LEUKEMIA2020-2021

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ASSOCIAZIONE ITALIANA CONTRO LE LEUCEMIE-LINFOMI E MIELOMA ONLUS

Ph+ Stem Cells and Marrow Microenvironment

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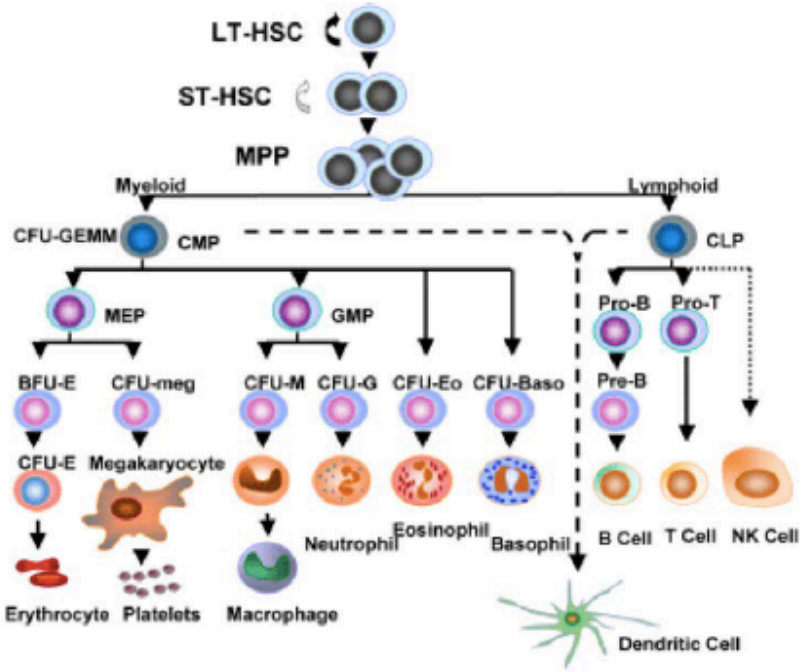
per la promozione e lo sviluppo della ricerca scientifica sulle malattie ematologiche. FRANCO MANDELLI

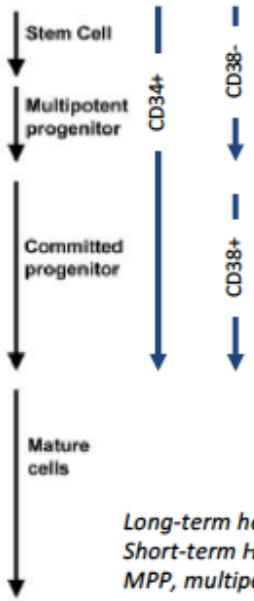
GITMO

SIES Società Italiana Ematologia Sperimentale

SIE - Società Italiana di Ematologia

Stem cell origin of CML

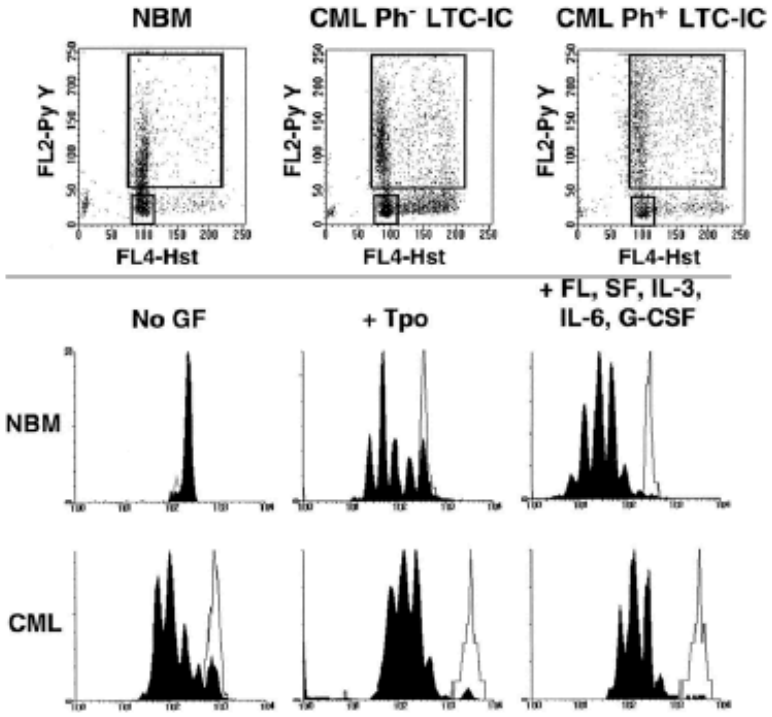




Long-term hematopoietic stem cells (LT-HSC) self-renew for life;
Short-term HSC(ST-HSC) self-renew for six to eight weeks;
MPP, multipotent progenitor;
CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte/macrophage progenitor;
MEP, megakaryocyte/erythrocyte progenitor

From: S Karlson, Lund University

A highly quiescent subpopulation of LSC is present in CML

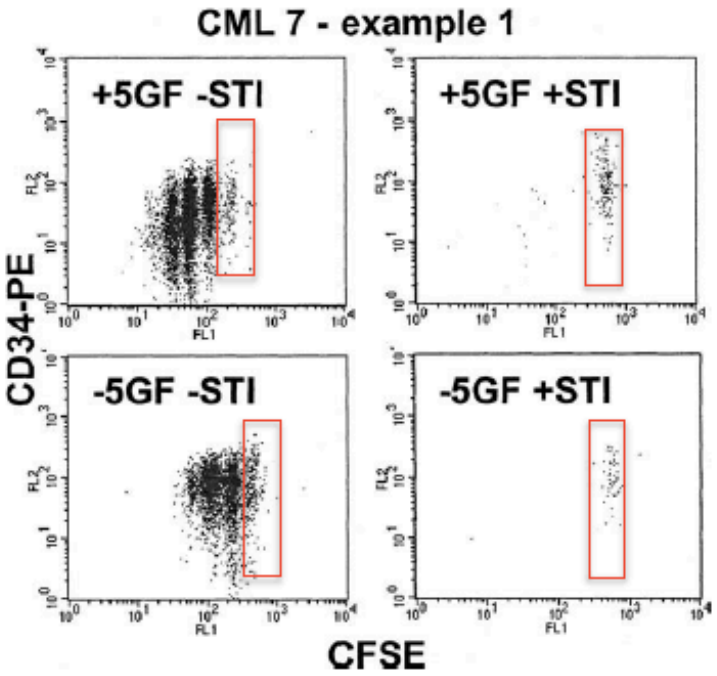


Direct evidence of a deeply quiescent subpopulation of leukemic cells with stem cell properties in patients with CML

Tessa Holyoake, Xiaoyan Jiang, Connie Eaves, Allen Eaves, Blood 1999 94:2056

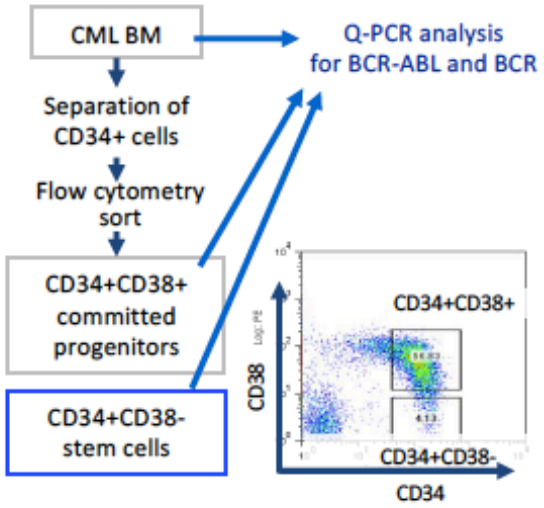
Quiescent CML LSCs survive TKI therapy

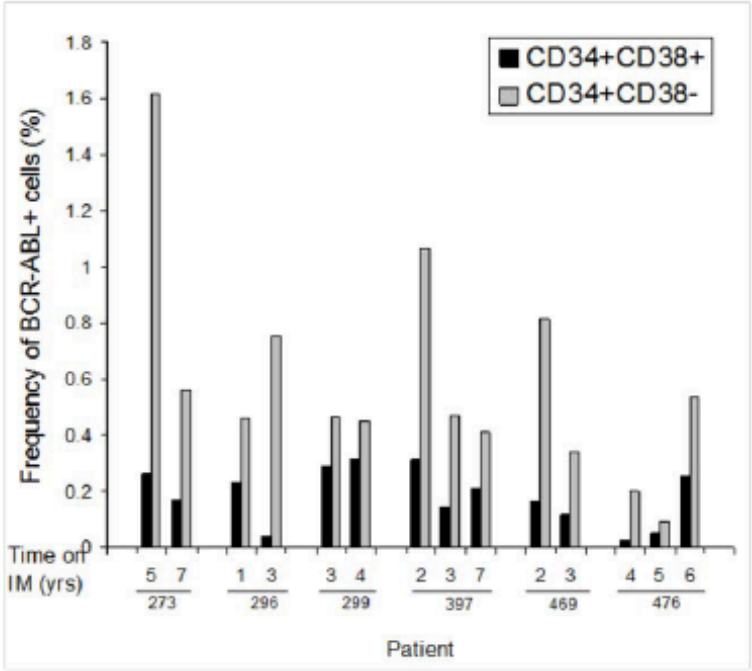
- TKIs have a strong antiproliferative effect on LSCs, but induce only modest levels of apoptosis
- Quiescent LSCs are especially resistant to TKI-induced apoptosis and elimination



Graham et al, Blood 2002; Holtz et al, Blood 2002

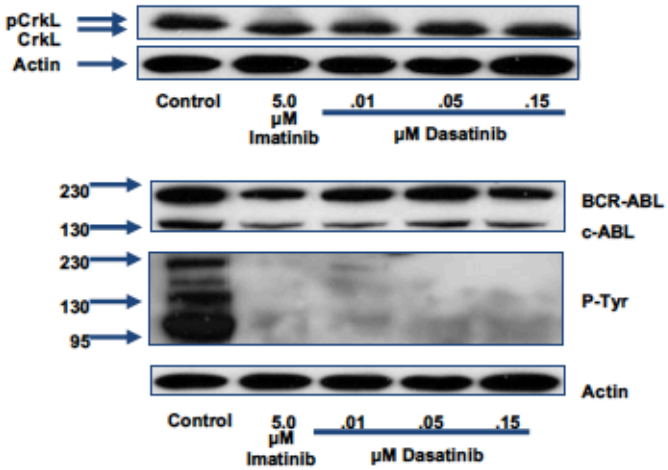
BCR-ABL+ CML LSCs persist in patients in long-term remission on imatinib



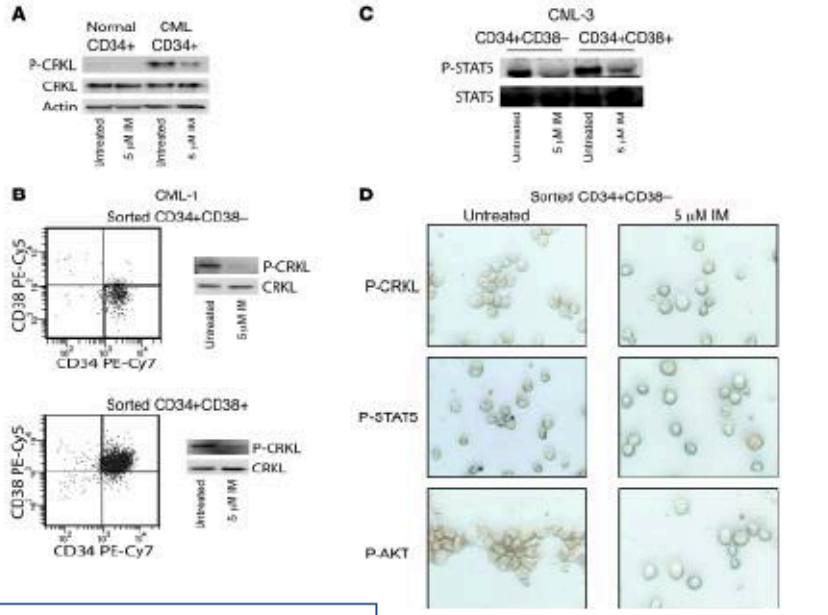


Chu S et al. Blood 2011

TKI treatment inhibits BCR-ABL kinase activity in CML LSCs



Konig H et al., Cancer Res. 2008.



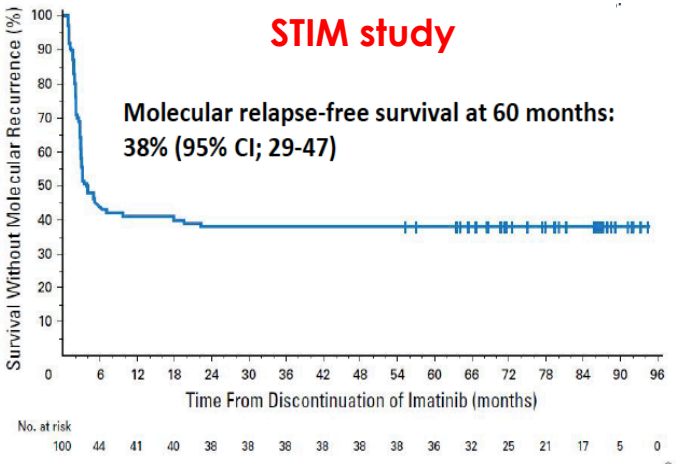
Corbin A et al., J Clin Invest., 2011

Survival of CML progenitors after TKI treatment is related to tyrosine kinase independent mechanisms

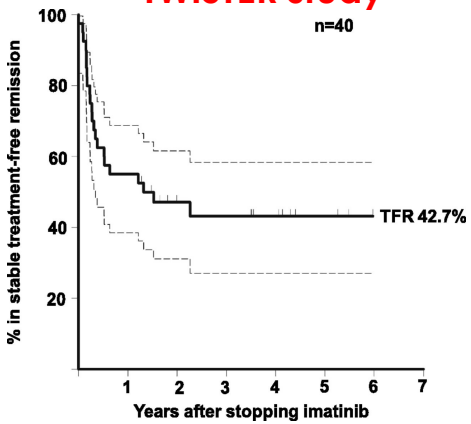
Only half of CML patients with DMR succeed in achieving TFR

At present, TFR is successful in 40-60% of patients (data from >30 studies)

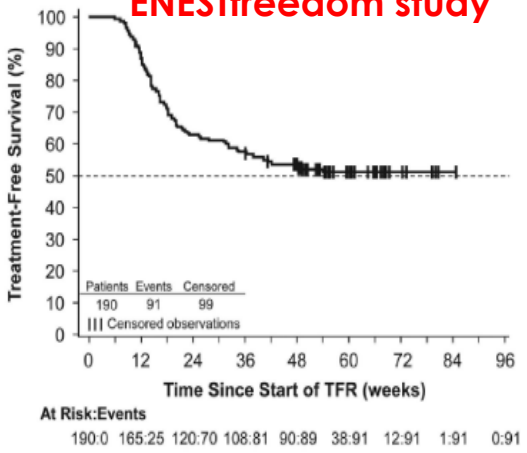
STIM study



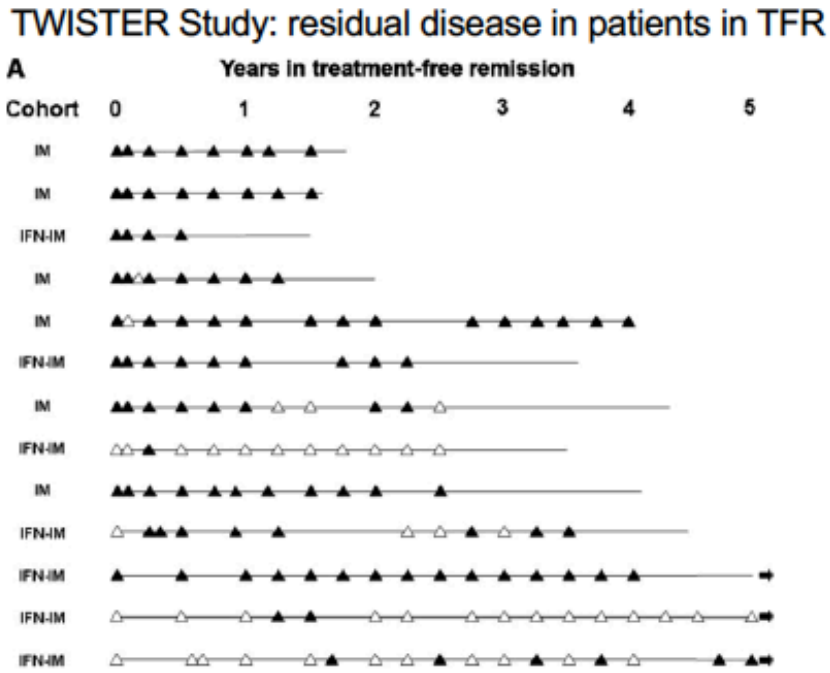
TWISTER study



ENESTfreedom study



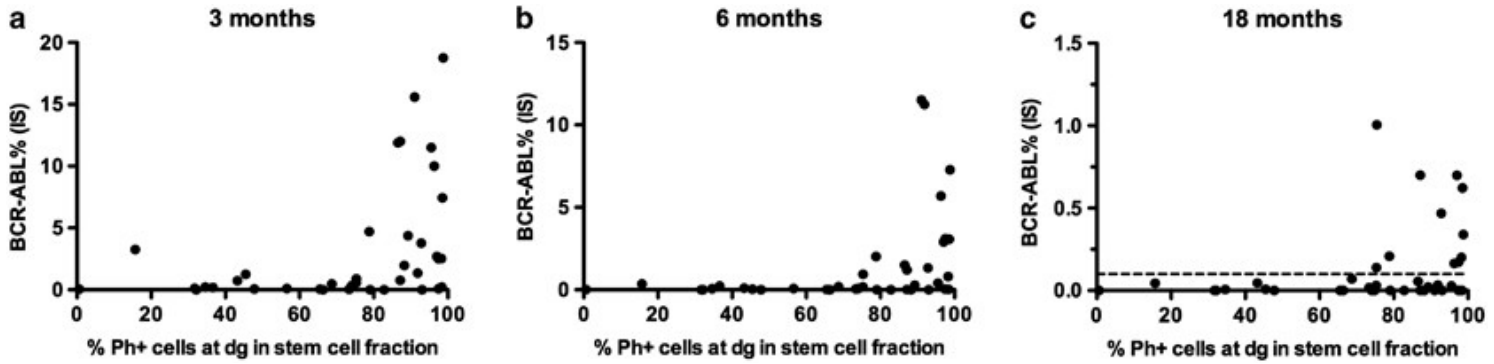
BCR-ABL+ cells persist in pts in TFR



Ross DM. Blood 2013

- Heterogeneity in residual LSCs in terms of their ability to behave as LICs?
- Quantitative or qualitative differences in the residual LSC reservoir and/or its niche?
- Role of the immune system?

Can CML LSC burden be a novel prognostic/predictive biomarker?



- Evaluated the LSC fraction defined as the fraction of BM cells with a Ph+CD34+CD38− phenotype at diagnosis and at +1, +3 and +6 months from start of treatment with imatinib or dasatinib in 46 CML pts
- All patients who did not achieve MMR at 18 months had >75% of Ph+ cells in the SC fraction at diagnosis
- Both patients who progressed during the study period had more than 90% of Ph+ cells in the SC compartment at diagnosis

Mustjoki et al, Leukemia 2013

Aberrant surface markers in CML LSCs and their diagnostic, predictive and therapeutic role

- In CP-CML, LSCs supposedly reside within the CD34+/CD38-/Lin- fraction

- CD34+/CD38- BCR-ABL+ LSCs exhibit an almost invariable aberration profile, defined as IL-2RA(CD25)+/CD26+/CD56 CD93+/IL-1RAP+

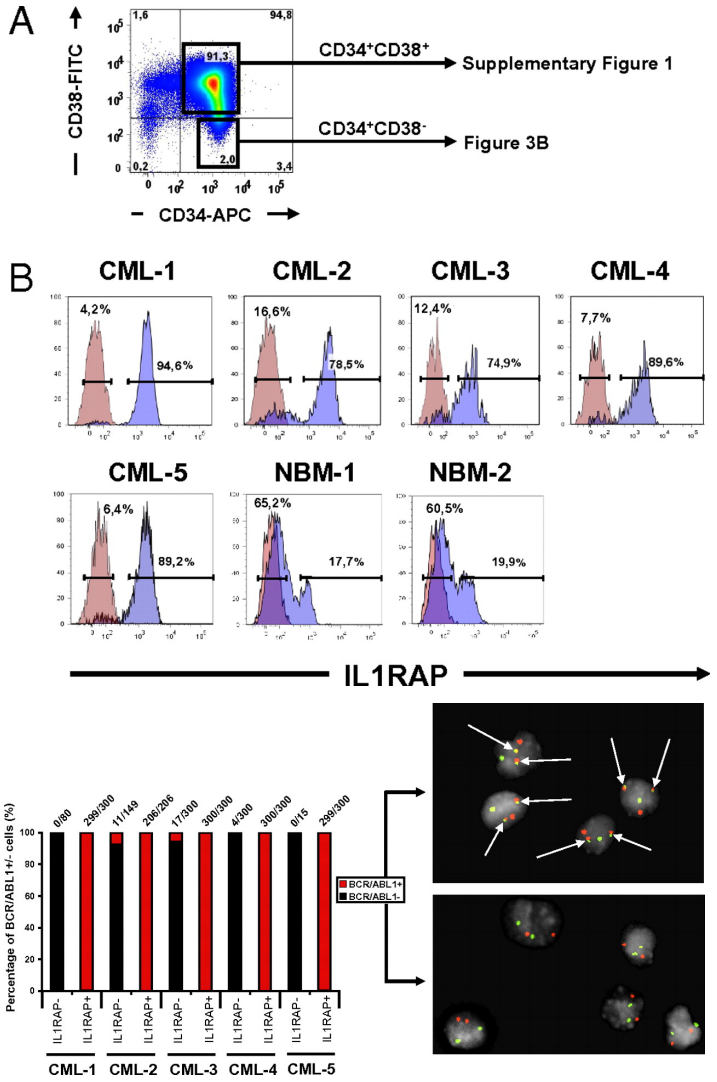
	Expressed on $CD34^+/CD38^-$ cells			
Target/Marker	CD	CML LSCs	AML LSCs	Normal stem cells
IL-2RA	CD25	+	+/–	–
DPPIV	CD26	+	–	–
Siglec-3	CD33	+	+	+/–
Pgp-1	[illegible]44	+	[illegible]	+
IAP				
				+
				–/+
			+	–/+

Immense application potential for
✓ Diagnosis
✓ isolation and biomolecular or functional LSC characterization
✓ Prediction of response, LSC tracking and quantitation during therapy
✓ therapeutic targeting of LSCs

Valent et al, Eur J Clin Invest 2014

IL1RAP

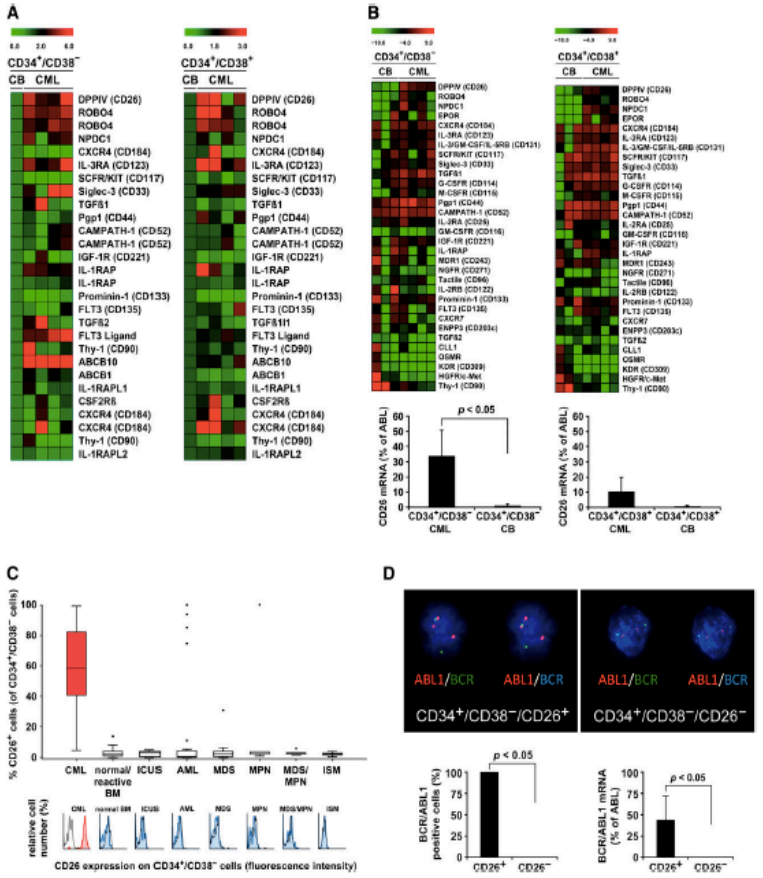
- Co-receptor of the interleukin 1 receptor (IL1R1) with unknown function
- Almost all CD34^{+}CD38^{low} BCR-ABL1+ cells express IL1RAP while BCR-ABL1- cells lack IL1RAP
- Estimation of the LSC burden at diagnosis by % IL1RAP-positive cells within the CD34^{+}CD38^{low} compartment predicts TKI response (CCyR, MMR)
- CML LSCs can be targeted by CAR-T cells directed against IL1RAP



Jaras et al, PNAS 2010; Landberg et al, Leukemia 2016; Warda et al, Cancer Res 2019

CD26 (dipeptidylpeptidase-IV)

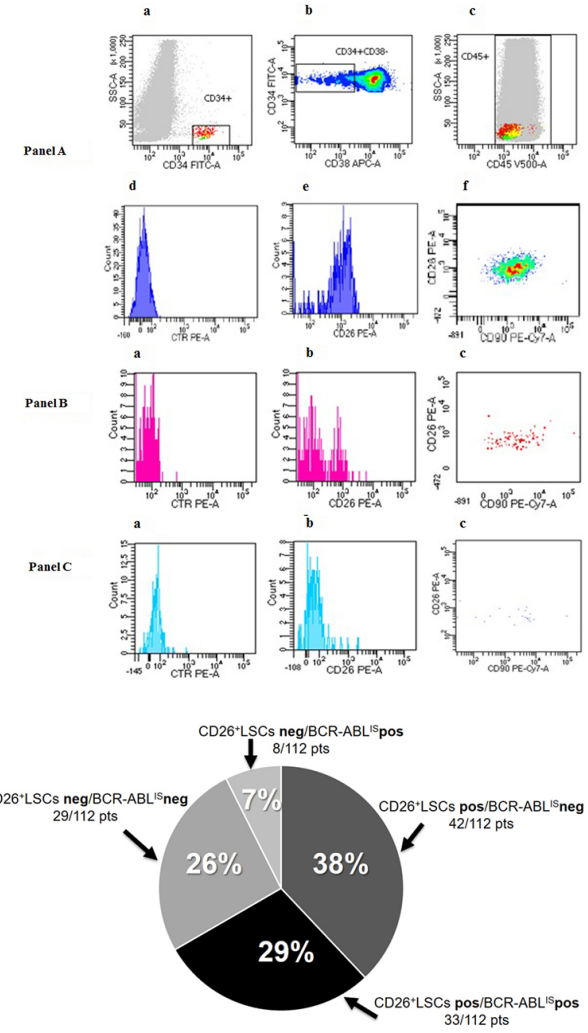
- Responsible for proteolytic degradation of various cytokines including IL-3, GM-CSF and SDF-1
- Nearly 100% of CD26^{+} LSC express BCR-ABL1, whereas the CD26^{-} SC from the same patients are BCR-ABL1^{-}
- Not detected on normal SC or LSC in other hematopoietic malignancies
- CD26^{+} LSC exhibit long-term proliferation and NSG repopulation activity
- Decrease in CD26+ SCs correlates with clinical responses to TKIs
- Well-known target of therapy in diabetes mellitus (gliptins)



Herrmann et al, Blood 2014; Culen et al, Oncotarget 2016

CD26 in PB: the FLOWERS study

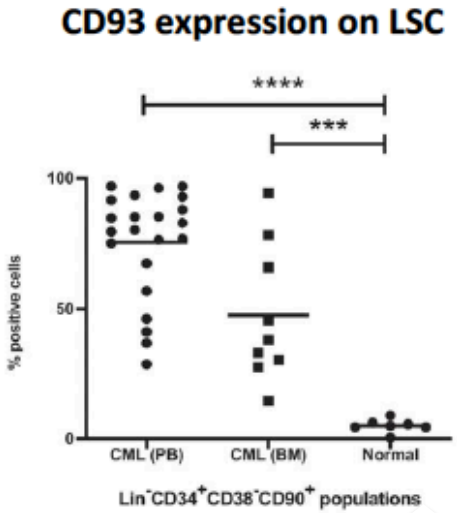
- The majority of CML patients on first line TKI treatment still harbored measurable residual LSCs, even when in stable DMR
- Residual circulating CD26^{+}LSC were detected in 66% of CML patients studied while in prolonged and stable TFR
- No correlation between the absolute number of persisting CD26+ LSCs and BCR-ABL1 copies
- However, at diagnosis, higher CD26+ LSCs number, PD-L1 positivity or both may correlated with a lower probability to achieve an optimal response

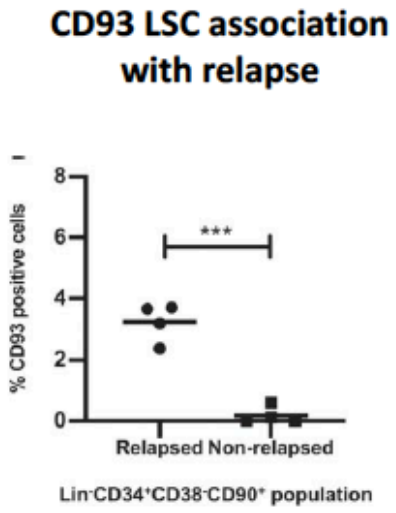


Bocchia, Front Oncol 2018; Raspadori et al, Cytometry B Clin Cytom. 2019; Bocchia et al, ASH 2020

LSC markers and relapse after stopping TKIs

- CD93 is consistently and selectively expressed on a lin⁻CD34⁺CD38⁻CD90⁺ CML LSC population
- CD93+ cells show robust engraftment in PDX models in comparison with CD93⁻ CML cells and show a SC signature
- CD93 expression was not eliminated by TKI and persisted in patients with prolonged TKI exposure (>3 yrs) who developed molecular recurrence upon TKI withdrawal





Predictive biomarker to distinguish those CML patients at high risk of molecular recurrence after discontinuation?

Kinstrie et al, Leukemia 2020

How to eradicate CML LSCs

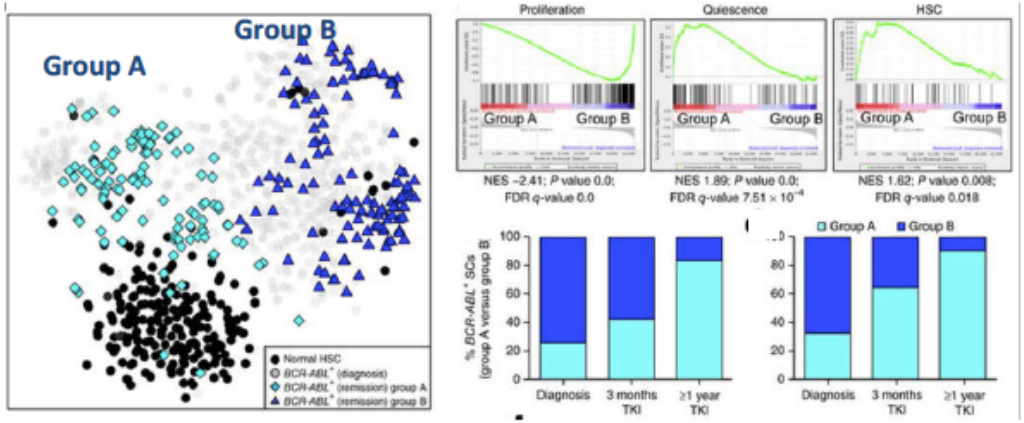
Cell-intrinsic and cell-estrinsic mechanisms of survival have been identified and probably cooperate:

- Identify and target CML LSC-specific survival pathways
- Inhibit the homing and engraftment of LSC within the BM niche, without affecting normal hematopoietic stem cells (HSC)

Cell intrinsic pathways/mechanisms traditionally known to support LSCs in CML

- Wnt
- Hedgehog
- β-catenin
- FOXO
- TGFβ
- PP2A
- Jak2
- p53/Myc
- Autophagy
- SIRT1
- ALOX5
- EZH2
- BCL6
- PML
- ADAR1
- miR126
- miR183

Single-cell analysis of CML LSC reveals the existence of different subgroups of LSCs

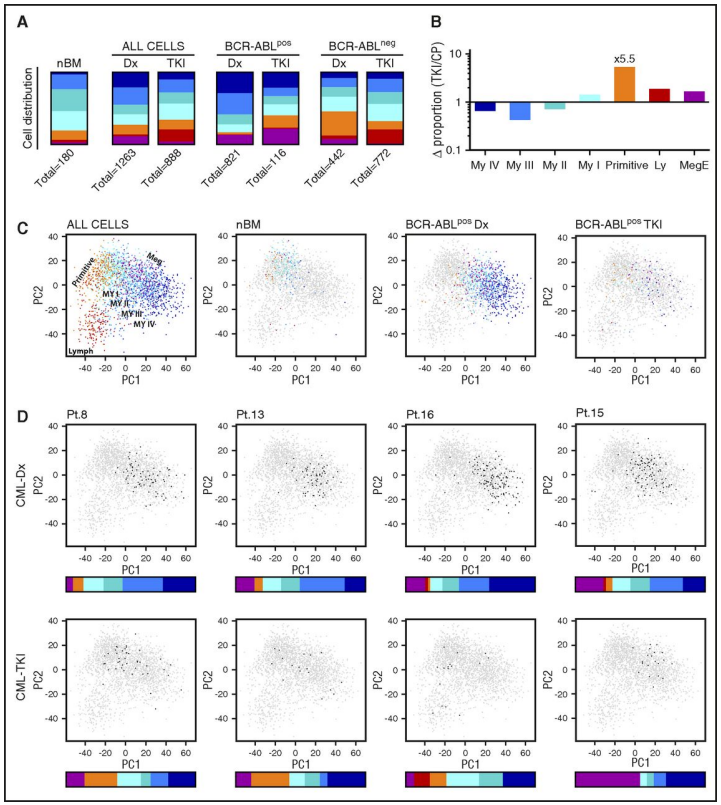


- LSCs in poor responders are already at diagnosis expressing more quiescence-associated genes than in pts who will later achieve MMR
- This was observed for both BCR-ABL+ and BCR-ABL− SCs, suggesting differences in cell-extrinsic, microenvironmental factors between pts
- TKI treatment results in the selective persistence of a distinct and highly quiescent BCR-ABL1+ LSC subset already present at diagnosis, that is transcriptionally distinct from quiescent normal HSCs, with dysregulation of specific genes and pathways (TGF-β, TNF-α, JAK–STAT..) that might be selectively targeted

Giustacchini et al, Nat Med 2017

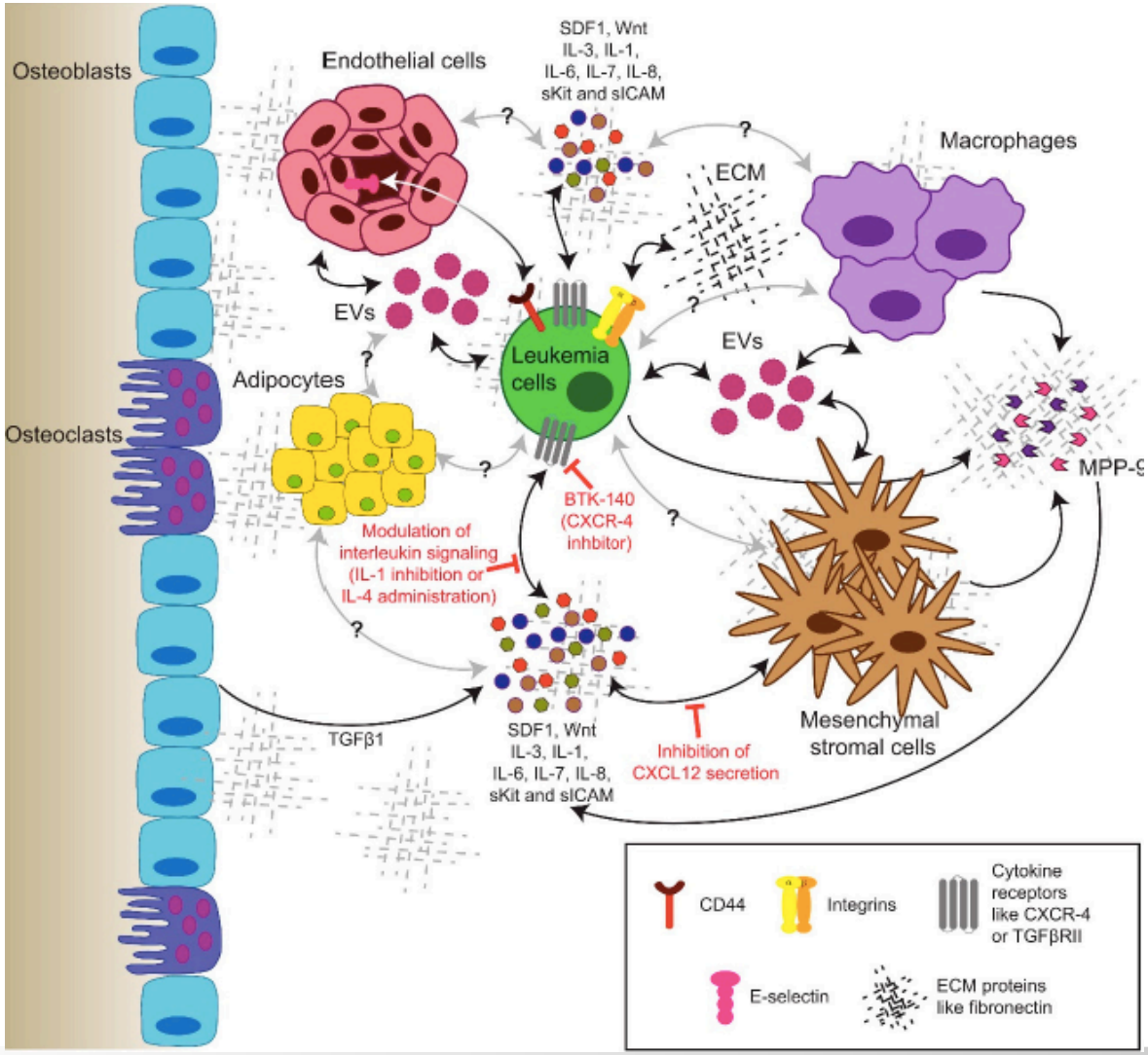
Single-cell analysis defines therapy response and immunophenotype of LSC subpopulations

- Substantial heterogeneity within the putative LSC population in CML at diagnosis and differences in response to subsequent TKI treatment between distinct subpopulations
- expansion of the *BCR-ABL1*$^{+}$ subpopulation with a quiescent, primitive molecular profile
- Despite heterogenous expression of surface markers, the most TKI-insensitive LSC subpopulation was found to be Lin^{-}CD34^{+}CD38$^{-/low}$CD45RA^{-}cKIT^{-} CD26^{+}, offering possibilities for characterization of therapy insensitivity in CML



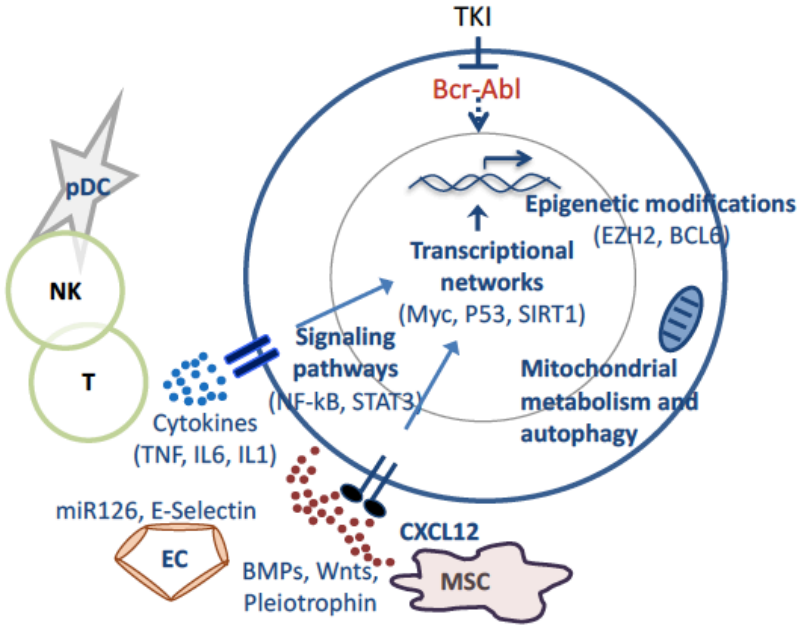
Warfvinge et al, Blood 2017

The BM microenvironment



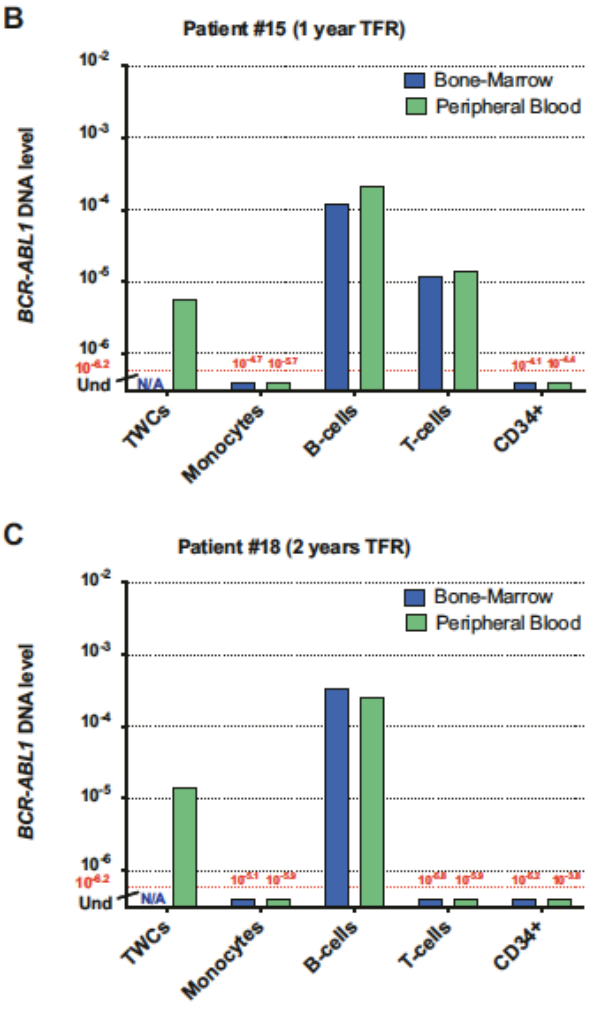
Minciacchi, Kumar and Krause, Cells 2021

Will improved knowledge of CML LSCs, microenvironment and immune effectors translate in more efficient LSC killing? At what cost? And do we really need it?



Lineage of MRD+ cells in pts in TFR

- FACS-sorting into granulocytes, monocytes, B cells, T cells, and NK cells of PB samples from 20 CML pts in TFR for >1 year, followed by DNA-PCR for BCR-ABL
- MRD was identified predominantly in the lymphoid compartment and never in granulocytes
- MRD in the blood of TFR patients does not necessarily imply the persistence of multipotent CML cells!



Pagani et al, Leukemia 2020

Take Home Messages

- It is believed that, by gaining a better understanding of the interactions between LSC and their microenvironment, it may be possible to identify factors that favor survival of the leukemic cells and identify targets for disease eradication
- This has led to hundreds of publications so far, and continues fostering studies

Take Home Messages

- Combining LSCs-targeting agents with TKIs is currently very challenging, given the high benchmark established with TKIs for patient care
- Further studies are needed to understand whether LSC persistence plays a role in molecular recurrence after TFR, or whether it is rather an immunological issue