

The management of CML:
current treatment paradigms and future perspectives
TKI in CML

New generation TK inhibitors in patients with treatment resistance

Giovanni Martinelli, MD
Institute of Hematology and Medical Oncology
"L. e A. Seragnoli"

Policlinico Universitario Federico II Università' di Napoli
18-19 May 2009

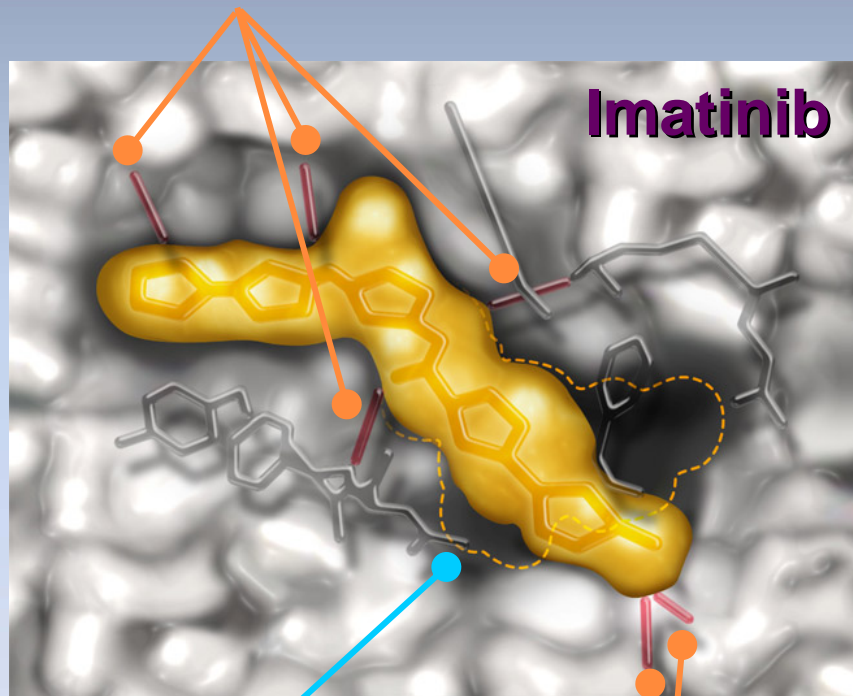
Novel TKIs in clinical trials for imatinib-resistant Ph+ leukemias

TK inhibitor	Company	Phase	Target(s)
Dasatinib (BMS-354825)	Bristol-Myers Squibb	Approved	Abl, Src
Nilotinib (AMN-107)	Novartis	II	Abl, kit, PDGFR
Bosutinib (SKI-606)	Wyeth	II	Abl, Src
MK-0457	Merck	I-II	Aurora
PHA-739358	Nerviano	II	Aurora
XL-228	Exelixis	I	Abl, Src, IGF1R
INNO-406 (NS-187)	Innovive	I	Abl, Lyn
SGX-70430	Sgx/Novartis	I	Abl
CCI779 (temsirolimus)	Wyeth	I	mTOR
RAD001 (everolimus)	Novartis	I	mTOR
Sorafenib (BAY 43-9006)	Bayer	I	Raf
UCN-01 (Staurosporine)	NCI	I	Chk1
KW-2449	Kyowa	I	Aurora
Deciphera	Decifera	I	Abl
AS703569	Merck Serono	I-II	Abl
AZD0530	Astra-Zeneca	I	Abl, Src

2. WHY New TKI?

Nilotinib Rationally Designed for a More Effective Binding to the Inactive Conformation of the ABL Kinase Domain

Hydrogen bonds form with specific amino acids lining the binding site

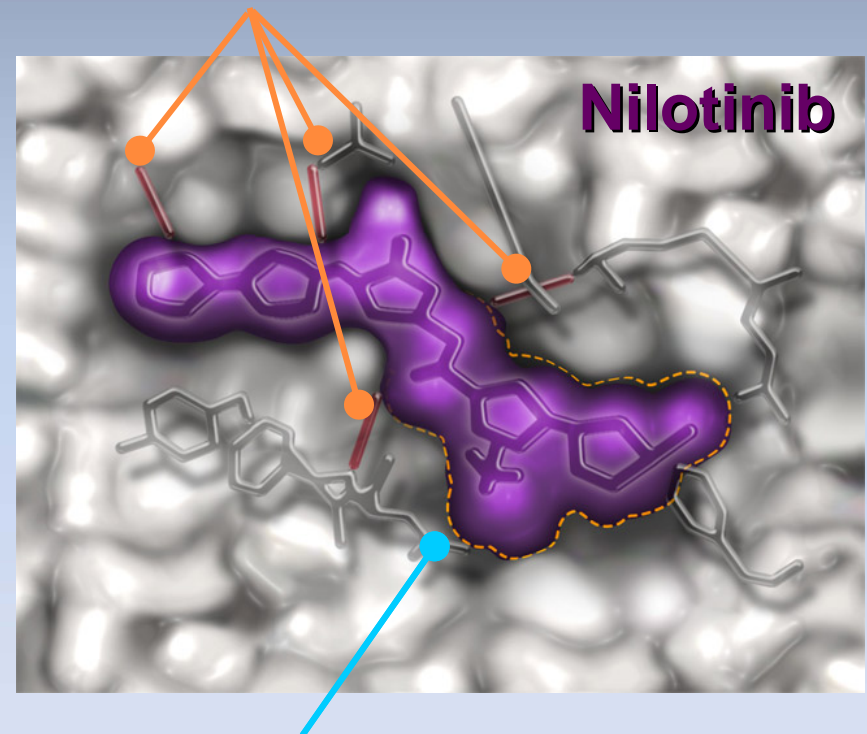


Imatinib

Auxiliary binding pocket

Hydrogen bonds with Ile360 & His361

Only maintains 4 hydrogen-bonds



Nilotinib

Improved fit to auxiliary pocket, via lipophilic interactions, **making it less susceptible to point mutations**

Nilotinib in Newly Diagnosed CML-CP (MDACC)

Response by Treatment

	Imatinib 400mg (MDACC) N=50	Imatinib 800mg (MDACC) N=205	Nilotinib (MDACC) N=47	Nilotinib (GIMEMA) N=41
Percent CCyR*				
3 months	37	62	97	84
6 months	54	82	100	97
12 months	65	86	95	100
Percent MMR**				
3 months	6	8	14	62
6 months	0	34	50	75
12 months	24	47	48	7

*Evaluable nilotinib patients: 39 at 3 mo, 33 at 6 mo, 19 at 12 mo

**Evaluable nilotinib patients: 42 at 3 mo, 36 at 6 mo, 21 at 12 mo Cortes J, et al. ASCO 2008. Abstract 7016.
Cortes JC, et al. European Hematology Association 2008. Abstract 0121.

Strategy to overcome resistance by use of new TKI

3 treatment strategies

1. BCR-ABL T315I active inhibitors
2. TKI active on leukemia stem cells
3. DNA repair inhibitors

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3 treatment strategies

1. BCR-ABL T315I active inhibitors

POST IMATINIB ERA: few mutations may confer resistance

	Cellular proliferation					
	Imatinib		AMN107		BMS-354825	
	IC ₅₀ (nmol/L)	Fold change	IC ₅₀ (nmol/L)	Fold change	IC ₅₀ (nmol/L)	Fold change
WT Bcr-Abl	260	1	13 (28) [†]	1	0.8 (2.6) [†]	1
M244V	2,000	8	38 (56)	3	1.3 (1.8)	2
G250E	1,350	5	48 (152)	4	1.8 (3)	2
Q252H	1,325	5	70 (156)	5	3.4 (11.2)	4
Y253F	3,475	13	125 (215)	10	1.4 (3)	2
Y253H	>6,400	>25	450 (1,024)	35	1.3 (3)	2
E255K	5,200	20	200 (415)	15	5.6 (12.8)	7
E255V	>6,400	>25	430 (2,000)	33	11 (27)	14
F311L	480	2	23 (48)	2	1.3 (2.8)	2
T315I	>6,400	>25	>2,000	>154	>200	>250
F317L	1,050	4	50 (116)	4	7.4 (15)	9
M351T	880	3	15 (31)	1.2	1.1 (3)	1.4
F359V	1,825	7	175 (385)	13	2.2 (4.8)	3
V379I	1,630	6	51 (115)	4	0.8 (1.6)	1
L387M	1,000	4	49 (115)	4	2 (4)	3
H396P	850	3	41 (69)	3	0.6 (1.2)	0.8
H396R	1,750	7	41 (73)	3	1.3 (2.7)	2
Parental Ba/F3	>6,400	>25	>2,000	>154	>200	>250

New BCR-ABL T315I TKI inhibitors

TK inhibitor	Company	Phase	Target(s)
MK-0457	Merck	Hold	Aurora, Jak2, Abl
PHA-739358	Nerviano	II	Aurora , Abl,
XL-228	Exelixis	I	Aurora, Abl, Src, IGF1R, Jak2
KW-2449	Kyowa	I	Aurora
Mserono AS703569	Merck Serono	I	Aurora, Jak2, Abl
AZD0530	Astra-Zeneca	I	Aurora, Abl, Src
SGX-70430	Sgx/ Novartis	I	Abl
Deciphera	Decifera	Pre clin.-I	Abl

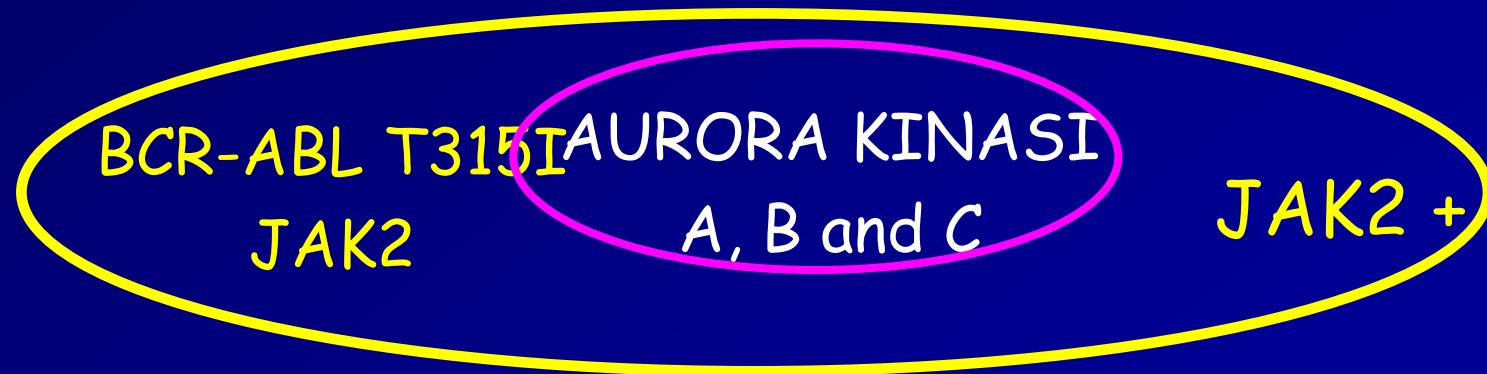
Razionale

PHA-739358

MK-0457

AS703569

AZD-1152



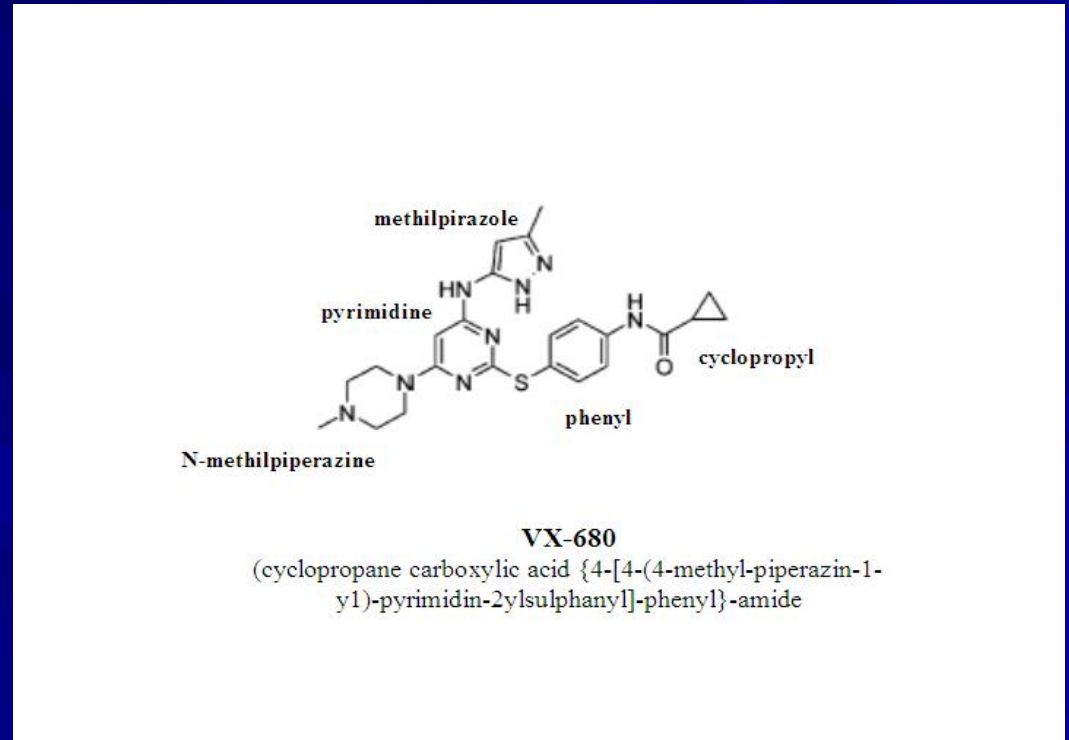
Ph⁺ ALL or CML

AML

PV TE and MF

MK-0457 (VX-680)

- ATP-competitive small molecule kinase inhibitor
- Inhibits aurora kinase A/B/C; FLT3; BCR-ABL; JAK2
- Induces apoptosis at nM levels in wide range of tumors
- Minimal toxicity in animals
- No effect on non-cycling cells



Harrington. Nat Med 10: 262, 2004 Carter.

PNAS 102: 11011, 2005;

Giles. Blood, 2006

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PHA-739358 (Nerviano Medical Sciences)

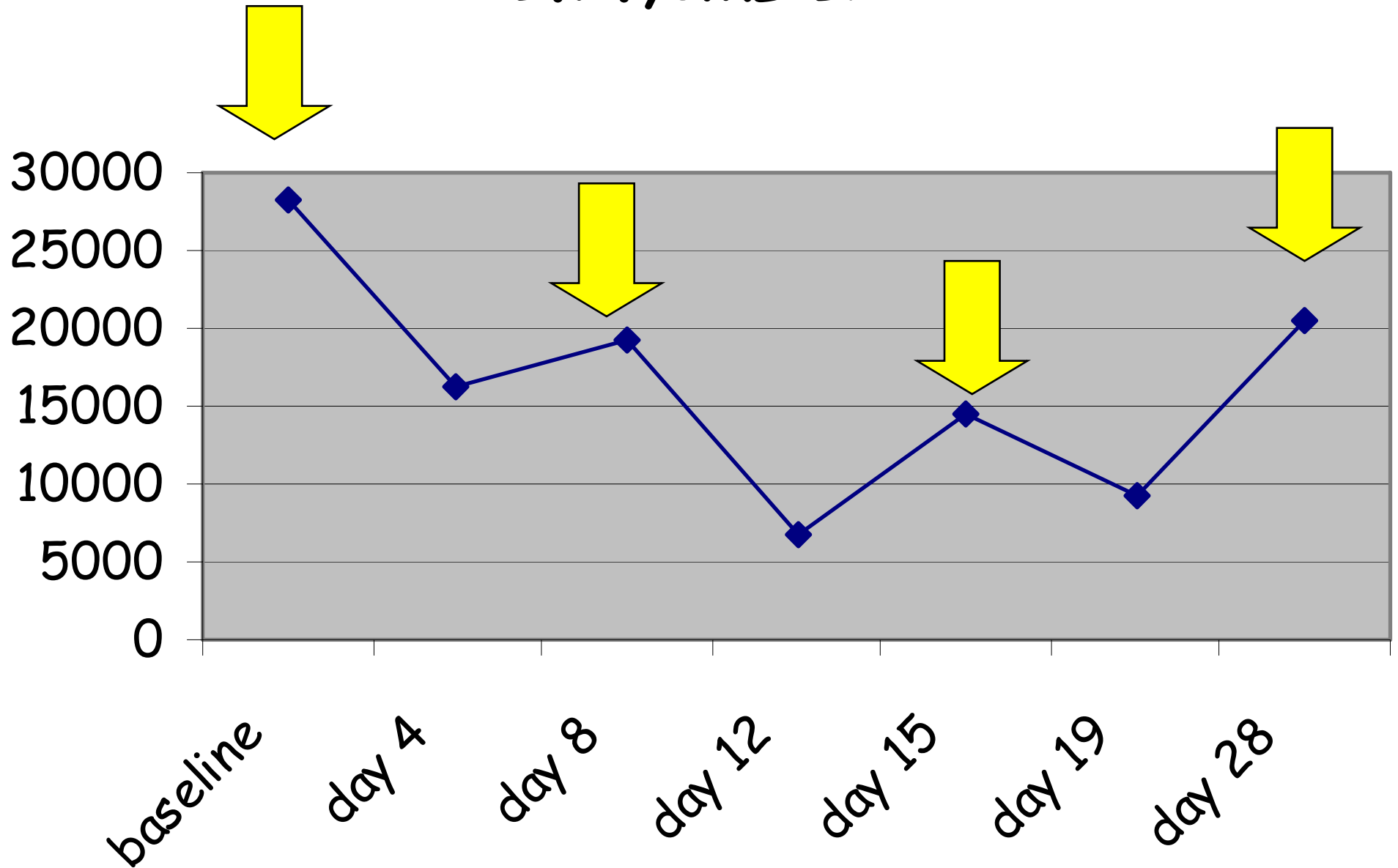


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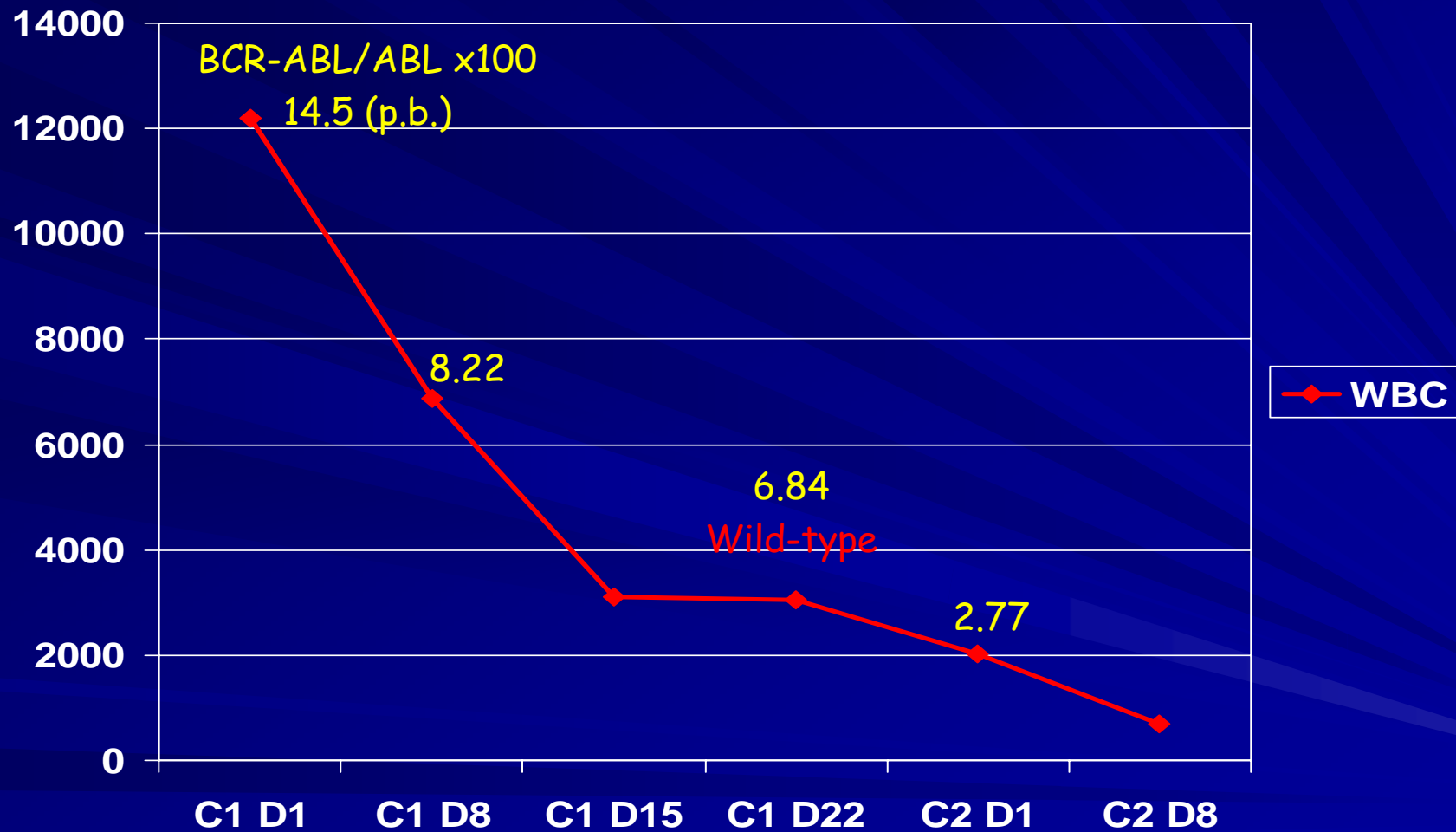
Mutational Status

11 patients	Wild-type
1 patient	Y253H
4 patients	T315I
1 patient	M351T
1 patient	T315A
1 patient	G250E, Y253H, F317L
1 patient	V299L
1 patient	V299L, H396P

I.R., CML BP



F.M., 70 aa, T315I mut



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Oral Pan-TK Inhibitor AS703569

**Study 27335:
Phase I-II in subjects with haematological
malignancies ...**

**TARGETS:
BCR-ABL including T315I
Jak2
Chk1-Chk2**

AS7035XX Kinase profiling on 127 kinases



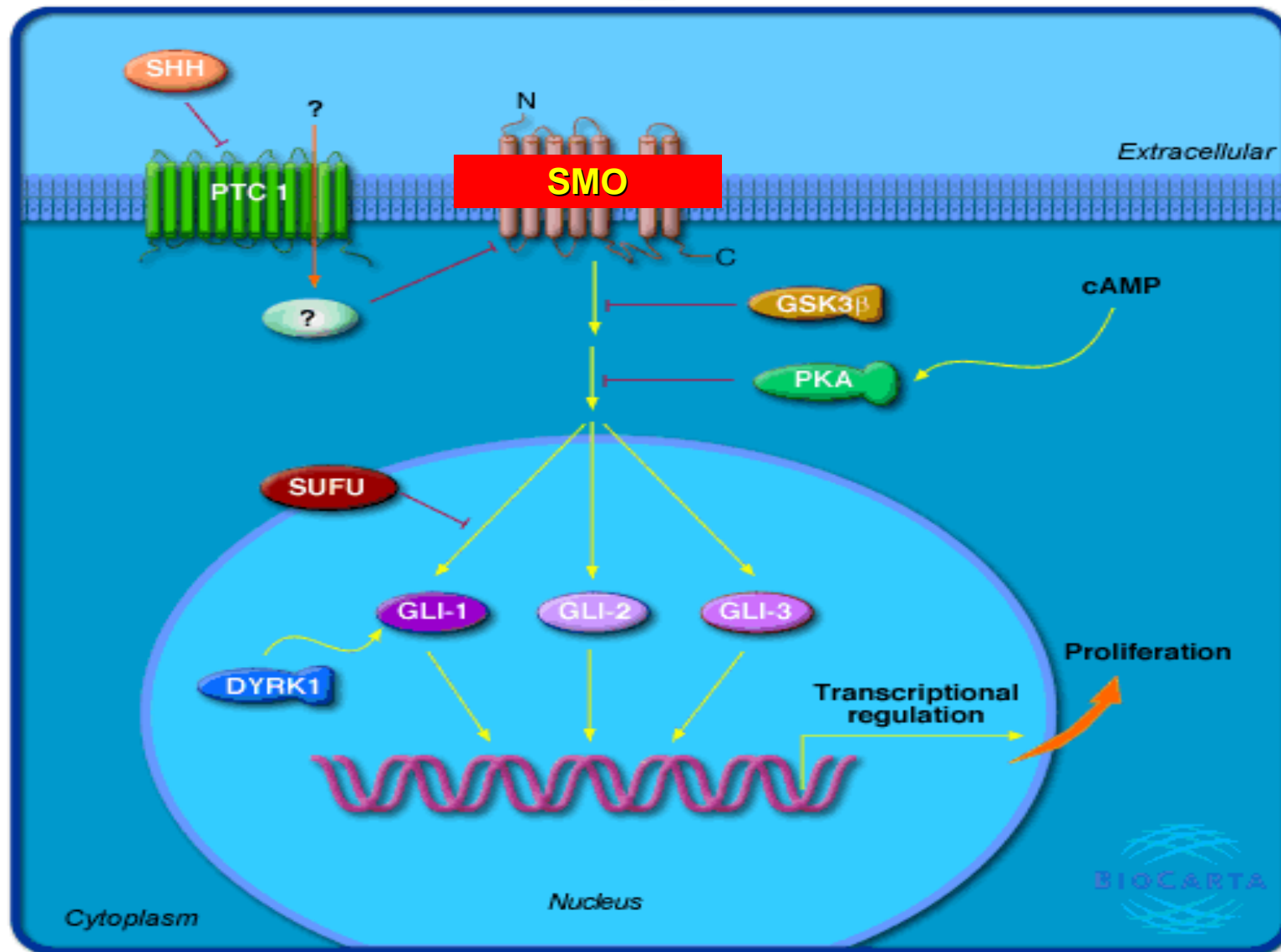
IC ₅₀ (nM)	N	Kinases
X <10	15	Aurora-A (6nM), Flt3, Blk, cSRC, FGFR1, Fgr, Flt1, Fyn, Lyn, Ret, Rsk1, Rsk2, Rsk3, Yes, AMPK
10 ≤ x <60	28	Abl, Abl(T315I), Arg, Axl, BRK, BTK, CHK2, FGFR3, FGFR4, Fms, JNK3, Flt3(D835Y), JNK1α1, Lck, Met, PKCμ, TAK1, ALK, CHK1, ErbB4, Hck, MINK, MST2, PAK2, PKCδ, PRK2, Pyk2, TrkA
60 ≤ x <600	36	Bmx, JNK2α2, MSK2, PKD2, Tie2, cKit, EphA2, Fer, Fes, IRAK4, PAR-1Bα, PDGFRβ, PKCα, PKCβII, PKCε, PKCθ, RIPK2, Ron, Syk, TrkB, CDK7, Rse, ASK1, CaMKII, CDK5, cKit(D816V), EGFR, EphB2, EphB4, MKK4, MKK7β, NEK2, p70S6K, PDGFRalpha, PDK1, ROCK-I
x ≥ 600	48	

Strategy to overcome resistance by use of new TKI

3 treatment activities

1. BCR-ABL T315I active inhibitors
2. TKI active on leukemia stem cells

Wnt and Hedgehog signaling sustain CML LSC self renewal



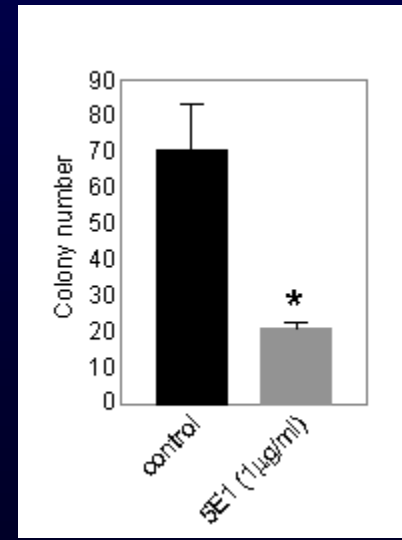
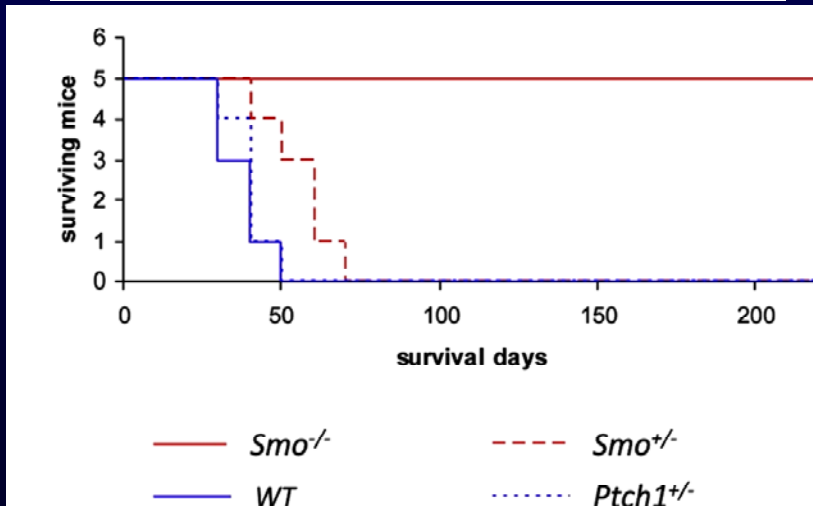
Deletion of Smo impairs the development of disease and depletes stem cells in a mouse model of BCR-ABL-induced CML

Fetal WBC D20 GFP analysis PB (B) 100 CML 10

Inhibition of Smo by TKI is a new target in Ph+ leukemia stem cell

Ptch1^{+/-}

Days Elapsed



Hh ligand-driven signaling:
Bcr-Abl + KLS (progenitor) cells respond to inhibition of Shh ligand

Dierks 2008 Cancer Cell 14: 238-249

Zhao 2009 Nature

Strategy to overcome resistance by use of new TKI

3 treatment strategy

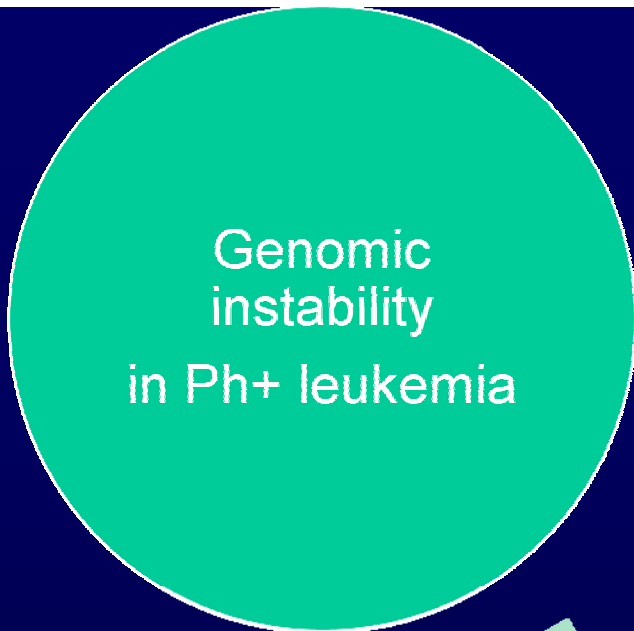
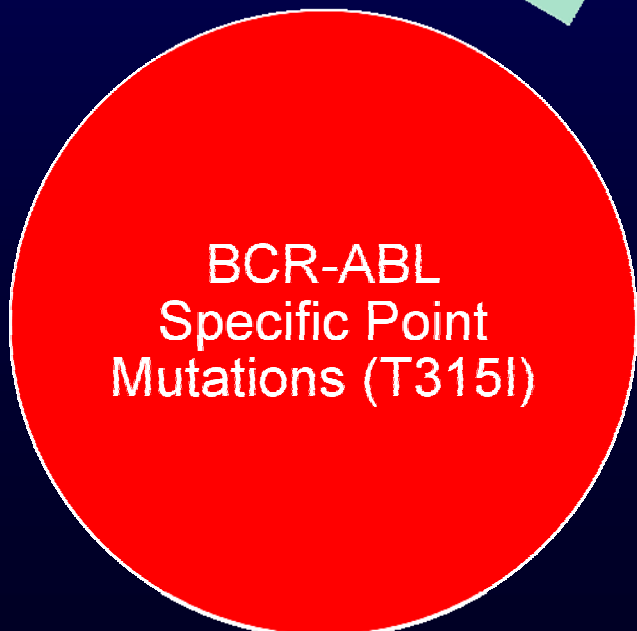
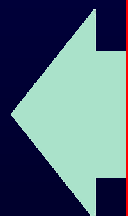
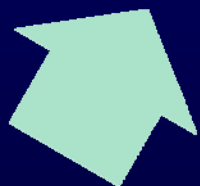
1. BCR-ABL T315I active inhibitors
2. TKI active on leukemia stem cells
3. DNA repair inhibitors

Genomic
instability
in Ph+ leukemia

BCR-ABL
Specific Point
Mutations (T315I)

AID
PARP
inhibitors

Over- expression



Take home message

- In response to different targeted treatment protocols, we can now characterize the in vivo emergence and spectrum of drug-resistant BCR-ABL mutations.
- **New TKI active on T315I mutation are in clinical development.**
- Self renewal pathway of CML leukemia stem cell, involving Smo and Wnt1 signaling, may be the next new targets for eradication of CD34+ LSC.
- **DNA repair system, which is over-expressed in CML, and contributes to genomic instability and leukemia progression, may be specifically targeted by new generation TKI inhibitors.**

ACKNOWLEDGMENTS

Michele Baccarani

Dpt of Hematology/Oncology "Seràgnoli", Bologna

Ilaria Iacobucci, Simona Soverini, Cristina Papayannidis, Emanuela Ottaviani, Annalisa Lonetti, Nicoletta Testoni, Carmen Baldazzi, Giulia Marzocchi, Sabrina Colarossi, Alessandra Gnani, Anna Ferrari, Federica Salmi, Stefania Paolini, Panagiota Giannoulia, Barbara Lama, Pier Paolo

Piccaluga

Pediatric Oncology and Hematology "Lalla Seragnoli"

Annalisa Astolfi, Serena Formica, Andrea Pession

Dpt di Scienze Cliniche e Biologiche, Orbassano (TO)

Francesca Messa, Francesca Arruga, Enrico Bracco, Daniela Cilloni, Giuseppe Saglio

University "La Sapienza", Rome

Robin Foà, Antonella Vitale, Sabina Chiaretti, Monica Messina, Giovanna Meloni

Department of Genetics and Microbiology University of Bari

Clelia Tiziana Storlazzi, Rocchi Mariano

Department of University of Naples

Fabrizio Pane