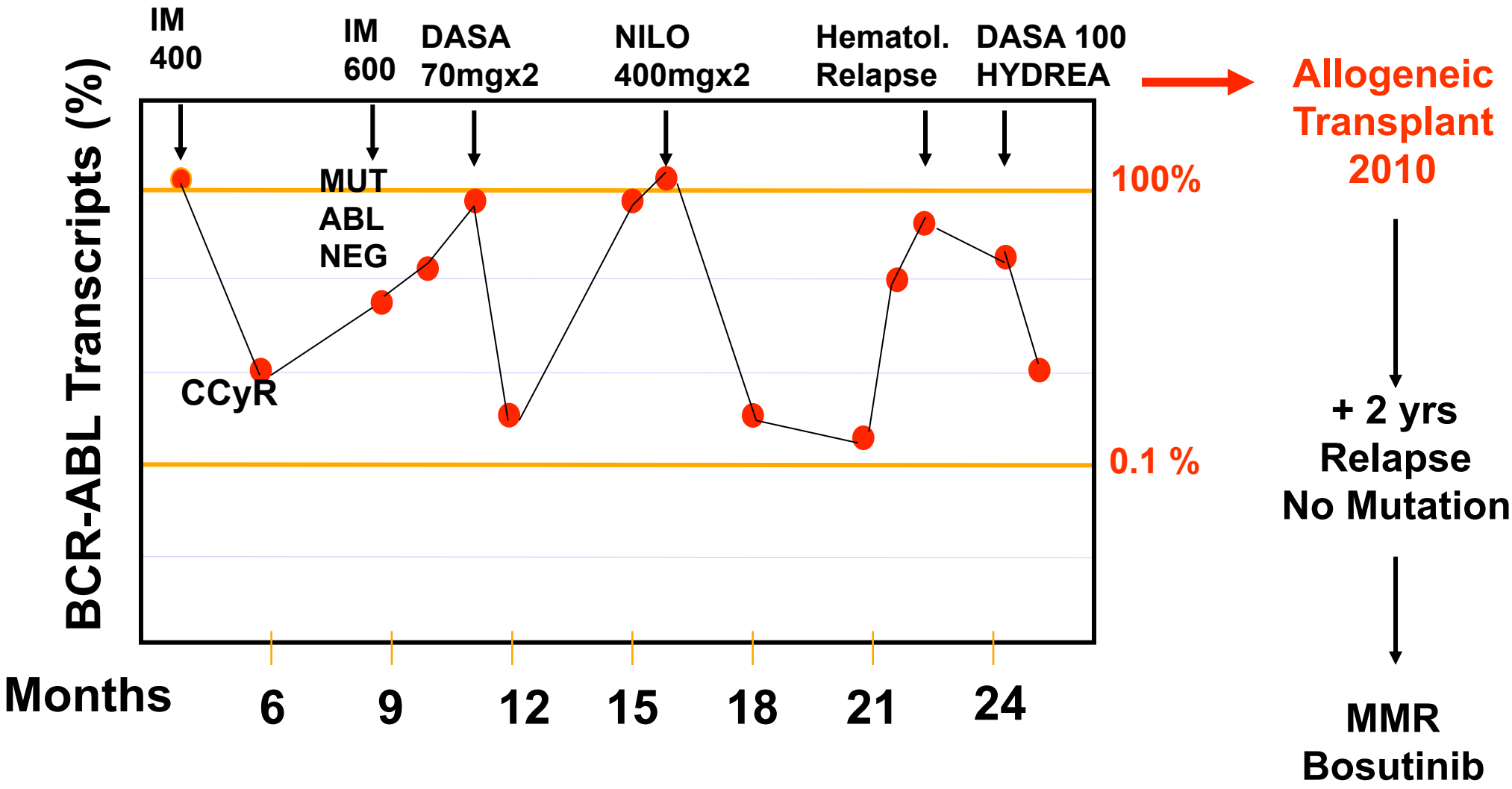


Persistent problems:
-Patients with primary resistance exist
-All data suggest CML stem cells are insensitive to TKI
-50-60 relapse upon TKI discontinuation. Others: some cures ?

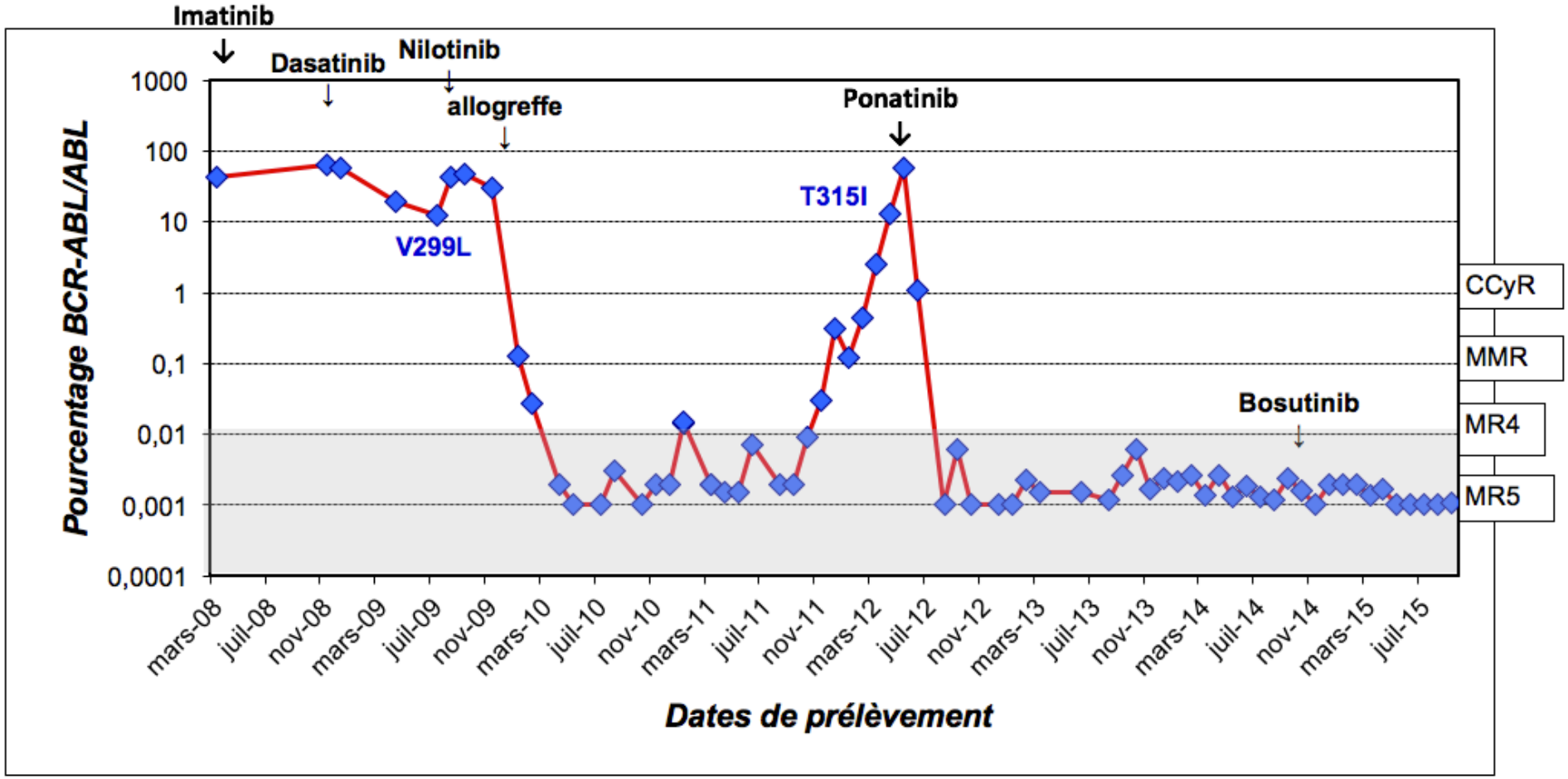
How can we improve existing therapies
-TKI+ Other agents

Can we find new targets ?

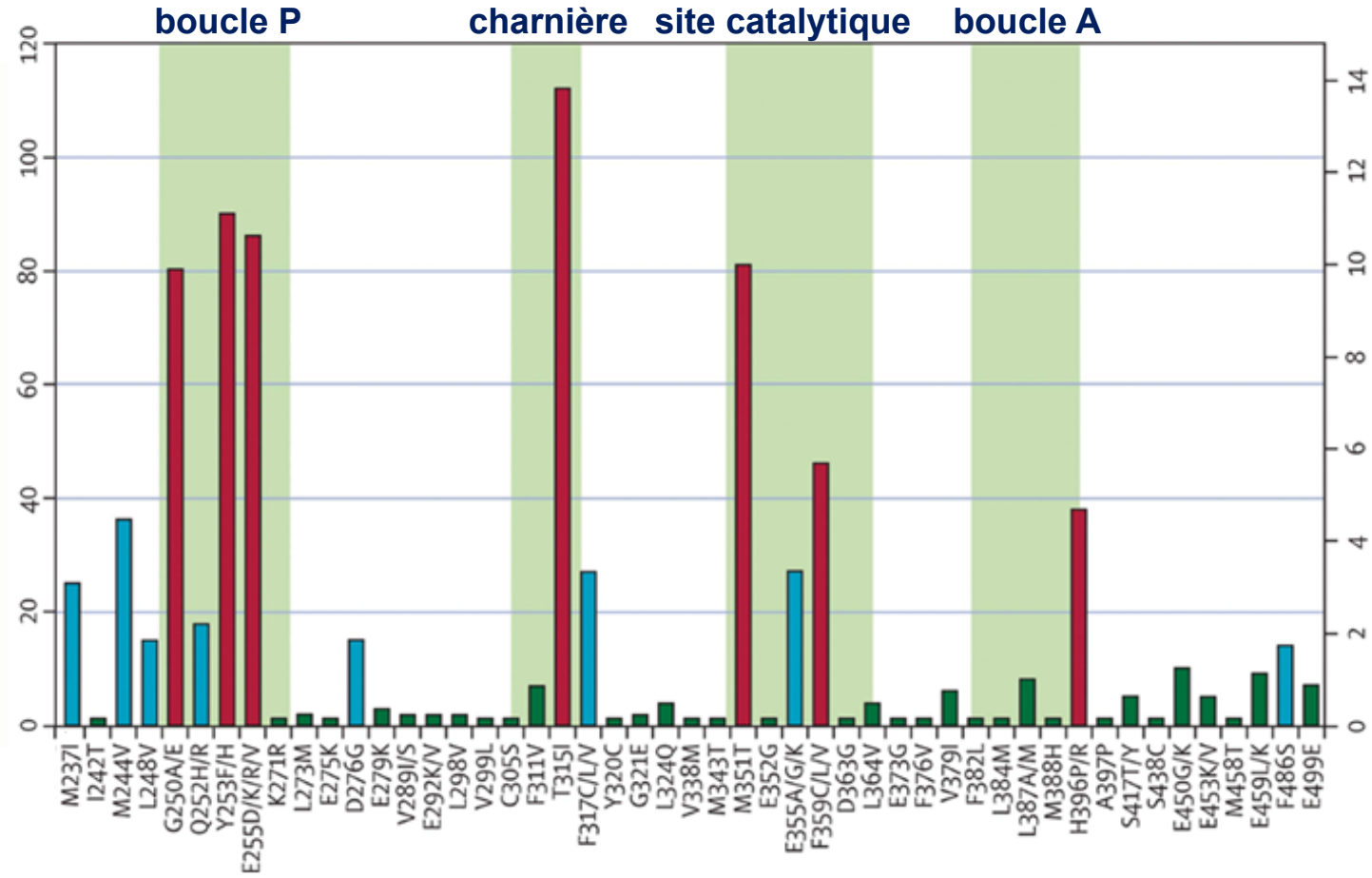
« PRIMARY » RESISTANCE PROFILE
Patient 52 yr old, CML-1st CP, Sokal Int, no ACA



« PRIMARY » RESISTANCE PROFILE WITH ABL-KINASE MUTATION



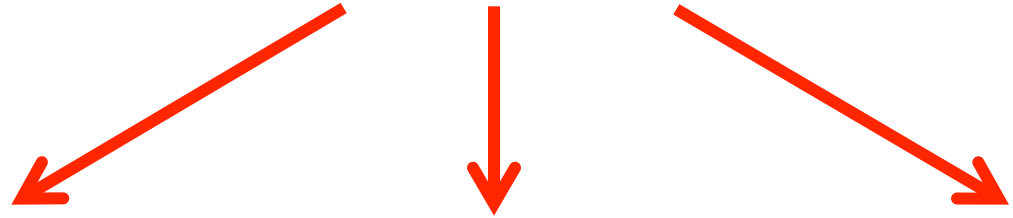
MUTATIONS ABL-KINASE AND RESISTANCE TO TKI



> 100 mutations affectant plus de 70 aa

Apperley, Lancet Oncol 2007

ABL-KINASE MUTATIONS AS A MECHANISM OF RESISTANCE: QUESTIONS



WHEN ?

Prior and/or
During TKI
Selection by TKI

HOW ?

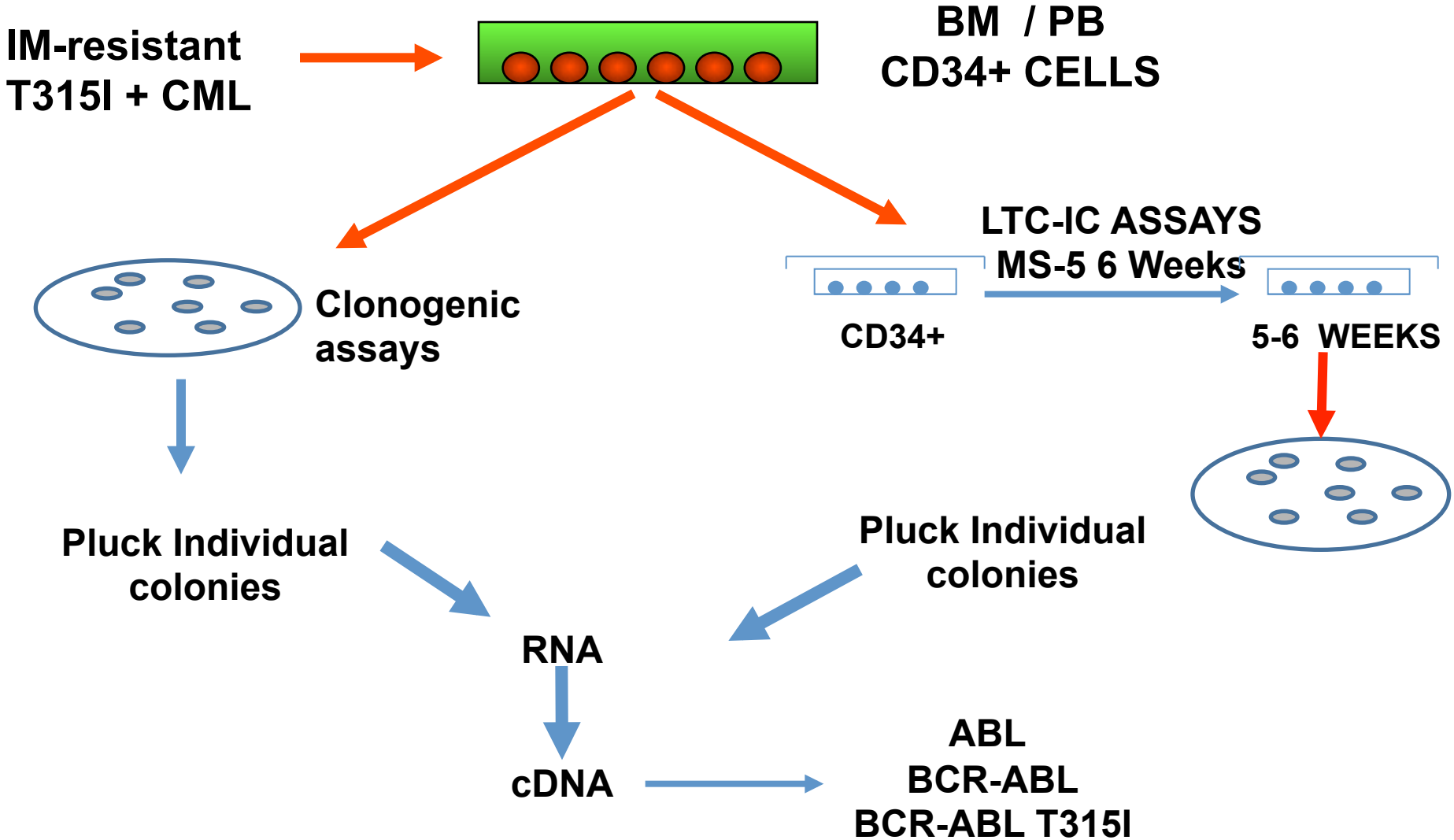
Oxydative stress
Genetic instabililiy
Reduced BRCA1 / DNA-PKcs

WHERE ?

- Progenitors ?
- Stem cells ?

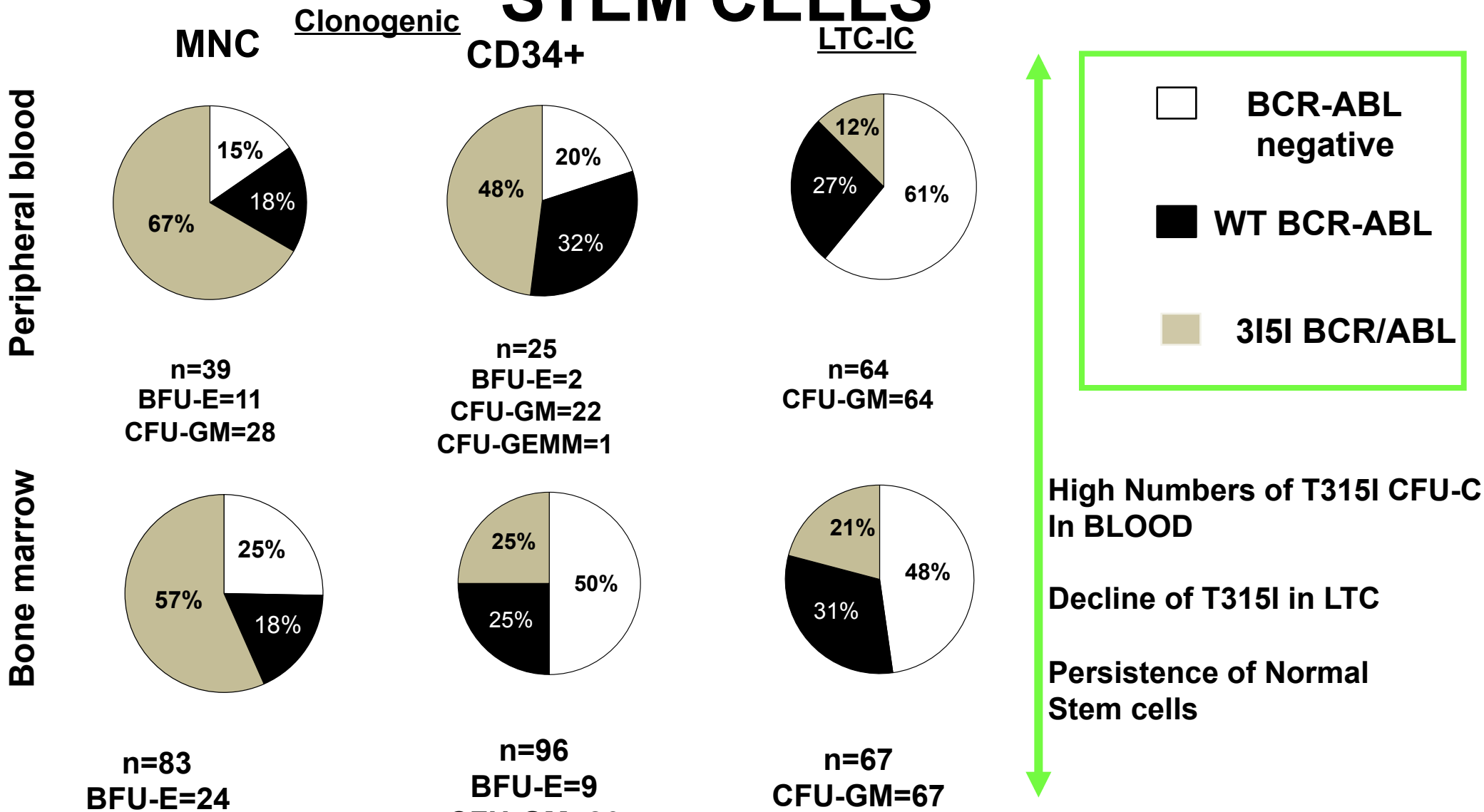
Canitrot et al 1999
Deutsch et al 2001, 2003
Nowicki et al2004
Slupaniek et al 2005
Koptyra et al 2006
Bolton-Gillespie et al 2013

CML WITH T315I MUTATION: WHAT IS THE HIERARCHICAL LEVEL OF STEM CELL ?



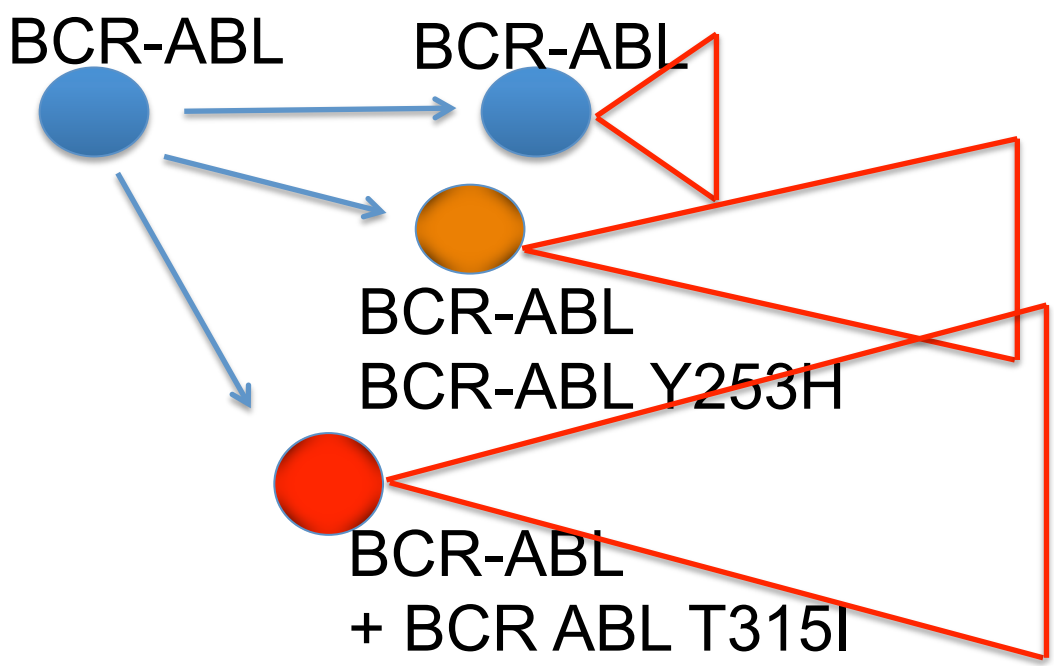
Chomel et al, Leuk Lymphoma 2010

CML WITH T315I: MUTATION OCCURS IN STEM CELLS



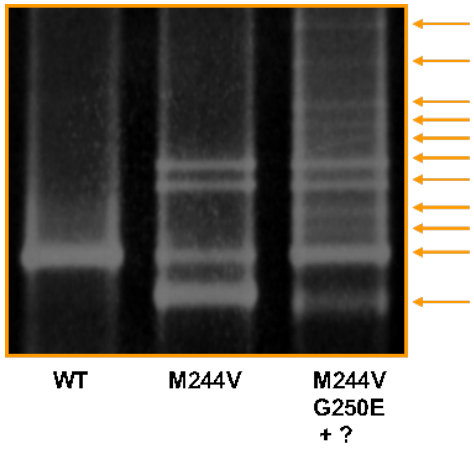
Chomel et al, Leuk Lymphoma 2010

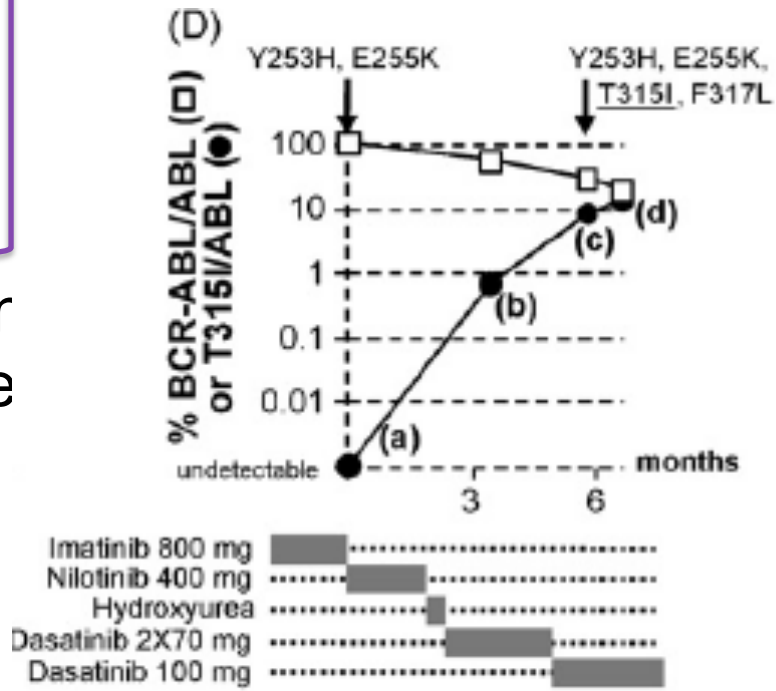
CML STEM CELLS IN THE ERA OF TARGETED THERAPIES: EVOLUTION OF MUTATED CLONES



TKI Therapies

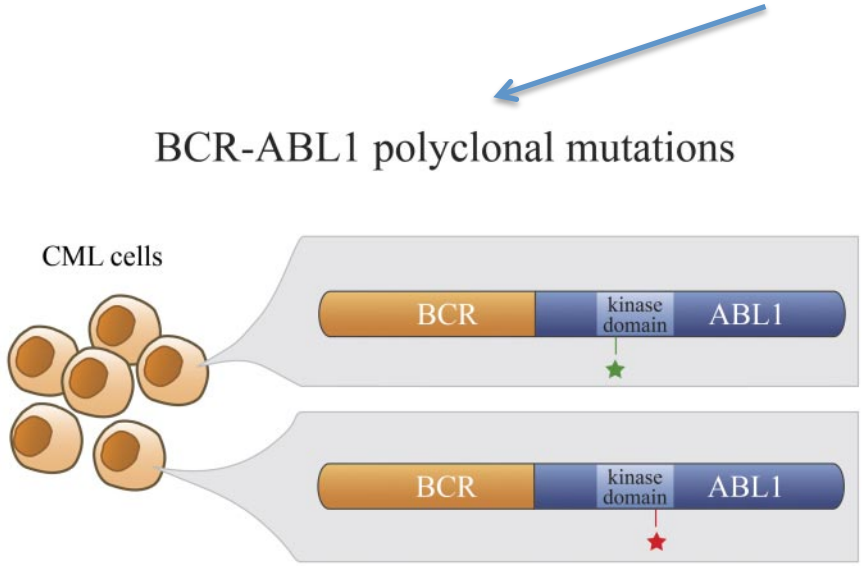
TKI: Expansior
Of T35I Mutate
clone

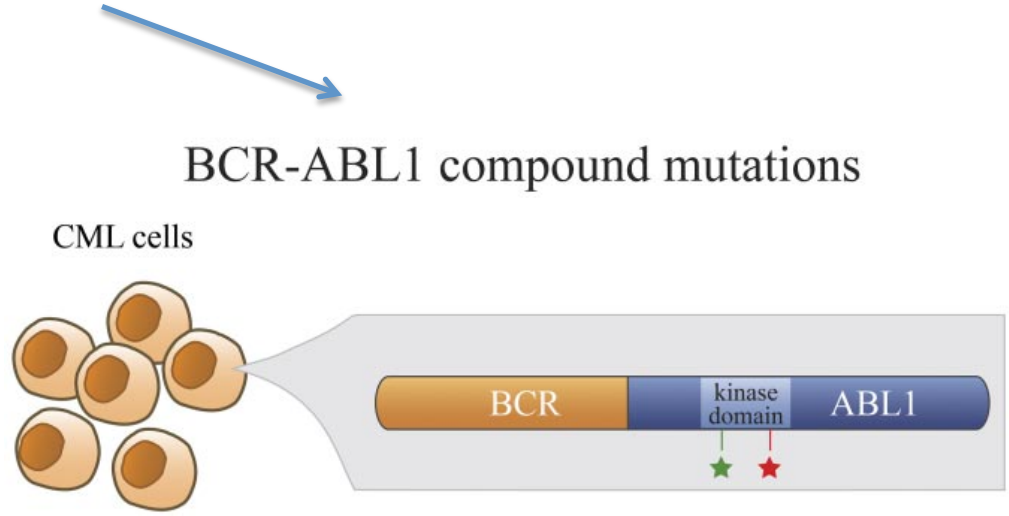




Chomel et al, Leuk Res 2009

POLYCLONAL VERSUS COMPOUND MUTATIONS AS MECHANISM OF RESISTANCE TO TKI





70% OF DOUBLE MUTATIONS CONFIRMED AFTER CLONING ARE CIS-COMPOUND MUTATIONS

Khorashad et al, 2013

POLYCLONAL VERSUS COMPOUND MUTATIONS AS MECHANISM OF RESISTANCE TO ALL TKI

Table 3. Mutations, TKIs, and disease phase

Patient ID no.	*BCR-ABL1* mutations	TKI therapy	Disease phase	Type of *BCR-ABL1* mutation
CML#23	G250E‡/T315I‡	Im	CP	Compound
CML#27	V338F/L384M†	Im	CP	Compound
CML#32	M244V/M351T	Im	CP	Compound
CML#40	M244V/E459K	Im	CP	Compound
CML#50	G250E‡/E459K	Im	CP	Compound
CML#51	F311L/H396R†	Im	CP	Compound
CML#24	G250E‡/T315A*	Das	BP	Compound
CML#36	T315I‡/H396R†	Pon	BP	Compound
CML#28	V299L*/E459K	Im, Das	BP	Compound
CML#31	M244V/F317L‡	Im, Das	CP	Compound
CML#41	E255K‡/T315I‡	Im, Das	BP	Compound
CML#43	F317L‡/M351T	Im, Das	AP	Compound
CML#44	T315I‡/L387M	Im, Das	BP	Compound
CML#49	G250E‡/V299L*	Im, Das	CP	Compound
CML#19	M351T/E255K‡	Im, Nil, Das	CP	Compound
CML#37	V299L*/F359V†	Im, Nil, Das	CP	Compound
CML#42	G250E‡/F317L‡	Im, Nil, Das	BP	Compound
CML#45	Y253H†/F317L‡	Im, Nil, Das	BP	Compound
CML#30	Y253H†/F317L‡	Im, Nil, Das, Bos	BP	Compound
CML#35	Y253H†/F359V†	Im, Nil, Das, Bos	CP	Compound
CML#33	M351T, F359V†	Im	CP	Polyclonal
CML#39	Y253H†, T315I‡	Im	BP	Polyclonal
CML#48	T315I‡, F359V†	Im	AP	Polyclonal
CML#20	H396R†, F317L‡	Das	CP	Polyclonal
CML#46	T315A*, F317C*, F317L‡, F317V*	Das	BP	Polyclonal
CML#34	L248V‡, G250E‡	Im, Das	BP	Polyclonal
CML#47	Y253H†, E255V‡	Im, Das	CP	Polyclonal
CML#38	V299L*, F359V†	Im, Nil, Das	CP	Polyclonal

Khorashad et al, 2013

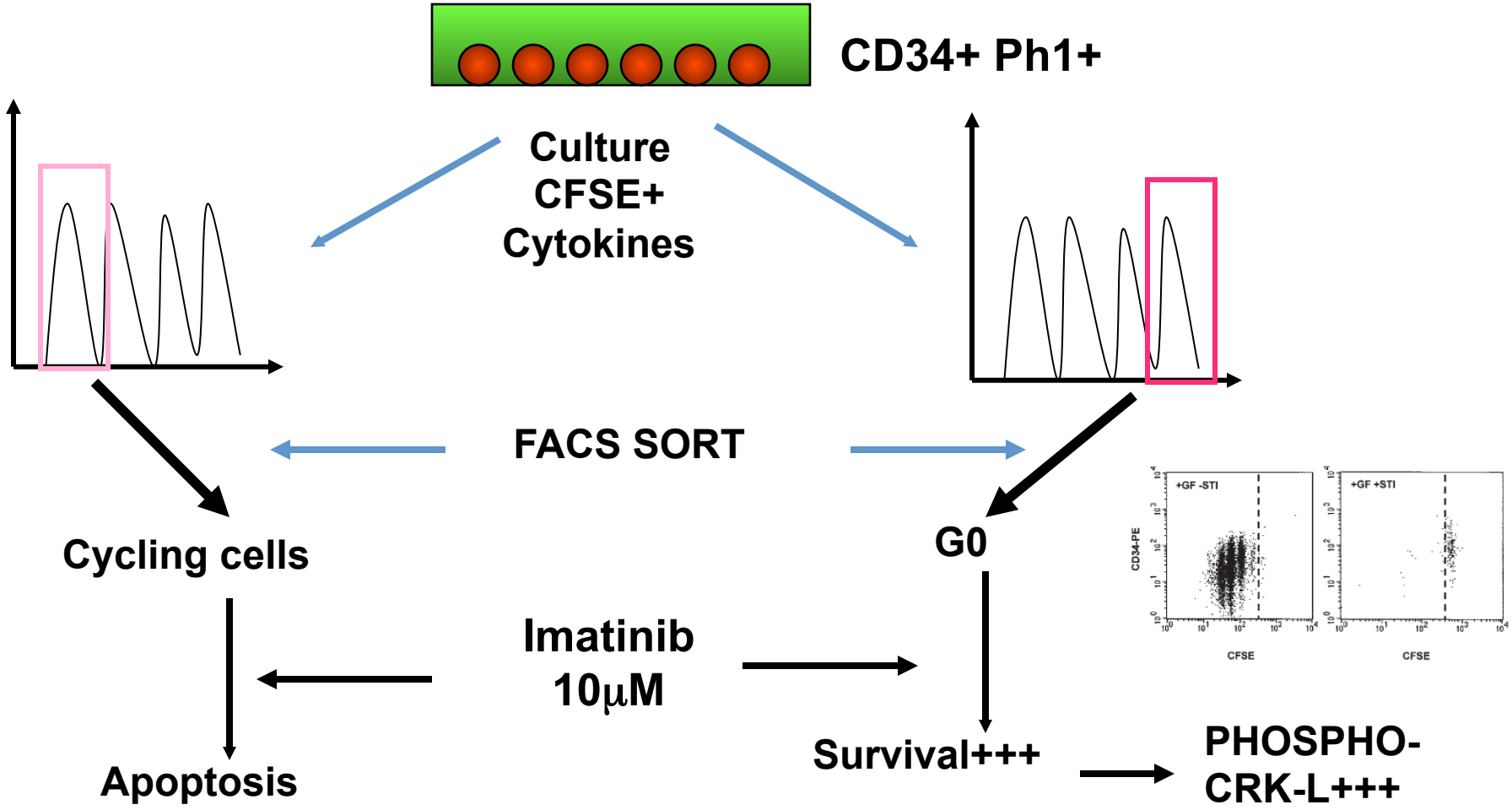
MÉTHODE DE DÉTECTION ET/OU DE CARACTÉRISATION DES MUTATIONS COMPOSÉES BCR-ABL KD

Méthode	Sensibilité	Détection des mutations composées	Caractérisation des mutations composées	Référence
Séquençage direct	**10-15%**	**NON**	**NON**	*Branford et al, Blood 2003*
Sous-clonage + séquençage	**5-10% ***	**OUI**	**OUI**	*Shah et al, Cancer Cell 2002*
Pyroséquençage	**5%**	**NON**	**NON**	*Khorashad et al, Leukemia 2006*
PCR allèle spécifique	**0,001-0,01%**	**NON**	**NON**	*Roche-Lestienne et al, Blood 2002* *Willis et al, Blood 2005* *Chomel et al, Leuk Res 2009*
DGGE	**2-5%**	**OUI ****	**NON**	*Sorel et al, Clin Chem 2005*
DHPLC	**1-2%**	**OUI ****	**NON**	*Deininger et al, Leukemia 2004* *Soverini et al, Clin Chem 2004*
HRM	**1-2%**	**OUI ****	**NON**	*Poláková et al, Leuk Res 2008*
NGS	**1%**	**OUI ****	**OUI ****	*Soverini et al, Blood 2013* *Poláková et al, J Canc Res Clin Onc 2014*
NGS longue distance	**1%**	**OUI**	**OUI**	*Kastner et al, Eur J Cancer 2014*

RESISTANCE AT THE STEM CELL LEVEL

PERSISTENCE

QUIESCENT Ph1+ STEM CELLS ARE RESISTANT TO IMATINIB



Graham et al, Blood 2002

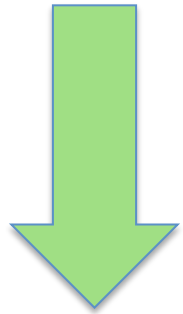
QUESTIONS

Do LSC persist in profound MR ?

What are the mechanisms of resistance of LSC ?

What are the mechanisms of persistence of LSC ?

WHAT IS THE NATURE OF THE CELLS AT THE ORIGIN OF RELAPSES ?



ANALYSIS OF LSC IN PATIENTS IN LONG-LASTING DEEP MOLECULAR RESPONSE

PILOT « PERSISTEM »STUDY

6 patients (1M / 5F)

(Age 66 – 78)

Therapies:

IFN–α (Pt 1, 2, 3) 13, 9, 6 years, Off Rx 11,16, 8 years

IFN-α + IM (Pt 4, 5) 8, 6 years, Off Rx 2 & 2 years

IM + DASATINIB (Pt 6) > 4 years, Dasatinib ON

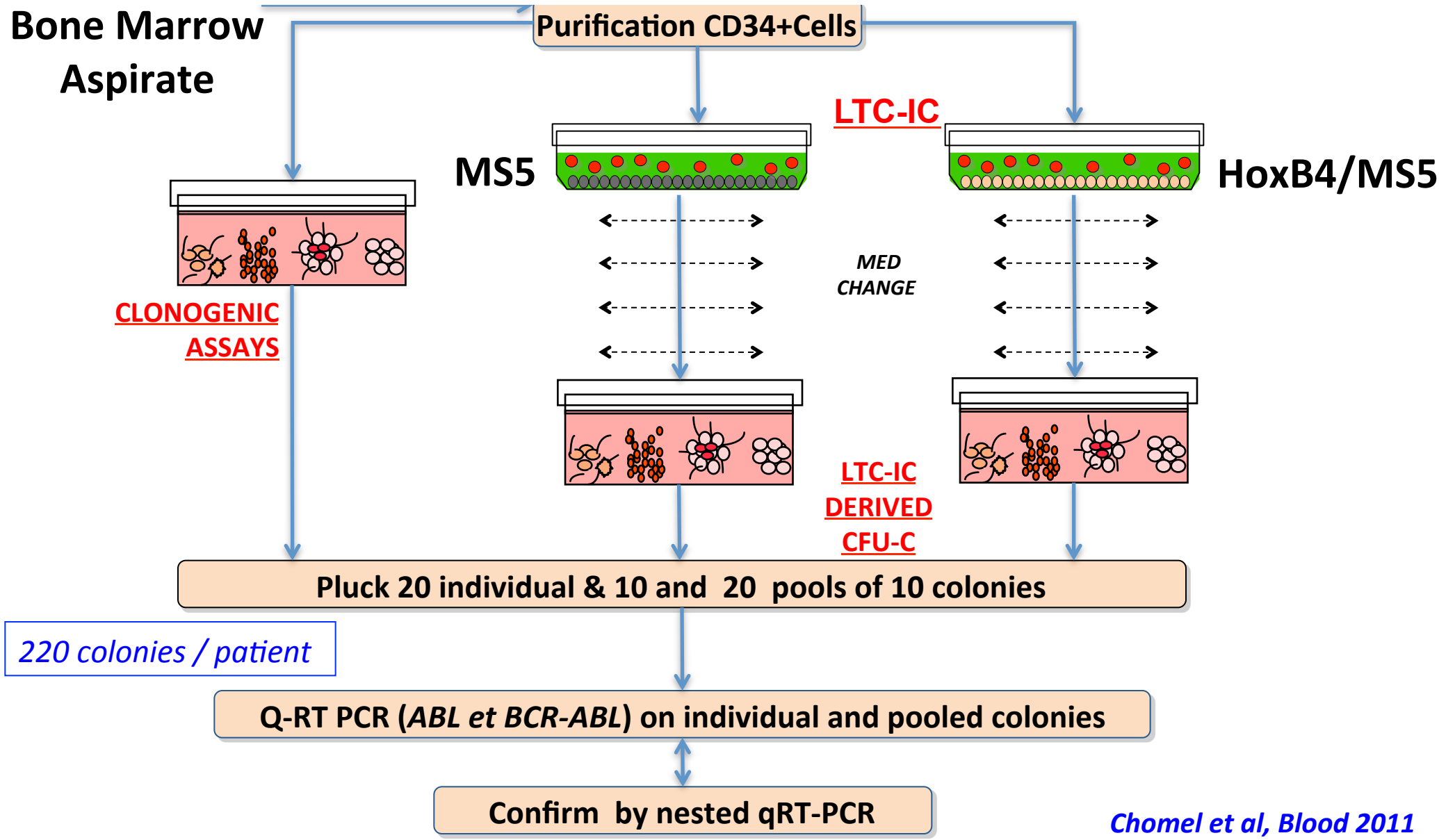
RQ-PCR-NEGATIVITY In Peripheral Blood

IFN-α patients: 5, 4 and 6 years

IFN-α + IM patients: 4 & 5 years

IM + Dasatinib : 3 years

EVALUATION OF BCR-ABL EXPRESSING LSC IN PATIENTS IN DEEP MR



Chomel et al, Blood 2011

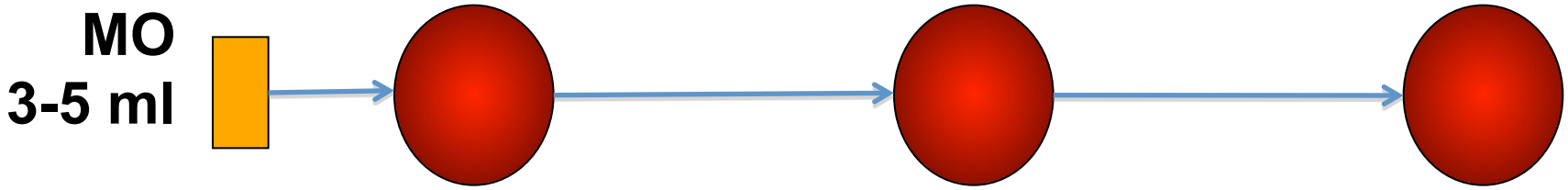
BCR-ABL EXPRESSING HSC AND CFC IN PATIENTS WITH UMRD

Pt	Previous Therapies	D0 : Clonogenic		Week 5: LTC-IC-derived CFU			
		CFU-Cs	Pools de 10 CFU-Cs	Individual		Pools of 10 LTC-ICs	
				MS-5	MS5/ HOXB4	MS-5	MS5/HOXB4
1	IFN-α	0/20	1/18	4/31		1/8	
2	IFN-α	0/20	1/19	2/39	4/40 *		
3	IFN-α	9/19	11/16	1/30	9/30 *		
4	IFN-α; IM	2/20	0/19	0/20		0/19	
5	IFN-α; IM	1/20	0/20	0/20	2/17 *	0/20	0/20
6	ON DASA	1/17		23/24	12/43		

N= 2000

Chomel et al, Blood 2011

« Charge » estimée des patients en MRI en cellules souches leucémiques BCR-ABL+



Patients	Nb de cellules CD34+	Fraction de CD34+ testées en culture à long terme	Fraction de LTC-Ics testées en méthylcellulose
1	0,1 10^6	1/2	1/4
2	0,7 10^6	1/10	1/5
3	1,2 10^6	1/20	1/4
4	0,2 10^6	1/5	1/1
5	3,5 10^6	1/20	1/6
6	0,03 10^6	1/2	1/1

X 20

X 4

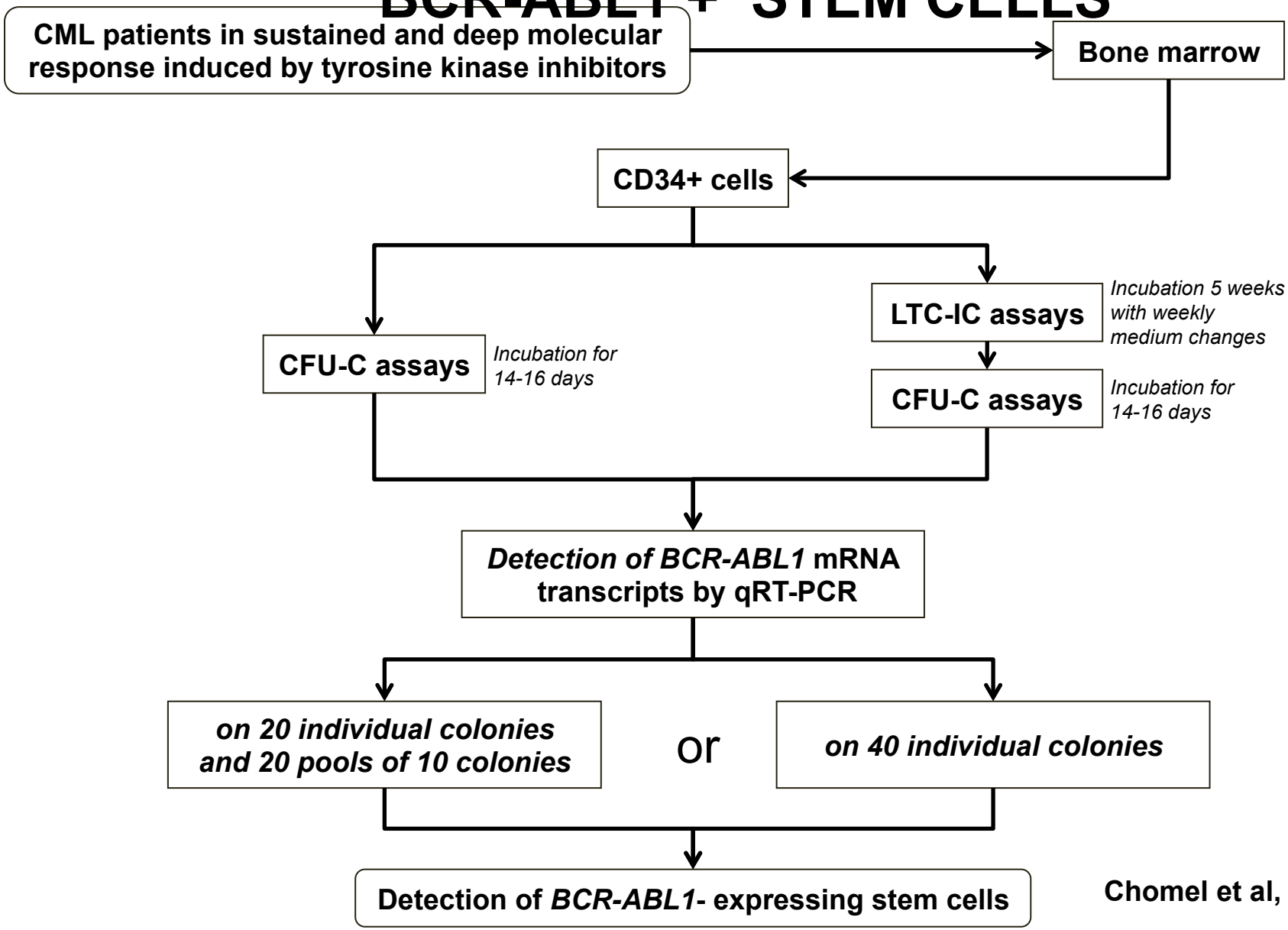
Persistance d'une quantité significative de cellules souches leucémiques *in vivo*

EVALUATION OF LSC PERSISTANCE ON MR 4.5 INDUCED BY TKI

21 PATIENTS ON MR 4.5 > 2 YEARS (Median 7)

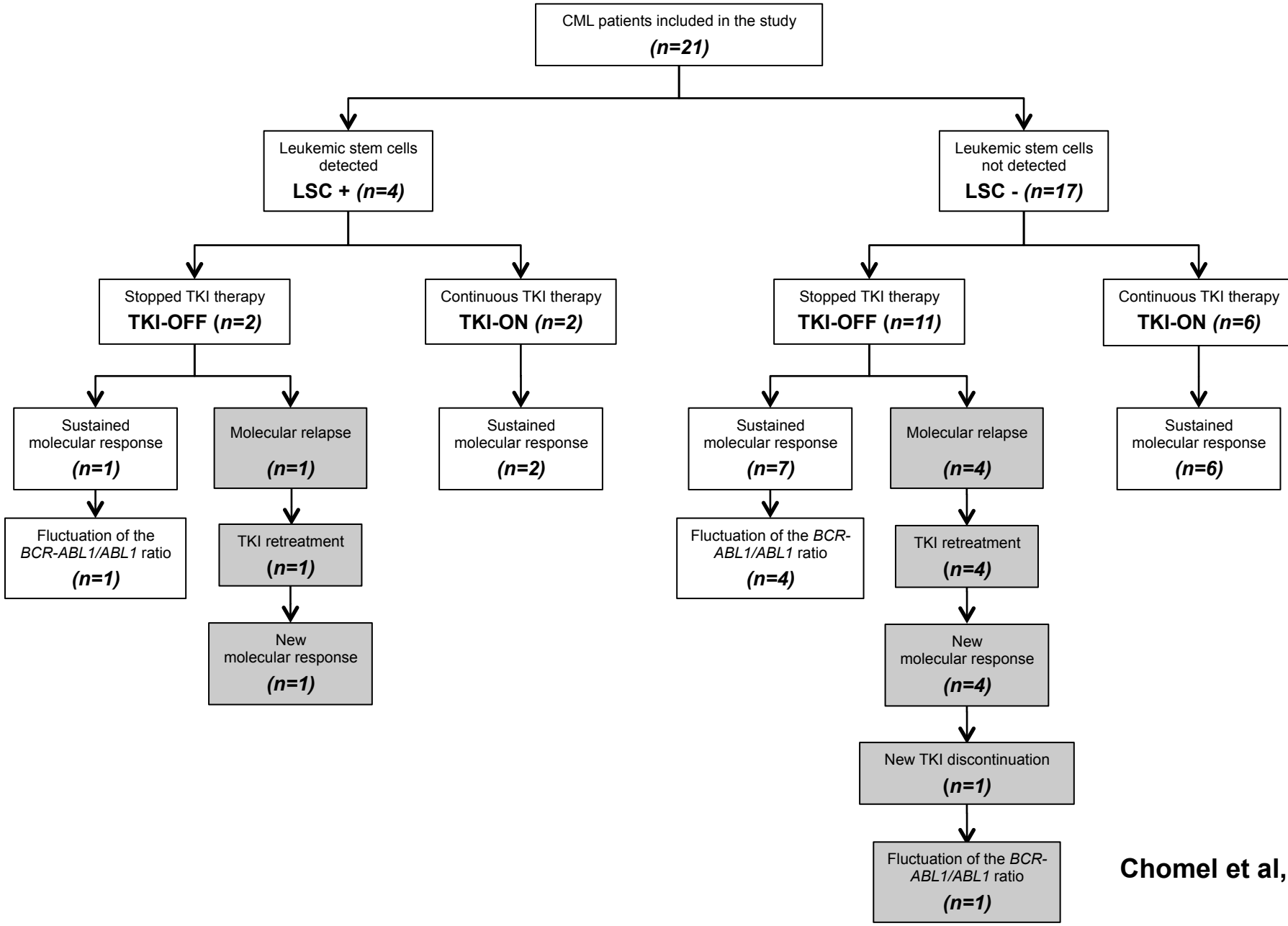
Chomel et al, Oncotarget 2016

STRATEGY USED FOR THE DETECTION OF BCR-ABL1 + STEM CELLS

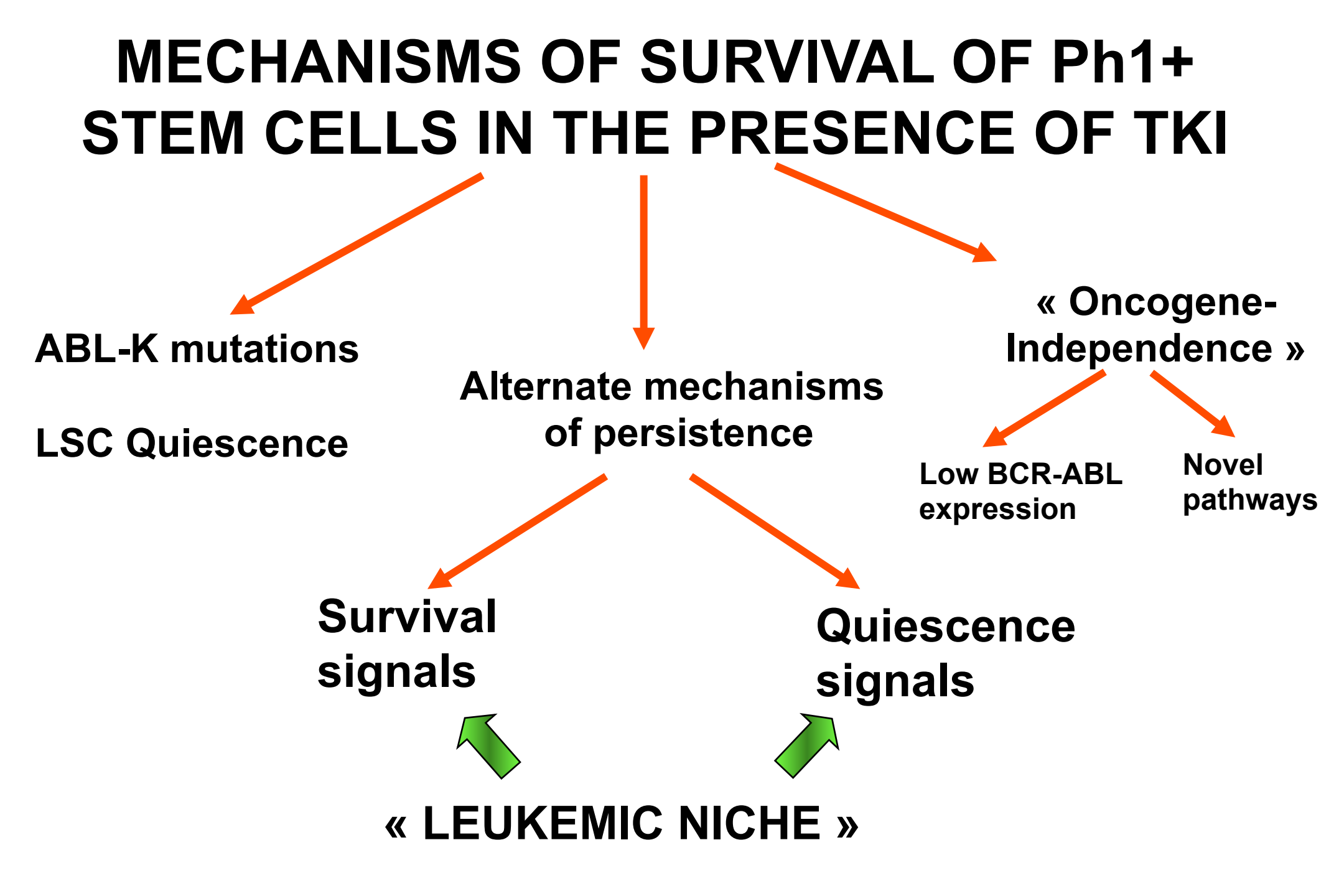


Chomel et al, Oncotarget 2016

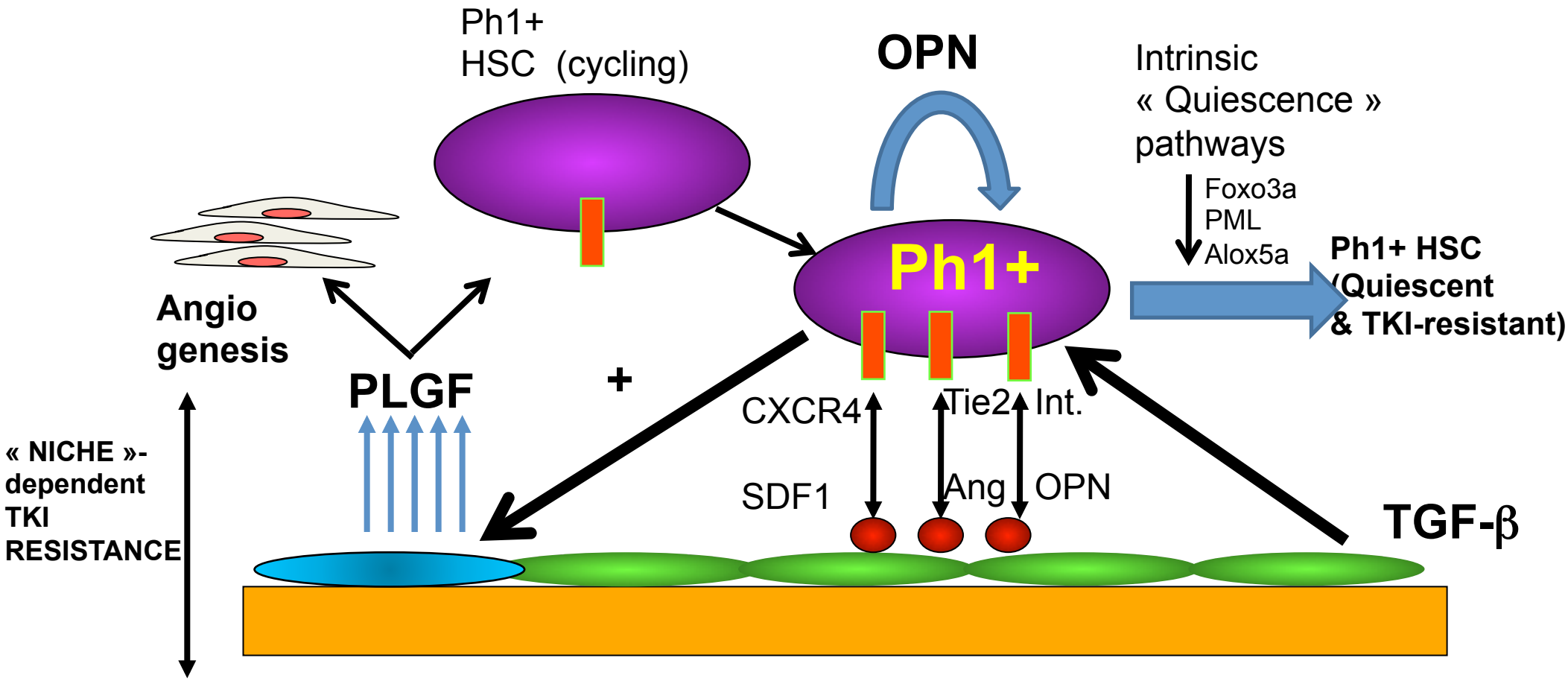
BCR-ABL+ STEM CELL PERSISTENCE AND OUTCOME



Chomel et al, Oncotarget 2016


MECHANISMS OF SURVIVAL OF Ph1+ STEM CELLS IN THE PRESENCE OF TKI
ABL-K mutations
LSC Quiescence
Alternate mechanisms of persistence
« Oncogene-Independence »
Low BCR-ABL expression
Novel pathways
Survival signals
Quiescence signals
« LEUKEMIC NICHE »

RESISTANCE TO TKI: NICHE-RELATED MECHANISMS?

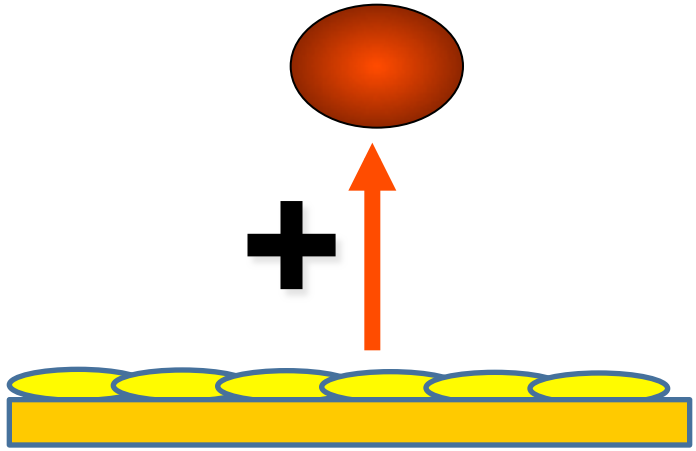


Turhan AG & Chomel JC : Oncotarget 2011

IS THE LEUKEMIC « NICHE » NORMAL ?

CML STEM CELLS AND THEIR NICHE: A CONSENSUAL CROSS-TALK ?

CML STEM Ph1

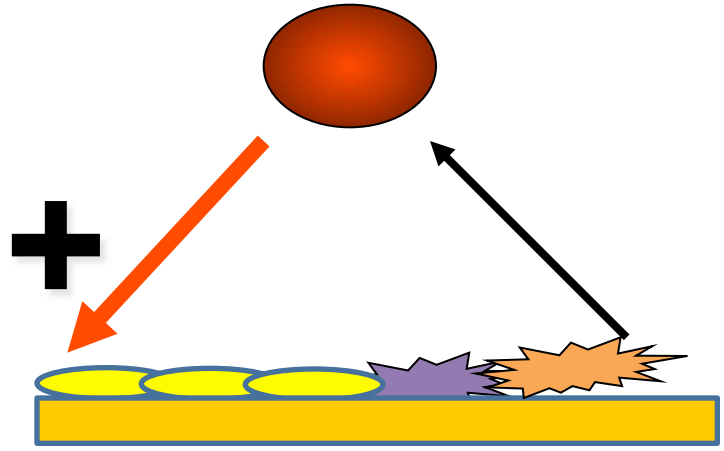


Marrow « Niche »

Protection from TKI toxicity
Induction of Quiescence

Weisberg et al. Mol Cancer Ther 2008

CML STEM Ph1

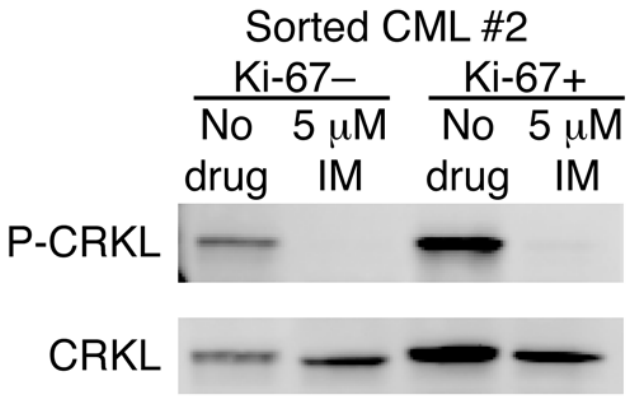


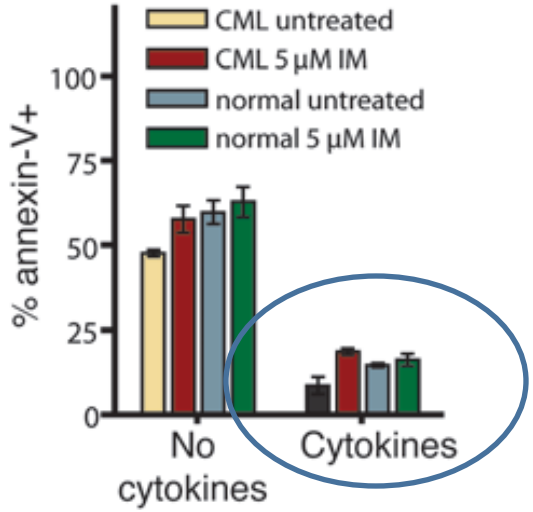
Normal Marrow Niche → « Abnormal » Marrow Niche

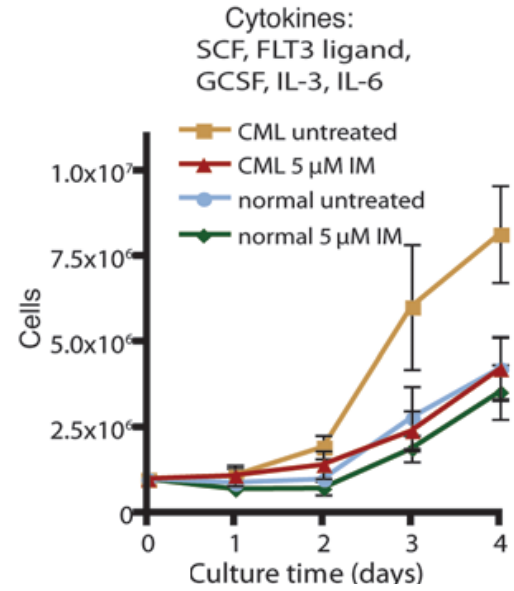
Contribution to CML resistance & persistence

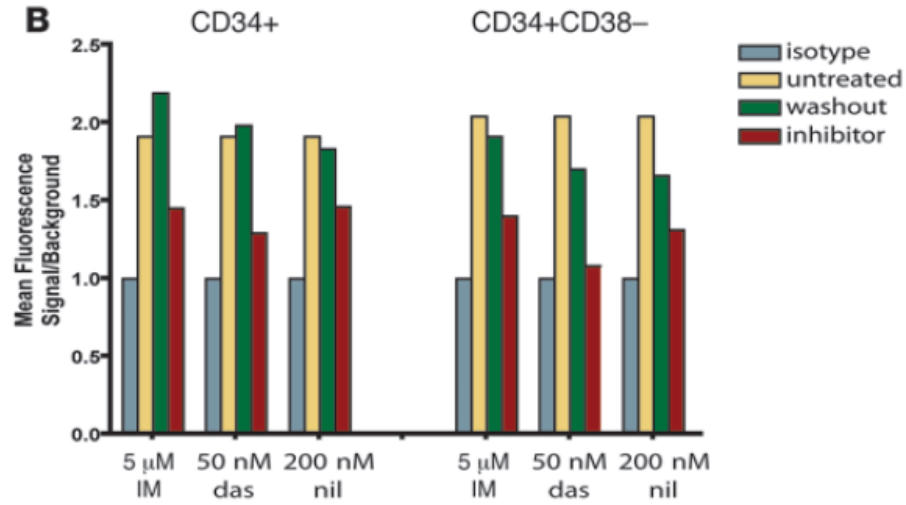
Schmidt et al, Cancer Cell 2011

ONCOGENE INDEPENDENCE AS A MECHANISM OF LSC RESISTANCE TO TKI



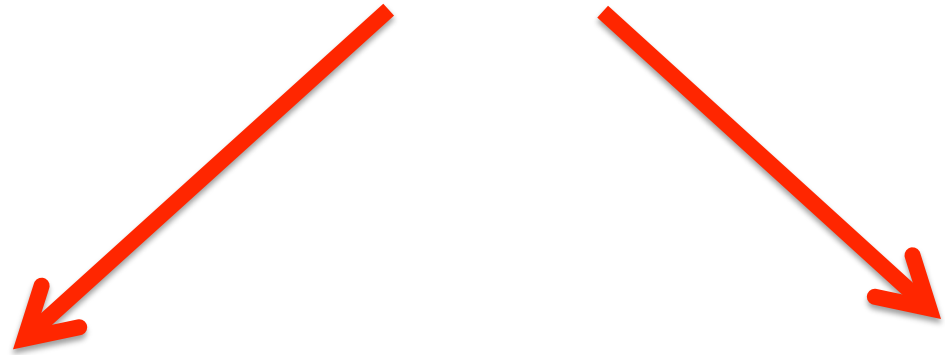






Corbin et al, JCI 2010

CML STEM CELLS: HOW TO BE « NON-ADDICT » TO BCR-ABL ?

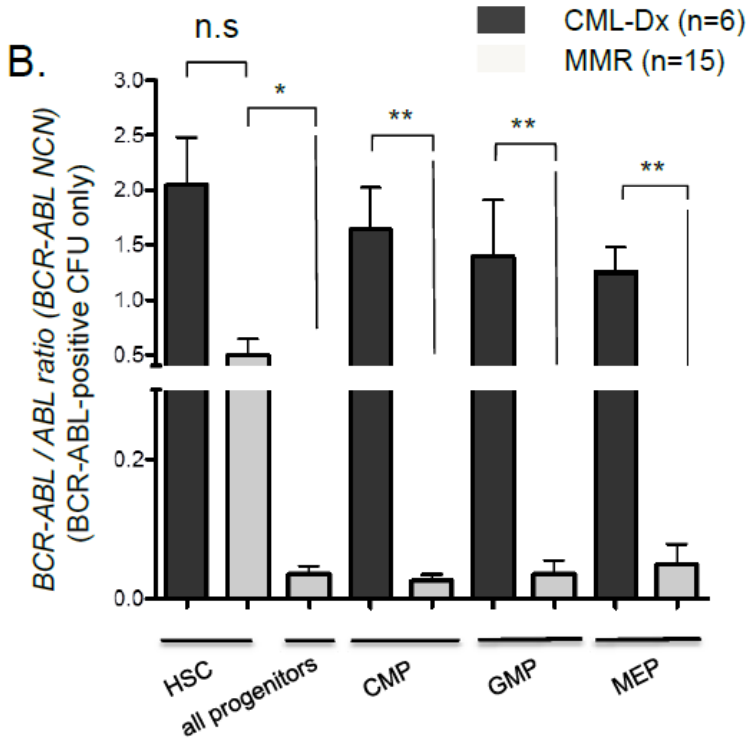


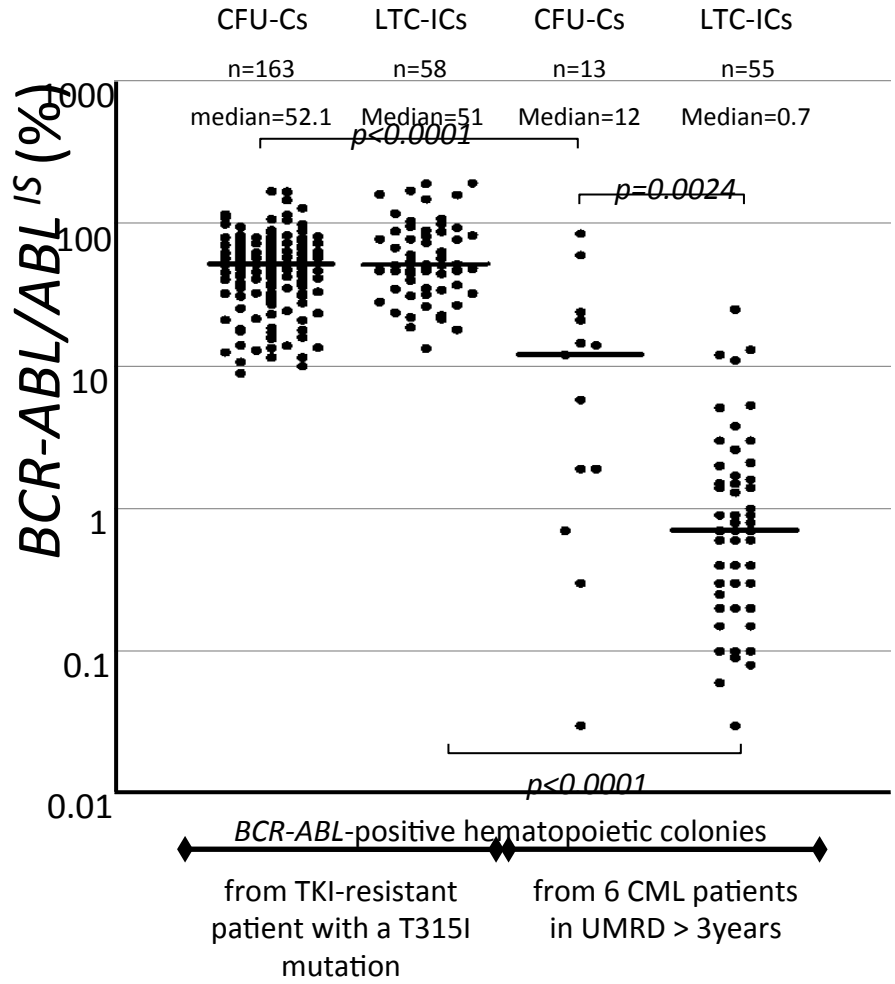
-Compensation of TK-induced signalling by other pathways

-Niche
-Intrinsic signalling

-Down-regulation of BCR-ABL expression

HIERARCHICAL AND TIME-DEPENDENT EXPRESSION OF BCR-ABL IN STEM CELLS

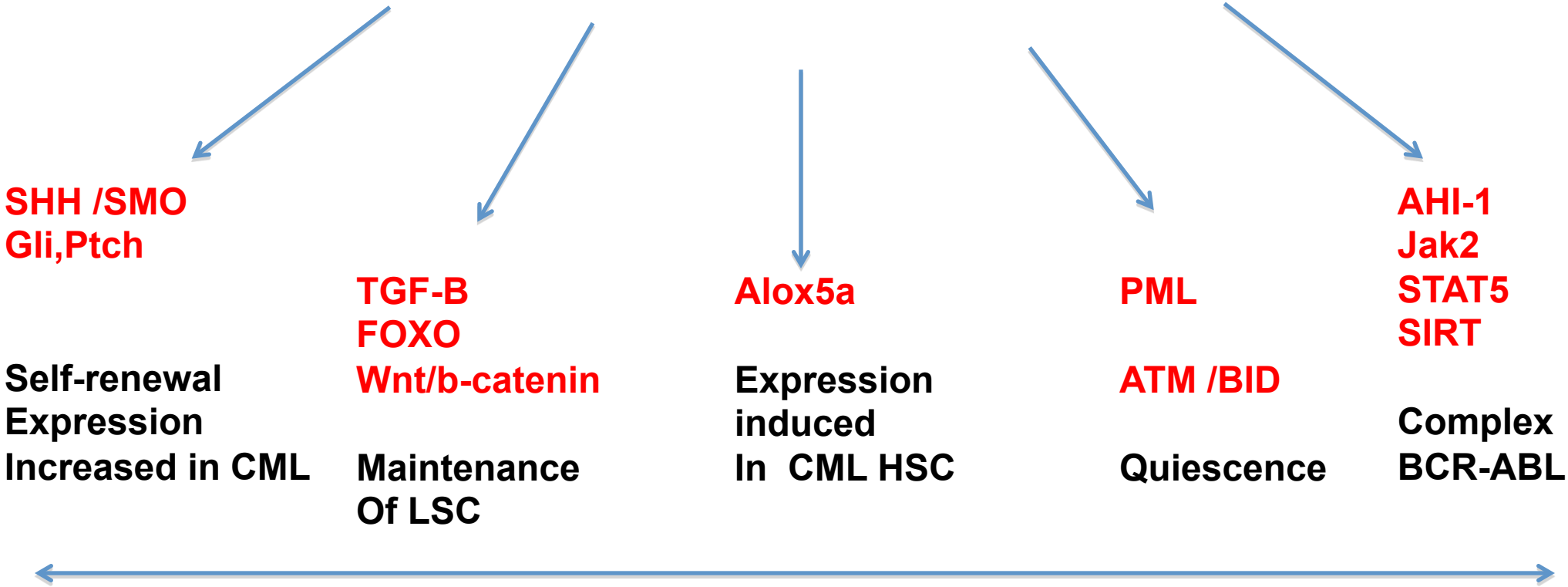




Kumari et al, Blood 2011
Chomel et al, Blood 2012

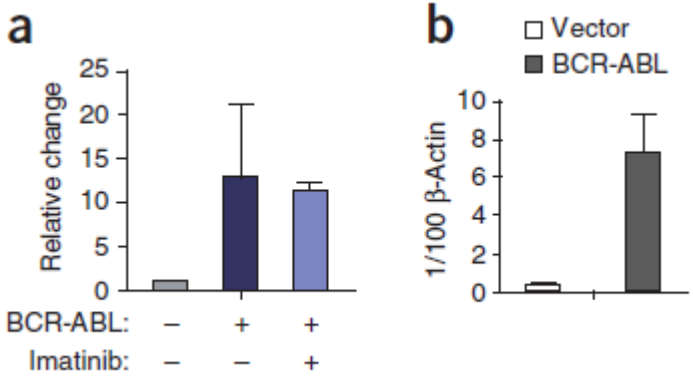
LOW LEVEL BCR-ABL-EXPRESSION IN MMR vs CMR:
LSC ARE NOT ADDICTED TO BCR-ABL

HOW TO TARGET CML HSC NON-DEPENDENT OF TK ACTIVITY OF BCR-ABL?

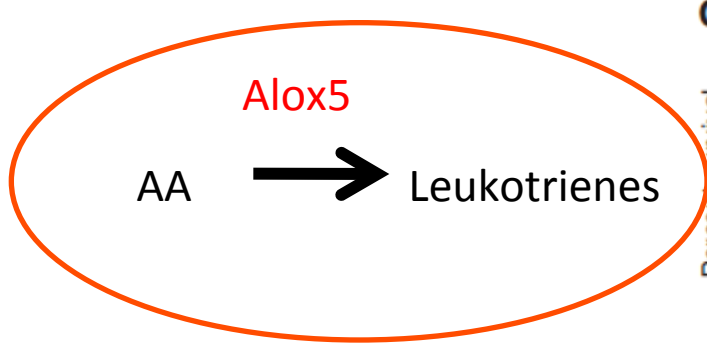


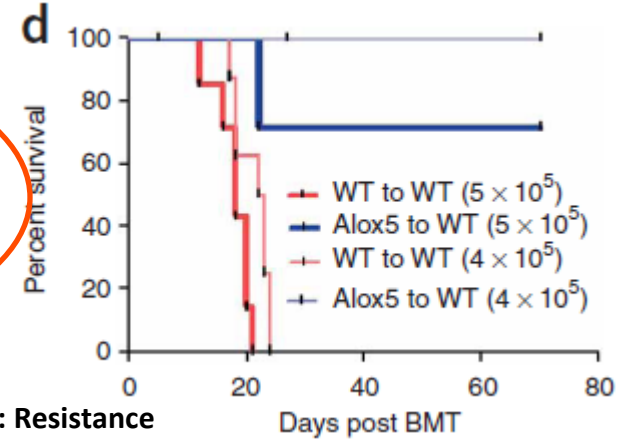
Naka et al, Nature 2010 ; Zhao et al, Cancer Cell 2007;
Dierks et al, Cancer Cell 2008; Ito et al, Nature 2008;
Chen Y et al, Nat Genet 2009; Zhao et al, Nature 2009
Zhou et al, J Exp Med 2008;

TARGETING ALOX5 GENE TO INHIBIT CML STEM CELLS



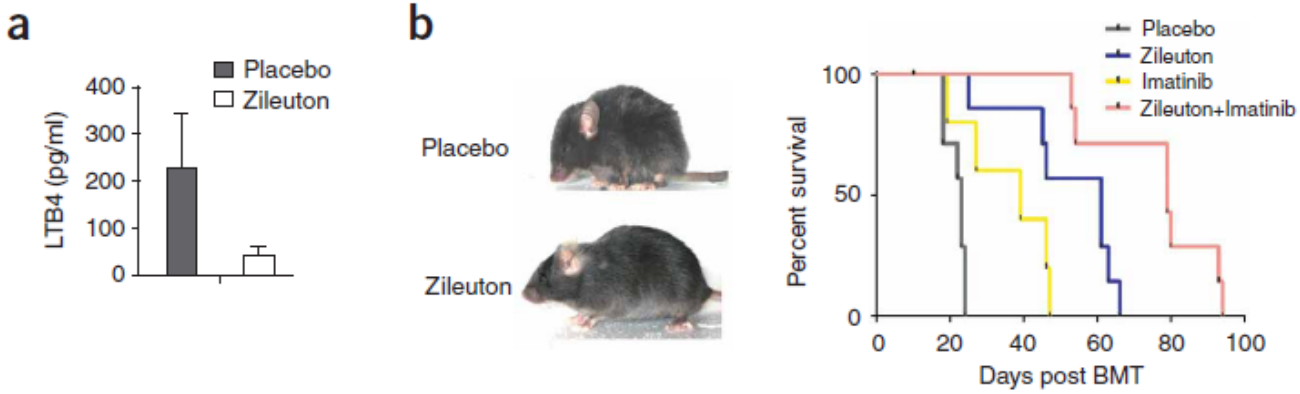
Arachidonate 5-Lipooxygenase(ALOX5) expression induced by BCR-ABL



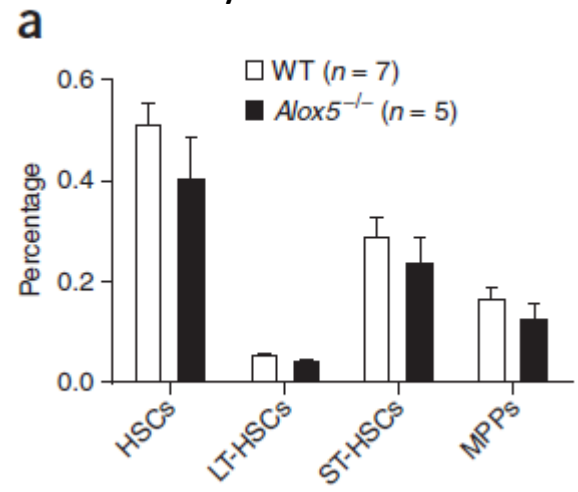


Souris Alox5-/-: Resistance À la leucemogenèse induite par BCR-ABL

Zileuton (ZYFLO) :
5-Lipooxygenase Inhibitor

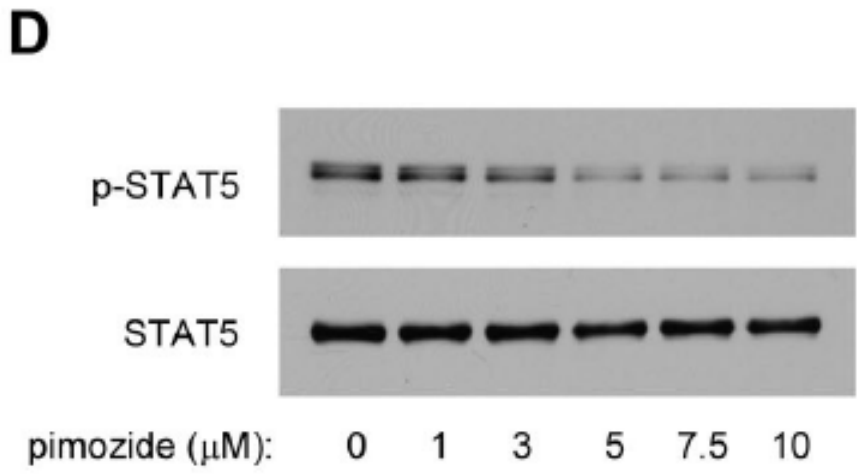


Alox5 deficiency: No effect on normal HSC



Chen et al, Nat Genetics, 2009

DUAL ACTIVATION OF STAT5PHOSPHORYLATION BY BCR-ABL AND JAK2 V617F



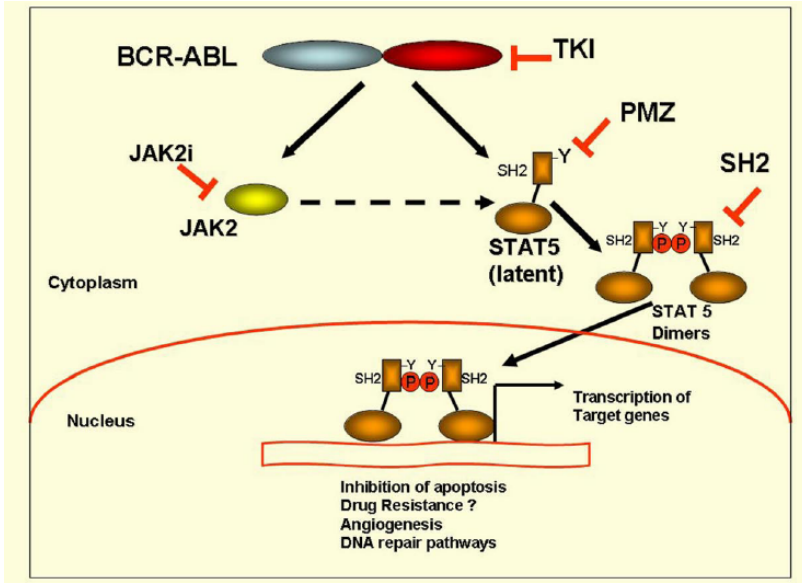


Table 2. The effect of pimozide on myeloid colony formation of CD34⁺ cells from CML patients and healthy donors

CD34 source/treatment	CFU-E	BFU-E	CFU-GM	CFU-GEMM
Healthy donors				
Vehicle	59 ± 10	139 ± 60	36 ± 12	12 ± 7
Pimozide	57 ± 8	107 ± 16	21 ± 10	12 ± 7
CML patients				
Vehicle	60 ± 12	17 ± 13	12 ± 14	0
Pimozide	0	0	0	0

Nelson et al, Blood 2011

STRATEGIES FOR CML STEM CELL TARGETING USING NOVEL MARKERS OF CML STEM CELLS ?

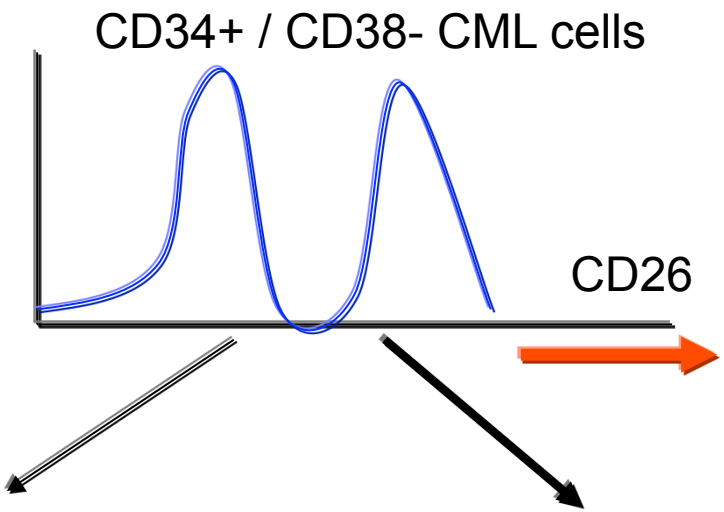
IL1-RAP

CD26

IL-2 R (CD25)

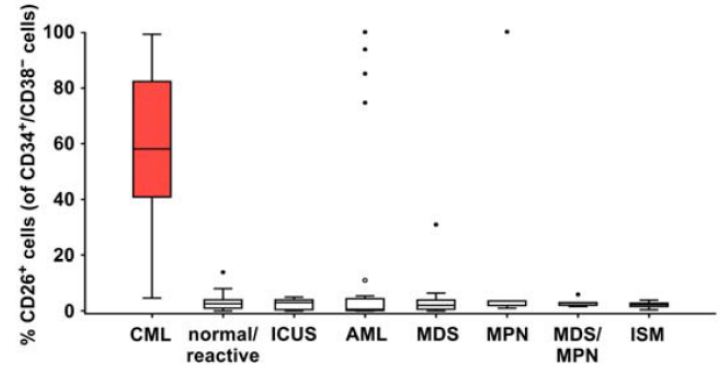
CD26 AS A CML STEM CELL MARKER

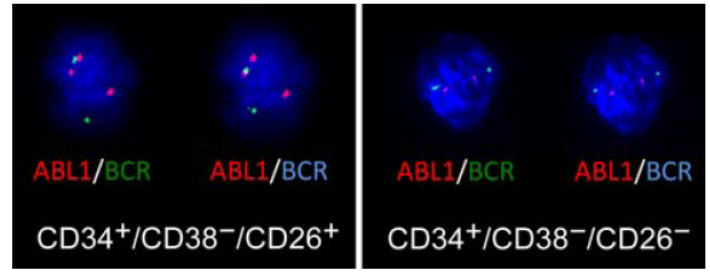
Highly expressed in CML CD34+ CD38- HSC
Not expressed in CML CD34+ CD38+ Cells
Expressed in AML
Not expressed in normal BM

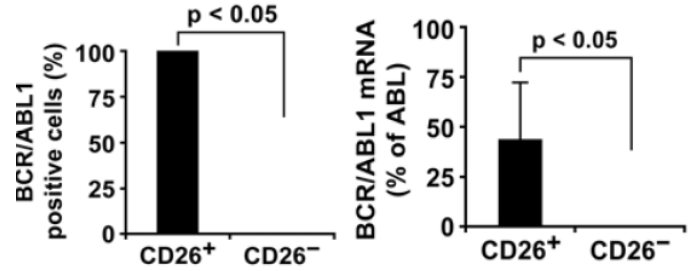


CD26-NEG FRACTION: FISH –NEG BCR-ABL

CD26-POS FRACTION: FISH –POS BCR-ABL

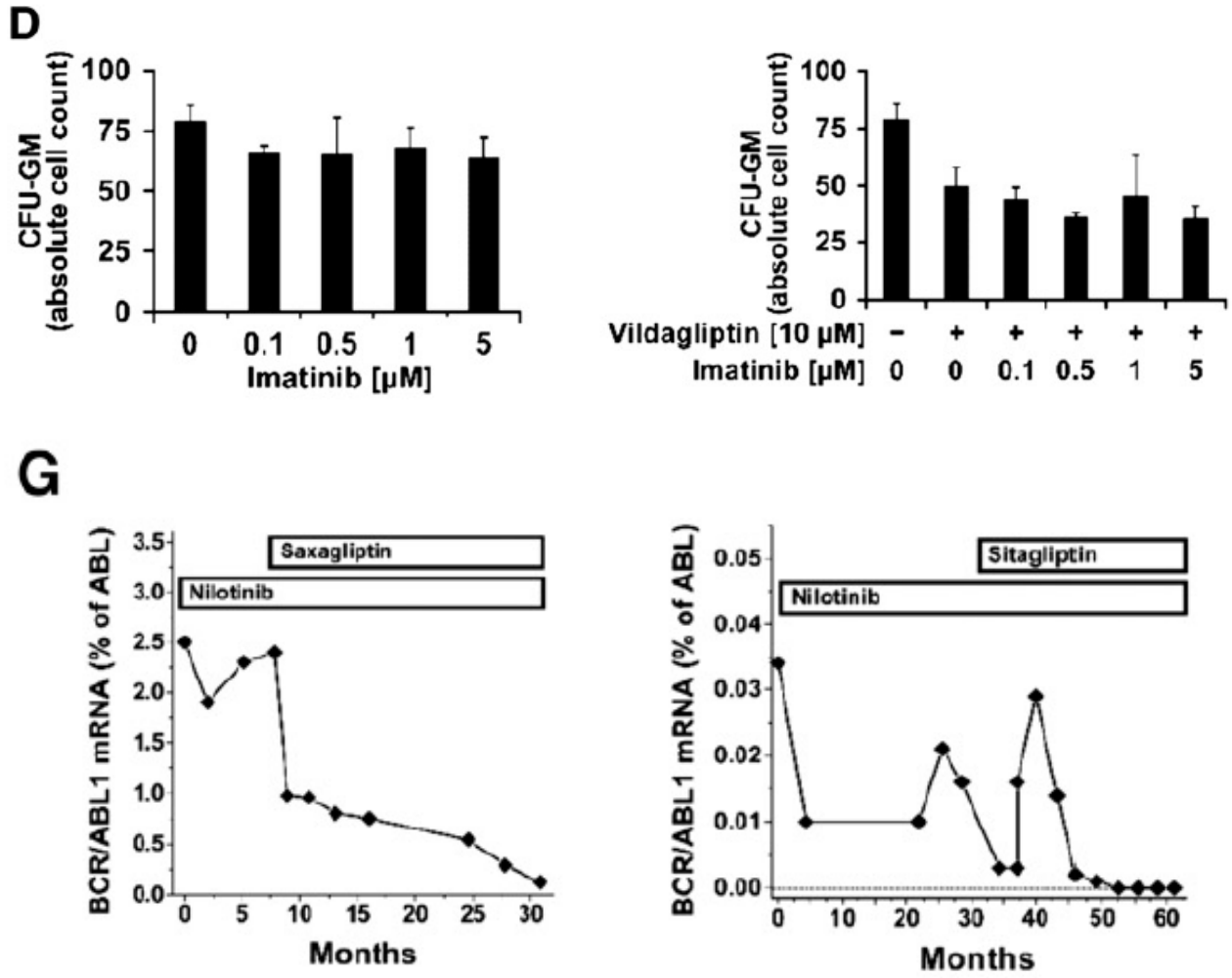






Herrmann et al, Blood 2014

CD26 INHIBITOR VILDAGLIPTIN TO TARGET CML STEM CELLS



Herrmann et al Blood 2014

Erosion of the chronic myeloid leukaemia stem cell pool by PPARγ agonists

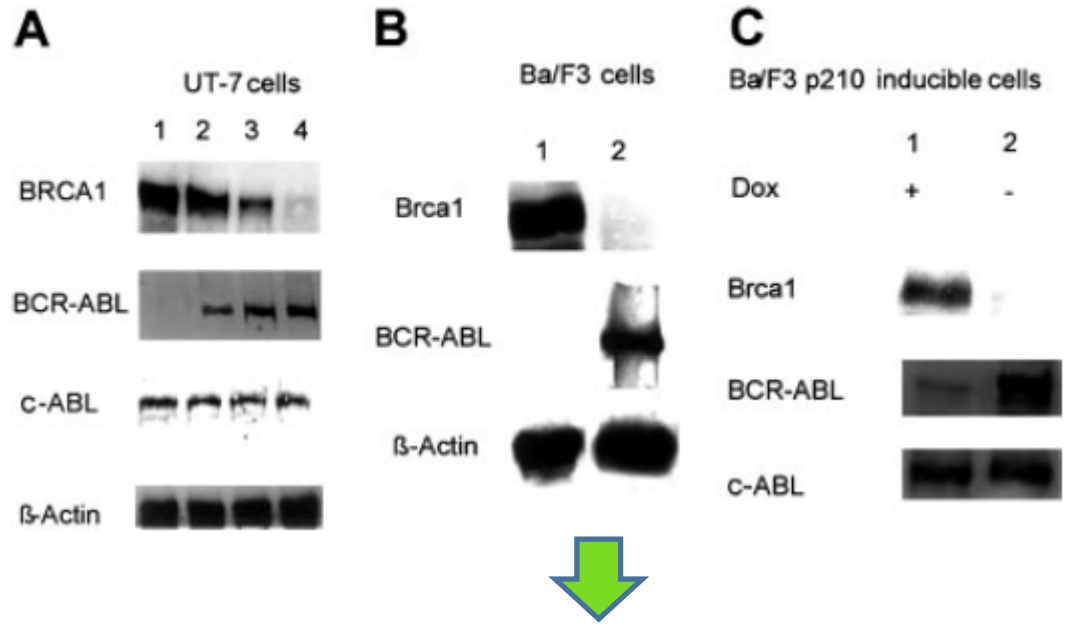
Stéphane Prost[1], Francis Relouzat[1], Marc Spentchian[2], Yasmine Ouzegdouh[1], Joseph Saliba[1], Gérald Massonnet[3], Jean-Paul Beressi[4], Els Verhoeyen[5,6], Victoria Raggueneau[7], Benjamin Maneglier[8], Sylvie Castaigne[9], Christine Chomienne[3], Stany Chrétien[1,10]*, Philippe Rousselot[3,9]* & Philippe Leboulch[1,11,12]*

Deregulated hedgehog pathway signaling is inhibited by the smoothened antagonist LDE225 (Sonidegib) in chronic phase chronic myeloid leukaemia

David A. Irvine[1,*], Bin Zhang[2,*], Ross Kinstrie[1], Anuradha Tarafdar[1], Heather Morrison[1], Victoria L. Campbell[1], Hothri A. Moka[1], Yinwei Ho[2], Colin Nixon[4], Paul W. Manley[3], Helen Wheadon[1], John R. Goodlad[5], Tessa L. Holyoake[1], Ravi Bhatia[6] & Mhairi Copland[1]

TARGETING LSC BY SYNTHETIC LETHALITY

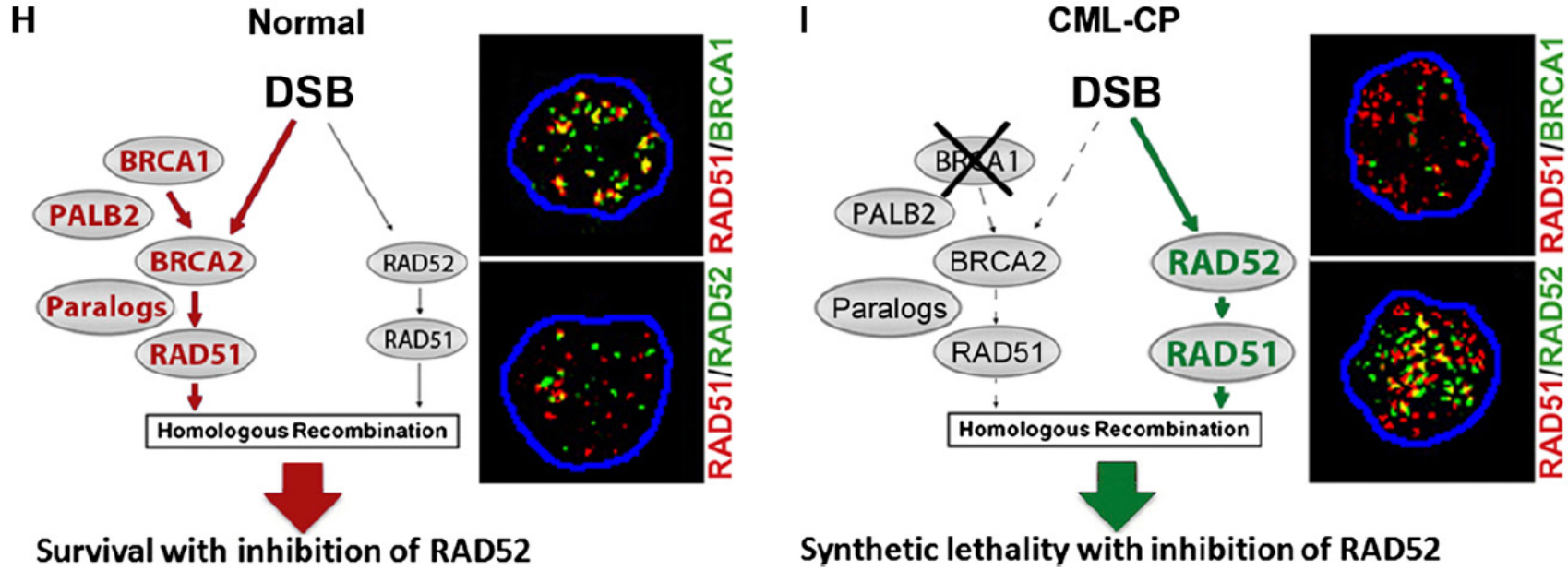
Down-regulation of BCR-ABL in BCR-ABL-expressing leukemic cells

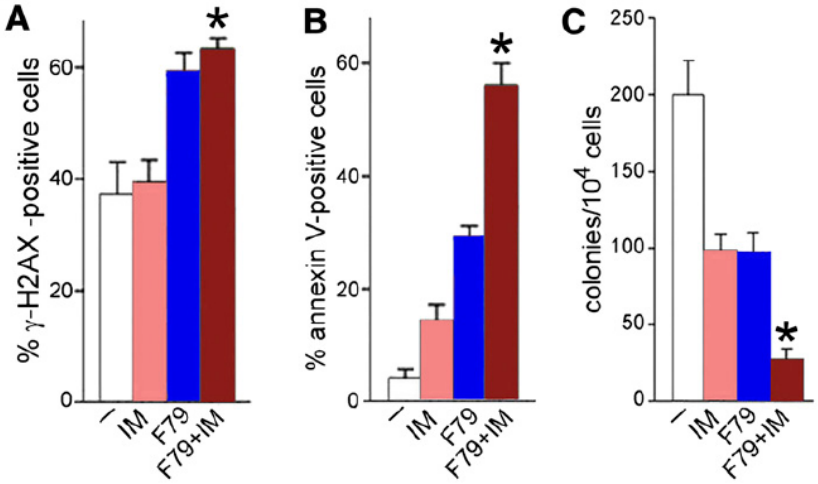


BCR-ABL-expressing Leukemic can not use BRCA1 for DNA repair

Deutsch et al, Blood 2003

TARGETING LSC VIA GENETIC INSTABILITY

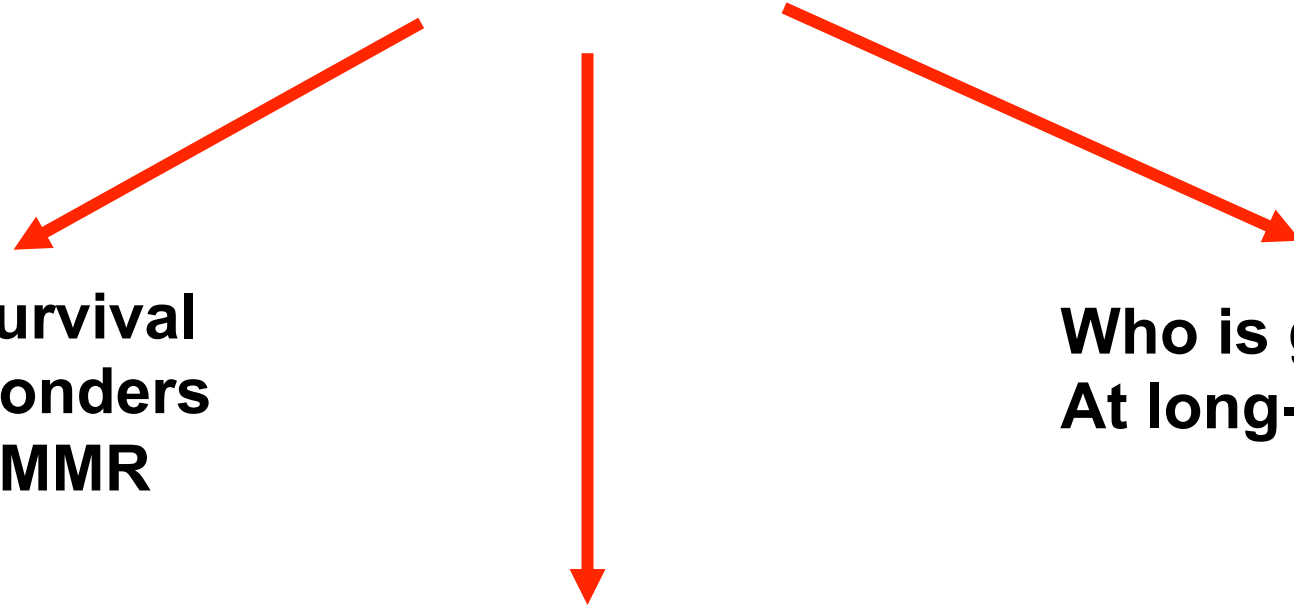




F79 APTAMER + TKI :
Synergistic effects on BCR-ABL-expressing stem cells

Cramer-Morales et al Blood 2013

CML IN THE ERA OF TARGETED THERAPIES: INCREASED SURVIVAL ON THERAPY BUT:

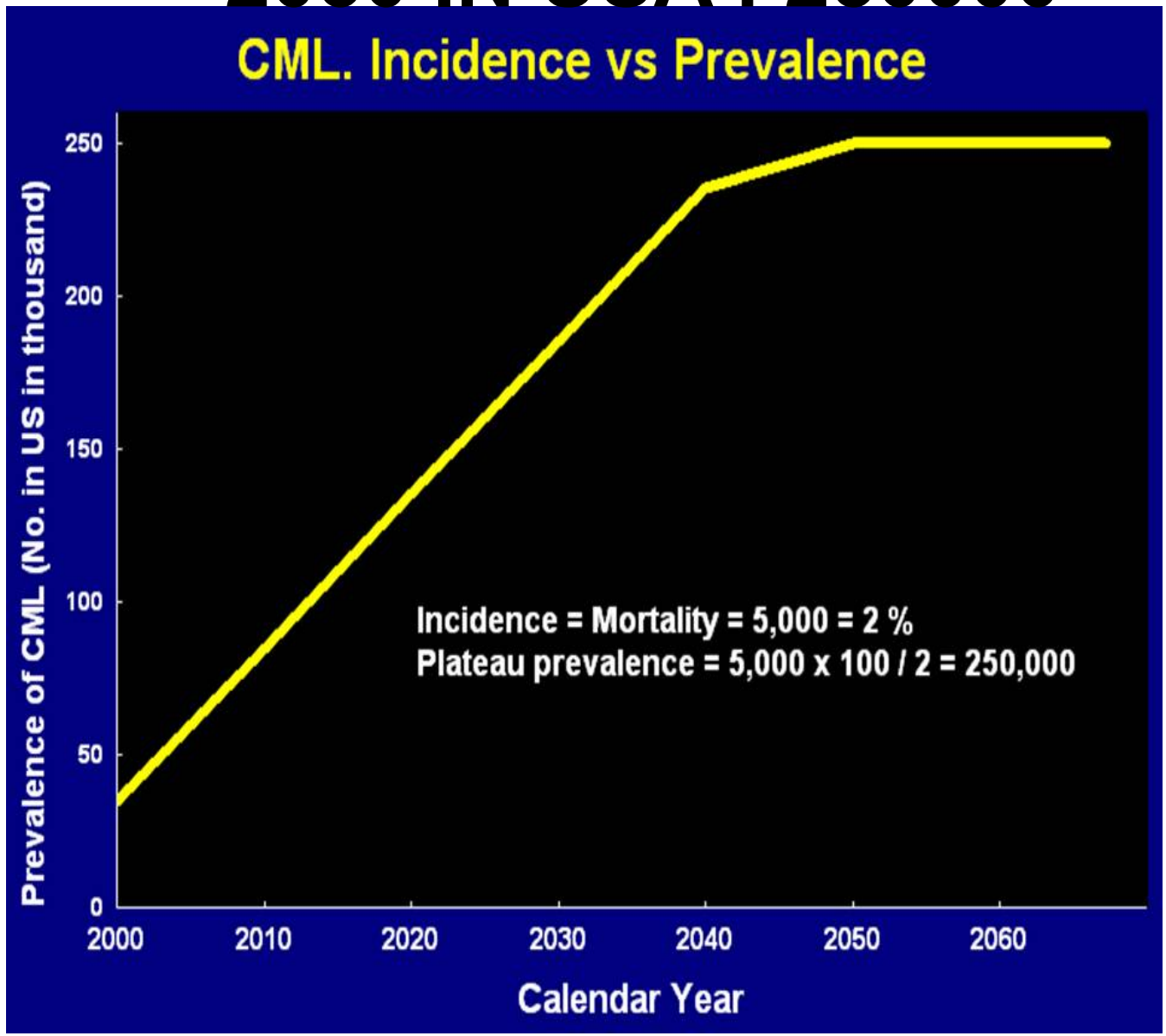


Increased survival only in responders (patients in MMR or less)

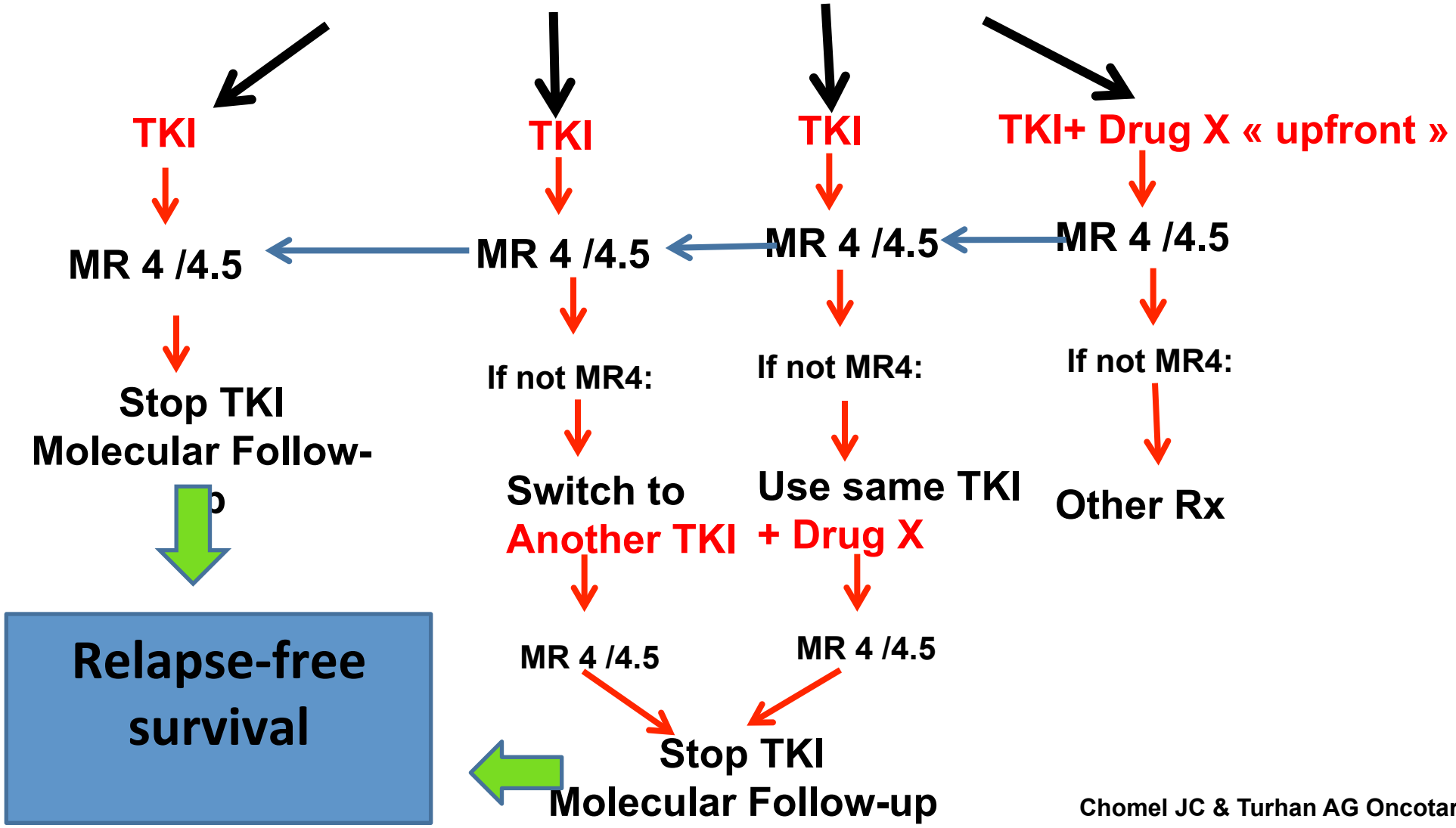
Long term effects of TKI are unknown

Who is going to pay At long-term ?

ESTIMATED PREVALENCE OF CML IN 2050 IN USA : 250000

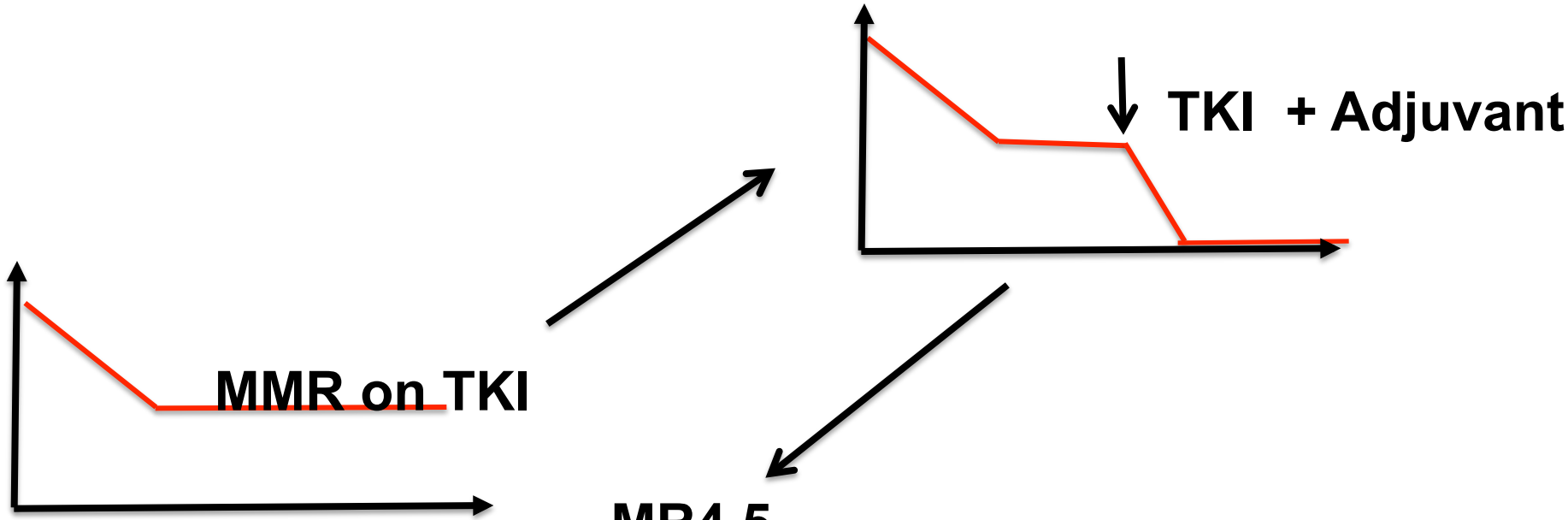


THERAPY-FREE REMISSION : WHAT IS THE BEST STRATEGY TO OBTAIN MR ?



Chomel JC & Turhan AG Oncotarget 2011

TFR and Concept of « Adjuvant » therapies of CML



D /C TKI: TFR ?

Chomel JC & Turhan AG Oncotarget 2012

POTENTIAL TARGETS FOR ADJUVANT THERAPIES….

COMPOUND	TARGET	STUDY
PIMOZIDE, PIOGLITAZONE	STAT5, PPAR-G	PIMOZIDE + IMATINIB* PIO + IMATINIB*
GLIPTINS	CD26	VILDAGLIPTINE + IMATINIB
INECALCITOL	VITAMIN D3 SIGNALLING	INECALCITOL + IMATINIB*
FTY720, FORSKOLIN (PAD)	PP2A	PAD + TKI
BP-5-087	STAT3	BP5 + TKI
TRAMETINIB	PRKCH	TRAMETINIB + TKI
F79 APTAMER	RAD52	F79 + TKI
EW-7197	TGF-b inhibitor	?

PAD: PP2A activating drugs
* Ongoing

Many runners but .. A winner ?

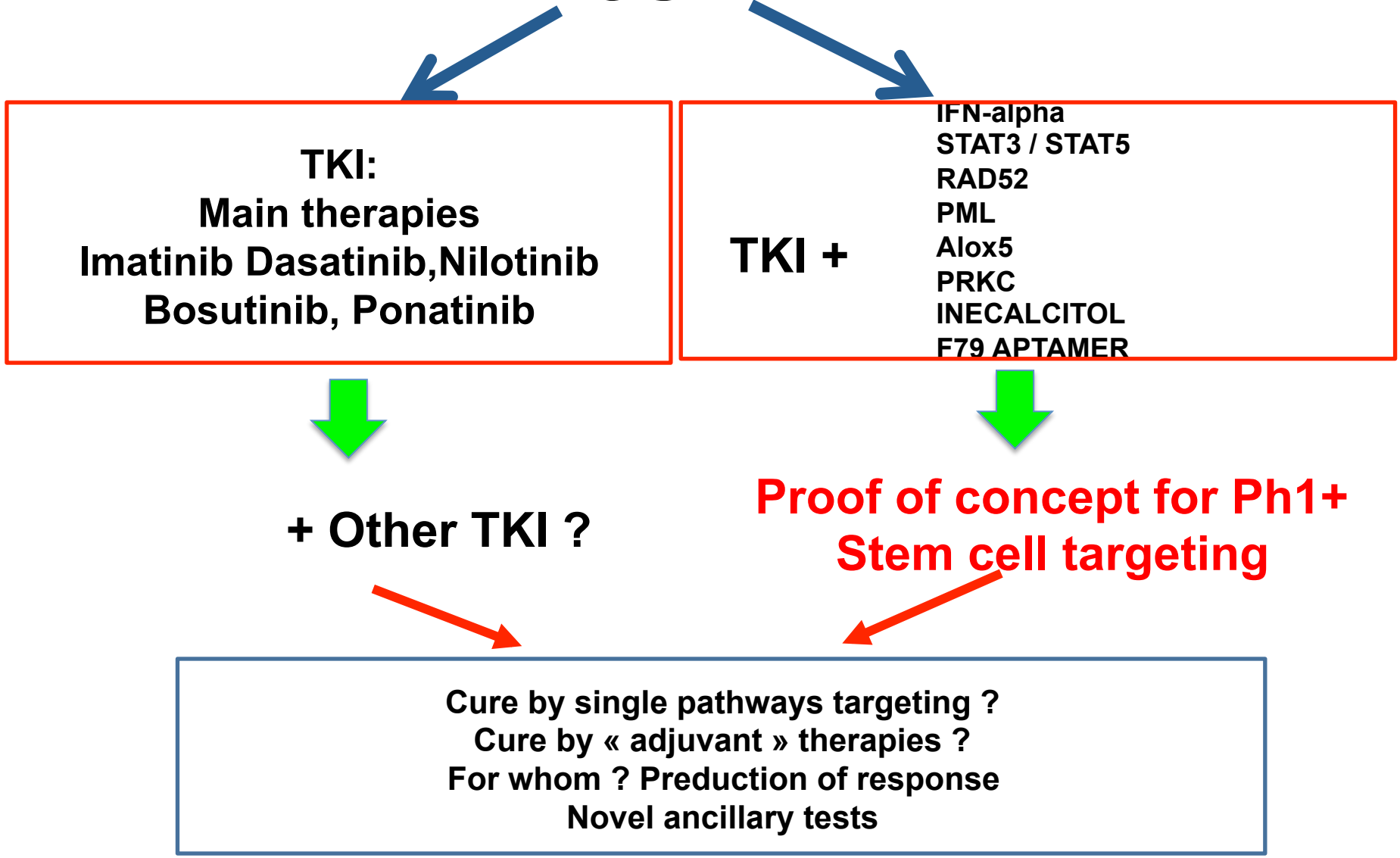
Chomel JC & Turhan AG Oncotarget 2011

TKI « COMBOS » UNDER CLINICAL INVESTIGATION

Combination	Phase
Imatinib/peg-IFN (Spirit)[1]	III
Nilotinib/Peg-IFN[2]	II
Dasatinib/Peg-IFN[3]	I
Dasatinib/zileuton[4]	IB
Dasatinib/nivolumab[5]	IB
Dasatinib/vorinostat[6]	I
Dasatinib/decitabine[7]	I/II
TKI + arsenic trioxide[8]	I
TKI + ruxolitinib[9]	I/II

1. Clinicaltrials.gov. NCT00219739. 2. Clinicaltrials.gov. NCT01866553. 3. Clinicaltrials.gov. NCT0172524. 4. Clinicaltrials.gov. NCT02047149.
5. Clinicaltrials.gov. NCT02011945. 6. Clinicaltrials.gov. NCT00816283. 7. Clinicaltrials.gov. NCT01498445. 8. Clinicaltrials.gov. NCT01397734.
9. Clinicaltrials.gov. NCT01914484.

TOWARDS HORIZON 2020: AIMING FOR CURE



INSERM U935

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