

**VIII Giornata Fiorentina  
dedicata ai pazienti con  
malattie mieloproliferative  
croniche**

**Sabato 28 aprile 2018**

**CRIMM**

Centro di Ricerca e Innovazione  
per le Malattie Mieloproliferative

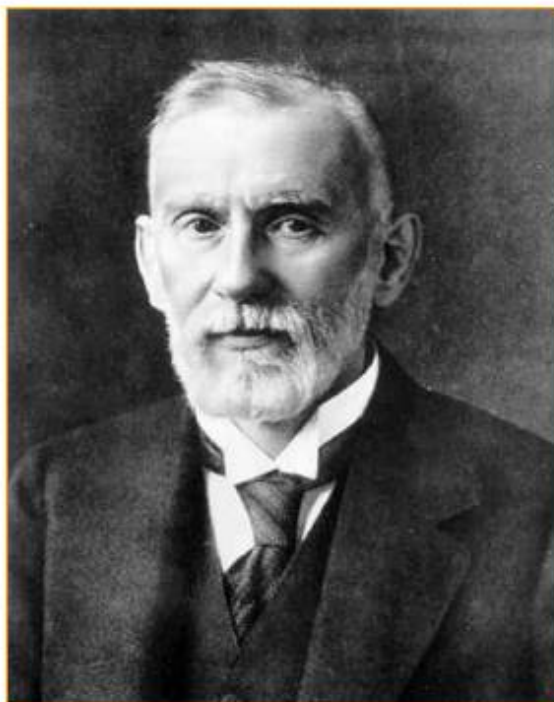


# Mastocitosi e Eosinofilie: Gli studi più recenti

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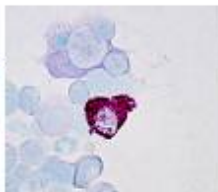




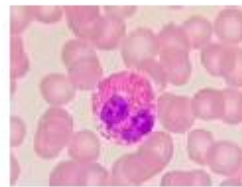
Paul Erlich  
Nobel Prize 1908 (Medicine)



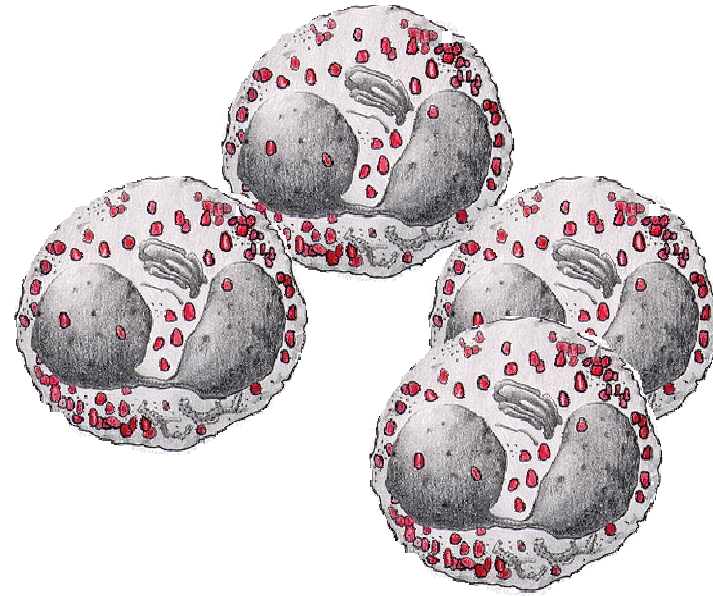
Described in 1879  
Metachromatic granules  
with toluidine blue



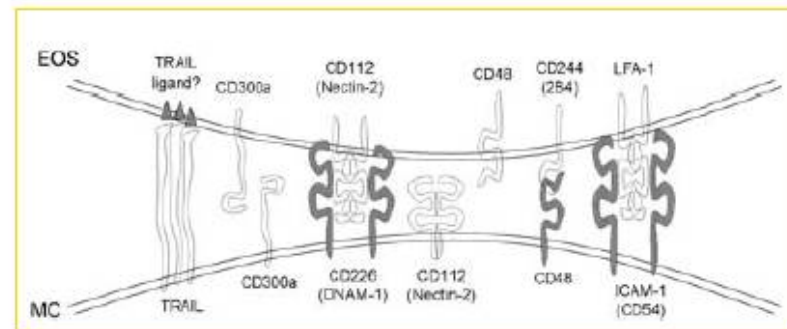
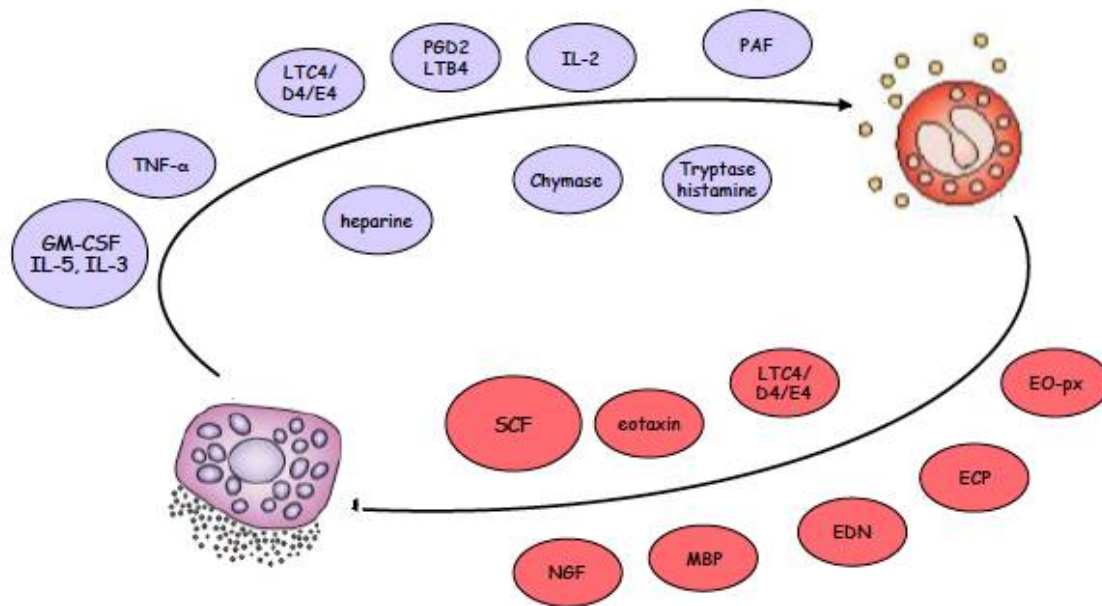
Described in 1879  
Granules taking acid staining  
(e.g. eosin)



# I pereosinofilie



# Mastociti ed eosinofili: unità effettrice nelle allergie

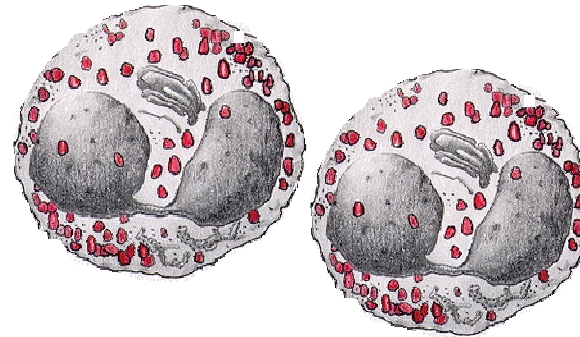


# Eosinofilia

Eosinofili  $\geq 0,5 \times 10^9/L$

Valori normali nel sp.:  $0,05-0,5 \times 10^9/L$

% eosinofili nel midollo normale: 1-6%

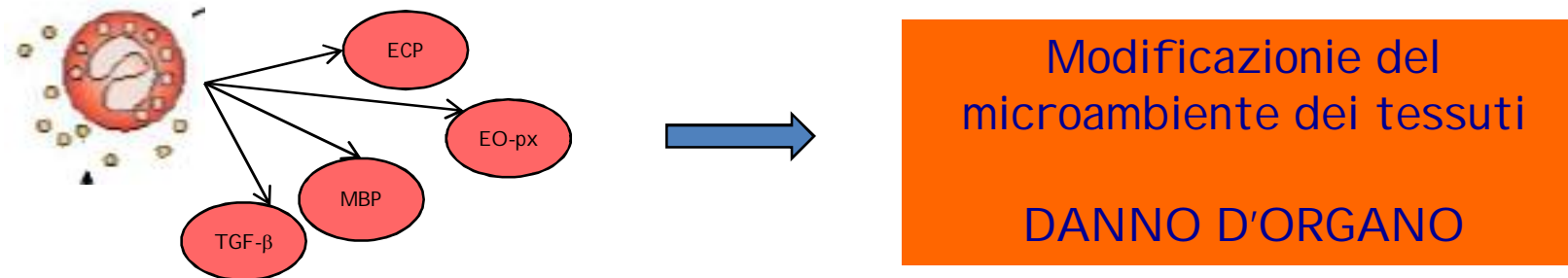


# I pereosinofilia

Eosinofili  $>1,5 \times 10^9/L$  per almeno 6 mesi

- Marcata  $1,5-5,0 \times 10^9/L$
- Massiva  $>5,0 \times 10^9/L$

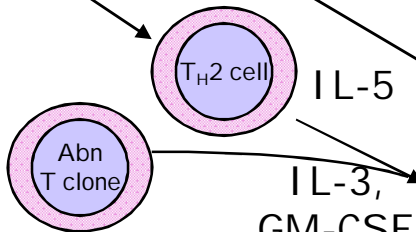
# Sindrome Iper eosinofila (HES) + danno/disfunzione d'organo (sec. Chusid)



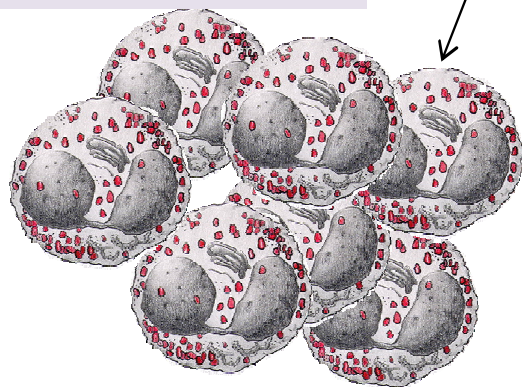
- FIBROSI : polmone, cuore (FIBROSI ENDOMI OCARDICA), intestino, cute, altri organi;
- TROMBOSI : con o senza tromboembolismo;
- CUTE: prurito, eritema, angioedema, ulcerazioni, eczema
- SISTEMA NERVOSO : polineuropatie, mononeuriti, vasculiti, neurite ottica, mielite acuta trasversa;
- SISTEMA GASTROINTESTINALE: epatosplenomegalia, gastroenteriti, colnagite sclerosante, ascite;
- SISTEMA EMATOPOIETICO : citopenie, Fibrosi midollare
- RENE: microangiopatia trombotica.

# I pereosinofilia

Malattie allergiche  
parassitosi  
Sindrome di Churg Strauss  
Mal infiammatorie del colon  
Linfoma di Hodgkin  
Lymphomi T e B  
Istiocitosi  
Tumori solidi,  
Etc...



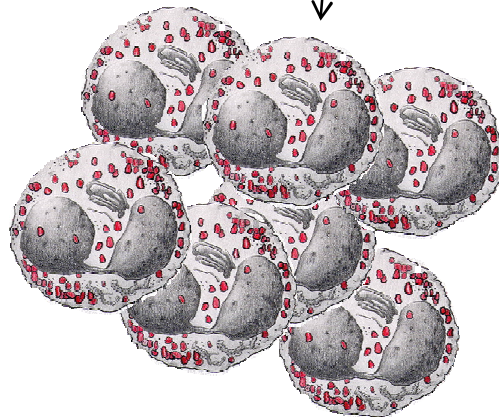
**IPEREOSINOFILIA  
VARIANTE LINFOCITICA**



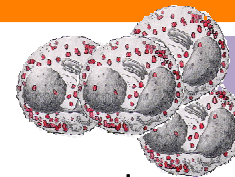
**I pereosinofilie  
secondarie**

Mutazione nei precursori  
midollari  
PDGFRA  
PDGFRB,  
FGFR1  
PCM1-JAK2  
(KIT)

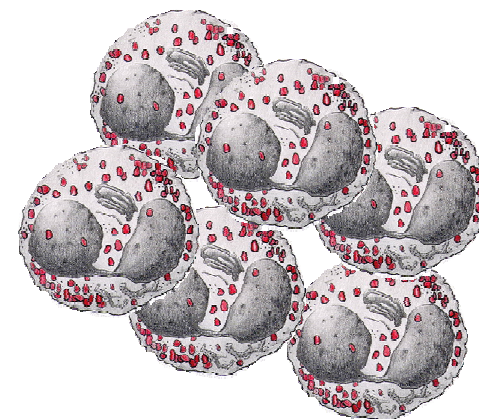
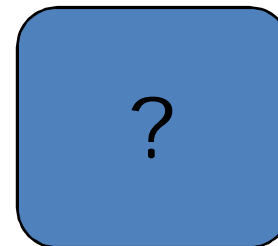
**IPER-EOSINOFILIA**



**I pereosinofilie  
clonali/neoplast**



**IPER-EOSINOFILIA  
FAMILIARE**



**I pereosinofilie  
di nnd.**

# Classificazione WHO 2016

## forme clonali

- 1. ....
- 2. Neoplasie mieloproliferative (MPN)
  - ...
  - **Leucemia eosinofila cronica, non altrimenti specificata (CEL-NOS)**
    - Eosinofili  $>1.5 \times 10^9/L$
    - Non soddisfatti i criteri WHO per CML BCR-ABL1-positive, PV, ET, PMF, CNL, CMML, or CML atipica
    - Assenti i riarrangimenti dei geni PDGFRA, PDGFRB, o FGFR1; assenti i geni di fusione PCM1-JAK2, ETV6-JAK2, or BCR-JAK2
    - La conta dei blasti nel sangue periferico e nel midollo è inferiore 20%, e sono assenti inv(16)(p13.1q22), t(16;16)(p13;q22) e altre aspetti diagnostici per leucemia mieloide acuta
    - sono **presenti anomalie clonali citogenetiche o genetiche molecolari, o blasti sono  $\geq 2\%$  nel sangue periferico o  $\geq 5\%$  nel midollo ma  $<20\%$**
- 3.....
- 4.....
- 5. Mastocitosi
- 6. Neoplasie Mieloidi/linfoidi associate ad eosinofilia e riarrangiamento dei geni PDGFRA, PDGFRB, FGFR1, o riarrangiamento PCM1-JAK2



# I pereosinofilie: forme "non clonali" (?)

## Sindrome ipereosinofila idiopatica o di incerto significato (HES-US)

- Eosinofili >1500/mmc (>6 mesi)
- Danno d'organo
- Esclusione di altre forme secondarie ("reattive")
- Esclusione di forme secondarie a proliferazioni T aberranti
- Esclusione di altre neoplasie mieloproliferative associate a eosinofilia

## I pereosinofilia idiopatica (HE-US)

- Come la precedente senza danno d'organo

## I pereosinofilia -Variante linfocitaria

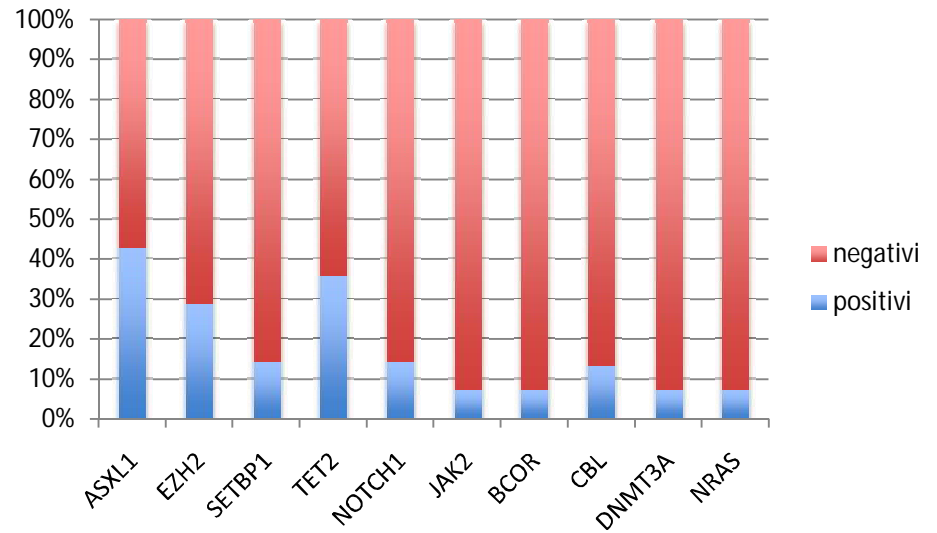
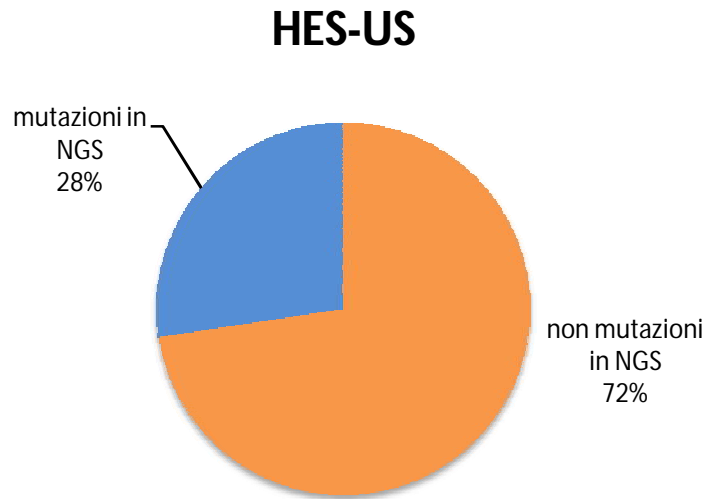
## I pereosinofilia familiare

## HE-US: screening molecolare

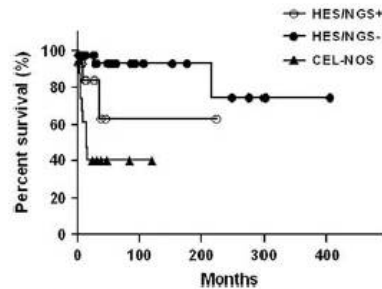
		%	diagnosi
negativi	340	80 %	HES-US
FIP1L1-PDGFR	55	13 %	CEL
KIT D816V	14	3 %	SM-eo (8), ASM .....
JAK2 V617F	17	4 %	MPN eo (3) ...

Schwaab J et al A JH 2016

# HES- US patologia non clonale?



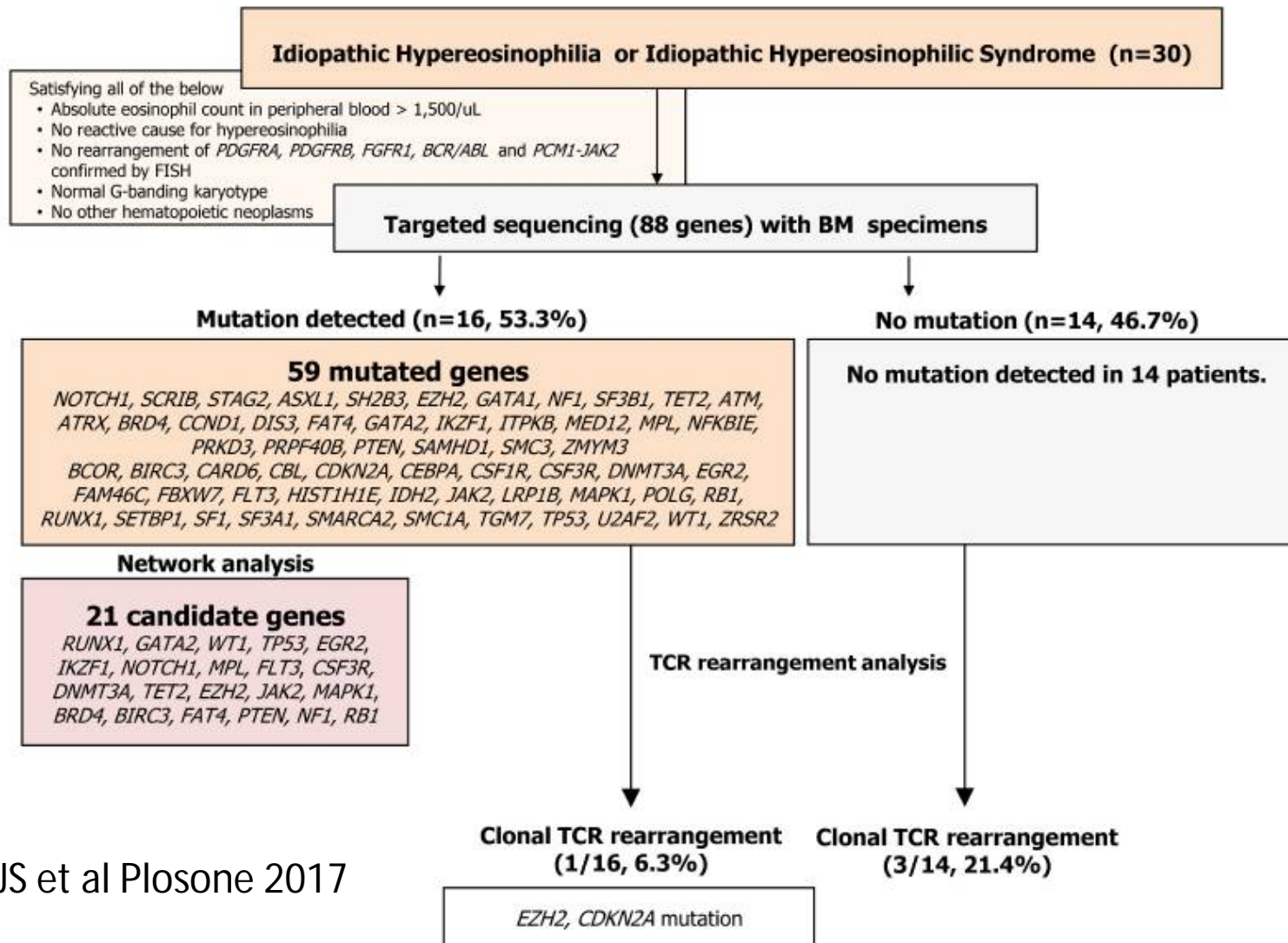
Mutations in idiopathic hypereosinophilic syndrome  
SA Wang *et al*



**Figure 2** Patients with chronic eosinophilic leukemia, not otherwise specified showed a median disease-specific survival of 14.4 months (1.0–120.1 months), significantly inferior to patients with idiopathic hypereosinophilic syndrome with no identifiable mutations (median disease-specific survival: not reached, 0.6–405.3 months,  $P < 0.001$ ), but not statistically different from idiopathic hypereosinophilic syndrome patients with mutations (not reached, 1.0–223.9 months,  $P = 0.117$ ).

Wang SA *et al* Mod Path 2016

# HES-US e HE-US patologia non clonale?



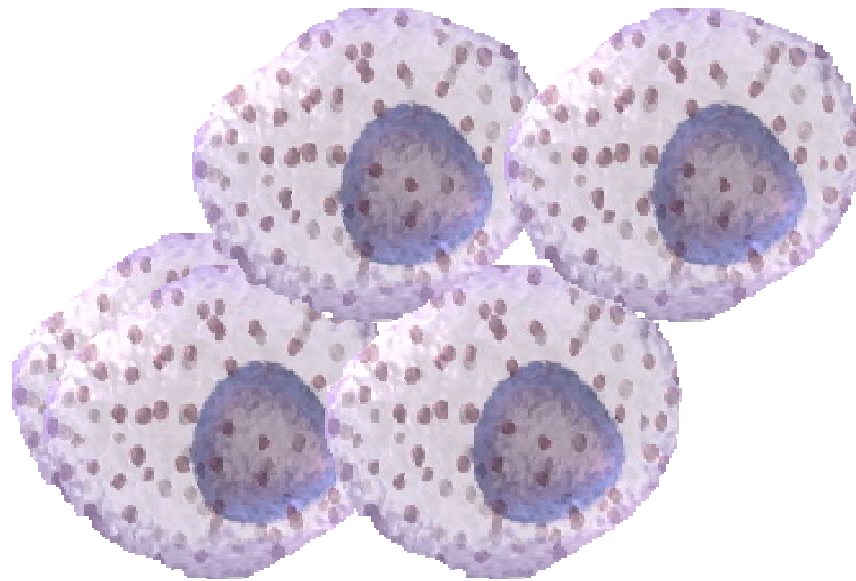
Lee JS et al Plosone 2017

**Fig 1. Flow of idiopathic hypereosinophilia study.** Flow diagram showing how patients were included and evaluated in this study.

# Biomarkers nelle HES

- **Conta assoluta degli eosinofili nel sp**
  - Valori assoluti >100.000/mmc peggiore prognosi
  - Rimane ancora da definire se la sola normalizzazione degli eosinofili sia prognosticamente favorevole
- **Eosinofilia tissutale** (difficile da quantificare), non si sono ancora studi sufficientemente ampi.
- **Dosaggio delle proteine rilasciate dagli eosinofili** durante l'attivazione e depositate nei tessuti (dosabili nel sangue, fluidi corporei, e nei tessuti : poco standardizzati).
  - major basic protein (MBP),
  - eosinophil cationic protein (ECP),
  - eosinophil peroxidase (EPO), specifica degli eosinofili
  - eosinophil-derived neurotoxin (EDN)EPO E EDN : alcuni dati di correlazione con la presenza di sintomi più che la conta degli eosinofili
- **Citochine sieriche, chemochine, recettori solubili**
  - Non informazioni dai livelli di IL-5 (indossabile)
  - Recettore solubile IL-5

# Mastocitosi



# Classificazione della Mastocitosi WHO-2016

## Mastocitosi cutanea

- Mastocitosi maculo-papulare = orticaria pigmentosa
- Mastocitosi cutanea diffusa
- Mastocitoma cutaneo

## Mastocitosi sistemica

- Mastocitosi sistemica indolente
- Mastocitosi Sistemica Smouldering
- Mastocitosi sistemica Aggressiva
- Mastocitosi sistemica sistemica associata ad altra neoplasia ~~ematologica non mastcellulare~~
- Leucemia mastcellulare

## Sarcoma mastcellulare

~~Mastocitoma extracutaneo~~

Arber A et al  
Blood 2016

# Fattori di rischio per l'anafilassi

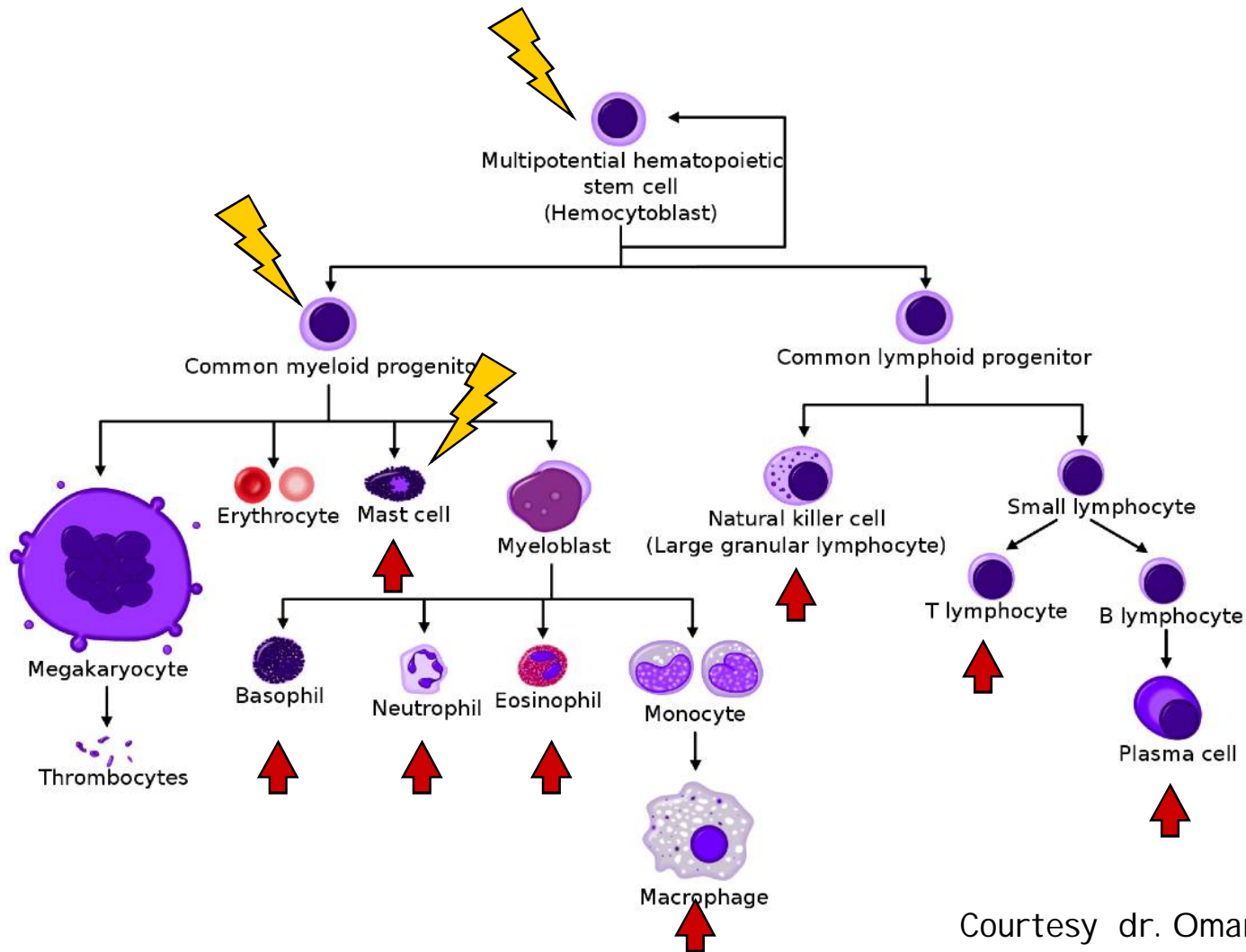
variabile		score
sesso	Maschile	1
	Femminile	0
Coinvolgimento cutaneo	Presente	0
	assente	3
Tryptasi sierica	< 40 ng/ml	2
	>= 40 ng/mL	0
Presenza di atopia	Presenza	1
	Assenza	0
IgE totali	> 15 kU/L	3
	< 15 kU/L	0
<b>Score &lt; 3</b>	<b>Bassa probabilità di anafilassi</b>	
<b>Score &gt;= 3</b>	<b>Alta probabilità di anafilassi</b>	

Sensibilità 86%, specificità 54%,  
potere predittivo negativo 82%  
Potere predittivo positivo 60%

Gulen et al  
J ALLERGY CLIN IMMUNOL PRACT 2017



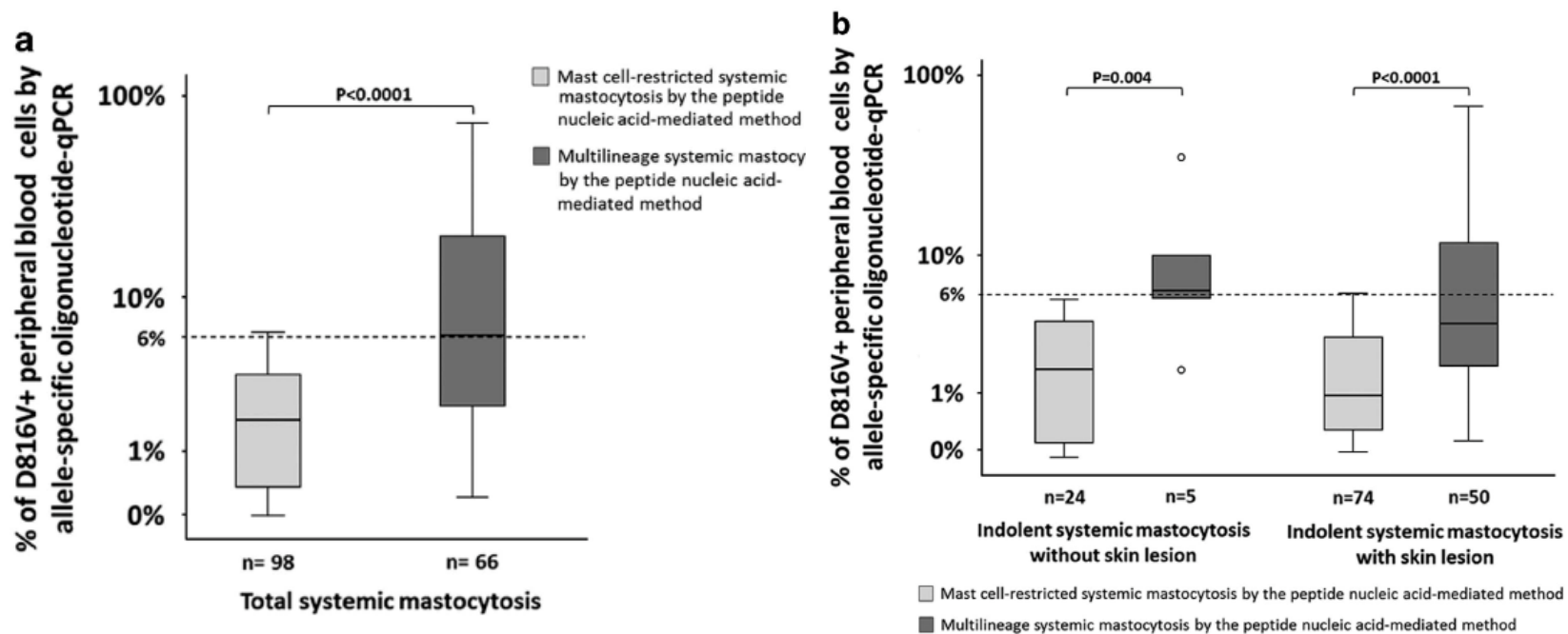
# Mastocitosi: Malattia Unilineale o Multilineale



Courtesy dr. Omar Perbellini

# "Allele burden" della mutazione D816V di KIT correla con la variante WHO e con la prognosi

LINEE GUIDA INTERNAZIONALI: Per l'identificazione della mutazione D816V di KIT è indicato il metodo PCR, allele-specifico, altamente sensibile e se possibile quantificare nel sangue periferico o nel midollo l'allele-burden



Jara-Acevedo et al 2015

Allele burden >6% = multilineage (in futuro CRITERIO B?)

Allele burden <6% e diagnosi di BMM = Mastocitosi unilineare

allele burden <6% non è dirimente se ISM con coinvolgimento cutaneo

# Mutazioni di geni non KI T aggiuntive

Mutated Gene	Cases with additional mutation
TET2	15
SRFS2	14
ASXL1	8
RUNX1	9
CBL	8
EZH2	2
JAK2	2
KRAS	4
NRAS	2
U2AF1	2
ETV6	1
SETB1	1

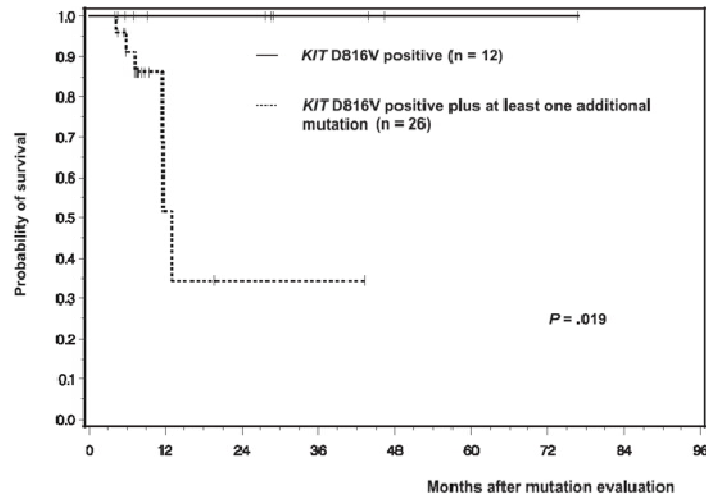
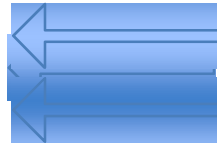


Figure 2. Kaplan-Meier estimates of overall survival of 38 SM patients with respect to the individual mutation status irrespective of disease subtype: 12 patients with *KIT* DB16V alone (ISM, n = 7; SSM, n = 2; ASM, n = 3) vs 26 patients with *KIT* DB16V + at least one additional mutation (ISM, n = 3; SM-AHNMD, n = 4; ASM, n = 19). Patients with *KIT* DB16V only are depicted by the continuous line; patients with additional mutations are depicted by the dashed line.

Schwaab J et al Blood 2013; 122: 2460-2466

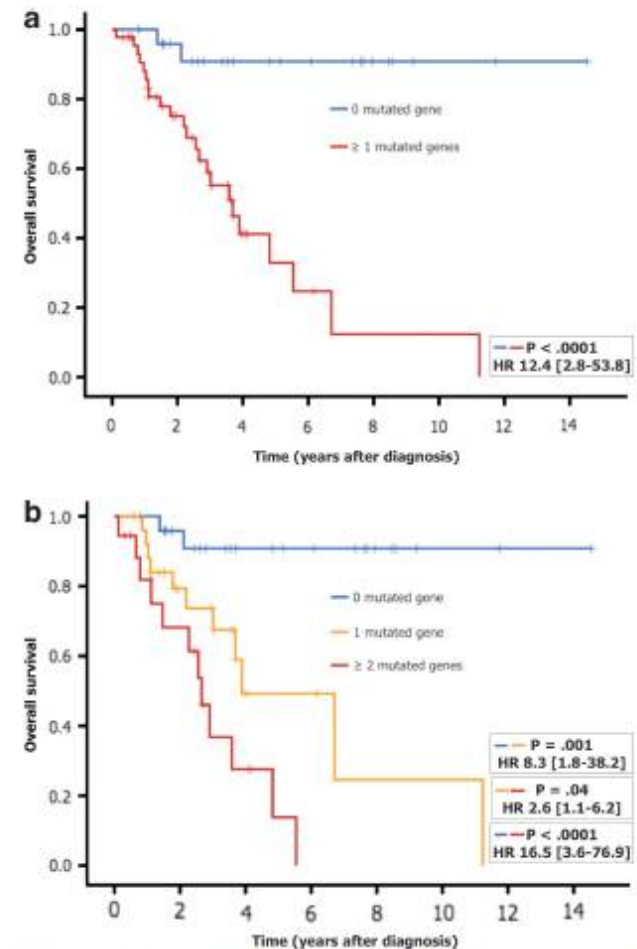


Figure 3. Kaplan-Meier estimates of OS of patients depending on the number of mutated genes in the *SRSF2/ASXL1/RUNX1* (S/A/R) panel. Pairwise significantly different OS probabilities were observed for the comparison (a) 0 (blue line, n = 25) vs  $\geq 1$  (red line, n = 45) mutated genes in the S/A/R panel and (b) 0 mutated gene (blue line, n = 25) vs 1 mutated gene (yellow line, n = 27) vs  $\geq 2$  mutated genes (red line, n = 18) in the S/A/R panel. The HR is presented together with the 95% CI.

# Espressione del CD30 nella mastocitosi

- Inizialmente individuato come marcatore delle forme aggressive
- Successivamente dimostrato come sia espresso in maniera aberrante come il CD25 in gran parte delle mastocitosi anche indolenti e in particolare nelle forme di mastocitosi bene differenziate
- In futuro nuovo criterio minore diagnostico?
- La sua inibizione ha un ruolo terapeutico? (in corso protocollo con BRENTUXIMAB, i risultati nei primi 10 pazienti mostrano una buona tollerabilità ma non sembrano incoraggianti in termini di risultati, (Gotlib et al Blood 2017))

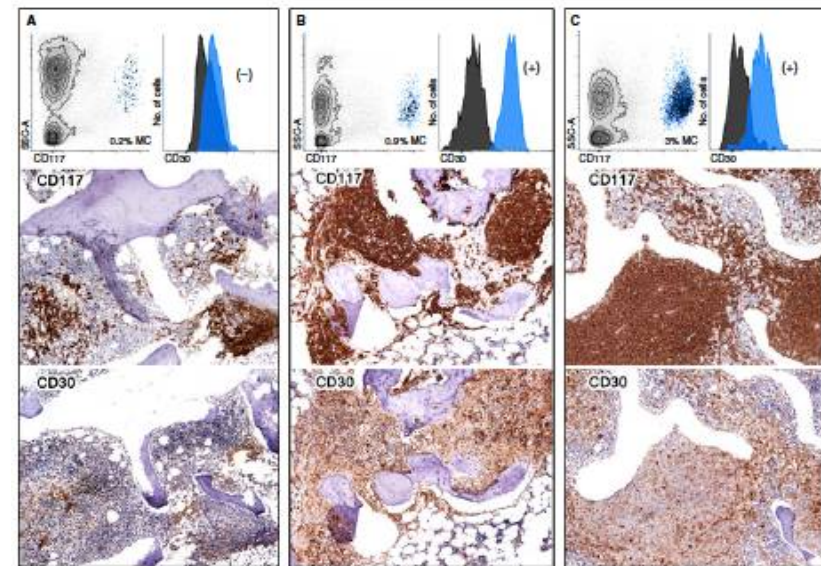
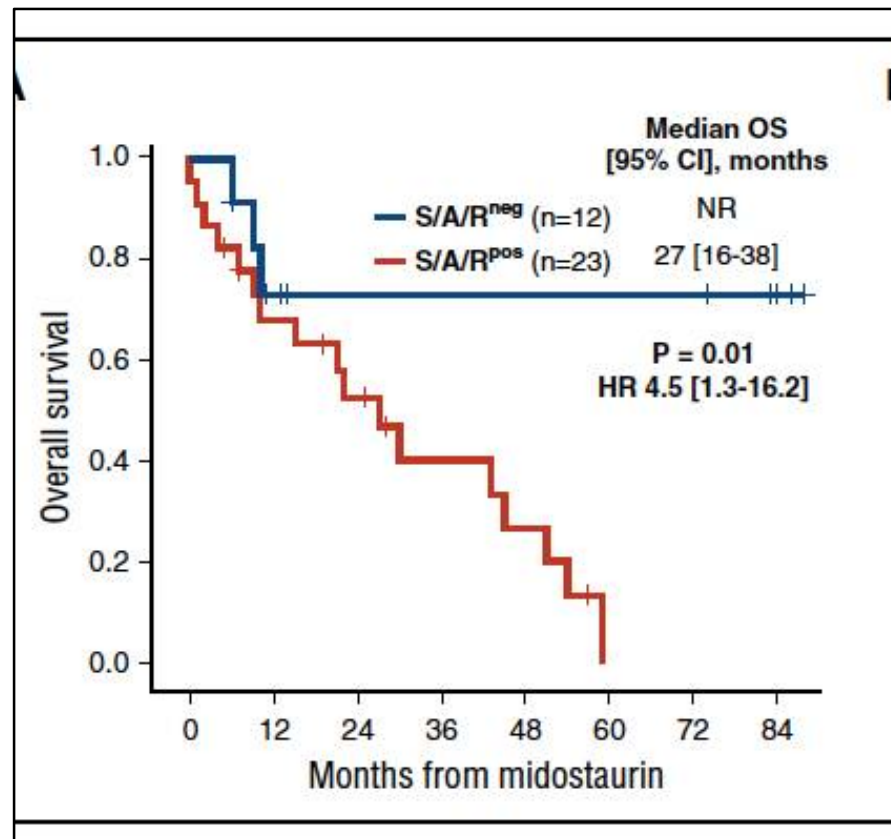
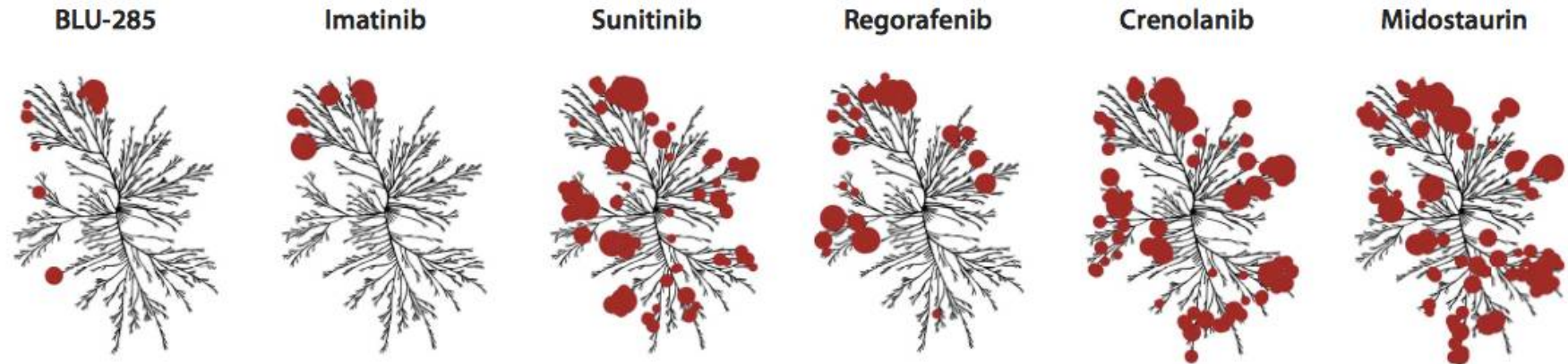


Figure 1. CD30 expression in two representative indolent systemic mastocytosis (ISM) and one aggressive systemic mastocytosis (ASM) bone marrow (BM) samples. A, Representative example of a CD30-negative ISM case; B, representative example of a CD30-positive ISM case; C, representative example of a CD30-positive ASM case. For each panel the top row includes a dot-plot representing a total of  $5 \times 10^4$  cellular events from erythrocyte-lysed whole BM aspirated samples together with a normalized single-parameter histogram containing information on mast cells autofluorescence levels (black histogram) and CD30 fluorescence intensity (blue histogram); representative microphotographs of sections stained immunohistochemically for CD117 (middle row) and CD30 (bottom row) from the same BM samples are also displayed.

## Mutazioni di SRFS2/ASXL1/RUNX1 e risposta alla midostaurina



# Nuove inibitori di KIT: BLU-285



**Fig. 2. BLU-285 is a potent and highly selective inhibitor of KIT and PDGFRA activation loop mutants.** (A) Chemical structure of BLU-285. (B) Biochemical potency against KIT WT and the KIT del557-558 (exon 11), D816V (exon 17), and PDGFRA D842V (exon 18) mutants for BLU-285 and several compounds in use or being explored for the treatment of GIST and SM. (C) Binding data for compounds screened at 3  $\mu$ M against 392 kinases are depicted as red circles on the kinome tree. The size of the circle indicates binding potency. Kinome illustration reproduced courtesy of Cell Signaling Technology ([www.cellsignal.com](http://www.cellsignal.com)).

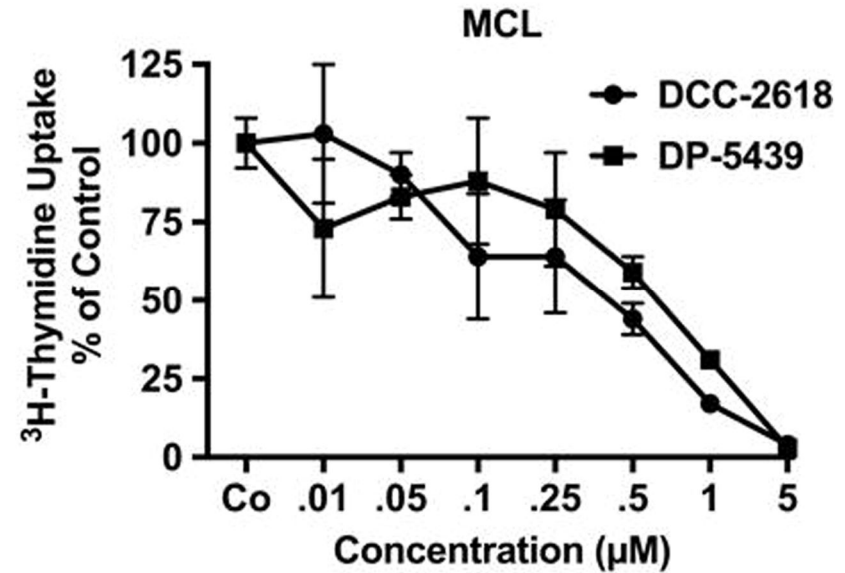
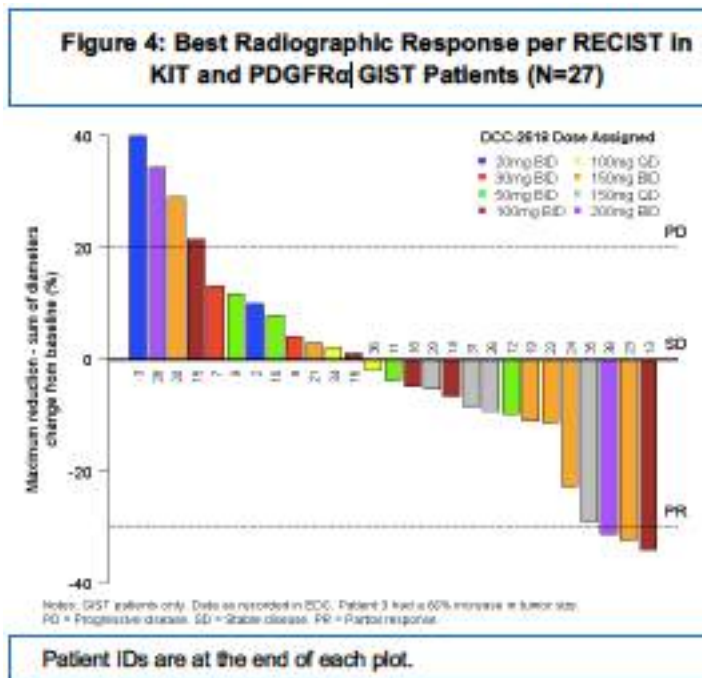
Table 1:

Measure	# Evaluable Patients (N)	Median (range) at baseline	Median (range) at best response	# with > 50% (> 35% for spleen volume) reduction n (%)
Serum tryptase (ug/L)	30	124 (14 - 1414)	11 (3 - 251)	28 (93%)
BM MC burden (%)	23	20 (2 - 95)	5 (0 - 60)	17 (74%)
Spleen volume (mL)	24	1014 (295 - 3069)	505 (143 - 1757)	13 (54%)
Blood/BM D816V allele burden (%)	23	4.5 (0.1 - 47)	1.1 (BLQ - 41)	15 (63%)

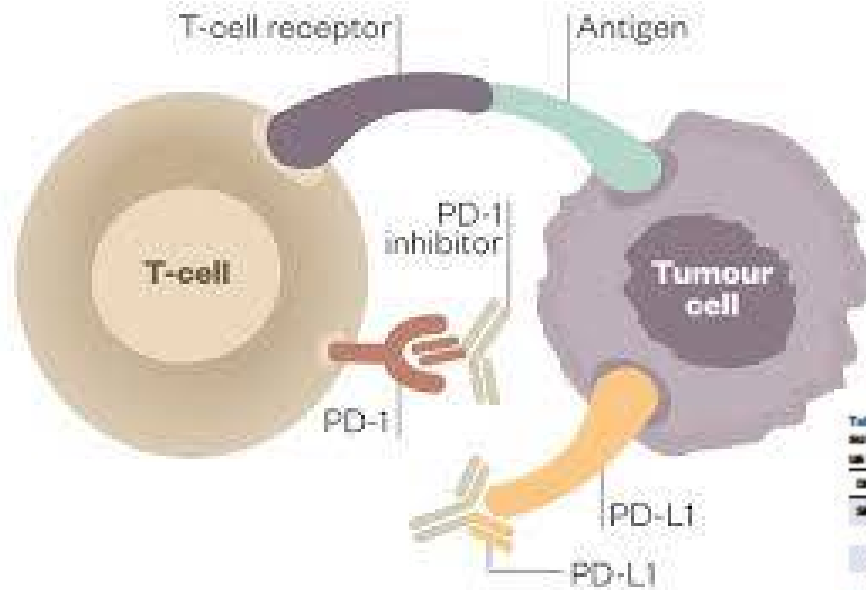
De Angelo et al Blood 2017  
30 pts

# Nuove inibitori di KIT: DCC-2618

DCC-2618: pan-KIT and PDGFR $\alpha$  inibitore



# Nuove Terapie nel futuro per la mastocitosi? Anti PD-1, anti PD-L1



**Table 2. Summary of expression of PD-1 and PD-L1 in mastocytosis subtypes, other myeloid neoplasms, and reactive/healthy BMs using IHC**

Diagnosis	PD-L1 expression (%)	PD-1 expression (%)
SM <sup>a</sup>	19/20 (95)	0/26 (0)
MCL <sup>b</sup>	3/3 (100)	0/4 (0)
AGM <sup>c</sup>	3/3 (100)	0/3 (0)
SM-AHN <sup>d</sup>	6/12 (50)	0/14 (0)
SM <sup>e</sup>	1/2 (50)	0/2 (0)
SM <sup>f</sup>	3/4 (75)	0/4 (0)
CM	23/25 (92)	4/22 (18)
MPCM <sup>g</sup>	15/17 (88)	2/18 (11)
Mastocytoma	0/3 (0)	3/3 (100)
MG	5/5 (100)	0/6 (0)
MEL	1/3 (33)	0/3 (0)
MNAG <sup>h</sup>	0/3 (0)	0/3 (0)
MPN <sup>i</sup>	0/18 (0)	0/17 (0)
MDS <sup>j</sup>	0/18 (0)	0/18 (0)
MDS/MPN <sup>k</sup>	0/5 (0)	0/5 (0)
Healthy and reactive BM <sup>l</sup>	0/15 (0)	0/21 (0)

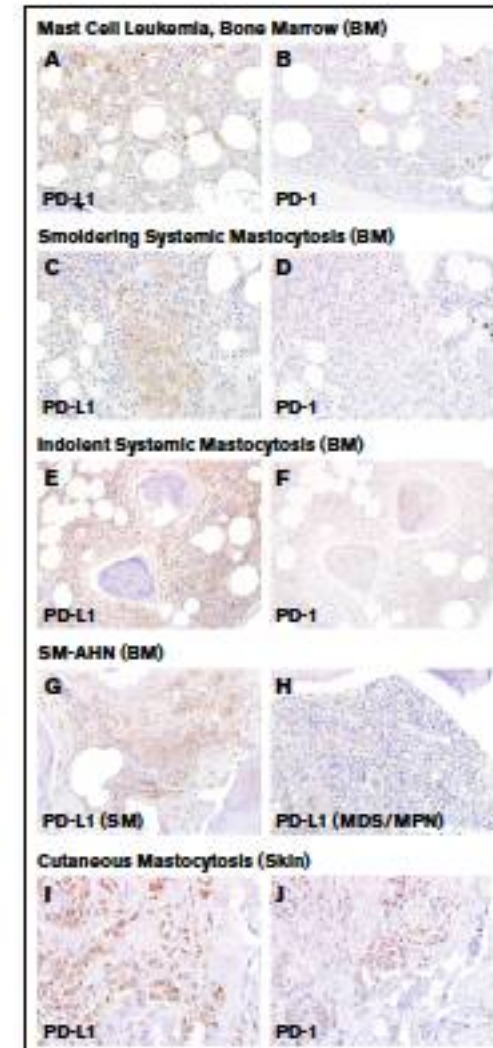
SM/MPN are all chronic myelomonocytic leukemia. MPNs included primary myelofibrosis (5), polycythemia vera (4), essential thrombocythemia (5), chronic myeloid leukemia (4), and chronic eosinophilic leukemia not otherwise specified (5). MDS included t(7; - syndrome) (9), MDS with excess blasts (5), MDS with single-lineage dysplasia (7A, erythrocytosis) (5), MDS with multilineage dysplasia (5), MDS with single-lineage dysplasia and ring sideroblasts (4), and MDS not otherwise specified (1). MDS refers to skin lesions of mastocytosis in patients with SM or MCL.

<sup>a</sup>PD-L1 positivity correlated with expression in SM ( $P = .0005$ ) and CM ( $P = .0147$ ), but did not correlate with subtype of SM or CM ( $P = \text{not significant}$ ).

<sup>b</sup>PD-1 positivity correlated with expression in CM ( $P = .0198$ ) and subtype of CM ( $P = .0285$ ).

<sup>c</sup>MCL and SM-AHN both include a single patient with MCL-MDS/MPN-U (patient 4 from Table 2). This patient is only included once in the overall total of SM patients.

<sup>d</sup>Gene-expression analysis of PD-L1 and PD-1 refers to expression in MCLs.





Grazie per l'attenzione!