2017

Progetto
Ematologia
Romagna

LMC: la malattia resistente
Basi biomolecolari

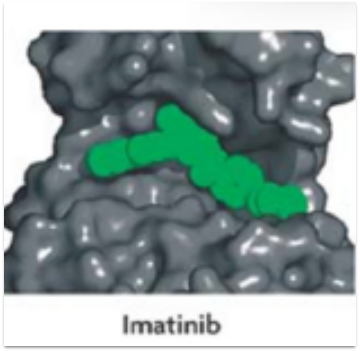
Manuela Mancini

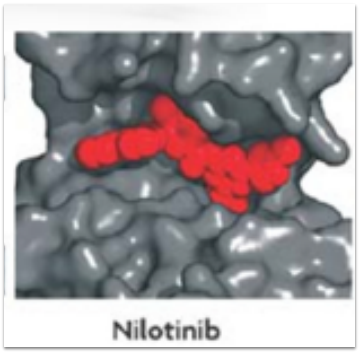
WHAT IS DRUG RESISTANCE?

Leukemic cells become resistant to the drug (TKI cannot inhibit BCR-ABL or kill BCR-ABL+ cells any more)

The TKI stops working for some reason

Look for other changes in the chromosomes or genes (mutations), so “the key” (TKI) does no longer fit into “the lock” (BCR-ABL)





A LOT ABOUT OTHER MECHANISMS ARE STILL UNKNOWN

Imatinib Resistance in Chronic Phase CML: Definitions

- Resistance can be defined as **primary** (lack of acceptable initial response) or **secondary** (loss of an established response)

Primary hematologic resistance refers to failure to achieve a CHR within 3-6 months of initiating imatinib (~2-4 % of cases*)

Primary cytogenetic resistance can be defined as:

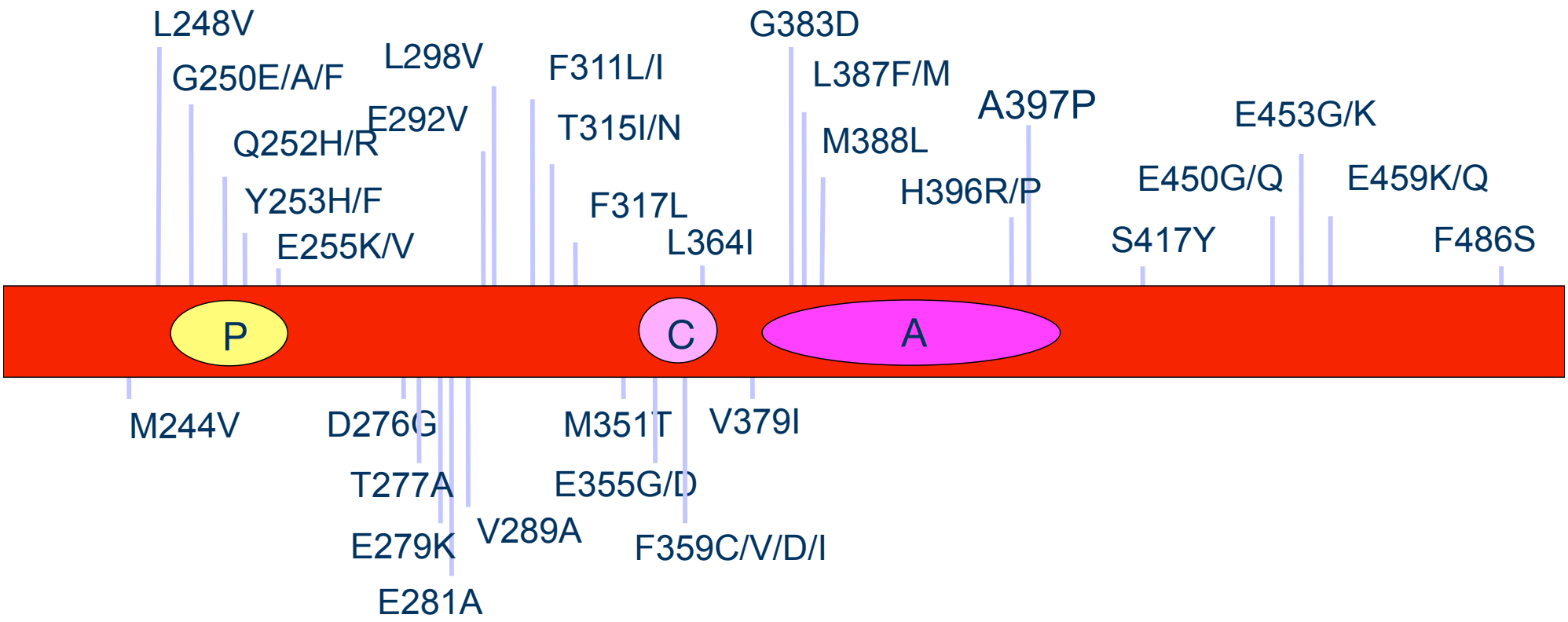
- Lack of any cytogenetic response by 6 months (~22% of cases* - IRIS study)
- **Lack of MCyR by 12 months** (~15% of cases* - IRIS)
- **Lack of CCyR by 18 months** (~25% of cases* - IRIS)

Secondary resistance refers to progression after an established hematologic or cytogenetic response

Clinical Resistance to Imatinib: Mechanisms

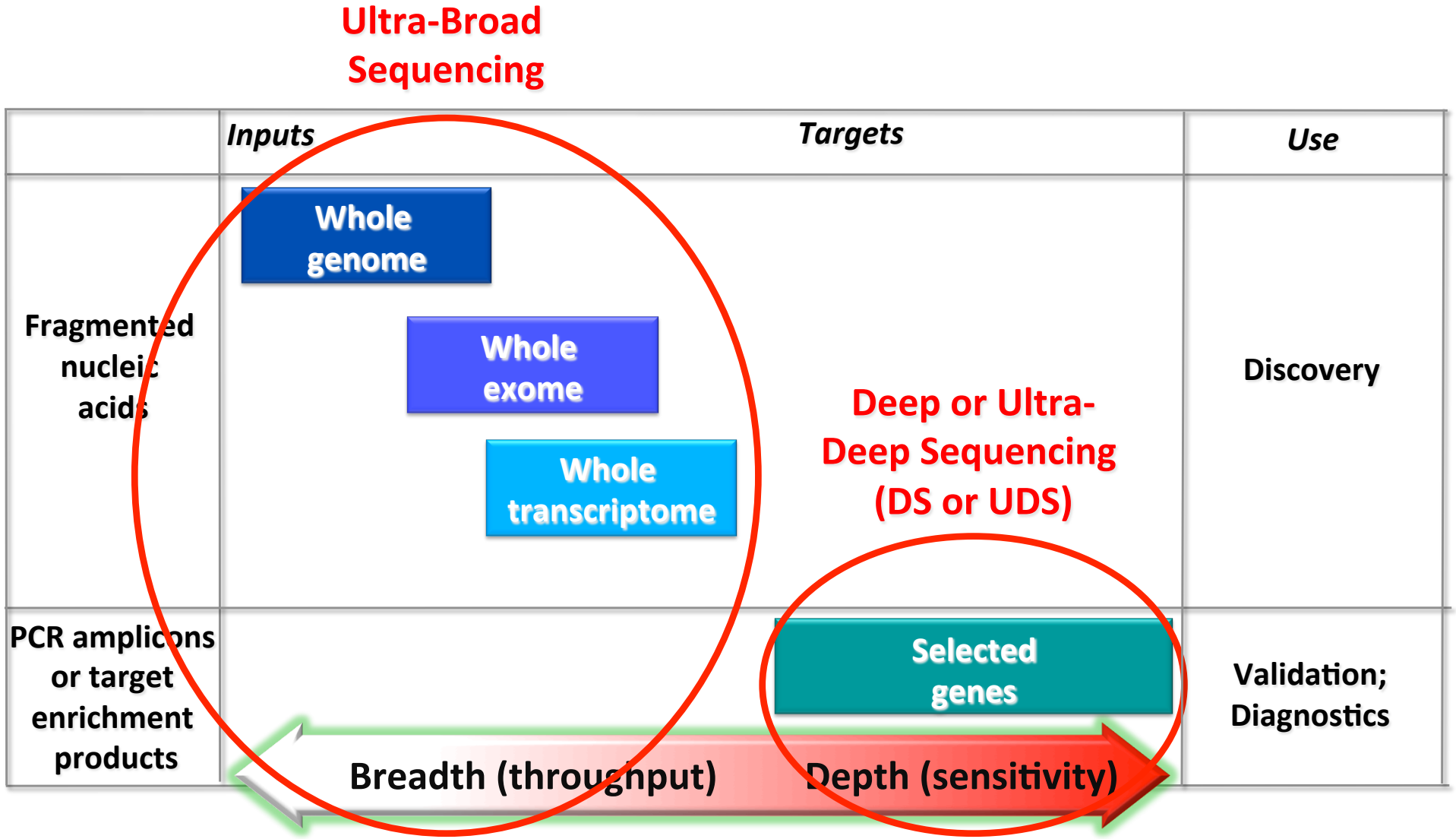
- **PRIMARY RESISTANCE**
  - Insufficient inhibition of BCR-ABL
  - Can be due to low plasma levels, activity of drug pumps, etc
  - Individual variation in normal bone marrow reserve (low levels of normal hematopoietic stem cells in some patients)
- **SECONDARY RESISTANCE**
  - Outgrowth of one or more clones harboring an imatinib-resistant BCR-ABL kinase domain mutation (most common)
  - Overproduction of BCR-ABL (e.g. via genomic amplification)
  - BCR-ABL-independent mechanisms (poorly understood)

2017 BCR-ABL Kinase Domain Mutations Associated with Clinical Resistance to Imatinib (Incomplete Map)

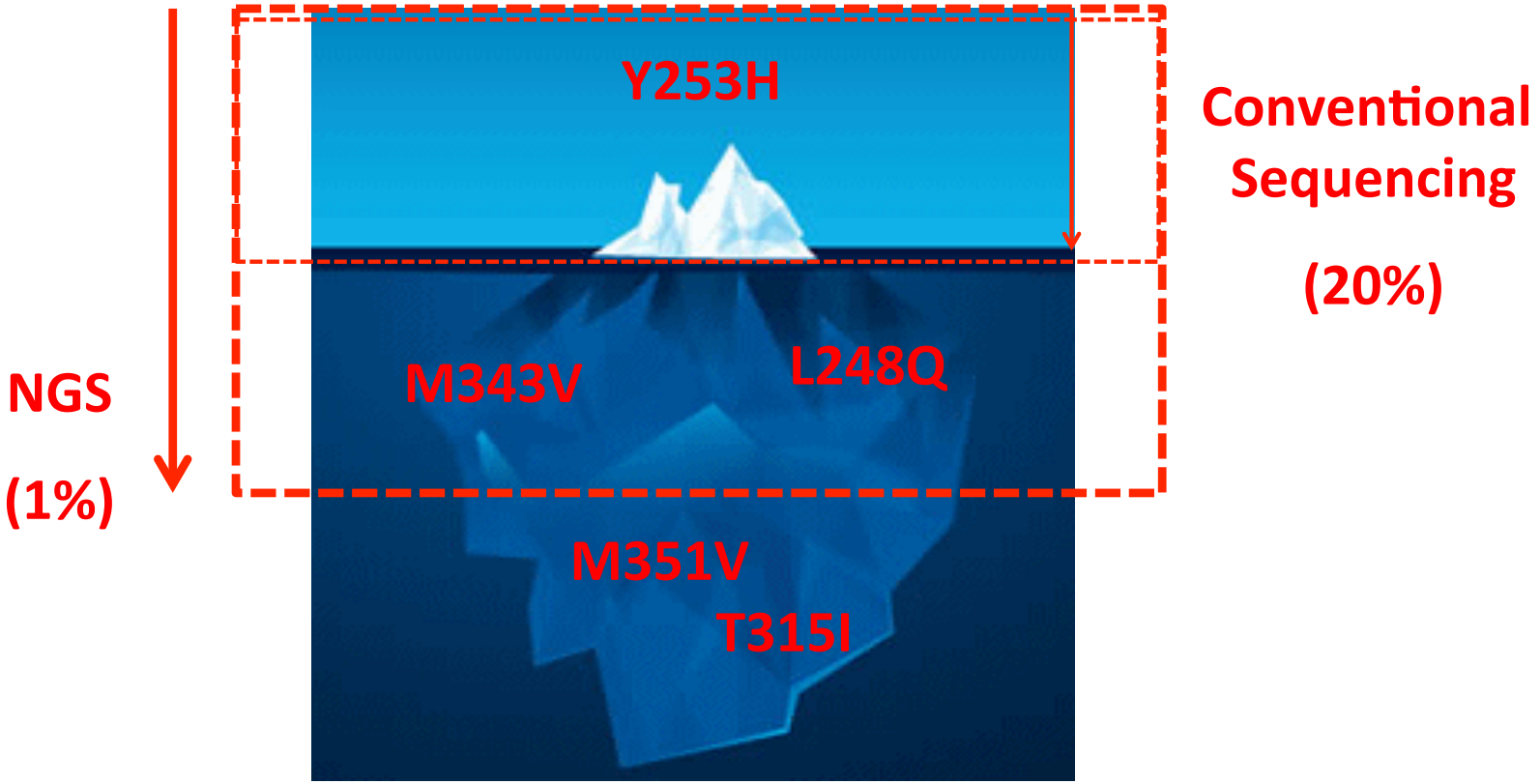


Gorre et al, 2001; von Bubnoff et al, 2002; Branford et al, 2002; Hofmann et al, 2002; Roche-L'Estienne et al, 2002; Shah et al, 2002; Hochhaus et al, 2002; Al-Ali et al, 2004

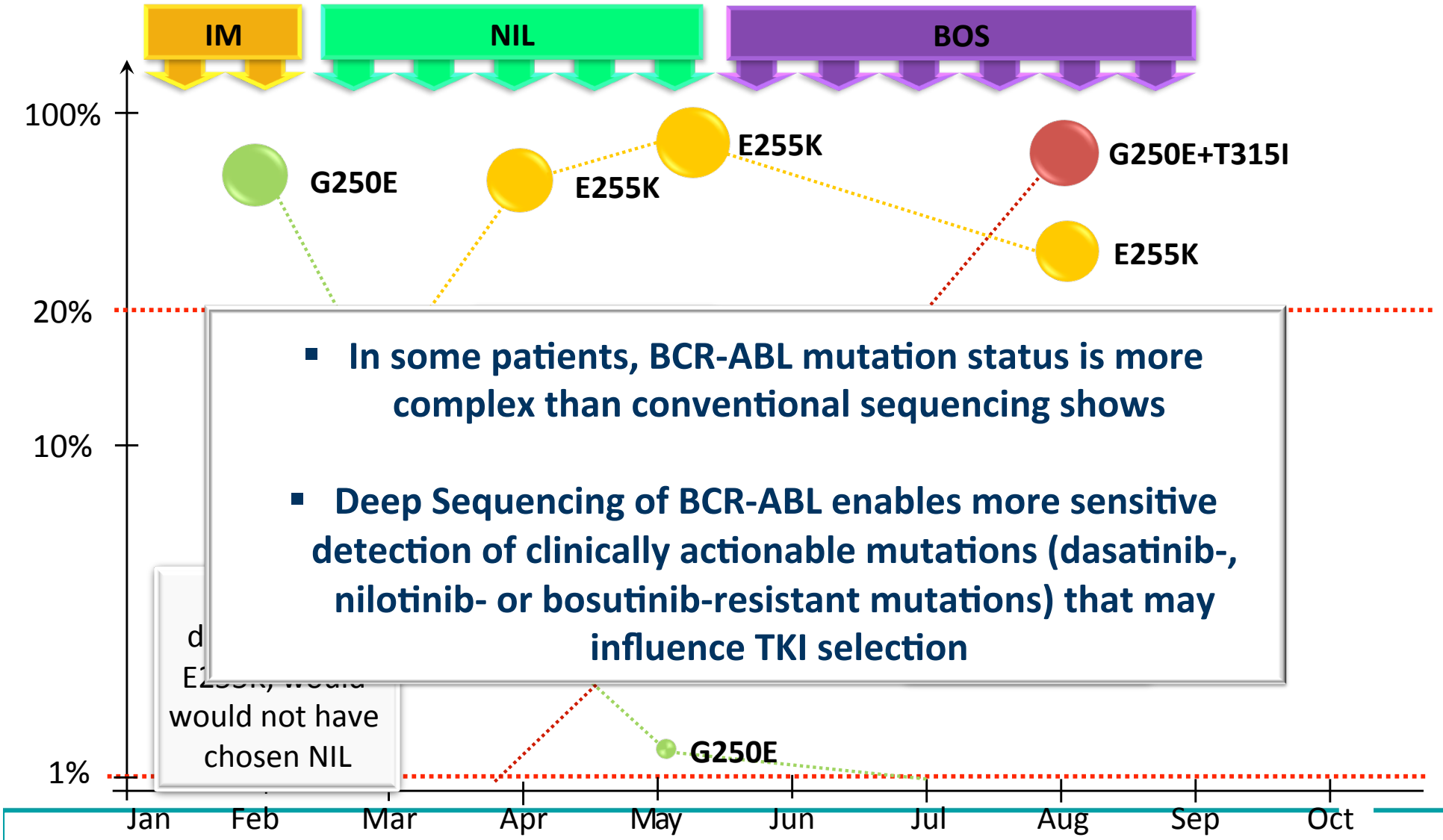
Next-Generation Sequencing: applications



Mutations detectable by conventional sequencing: the tip of the iceberg



The dynamic landscape of mutant populations can be best followed by NGS



- In some patients, BCR-ABL mutation status is more complex than conventional sequencing shows
- Deep Sequencing of BCR-ABL enables more sensitive detection of clinically actionable mutations (dasatinib-, nilotinib- or bosutinib-resistant mutations) that may influence TKI selection

would not have chosen NIL

Soverini et al, Blood 2013

2017

ONE STEP FURTHER: THE 'NEXT-IN-CML' STUDY

We have initiated a multicenter, multilaboratory prospective study ('NEXT-IN-CML') aimed to assess the feasibility, cost, turnaround times and clinical utility of a NGS-based BCR-ABL KD mutation screening approach

STUDY TITLE:

"NEXT-GENERATION SEQUENCING FOR BCR-ABL KD MUTATION SCREENING IN PHILADELPHIA CHROMOSOME-POSITIVE LEUKEMIAS"

STUDY ACRONYM: "NEXT-IN-CML"

Prospective Investigational Multi-Center Tissue Study

- **4 labs (Bologna, Catania, Orbassano, Napoli)**
- **54 clinical centers**

NEXT IN CML

THE 'NEXT-IN-CML' STUDY

PHASE A | **PHASE B**

- create a network of reference labs sharing a common NGS workflow, a joint database for clinical and mutational data storage and a common pipeline of data analysis, interpretation and reporting

- verify accuracy and inter-laboratory reproducibility of results on a common set of samples with known mutation status and mutation load

THE 'NEXT-IN-CML' STUDY

PHASE A | **PHASE B**

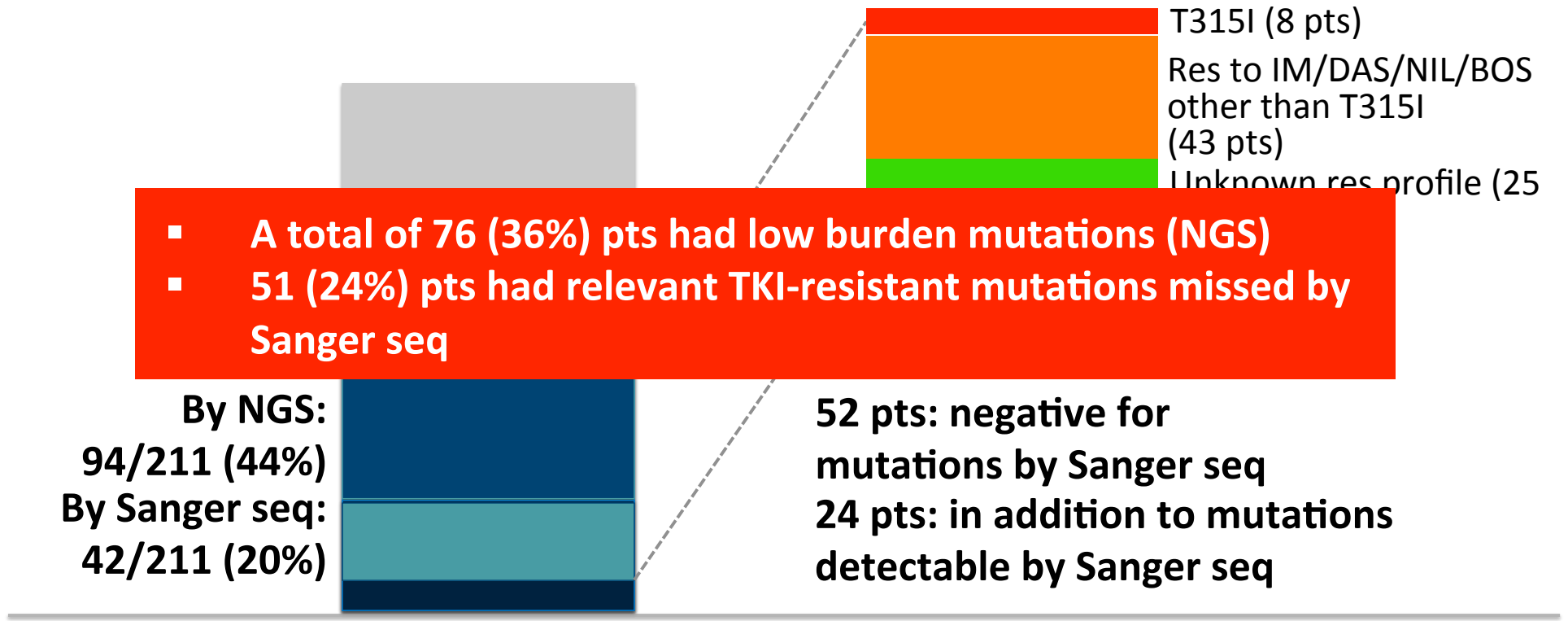
- prospectively assess the frequency of low burden mutations undetectable by Sanger sequencing in patients with failure or warning to TKI therapies

- correlate NGS data with:

1) baseline disease features, previous therapy and level of response at the time of sampling

2) response to subsequent therapy(ies) and 12-month outcome

PHASE B


T315I (8 pts)
Res to IM/DAS/NIL/BOS other than T315I (43 pts)
Unknown res profile (25
A total of 76 (36%) pts had low burden mutations (NGS)
51 (24%) pts had relevant TKI-resistant mutations missed by Sanger seq
By NGS: 94/211 (44%)
By Sanger seq: 42/211 (20%)
52 pts: negative for mutations by Sanger seq
24 pts: in addition to mutations detectable by Sanger seq
Any mutation
Low burden mutations detectable by NGS only

CONCLUSIONS – NEXT-IN-CML STUDY

- **Robust and reproducible NGS-based BCR-ABL1 KD mutation screening can successfully be implemented in national diagnostic lab networks and is feasible with turnaround times and costs comparable to those of Sanger seq**

- **In a large, prospective series of CML pts with Failure or Warning, known IM/DAS/NIL/BOS resistant mutations were missed by Sanger seq in 24% of the pts**

- **Low burden (≥3%) TKI-resistant mutations were found to be sufficient to drive clonal expansion, whereas more data are needed to understand the clinical significance of those with an unknown resistance profile**

Clinical Resistance to Imatinib: Mechanisms

- **PRIMARY RESISTANCE**
  - Insufficient inhibition of BCR-ABL
  - Can be due to low plasma levels, activity of drug pumps, etc
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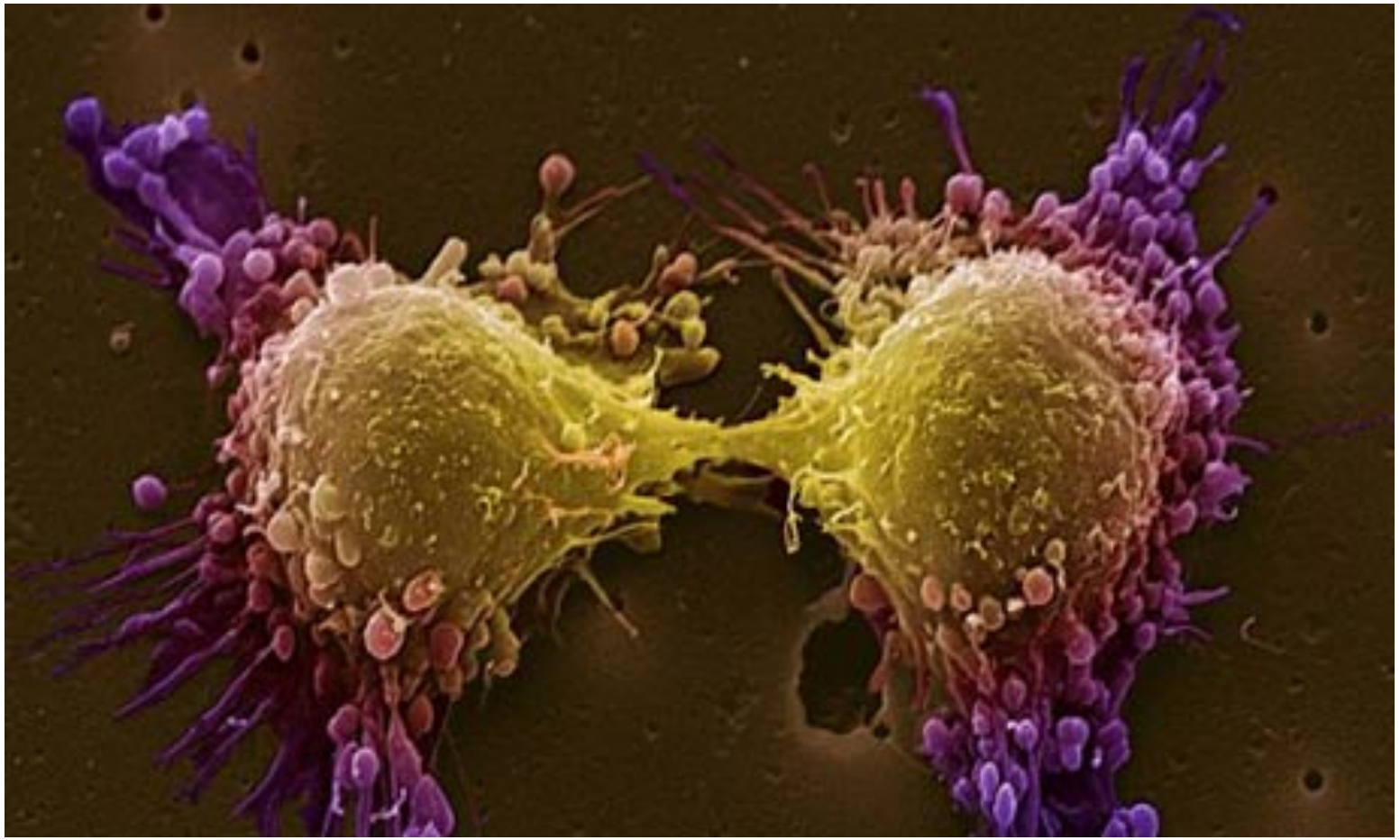
  - BCR-ABL-independent mechanisms (poorly understood)

2017 BCR-ABL-INDEPENDENT MECHANISMS OF DRUG RESISTANCE

2017

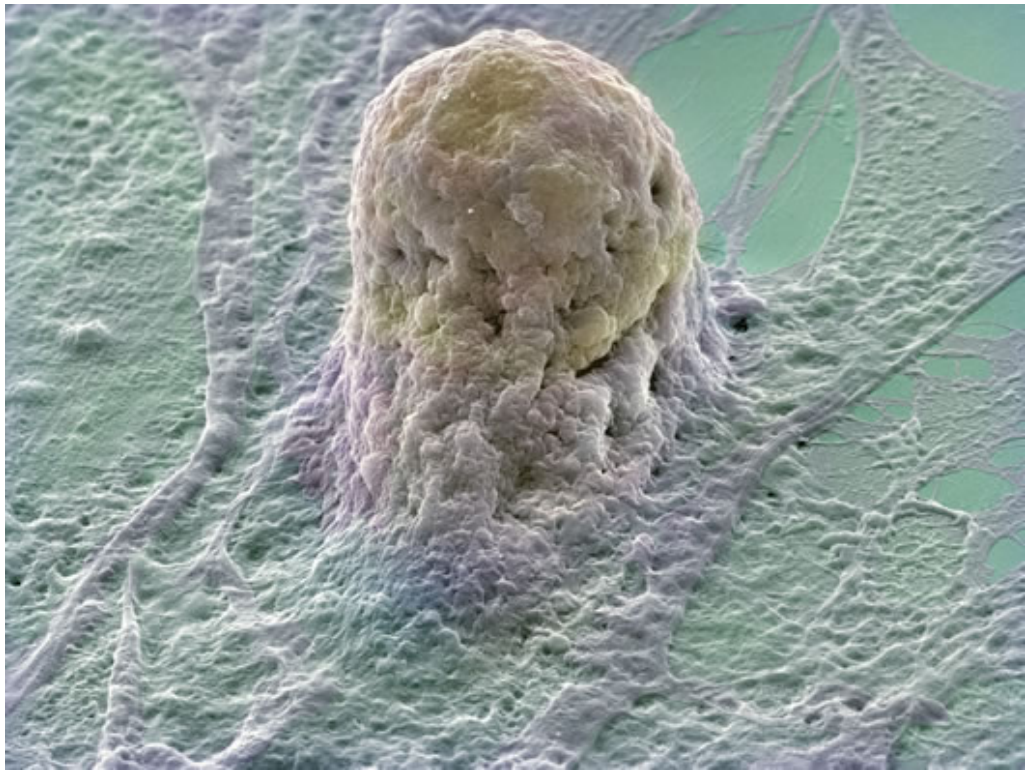
ROLE OF LEUKEMIC STEM CELL



2017

BCR-ABL-INDEPENDENT MECHANISMS OF DRUG RESISTANCE

"Long before the worldwide obsession with all type of stem cell started, the stem cells was fully accepted in the field of hematology"



Alexander Alexandrowitsch Maximow in a scientific presentation in Berlin in 1909: "Stamzellen"

IMPORTANCE OF STEM CELL RESEARCH

"Science has presented us with a hope called stem-cell research, which may provide our scientists with answers that have so long been beyond our grasp."

Nancy Reagan

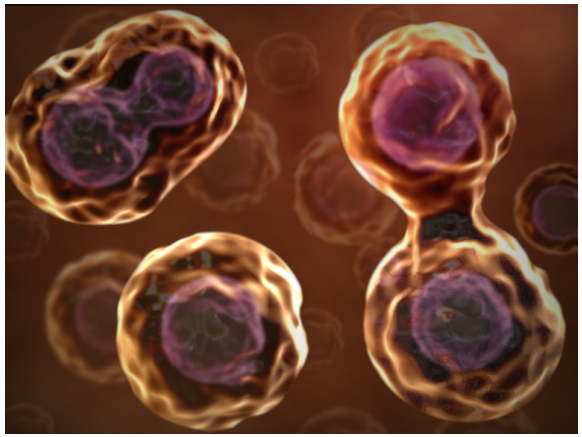
"If the potential of stem cell research is realized, it would mean an end to the suffering of millions of people. If stem cell research succeeds, there isn't a person in the country who won't benefit, or know somebody who will."

Michael J. Fox

HOW STEM CELL COULD IMPROVE OUR LIFE?

"Your radiator is leaking. Dont worry, I can patch it up with stem cells."

STEM CELL – DEFINITION and CHARACTERISTICS



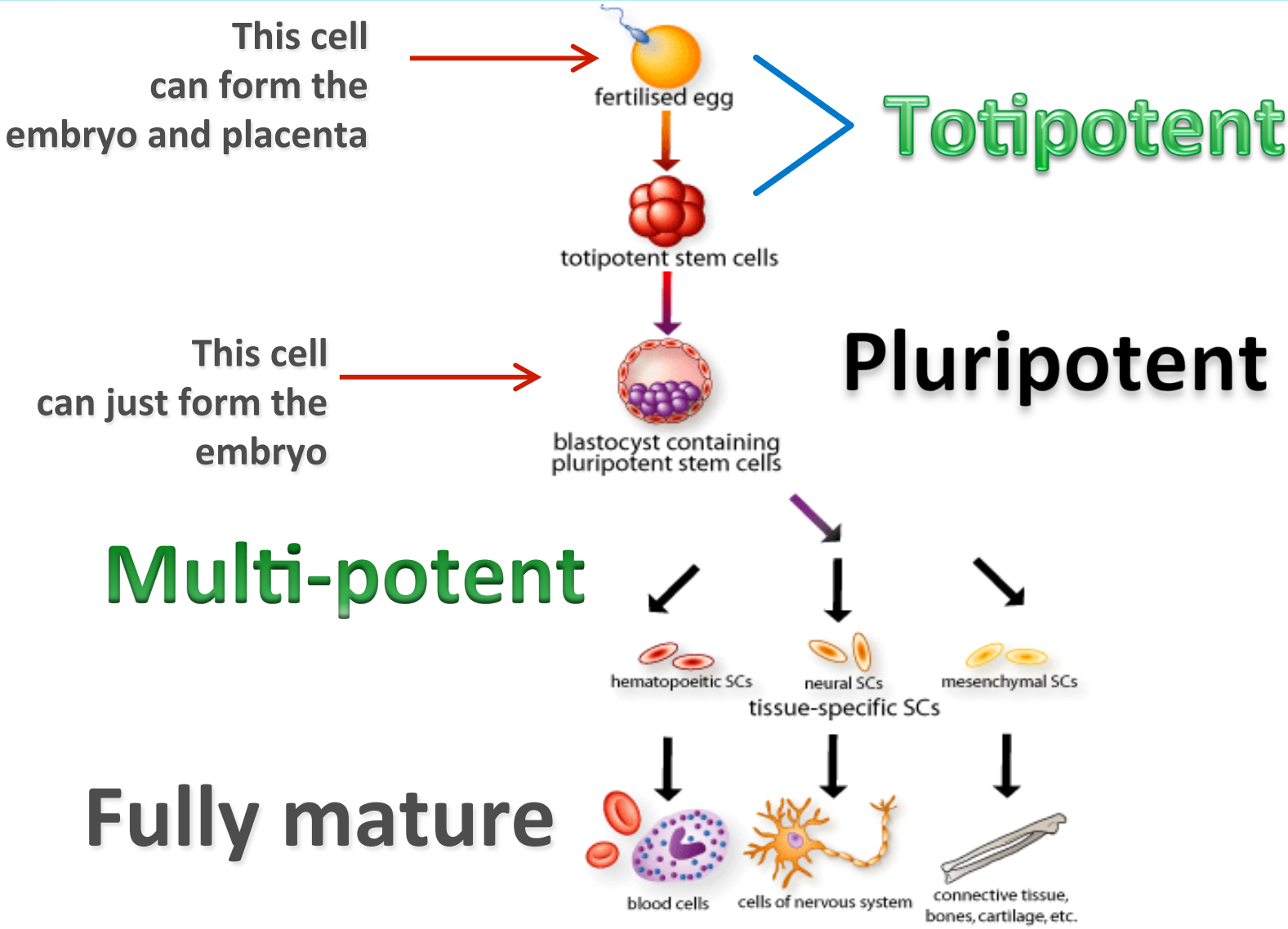
A cell that has the ability to continuously divide and differentiate (develop) into various other kind(s) of cells/tissues

'Blank cells' (unspecialized)

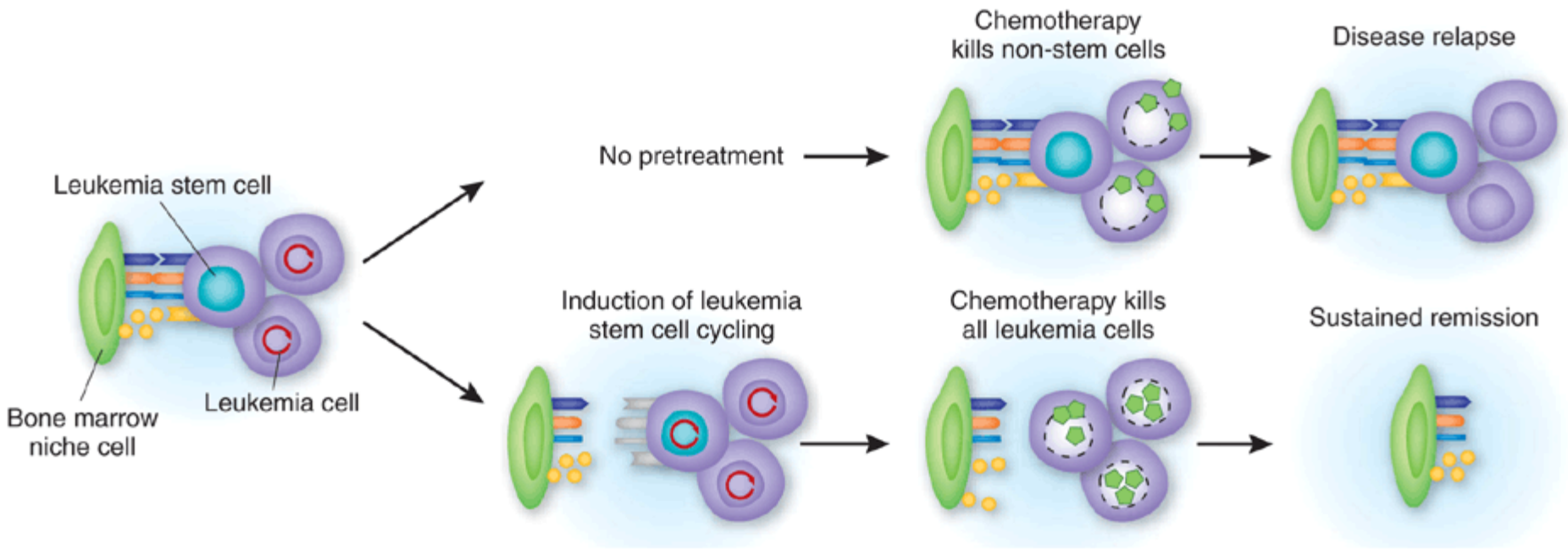
Capable of dividing and renewing themselves for long periods of time (proliferation and renewal)

Have the potential to give rise to specialized cell types (differentiation)

KINDS OF STEM CELLS

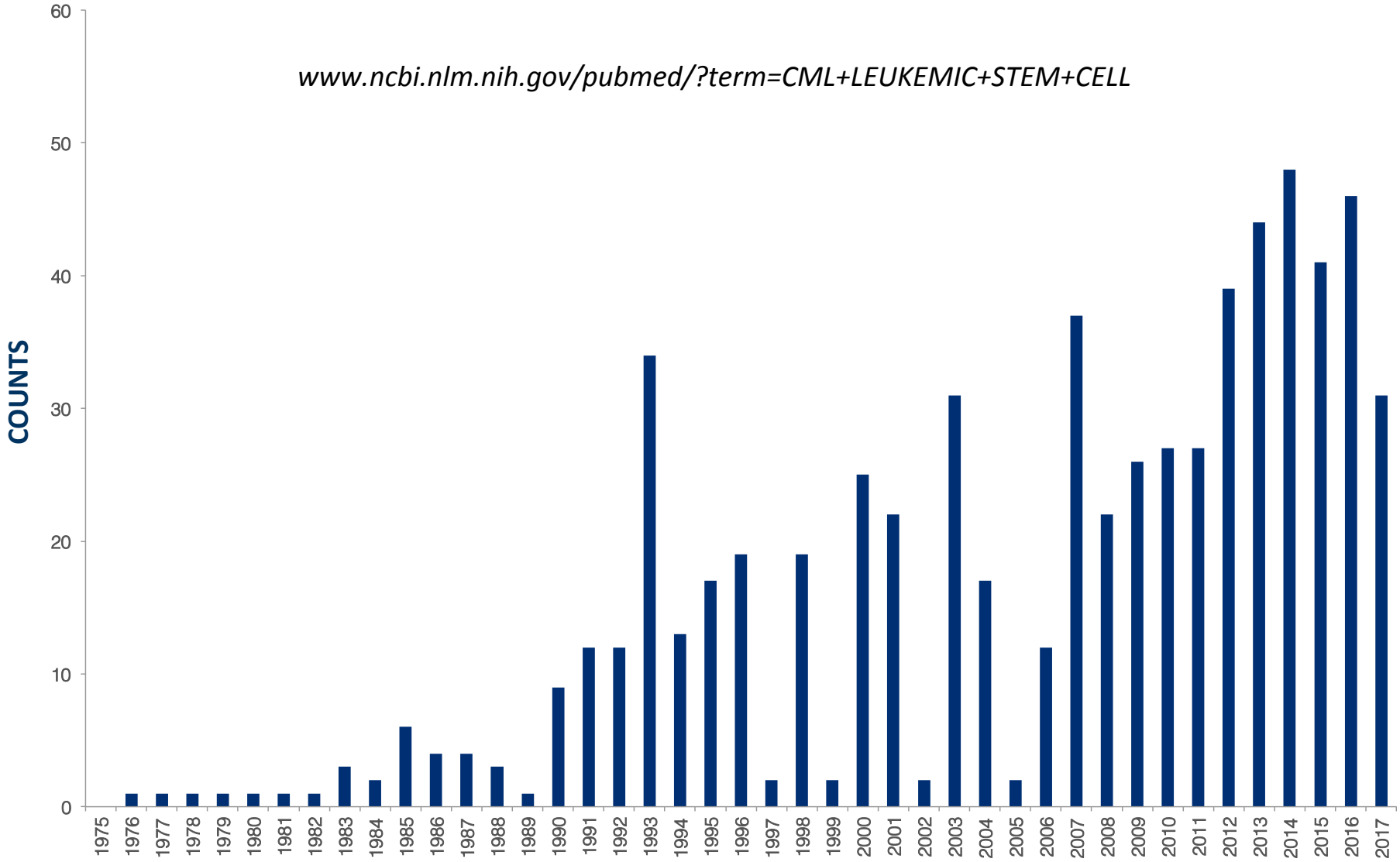


WHY IS STEM CELL RESEARCH SO IMPORTANT TO ALL OF US?



THE IMPORTANCE OF LSC RESEARCH IN THE LAST YEARS

www.ncbi.nlm.nih.gov/pubmed/?term=CML+LEUKEMIC+STEM+CELL



Current Drug Targets

Volume 18, Number 4, 2017

Contents

Thematic Issue

CML HSCs: Are the True Enemies for the Patient?

Guest Editor: Manuela Mancini

[Cell Cycle 8:9, 1338-1343; 1 May 2009]; ©2009 Landes Bioscience

Perspective

The CML stem cell

Evolution of the progenitor

Scott A. Stuart,[1-3] Yosuke Minami[4] and Jean Y.J. Wang[1-3,*]

[1]Division of Hematology-Oncology; Department of Medicine; [2]Moores Cancer Center;

blood

2012 119: 1501-1510
Prepublished online December 19, 2011;
doi:10.1182/blood-2010-12-326843

Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival

Ashley Hamilton, G. Vignir Helgason, Mirle Schemionek, Bin Zhang, Svetlana Myssina, Elaine K. Allan, Franck E. Nicolini, Carsten Müller-Tidow, Ravi Bhatia, Valerie G. Brunton, Steffen Koschmieder and Tessa L. Holyoake

Contents lists available at SciVerse ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

ELSEVIER

Review

Signaling pathways in chronic myeloid leukemia and leukemic stem cell maintenance: Key role of stromal microenvironment

P.F. Seke Etet [a], L. Vecchio [b], A.H. Nwabo Kamdje [c,d,*]

Bcr-Abl stabilizes β-catenin in chronic myeloid leukemia through its tyrosine phosphorylation

Addolorata Maria Luce Coluccia[1,2,*], Angelo Vacca[2], Mireia Duñach[3], Luca Mologni[1], Sara Redaelli[1], Victor H Bustos[4], Daniela Benati[1], Lorenzo A Pinna[4,5] and Carlo Gambacorti-Passerini[1,6]

THE EMBO JOURNAL

2007

The NEW ENGLAND JOURNAL of MEDICINE

Granulocyte–Macrophage Progenitors as Candidate Leukemic Stem Cells in Blast-Crisis CML

2004

Catriona H.M. Jamieson, M.D., Ph.D., Laurie E. Ailles, Ph.D., Scott J. Dylla, Ph.D., Manja Muijtjens, M.S., Carol Jones, B.A., James L. Zehnder, M.D., Jason Gotlib, M.D., Kevin Li, Ph.D., Markus G. Manz, M.D., Armand Keating, M.D., Charles L. Sawyers, M.D., and Irving L. Weissman, M.D.

Leukemia 2009

β-Catenin is essential for survival of leukemic stem cells insensitive to kinase inhibition in mice with BCR-ABL-induced chronic myeloid leukemia.
Essential role of β-catenin in survival of CML stem cells

Y Hu, Y Chen, L Douglas and SLi

2017

Seràgnoli AND CML STEM CELL

OPEN ACCESS Freely available online

PLOS ONE

BCR-ABL1-Associated Reduction of Beta Catenin Antagonist Chibby1 in Chronic Myeloid Leukemia

Elisa Leo*, Manuela Mancini, Michela Aluigi, Simona Luatti, Fausto Castagnetti, Nicoletta Testoni, Simona Soverini, Maria Alessandra Santucci, Giovanni Martinelli

Istituto di Ematologia "Lorenzo e Ariosto Seràgnoli", Dipartimento di Medicina Specialistica Diagnostica e Sperimentale - DIMES, University of Bologna - Medical School, Bologna, Italy

ELSEVIER

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journal homepage: www.elsevier.com/locate/cellsig

A calpain-cleaved fragment of β-catenin promotes BCRABL1 + cell survival evoked by autophagy induction in response to imatinib

Manuela Mancini *, Elisa Leo, Virginia Campi, Fausto Castagnetti, Luca Zazzeroni, Gabriele Gugliotta, Maria Alessandra Santucci, Giovanni Martinelli

Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale DIMES, Istituto di Ematologia "L. e A. Seràgnoli," University of Bologna, Medical School, via Massarenti, 940138 Bologna, Italy

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journal homepage: www.elsevier.com/locate/cellsig

Chibby drives β catenin cytoplasmic accumulation leading to activation of the unfolded protein response in BCR-ABL1 + cells

Manuela Mancini [a,*], Elisa Leo [a], Ken-Ichi Takemaru [b], Virginia Campi [a], Enrica Borsi [a], Fausto Castagnetti [a], Gabriele Gugliotta [a], Maria Alessandra Santucci [a], Giovanni Martinelli [a]

[a] *Department of Experimental Diagnostic and Specialty Medicine – DIMES – Institute of Hematology "L. and A. Seràgnoli", University of Bologna-Medical School, Italy*
[b] *Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, NY, USA*

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2017

Chibby 1: a new component of β-catenin-signaling in chronic myeloid leukemia

Manuela Mancini[1], Simona Soverini[1], Gabriele Gugliotta[1], Maria Alessandra Santucci[1], Gianantonio Rosti[1], Michele Cavo[1], Giovanni Martinelli[1], Fausto Castagnetti[1]

[1]Department of Experimental Diagnostic and Specialty Medicine–DIMES–Institute of Hematology "L. and A. Seràgnoli", University of Bologna-Medical School, Italy

PLOS ONE

RESEARCH ARTICLE

14-3-3 Binding and Sumoylation Concur to the Down-Modulation of *β-catenin* Antagonist *chibby 1* in Chronic Myeloid Leukemia

Manuela Mancini[1]*, Elisa Leo[1], Ken-Ichi Takemaru[2], Virginia Campi[1], Fausto Castagnetti[1], Simona Soverini[1], Caterina De Benedittis[1], Gianantonio Rosti[1], Michele Cavo[1], Maria Alessandra Santucci[1], Giovanni Martinelli[1]

1 Department of Experimental Diagnostic and Specialty Medicine—DIMES—Institute of Hematology "L. and A. Seràgnoli". University of Bologna-Medical School, Bologna, Italy, 2 Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, New York, United States of America

CrossMark

ARTICLE

Journal of Cellular Biochemistry 116:589–597 (2015)

Journal of Cellular Biochemistry

DNA Methyltransferase 1 Drives Transcriptional Down-Modulation of β Catenin Antagonist Chibby1 Associated With the *BCR-ABL1* Gene of Chronic Myeloid Leukemia

Elisa Leo,* Manuela Mancini, Fausto Castagnetti, Gabriele Gugliotta, Maria Alessandra Santucci, and Giovanni Martinelli

Dipartimento di Medicina Specialistica, Diagnostica a Sperimentale-DIMES, Istituto di Ematologia "L. e A. Seràgnoli" – University of Bologna – Medical School, Bologna, Italy

ARTICLE

Journal of Cellular Biochemistry 9999:1–8 (2017)

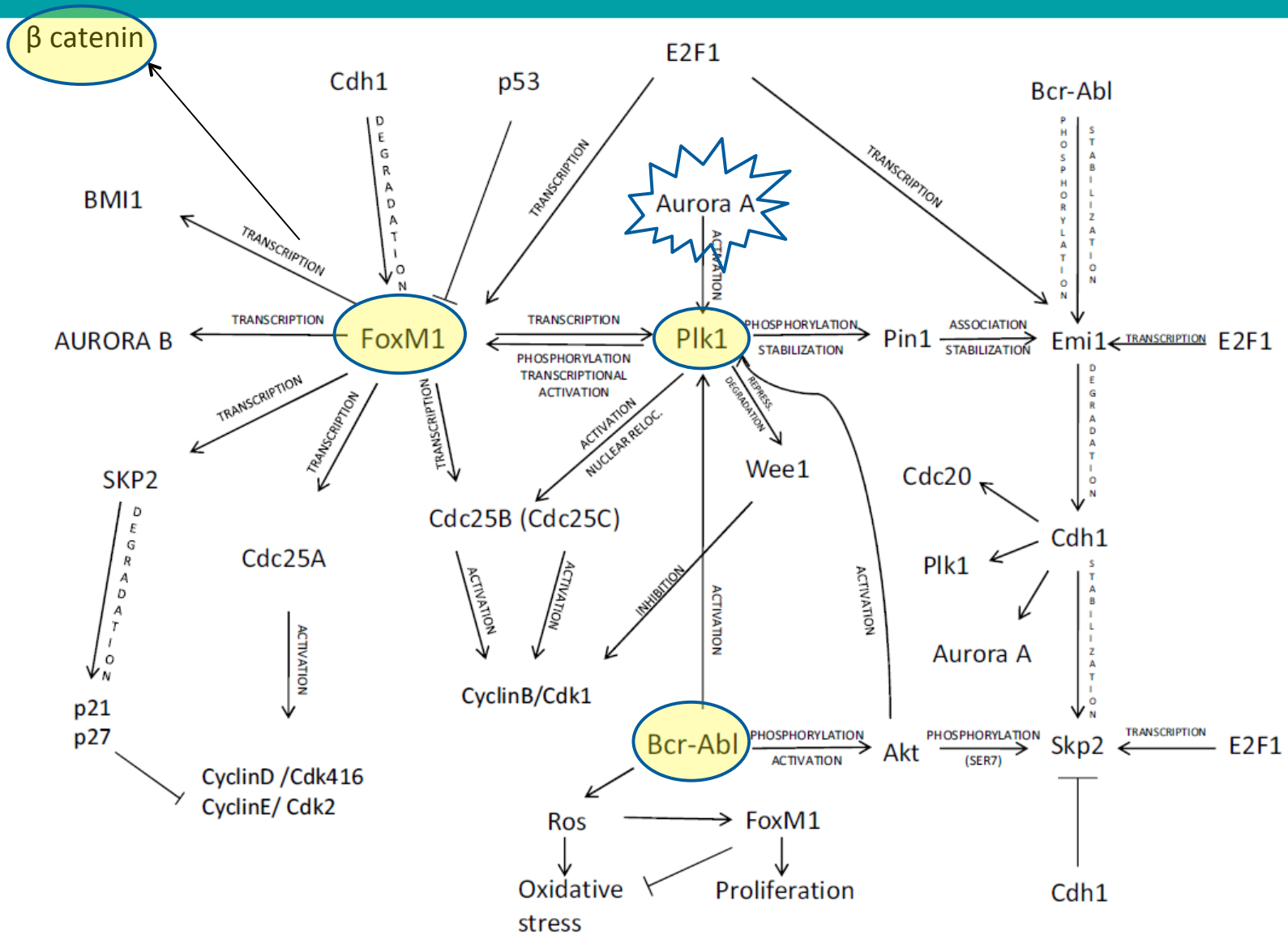
Journal of Cellular Biochemistry

FOXM1 Transcription Factor: A New Component of Chronic Myeloid Leukemia Stem Cell Proliferation Advantage

Manuela Mancini,* Fausto Castagnetti, Simona Soverini, Elisa Leo, Caterina De Benedittis, Gabriele Gugliotta, Gianantonio Rosti, Luana Bavaro, Sara De Santis, Cecilia Monaldi, Margherita Martelli, Maria Alessandra Santucci, Michele Cavo, and Giovanni Martinelli

Department of Experimental, Diagnostic and Specialty Medicine–DIMES, Institute of Hematology L. and A. Seràgnoli-University of Bologna, Bologna, Italy

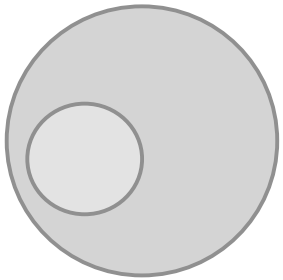
FOXM1 SIGNALING NETWORK



AIM OF OUR STUDIES...

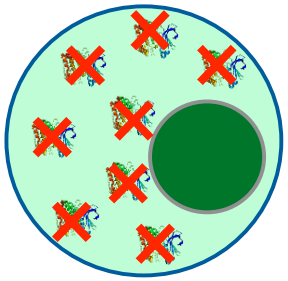
TARGET THERAPY

HSC



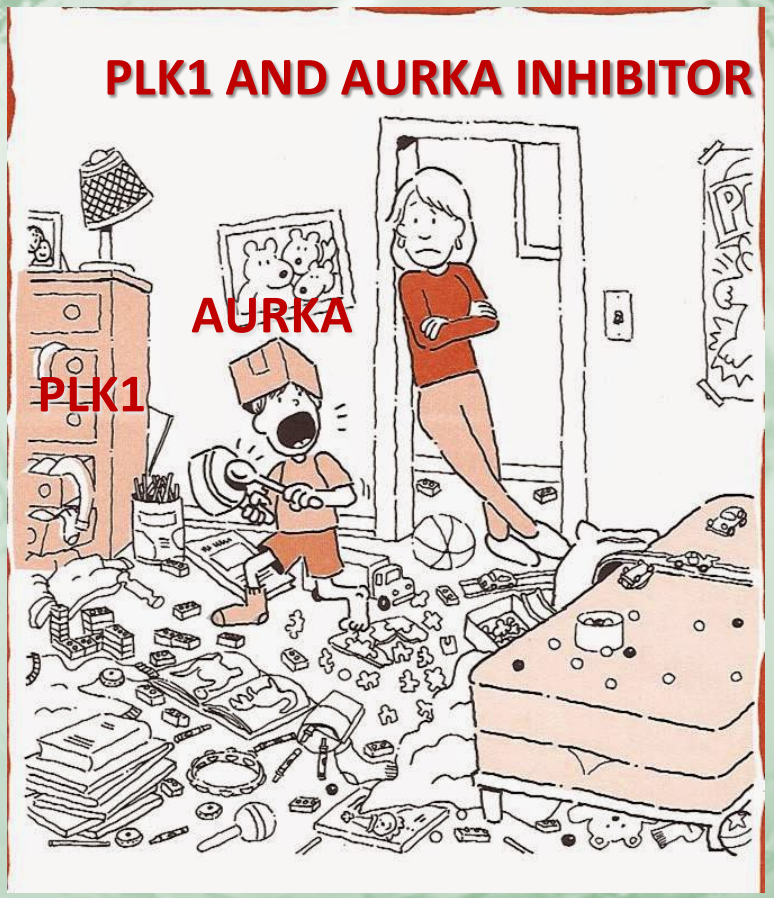
SURVIVAL

LSC



APOPTOSIS

CONCLUSIONS



Thank you for attention!