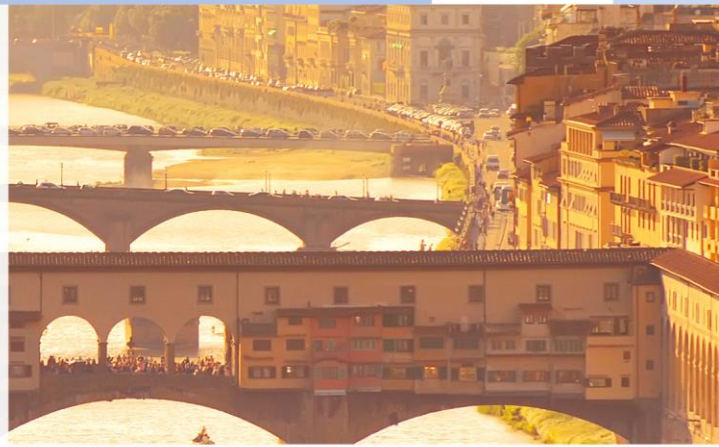
8° WORKSHOP IN EMATOLOGIA TRASLAZIONALE

DELLA SOCIETÀ ITALIANA DI EMATOLOGIA SPERIMENTALE

Firenze - Auditorium CTO - A.O.U. Careggi, 22-23 giugno 2023



Cellule staminali leucemiche residue dopo terapia con TKIs: do they matter?

Anna Sicuranza

Disclosures of Anna Sicuranza

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

Dipeptidylpeptidase IV (CD26) defines leukemic stem cells (LSC) in chronic myeloid leukemia

Harald Herrmann,[1] Irina Sadovnik,[2] Sabine Cerny-Reiterer,[1,2] Thomas Rülicke,[3] Gabriele Stefanzl,[2] Michael Willmann,[4] Gregor Hoermann,[5] Martin Bilban,[5] Katharina Blatt,[2] Susanne Herndlhofer,[2] Matthias Mayerhofer,[5] Berthold Streubel,[6] Wolfgang R. Sperr,[1,2] Tessa L. Holyoake,[7] Christine Mannhalter,[5] and Peter Valent[1,2]

BLOOD, 19 JUNE 2014 • VOLUME 123, NUMBER 25

DPPIV (CD26) as a novel stem cell marker in Ph+ chronic myeloid leukaemia

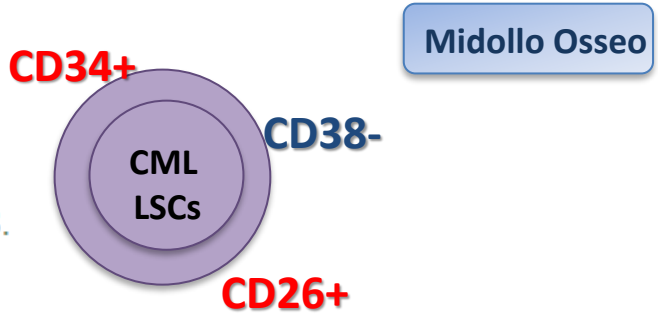
Peter Valent*,†, Irina Sadovnik*, ZdeněkRáčil‡,§, Harald Herrmann*,†,¶, Katharina Blatt*, Sabine Cerny-Reiterer*,†, Gregor Eisenwort*,†, Thomas Lion**, Tessa Holyoake†† and Jiří Mayer‡,§

Eur J Clin Invest 2014; 44 (12): 1239–1245

Distinguishing CML LSCs from HSCs using **CD26**.

Jiang X.

Blood. 2014 Jun 19;123(25):3851-2. doi: 10.1182/blood-2014-05-574293.



DPPIV (**CD26**) as a novel **stem** cell marker in Ph+ chronic myeloid leukaemia.

Valent P, Sadovnik I, Ráčil Z, Herrmann H, Blatt K, Cerny-Reiterer S, Eisenwort G, Lion T, Holyoake T, Mayer J.

Eur J Clin Invest. 2014 Dec;44(12):1239-45. doi: 10.1111/eci.12368.

Quantitative assessment of the **CD26+ leukemic stem** cell compartment in chronic myeloid leukemia: patient-subgroups, prognostic impact, and technical aspects.

Culen M, Borsky M, Nemethova V, Razga F, Smejkal J, Jurcek T, Dvorakova D, Zackova D, Wei B, Semerad L, Sadovnik I, Eisenwort G, Herrmann H, Valent P, Mayer J, Racil Z.

Oncotarget. 2016 May 31;7(22):33016-24. doi: 10.18632/oncotarget.9108.

studio retrospettivo

Sangue periferico

frontiers in Oncology

Residual Peripheral Blood CD26+ Leukemic Stem Cells in Chronic Myeloid Leukemia Patients During TKI Therapy and During Treatment-Free Remission

OPEN ACCESS

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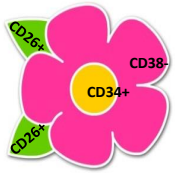
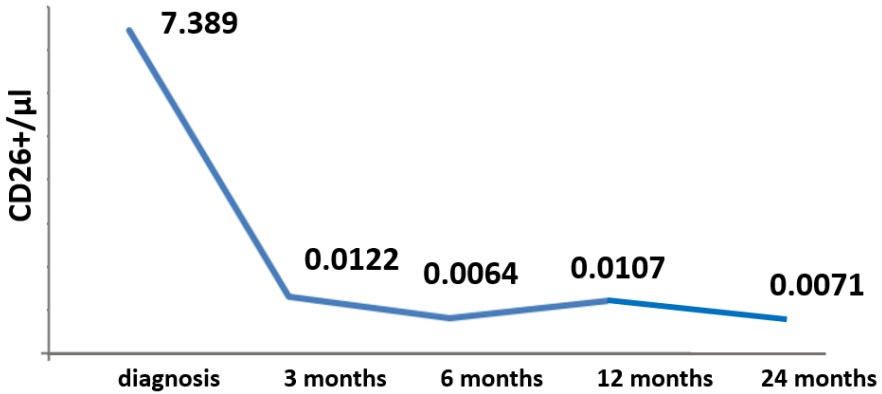
- Valutazione citofluorimetrica delle CD26+ LSCs nel sangue periferico di pazienti LMC alla diagnosi, durante il follow-up, e durante il TFR
- Persistenza di cellule CD26+ circolanti residue anche in LMC con trascritto BCR-ABL1 undetectable
- Nessuna correlazione tra la persistenza delle cellule staminali circolanti e il tipo di TKI
- Nessuna correlazione tra il numero assoluto di cellule CD26+ e il numero di copie di BCR::ABL1 e la risposta molecolare

Studio prospettico multicentrico

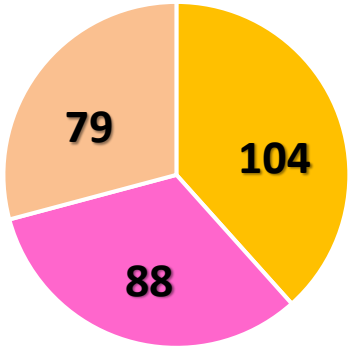
PROSPECTIVE FLOW-CYTOMETRY EVALUATION OF RESIDUAl STEM CELLS IN CML PATIENTS DURING TKI TREATMENT (PROSPECTIVE FLOWERS STUDY)



Median CD26+LSCS/µL



271 pazienti LMC



IMATINIB NILOTINIB DASATINIB

Nessuna differenza tra TKIs

Risposta molecolare vs CD26+ LSCs durante TKI

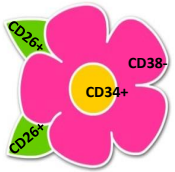
	# of patients	BCR-ABL/ABL^{IS} median values	CD26+LSCs/µl median values
at 3 months of TKI treatment	171/182 (94%)	1.25 (0.0029-82.49)	0.014 (0-6.4901)
• BCR-ABL/ABL^{IS} >10%	23/171 (13.4%)	18.08 (10.2-82.49)	0.0215 (0-0,2516)
• BCR-ABL/ABL^{IS} <10%	148/171 (86.6%)	0.6117 (0-9.3)	0.0132 (0-6.4901)
at 6 months of TKI treatment	150/182 (82.4%)	0.106 (0-23.35)	0.0084 (0-1.1889)
• BCR-ABL/ABL^{IS} >1%	29/150 (19%)	4 (1,113-23.35)	0.01205 (0-1.1889)
• BCR-ABL/ABL^{IS} <1%	121/150 (81%)	0.0595 (0-0.981)	0.00645 (0-0.142)
at 12 months of TKI treatment	124/182 (68%)	0.0291 (0-8.6)	0.0128 (0-0.5888)
• BCR-ABL/ABL^{IS} >0,1%	35/124 (28.2%)	0.491 (0.1-8.6)	0.013 (0-0.1824)
• BCR-ABL/ABL^{IS} <0,1%	89/124 (71.8%)	0.0111 (0-0.0955)	0.0112 (0-0.0878)

Nessuna correlazione Tra il numero di CD26+LSCs residue E il numero di copie di BCR::ABL1

What happens to CD26+LSCs during TREATMENT FREE REMISSION?

Studio prospettico multicentrico

PROSPECTIVE FLOW-CYTOMETRY EVALUATION OF RESIDUAL STEM CELLS IN CML PATIENTS DURING TFR (PROSPECTIVE FLOWERS-TFR)



frontiers | Frontiers in Pharmacology

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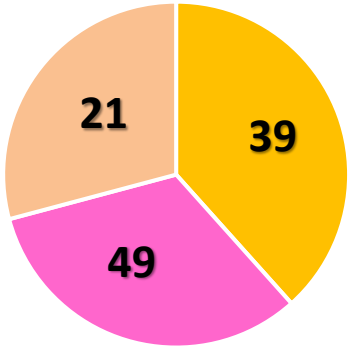
Prospective monitoring of chronic myeloid leukemia patients from the time of TKI discontinuation: the fate of peripheral blood CD26$^+$ leukemia stem cells

Paola Pacelli[1*†], Adele Santoni[1*†], Anna Sicuranza[1*†], Elisabetta Abruzzese[2], Valentina Giai[3], Monica Crugnola[4], Mario Annunziata[5], Sara Galimberti[6], Alessandra Iurlo[7], Luigiana Luciano[8], Federica Sorà[9], Carmen Fava[10], Elena Bestoso[1], Cristina Marzano[1], Alessandra Cartocci[11], Marzia Defina[1], Vincenzo Sammartano[1], Emanuele Cencini[1], Donatella Raspadori[1] and Monica Bocchia[1]

109 pazienti



IMATINIB **NILOTINIB** **DASATINIB**

EMATOLOGIA

PATIENT'S CHARACTERISTICS	Whole cohort (n = 109)
Median age at diagnosis (range)	53 years (19-76 yr)
Sex	
Male	62 (57%)
Female	47 (43%)
Median WBC at discontinuation (range)	6000/mmc (3342-12680/mmc)
Median LY at discontinuation (range)	1680,5/mmc (1000-3465/mmc)
Sokal score	
High	16 (14,5%)
Intermediate	38 (35%)
Low	49 (45%)
Unknown	6 (5,5%)
EUTOS score	
High	15 (14%)
Intermediate	6 (5,5%)
Low	82 (75%)
Unknown	6 (5,5%)
Median time to complete cytogenetic response (range)	3 months (2-21 mos)
Molecular Response after starting therapy 12 months	
MR ≤ 3	56 (51,4%)
MR = 4	16 (14,7%)
MR = 4,5	14 (12,8%)
MR = 5	16 (14,7%)
Unknown	7 (6,4%)

109 pazienti

38/109 (35%) perso TFR

71/109 (65%) mantenuto TFR

PATIENT'S CHARACTERISTICS	Whole cohort (n = 109)	TFR loss (n = 38)	TFR sustained (n = 71)
Median age at diagnosis (range)	53 years (19-76 yr)	51,5 years (27-76 yrs)	54 years (19-76 yrs)
Sex			
Male	62 (57%)	20 (53%)	42 (59%)
Female	47 (43%)	18 (47%)	29 (41%)
Median WBC at discontinuation (range)	6000/mmc (3342-12680/mmc)	6000/mmc (3767-12680/mmc)	6040/mmc (3342-9800/mmc)
Median LY at discontinuation (range)	1680,5/mmc (1000-3465/mmc)	1680,5/mmc (1000-3465/mmc)	1700/mmc (1000-3400/mmc)
Sokal score			
High	16 (14,5%)	4 (10,5%)	12 (17%)
Intermediate	38 (35%)	12 (31,5%)	26 (36,5%)
Low	49 (45%)	20 (53%)	29 (41%)
Unknown	6 (5,5%)	2 (5%)	4 (5,5%)
EUTOS score			
High	15 (14%)	4 (10,5%)	11 (15,5%)
Intermediate	6 (5,5%)	4 (10,5%)	2 (3%)
Low	82 (75%)	28 (74%)	54 (76%)
Unknown	6 (5,5%)	2 (5%)	4 (5,5%)
Median time to complete cytogenetic response (range)	3 months (2-21 mos)	3 months (2-21 mos)	3 months (2-18 mos)
Molecular Response after starting therapy 12 months			
MR $\leq$ 3	56 (51,4%)	15 (39,5%)	41 (57,7%)
MR = 4	16 (14,7%)	8 (21,1%)	8 (11,3%)
MR = 4,5	14 (12,8%)	8 (21,1%)	6 (8,5%)
MR = 5	16 (14,7%)	4 (10,5%)	12 (16,9%)
Unknown	7 (6,4%)	3 (7,8%)	4 (5,6%)

PATIENT'S CHARACTERISTICS	Whole cohort (n = 109)	TFR loss (n = 38)	TFR sustained (n = 71)
TKI therapy before discontinuation			
Imatinib	39 (35,8%)	19 (50,0%)	20 (28,2%)
Nilotinib	49 (45%)	11 (28,9%)	38 (53,5%)
Dasatinib	21 (19,2%)	8 (21,1%)	13 (18,3%)
Median TKI treatment duration (range)	7 years (3-18 yrs)	7 years (7-18 yrs)	7,5 years (3-17 yrs)
Median TKI treatment duration according to TKI			
Imatinib	8 years (4-18 yrs)	8 years (4-18 yrs)	8 years (4-16 yrs)
Nilotinib	7 years (3-17 yrs)	7 years (3-12 yrs)	7 years (3-17 yrs)
Dasatinib	8 years (3-15 yrs)	8 years (4-14 yrs)	8 years (3-15 yrs)
Median observation time from TKI discontinuation (range)	33 months (2-63 mos)	35 months (4-60 mos)	32 months (2-63 mos)

PATIENT'S CHARACTERISTICS	Whole cohort (n = 109)	TFR loss (n = 38)	TFR sustained (n = 71)
TKI therapy before discontinuation			
Imatinib	39 (35,8%)	19 (50,0%)	20 (28,2%)
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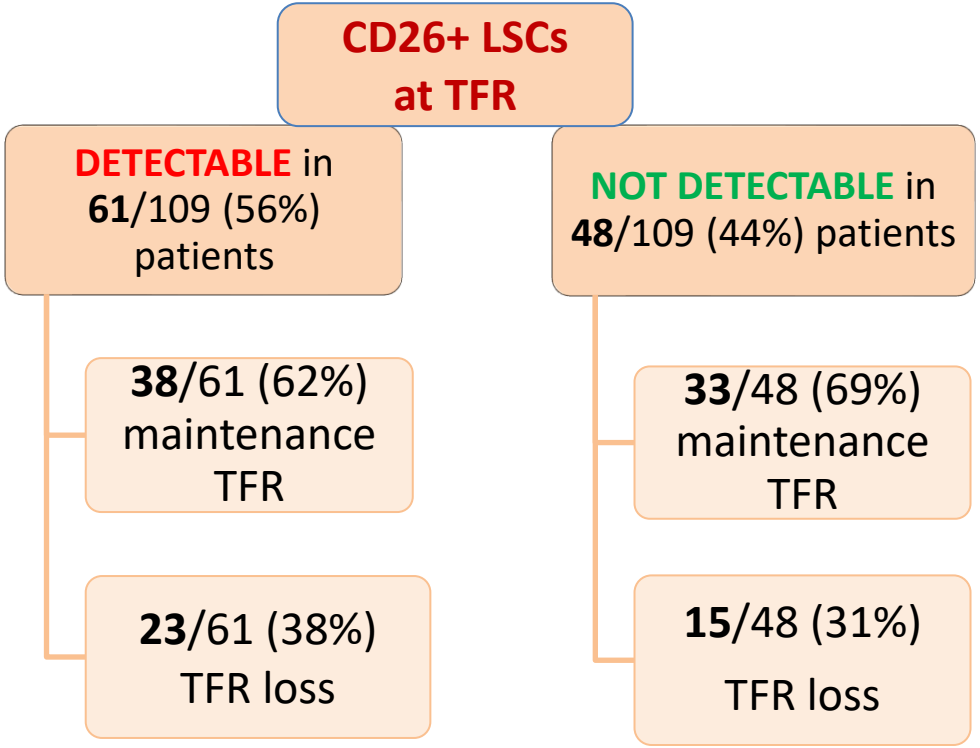
Dasatinib	21 (19,2%)	8 (21,1%)	13 (18,3%)
Median TKI treatment duration (range)			
Median TKI treatment duration according to TK			
Imatinib			
Nilotinib			
Dasatinib			
Median observation time from TKI discontinuation (range)	33 months (2-63 mos)	35 months (4-60 mos)	32 months (2-63 mos)

20/39 imatinib (51%) sustained TFR

38/49 nilotinib (77%) sustained TFR — P=<0.05

13/21 dasatinib (62%) sustained TFR — P=N.S. — P=N.S.

- Tempo mediano di ricaduta dopo sospensione TKI è di 4 mesi



- Numbero mediano di CD26+LSCs: 0.007/µL (range 0.0001-0.1184)

Detectable/undetectable CD26+LSCs vs detectable/undetectable BCR::ABL1

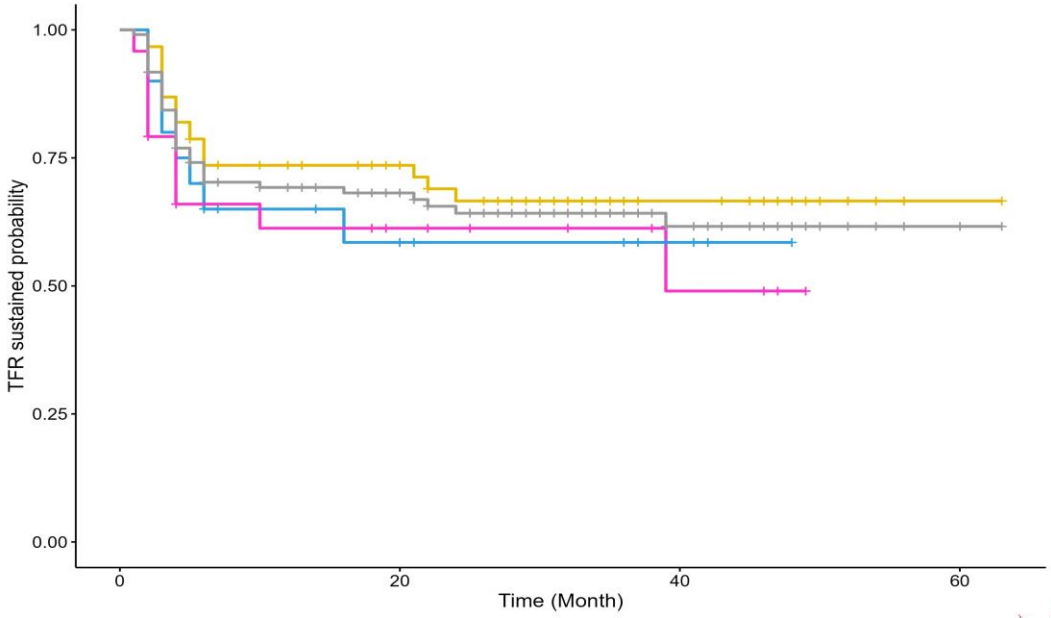
	CD26+ LSCs detectable BCR::ABL1 detectable	CD26+ LSCs undetectable BCR::ABL1 undetectable	CD26+ LSCs detectable BCR::ABL1 undetectable	CD26+ LSCs undetectable BCR::ABL1 detectable
Patients (n = 109)	25/109 (22,9%)	21/109 (19,3%)	36/109 (33,0%)	27/109 (24,7%)
TFR LOSS (n = 38)	11/38 (29,0%)	8/38 (21,0%)	12/38 (31,6%)	7/38 (18,4%)
TFR SUSTAINED (n = 71)	14/71 (19,7%)	13/71 (18,3%)	24/71 (33,8%)	20/71 (28,2%)

Sopravvivenza senza ricaduta molecolare dal momento della discontinuazione

DOUBLE POSITIVE =“positive” for both CD26+LSCs and BCR::ABL1 copies

DOUBLE NEGATIVE= negative for both CD26+LSCs and BCR::ABL1 copies

DISCORDANT= either way positive only for CD26+LSCs or BCR::ABL1

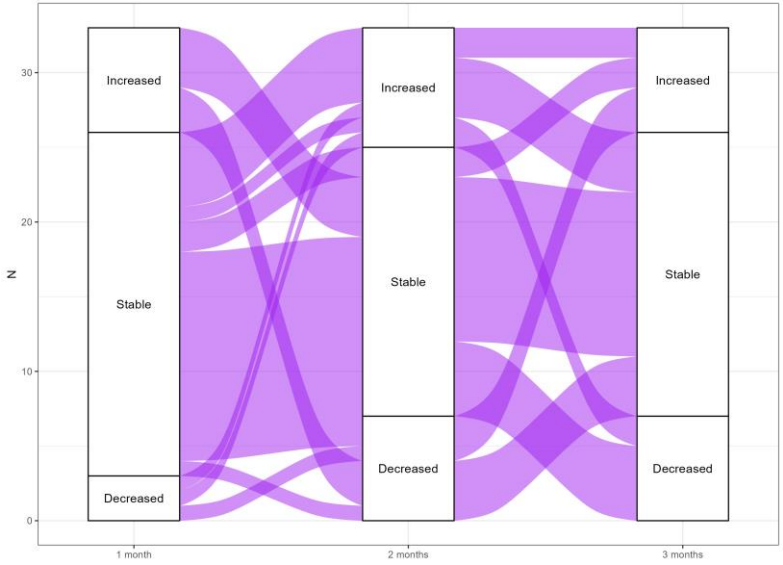


PB CD26+LSCs vs BCR::ABL1 alla sospensione

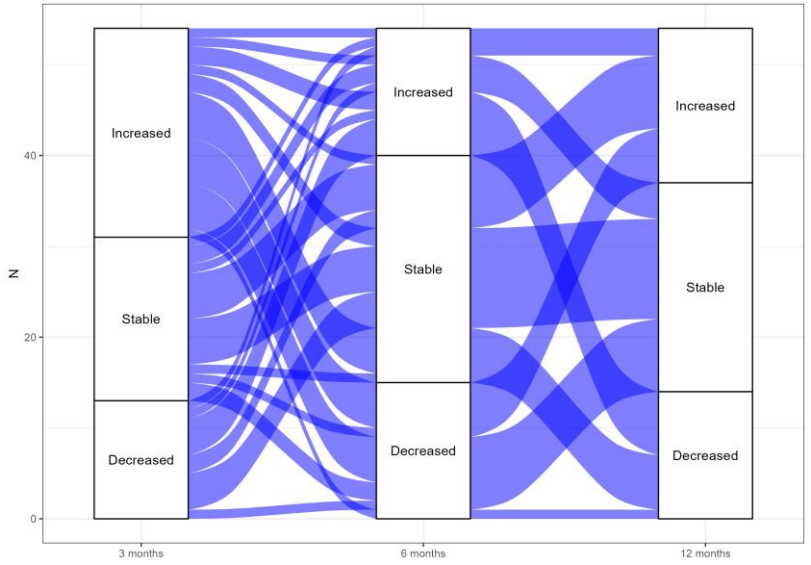
Type of TKI before discontinuation	IMATINIB	NILOTINIB	DASATINIB
Patients whole cohort (n = 109)	39 (35,8%)	49 (45,0%)	21 (19,2%)
CD26+LSCs at TKI discontinuation:			
POSITIVE (range)	19/39 (48,7%) (0,0048-0,1184 cells/µl)	30/49 (61,2%) (0,0001-0,1039 cells/µl)	12/21 (57,1%) (0,0063-0,0695 cells/µl)
NEGATIVE	20/39 (51,3%)	19/49 (38,8%)	9/21 (42,9%)
BCR::ABL1 transcript at TKI discontinuation:			
POSITIVE (range)	19/39 (48,7%) (0,001-0,0046 copies)	26/49 (53,1%) (0,00024-0,007 copies)	10/21 (47,6%) (0,001-0,029 copies)
NEGATIVE	20/39 (51,3%)	23/49 (46,9%)	11/21 (52,4%)

Andamento CD26+LSCs

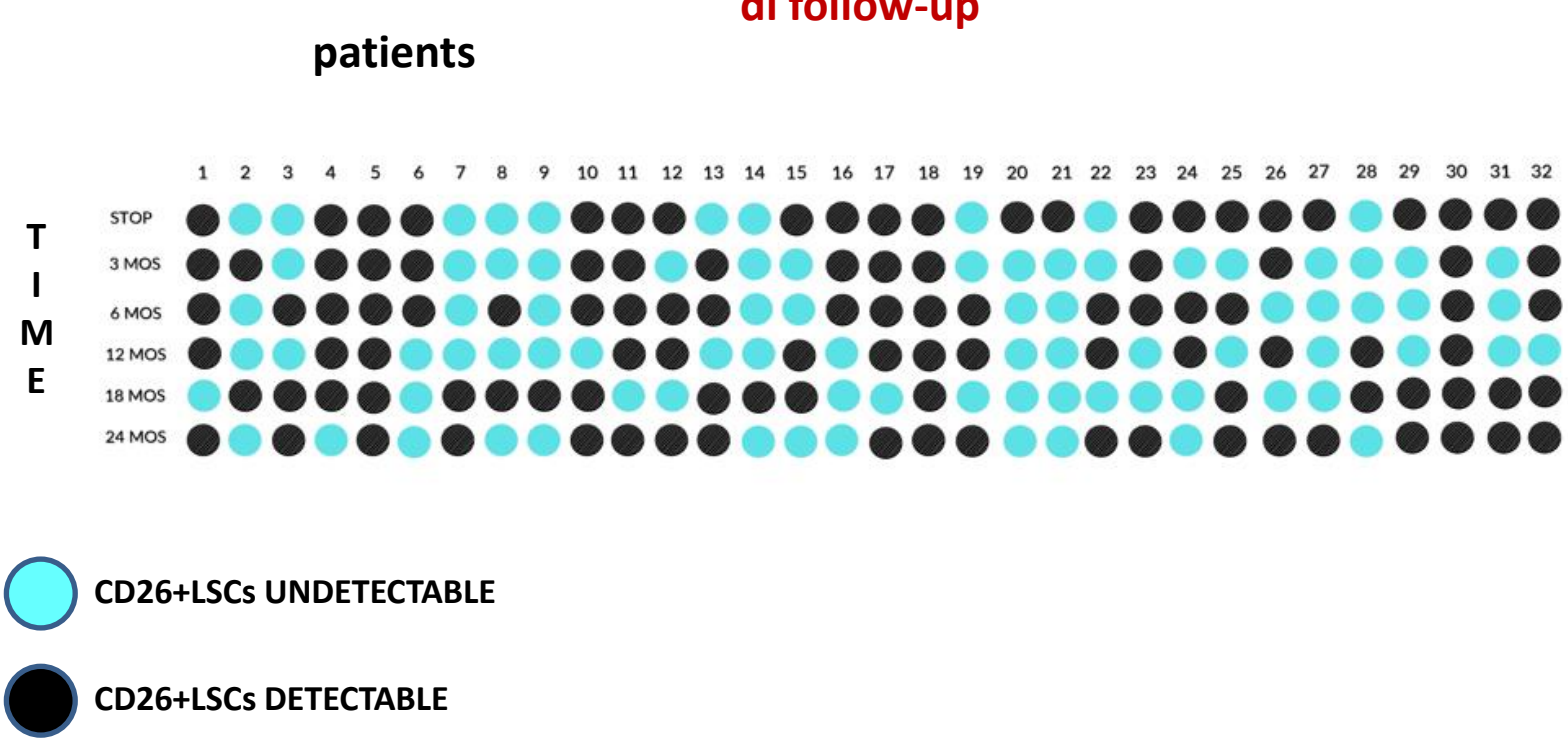
Pazienti che hanno perso il TFR (#38)



Pazienti che hanno mantenuto il TFR (#71)



Andamento CD26+LSCs in 32 pazienti che hanno mantenuto TFR fino a 24 mesi di follow-up



Persistenza delle CD26+ LSCs: perchè?

BANDO SALUTE REGIONE TOSCANA

Progetto StemCMLcure

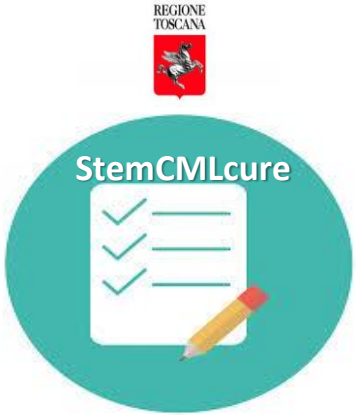
«The achievable cure in Chronic Myeloid Leukemia: unravelling CD26+ leukemia stem cell features to enable safe tyrosine kinase inhibitor discontinuation.»

SIENA
PISA
FIRENZE

Centri partecipanti:
Roma S.Eugenio
Torino
Milano

Time points:
Diagnosi, +3, +6 e +12 mesi di trattamento

Al TFR e durante TFR (+1, +2, +3, +4, +5, +6 mesi)



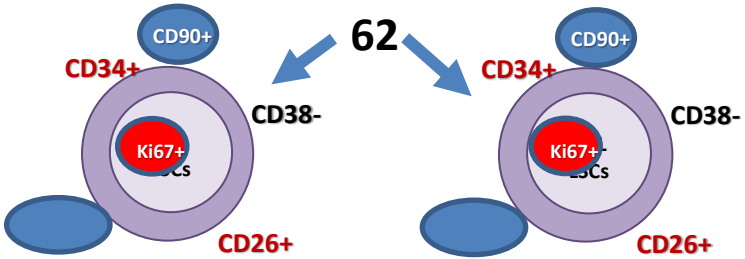
Caratteristiche specifiche di «staminalità»

- Espressione di CD90
- Espressione di Ki67 e BMI1 (assetto proliferativo)
- capacità di sopravvivere in condizioni di ipossia

Controllo del sistema immunitario sulle cellule staminali leucemiche

- Espressione e polimorfismi di PDL1
- Titolo plasmatico di Torquetenovirus come indice di immunocompetenza

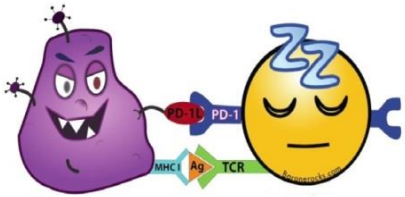
62 CP CML alla diagnosi

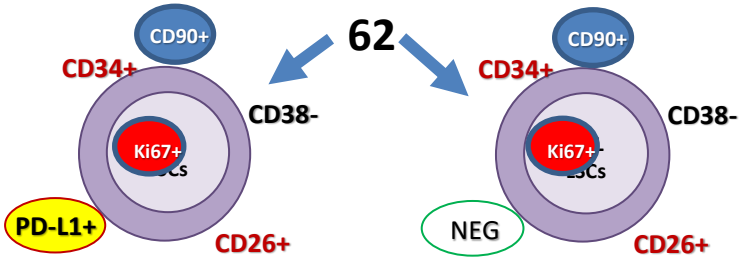


100% CD90+
CD26+LSCs are true stem cells

100% ki67+
CD26+LSCs are cycling?

62 CP CML alla diagnosi



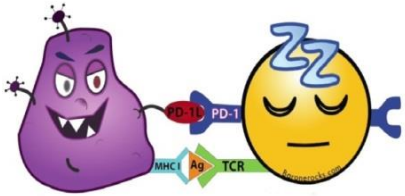


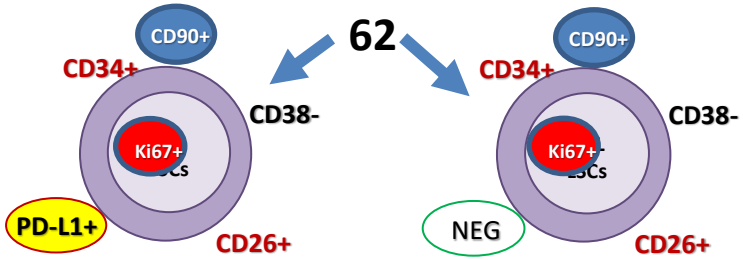
PD-L1 **40**/62 (65%) **22**/62 (35%)

100% CD90+
CD26+LSCs are true stem cells

100% ki67+
CD26+LSCs are cycling?

62 CP CML alla diagnosi





PD-L1 **40**/62 (65%) **22**/62 (35%)

23/40 (57.5%) HIGH INTENSITY

17/40 (43.5) LOW INTENSITY

100% CD90+
CD26+LSCs are true stem cells

100% ki67+
CD26+LSCs are cycling?

TAKE HOME MESSAGE

- Le cellule staminali CD26+ sono detectabili al momento della sospensione del TKI e durante il TFR nella maggior parte dei pazienti
- La persistenza di “valori oscillanti” di CD26+LSCs residue non ostacola il mantenimento di un TFR stabile
- Non è stato individuato un “cut-off” di numero di cellule residue che possa predire la perdita del TFR
- Nessuna correlazione tra il BCR::ABL1 ratio e il numero delle CD26+ LSCs residue
- Nessuna differenza statistica in termini di “relapse rate” tra pazienti doppio positivi CD26+LSCs e BCR::ABL1, o doppio negativi oppure discordanti

TAKE HOME MESSAGE

- valori fluttuanti di CD26+ LSCs residue con grande variabilità tra i pazienti (sia nel sottogruppo che ha perso TFR sia nei 71 pazienti che hanno mantenuto TFR)

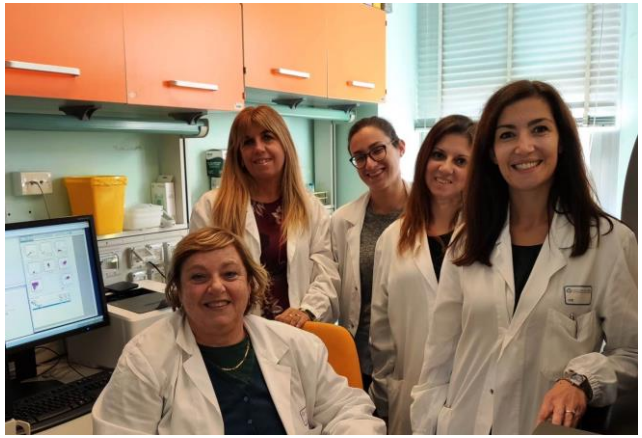
- La persistenza di lunga durata di CD26+LSCs e la fluttuazione del loro valore è stata confermata anche in 32 pazienti con TFR stabile in cui è stata monitorata la valutazione citofluorimetrica delle LSC residue fino a 24 mesi

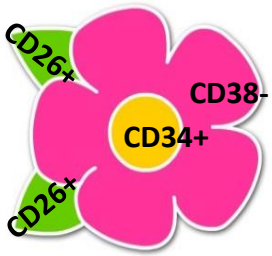
- Le CD26+LSCs hanno un'espressione diversa di PD-L1 tra i pazienti: un follow-up più lungo potrebbe svelare se questa evidenza ha un ruolo per il raggiungimento e il mantenimento del TFR

PROGETTI FUTURI…

in collaborazione con Simona Bernardi, Domenico Russo, Sara Galimberti, Luigiana Luciano, Elisabetta Abruzzese, Carmen Fava e altre/i…

- Valutazione di una possibile correlazione tra le CD26+LSCs e il contenuto di BCR::ABL1 nelle vescicole extracellulari circolanti
- Confronto fra CD26+LSCs, digital-PCR e RT-qPCR nei pazienti LMC in Deep Molecular Response (DMR)
- Confronto fra CD26+LSCs, digital-PCR e RT-qPCR nei pazienti in long TFR





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