Contribution of molecular diagnosis in eosinophilia/hypereosinophilia

Eosinophilia
Hypereosinophilia
Hypereosinophilic syndrome

Immune mediated hypereosinophilia
Chronic eosinophilic leukemia (NOS)/ Idiopathic hypereosinophilic syndrome*
Myeloid and lymphoid neoplasms with abnormalities of PDGFRA, PDGFRB, FGFR1*

*** WHO 2008**

Supervisor:
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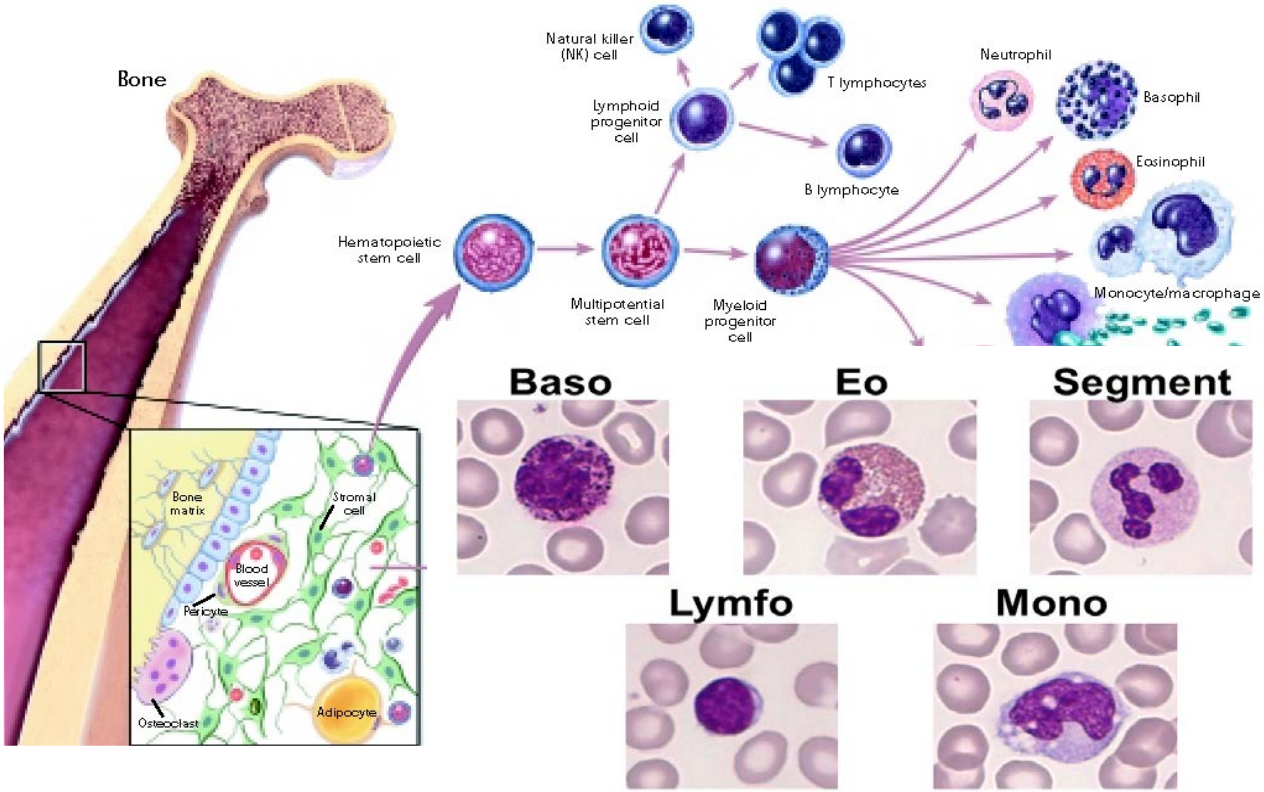
UZ LEUVEN

Review of the literature

Overview of the 5 different WBC (leucocytes)



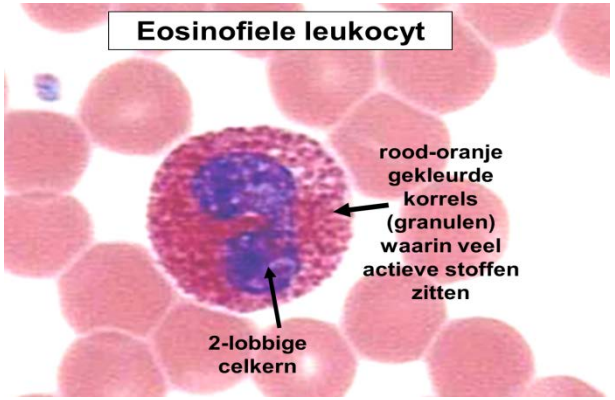
WBC range in UZ-Leuven : 4000 – 10 000/µL

Basophil :	≤ 1% / ≤ 100/µL
Eosinophil:	**≤ 6% / ≤ 400/µL**
Segment/Neutrophil:	38-77% / 2500-7800/µL
Lymphocyte:	20-50% / 1200-3600/µL
Monocyte:	2-10% / 200-800/µL

100%

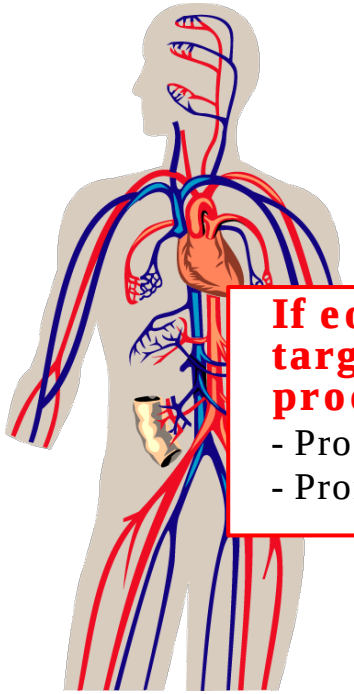
Eosinophils

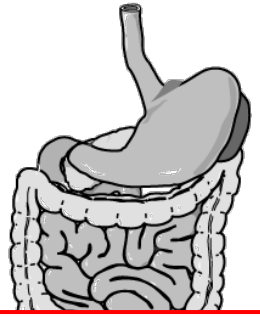
- Specialized granulocytic effector cells
  - **Produce diverse biologically active molecules** : cytotoxic, cytostimulatory proteins, lipid mediators, chemotactic petides and cytokines
- Nucleus divided into two tear-shaped lobes
- Cytoplasma with pink-orange granules



The natural history of eosinophils

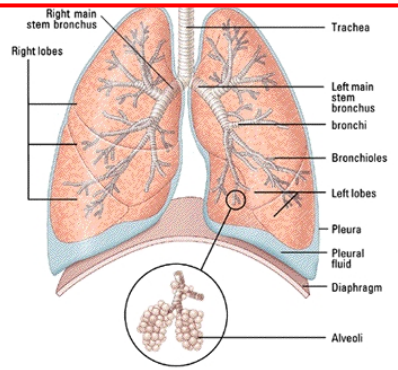
Bone marrow derived granulocytes (myeloid lineage)





If eosinophils ↑ in PB and/or invade target organs → they may secrete their products :

- Promote hypercoagulability : ↑ tromboembolic risk
- Promote local inflammation → tissue remodeling



IL-3, GM-CSF, IL-5
=Eosinophils growth factors

days to weeks

Normal eosinophil count

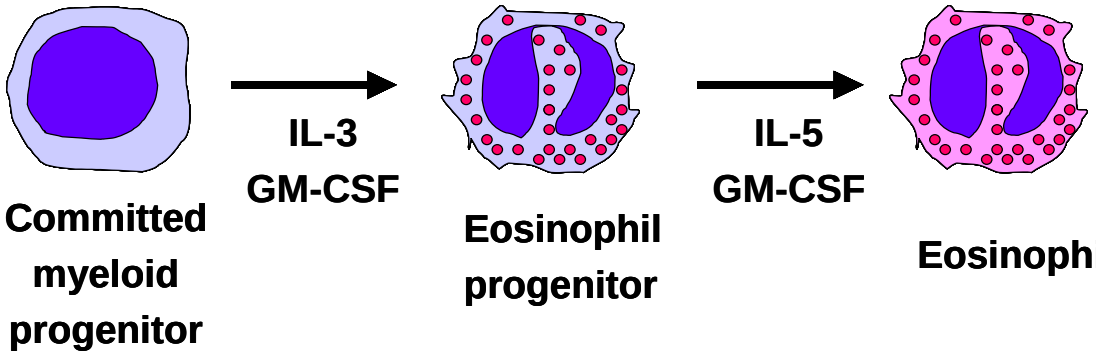
- Peripheral blood : 50-500/µL
- Bone marrow : 1% - 6%
- Tissues : unknown

Blood eosinophilia

We can divide peripheral blood eosinophilia into :

1. Mild eosinophilia : 500 – 1500/µL
2. Marked eosinophilia : > 1500/µL
3. Massive eosinophilia : > 5000/µL

Causes of hypereosinophilia



Malignant / clonal eosinophilia

- CML-eo
- MPN-eo
- MDS-eo
- AML-M4 eo
- Systemic mastocytosis

- CEL - NOS
- Hematopoietic neoplasms with eosinophils and abnormalities in PDGFRA, PDGFRB, FGFR1

"Immunological" or "reactive" eosinophilia

- Allergy or atopic diseases
- Drug hypersensitivity
- Parasitic infections
- Dermatological diseases
- Autoimmune and connective tissue diseases
- Pulmonary, gastrointestinal diseases
- B- or T-Lymphoproliferative disorders (ALL, T-LGL, HL)
- Solid tumors/malignancy

..... unknown (idiopathic) .....

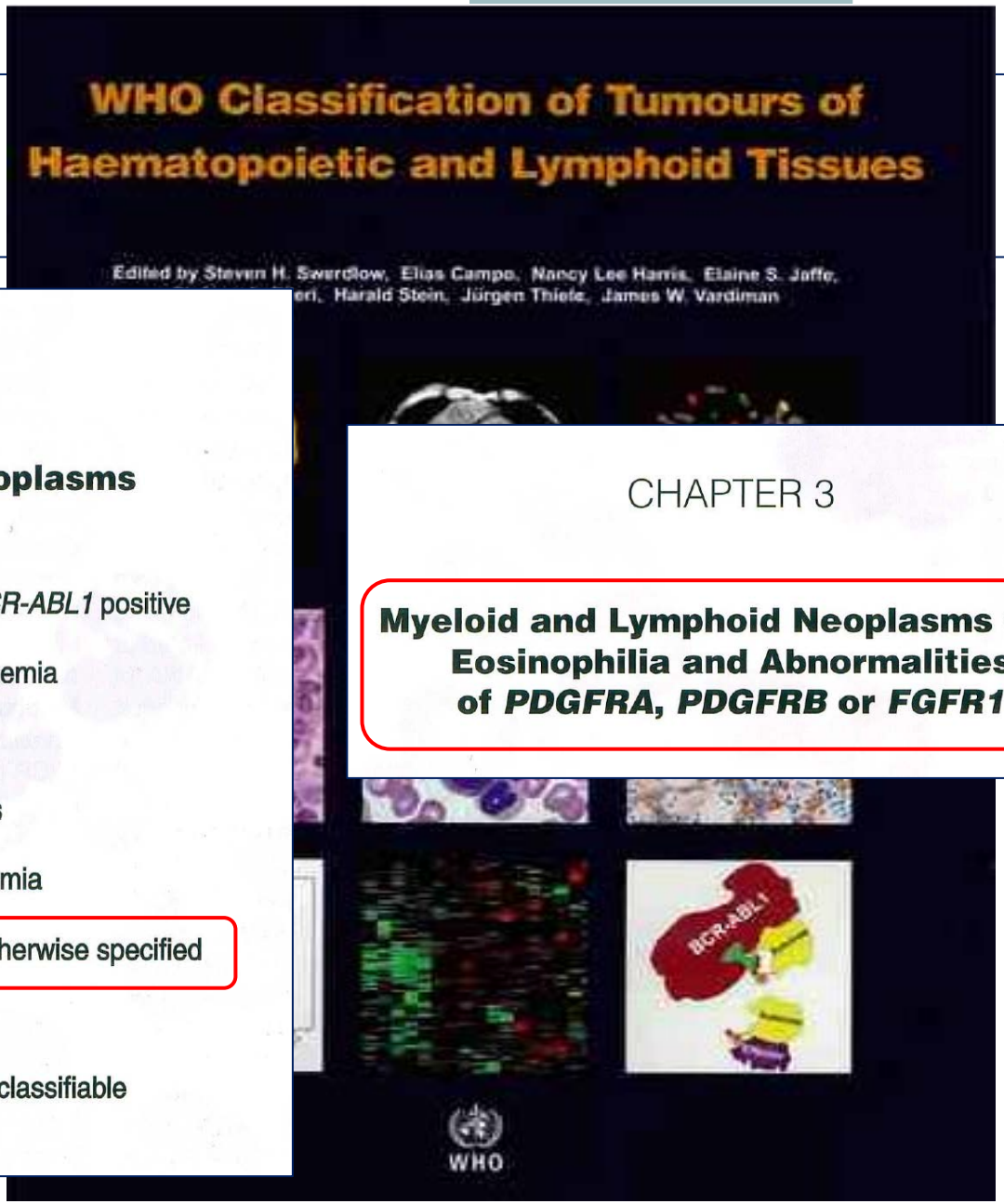
Causes of hypereosinophilia

Neoplastic

? Idiopathic?

Reactive/immune

WHO 2008
4th Edition



CHAPTER 2

Myeloproliferative Neoplasms

Chronic myelogenous leukaemia, *BCR-ABL1* positive

Chronic neutrophilic leukaemia

Polycythaemia vera

Primary myelofibrosis

Essential thrombocythaemia

Chronic eosinophilic leukaemia, not otherwise specified

Mastocytosis

Myeloproliferative neoplasm, unclassifiable

CHAPTER 3

Myeloid and Lymphoid Neoplasms with Eosinophilia and Abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*

Chronic eosinophilic leukaemia, not otherwise specified

- Chronic myeloproliferative neoplasm
- Criteria:
  - Eosinopl
  - **NO** gene ne or rearrang
  - Blasts in **UT** < 20%

= Morphology criteria

IF no increase in blast cells

“idiopathic hypereosinophilic syndrome”

Myeloid and Lymphoid Neoplasms with Eosinophilia and Abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*

- Rare specific
- Formation o osine kinase
- Chronic mye t eosinophilia

  (but the freq oplasm varies)

= Genetic criteria

2 pitfalls with WHO 2008

1. Classification criteria: morphology or genetic criteria

2. Classification of neoplasms : What to do with no neoplastic diseases?

Classification of the major causes of hypereosinophilia (HE)

Blood eosinophilia can be increased under various conditions

- Difficult differential diagnosis
- In **2011** P. Valent proposed a new terminology for the classification of the major causes of HE **(eo > 1500/µl)**

Contemporary consensus proposal on criteria and classification of eosinophilic disorders and related syndromes

Peter Valent, MD,[a] Amy D. Klion, MD,[b] Hans-Peter Horny, MD,[c] Florence Roufosse, MD, PhD,[d] Jason Gotlib, MD,[e] Peter F. Weller, MD,[f] Andrzej Hellmann, MD,[g] Georgia Metzgeroth, MD,[h] Kristin M. Leiferman, MD,[i] Michel Arock, PharmD, PhD,[j] Joseph H. Butterfield, MD,[k] Wolfgang R. Sperr, MD,[a] Karl Sotlar, MD,[l] Peter Vandenberghe, MD, PhD,[m] Torsten Haferlach, MD,[n] Hans-Uwe Simon, MD, PhD,[o] Andreas Reiter, MD,[h] and Gerald J. Gleich, MD[f,p] *Vienna, Austria, Bethesda, Md, Ansbach, Mannheim, and Munich, Germany, Brussels and Leuven, Belgium, Stanford, Calif, Boston, Mass, Gdansk, Poland, Salt Lake City, Utah, Cachan, France, Rochester, Minn, and Bern, Switzerland*

Classification of the major causes of HE

Table 1. Classification of the major causes of hypereosinophilia (HE)

- Secondary/reactive HE (nonclonal cells) : **HE_R**
  - ✓ Helminth infections
  - ✓ Drug reactions (allergic or toxic)
  - ✓ Other allergic reactions
  - ✓ Atopic diseases
  - ✓ Scabies, other infestations
  - ✓ Allergic bronchopulmonary aspergillosis
  - ✓ Chronic inflammatory disorders (eg, IBD)
  - ✓ Autoimmune diseases (eg, skin diseases)
  - ✓ Connective tissue diseases
  - ✓ Metabolic abnormalities
  - ✓ Solid tumors/malignancy
  - ✓ Chronic graft-versus-host disease
  - ✓ Hodgkin disease
  - ✓ B- or T-cell lymphoma/leukemia
  - ✓ Langerhans cell histiocytosis
  - ✓ Indolent systemic mastocytosis
- Hereditary/familial form : **HE_{FA}**
- Clonal (neoplastic) hypereosinophilia : **HE_N**
  - ✓ Chronic eosinophilic leukemia – Not Otherwise Specified (NOS)
  - ✓ Hematopoietic neoplasms with eosinophilia and abnormalities in PDGFRA
  - ✓ Hematopoietic neoplasms with eosinophilia and abnormalities in PDGFRB
  - ✓ Hematopoietic neoplasms with eosinophilia and abnormalities in PGFR1
  - ✓ CML with eosinophilia (CML-eo)
  - ✓ AML with inv(16) and eosinophilia (AML-M4-eo)
  - ✓ JAK2 V617F$^+$ MPN with eosinophilia (MPN-eo)
  - ✓ ASM with eosinophilia (ASM : Aggressive systemic mastocytosis)
  - ✓ MDS with eosinophilia (MDS-eo)
  - ✓ MPN/MDS overlap syndromes with eosinophilia
- HE of undetermined significance : **HE_{US}**

For the moment it is a proposal

1. Secondary/reactive HE and hereditary HE

2. Clonal - malignant

3. Idiopathic

Hypereosinophilic Syndrome (HES)

- **1975** : Chusid described the first diagnostic criteria for **"hypereosinophilic syndrome"** (HES) (Chusid & al., Medicine, 1975, 54:1)

The hypereosinophilic syndrome (HES) is characterized by the presence of marked unexplained blood and tissue eosinophilia associated with a variety of clinical manifestations. Since 1975, 3 criteria have been used to define HES: (1) blood eosinophilia ≥1500/mm^3 for longer than 6 months (or death before 6 months associated with signs and symptoms of hypereosinophilic disease), (2) lack of evidence for parasitic, allergic, or other known causes of eosinophilia, and (3) presumptive signs of organ involvement, such as heart failure, gastrointestinal dysfunction, central nervous system abnormalities, fever, or weight loss.

- **2002**: Gleich discovered **efficacy of imatinib for R/ of HES**

THE LANCET
Volume 359, Issue 9317, 4 May 2002, Pages 1577–1578

Research Letters

Treatment of hypereosinophilic syndrome with imatinib mesilate

Dr Gerald J Gleich, MD[a, b], Kristin M Leiferman, MD[a, b], Animesh Pardanani, MD[a], Ayalew Tefferi, MD[a], Joseph H Butterfield, MD[a]

- **2003** : Cools discovered an interstitial deletion in chromosome 4 that leads to the formation of a novel gene by **fusion of PDGFRA and FIP1-L1 genes** → coding for a **tyrosine kinase** that can be inhibited by **imatinib** in **HES**

The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 MARCH 27, 2003 VOL. 348 NO. 13

A Tyrosine Kinase Created by Fusion of the *PDGFRA* and *FIP1L1* Genes as a Therapeutic Target of Imatinib in Idiopathic Hypereosinophilic Syndrome

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2011: P. Valent proposed "new diagnostic criteria" for hypereosinophilic syndrome

Contemporary consensus proposal on criteria and classification of eosinophilic disorders and related syndromes

Proposed term	Proposed abbreviation	Definition and criteria
Blood eosinophilia	—	>0.5 Eosinophils × 10^9/L blood
Hypereosinophilia	HE	>1.5 Eosinophils × 10^9/L blood on 2 examinations (interval ≥1 month*) and/or tissue HE defined by the following†: 1. Percentage of eosinophils in BM section exceeds 20% of all nucleated cells and/or 2. Pathologist is of the opinion that tissue infiltration by eosinophils is extensive and/or 3. Marked deposition of eosinophil granule proteins is found (in the absence or presence of major tissue infiltration by eosinophils).
Hypereosinophilic syndrome	HES	1. Criteria for peripheral blood HE fulfilled* and 2. Organ damage and/or dysfunction attributable to tissue HE‡ and 3. Exclusion of other disorders or conditions as major reason for organ damage.
Eosinophil-associated single-organ diseases		1. Criteria of HE fulfilled and 2. Single-organ disease (see Table III and Tables E4 and E5 for specific entities)

*In the case of evolving life-threatening end-organ damage, the diagnosis can be made immediately to avoid delay in therapy.

†Validated quantitative criteria for tissue HE do not exist for most tissues at the present time. Consequently, tissue HES is defined by a combination of qualitative and semiquantitative findings that will require revision as new information becomes available.

‡HE-related organ damage (damage attributable to HE): organ dysfunction with marked tissue eosinophil infiltrates and/or extensive deposition of eosinophil-derived proteins (in the presence or absence of marked tissue eosinophils) and 1 or more of the following: (1) fibrosis (lung, heart, digestive tract, skin, and others); (2) thrombosis with or without thromboembolism; (3) cutaneous (including mucosal) erythema, edema/angioedema, ulceration, pruritus, and eczema; and (4) peripheral or central neuropathy with chronic or recurrent neurologic deficit. Less commonly, other organ system involvement (liver, pancreas, kidney, and other organs) and the resulting organ damage can be judged as HE-related pathology, so that the clinician concludes the clinical situation resembles HES. Note that HES can manifest in 1 or more organ systems.

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P. Valent et al. 2011

MLN-eo/PDGFRA + : most frequent cause of clonal /neoplastic hypereosinophilia

WHO 2008

CEL-NOS

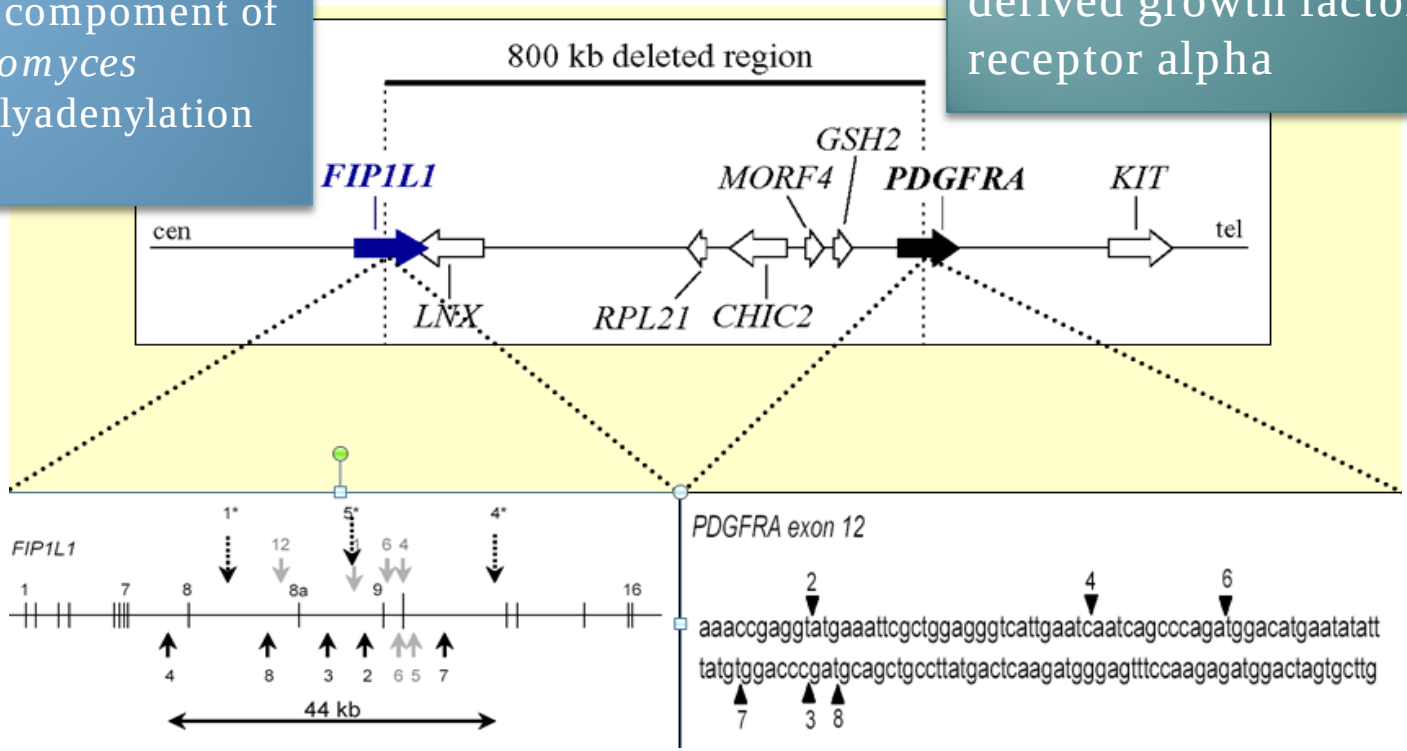
MLN –eo PDGFRA/ PDGFRB/PGFR1

FIP1L1-PDGFRA mutation (F/P)

= Novel fusion protein (discoverd in 2003)

FIP1L1 = FIP1-like1 (FIP1 = an essential compoment of the *Sacchoromyces cervisiae* polyadenylation machinery)

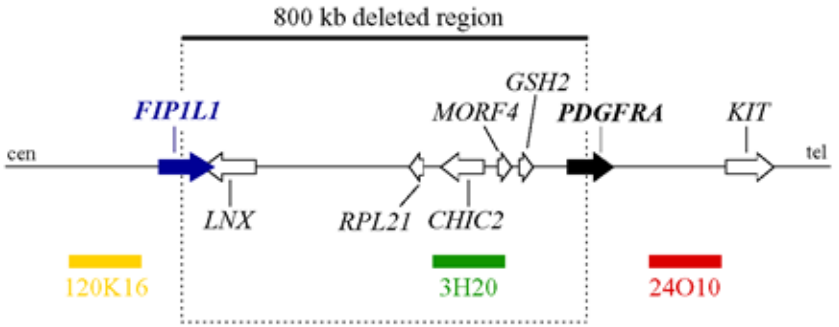
PDGFRA = platelet-derived growth factor receptor alpha

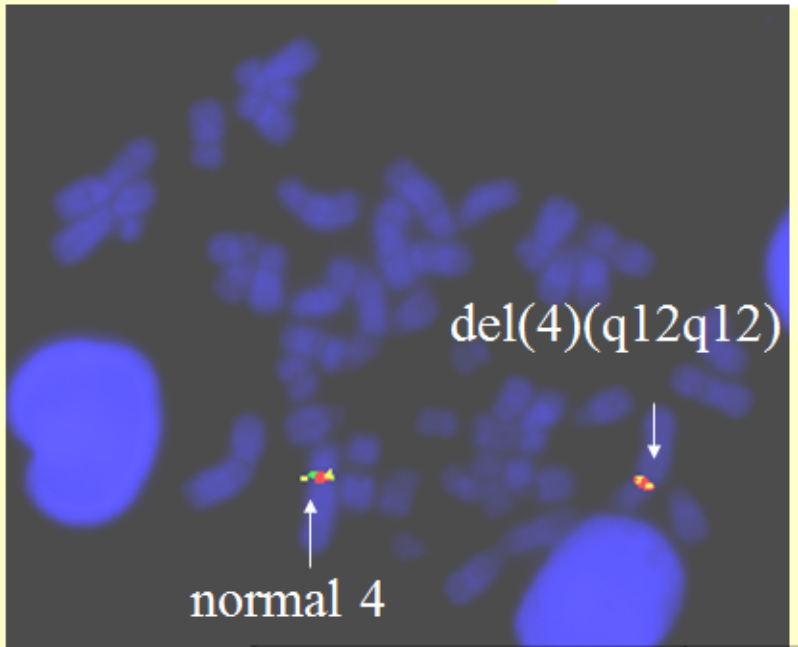


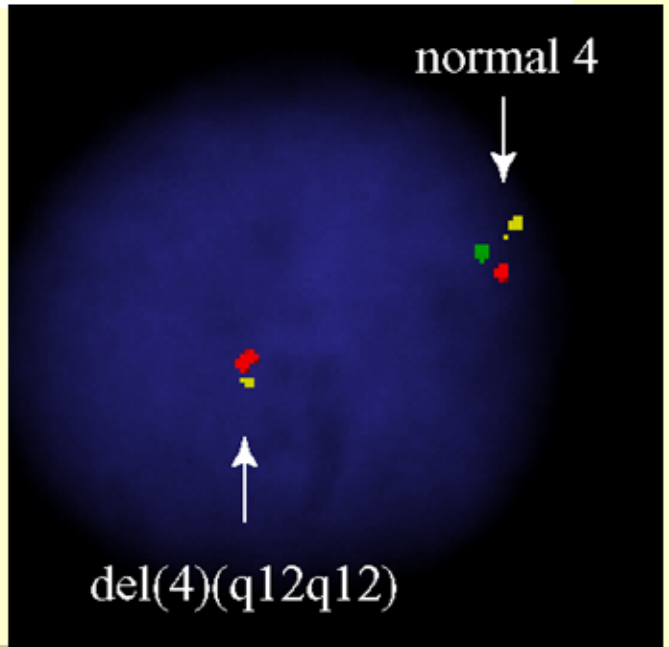
FIP1L1-PDGFRA mutation (F/P)

Detection by FISH in bone marrow

3-color FISH







FIP1L1-PDGFRA mutation (F/P)

Detection by RT-PCR in peripheral blood of in bone marrow

MLN-eo/FIP1L1-PDGFRA +

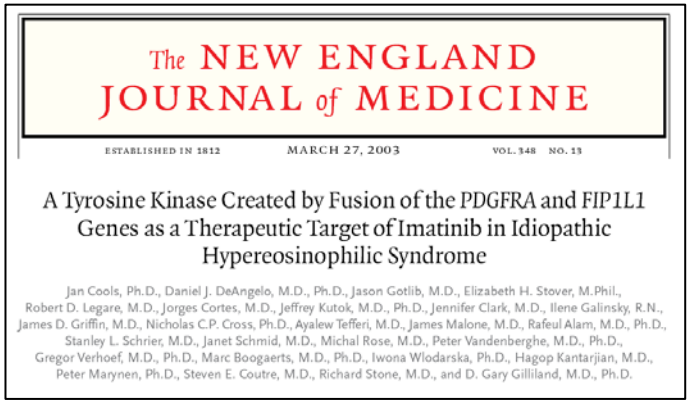
- Rare chronic disorder
- PB eosinophil count markedly elevated : ≥ 1500/µL
- < 20% blasts in the PB and BM
- *FIP1L1-PDGFRA* positive = F/P+

  - High probability of organ involvement & disease-related death
  - Does not respond well to conventional therapy, with median survival ~4-8 years
  - Rapid & complete hematological responses to imatinib (Glivec®)
  - Complete molecular response in 2/3 evaluable cases

MLN-eo/FIP1L1-PDGFRA +

HE + molecular abnormality FIP1L1-PDGRA
= HE F/P +

R/ IMATINIB



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Study UZ Leuven

FIP1L1-PDGFRA rearrangement

Diagnosis in UZ Leuven :

- Molecular analysis for FIP1L1-PDGFRA is available since 2003
- Methode : RT-PCR (peripheral blood of bone marrow) or/and FISH (bone marrow)

Difficulties :

- **Not generally available test**
- **In most of cases : negative**

FIP1L1-PDGFRA rearrangement

lab_name	test_name	contact	comments
AZ Sint-Jan AV Brugge	FIP1L1/PDGFRA qualitative analysis	Johan Billiet/Friedel Nollet	nested RT-PCR; primers from Cool-J et al. NEJM (2003)
CMG-UZGent	FIP1L1/PDGFRA qualitative analysis	nadine van roy	FISH analysis
CHU Liège - Hemato	FIP1L1/PDGFRA qualitative analysis	Dr Frédéric Lambert	As described by Cools et al., NEJM, 2003. Please, provide us with EDTA anticoagulated blood (10 ml) or bone marrow (3 ml) within 16H post-sampling OR cells within Trizol/Tripure solution OR preserved cells within RNALater solution.
UZ-KULeuven CEMOL	FIP1L1/PDGFRA qualitative analysis	Peter Vandenberghe / Els Lierman	Sample type: EDTA-blood / EDTA-bone marrow, minimal volume: 5ml Frequency: 2x/week Test type: nested PCR on cDNA, based on Cools et al, NEJM 2003.

http://www.moleculardiagnostics.be/

STUDY

We aimed to establish a **scoring system** that includes symptoms and/or laboratory tests in order to establish this molecular test.

Necessary in UZ Leuven for :

- Clinical
- Organizational/logistic
- Economic
- Strategic reasons

Introduction

In the literature, HE F/P+ is described as :

- Men > women (M:F ratio ± 17:1)
- Palpable splenomegaly and/or hepatomegaly
- Cardiac damage
- Thrombus formation
- Serum vitamin B12 ↑
- Serum tryptase ↑
- Serum IgE normal
- Marrow biopsy : hypercellular/myeloproliferative aspect
- Periferal blood cytology : not specific
- Marrow cytology : not specific
- Normal karyotype

Patients' selection

- Single-institution retrospective study (UZ Leuven)
- Between 2004 – 2013 : **157 patients**
  - **2 patients F/P + (2/157 = 1,3% (prevalence))**
  - Review of 40 patients over 155 F/P (-)
  - 18 patients FIP1L1-PDGFRA (-) included in our study

Total of 20 patients included in our study : 2 F/P+ and 18 F/P-

Selection criteria

Clinical symptoms :

- Dominant signs = not specific
- Palpable splenomegaly/hepatomegaly = not specific
- Endomyocardial pathology = not specific
- Thrombus formation = not specific

Selection criteria

Laboratory tests :

- Hemoglobin = not specific
- WBC count = not specific
- Platelet count = not specific
- **Absolute Eosinophil count = specific**
- Absolute basophil count = not specific

Selection criteria

Laboratory tests :

- **Serum Vitamin B12 level = specific**
- **Serum tryptase level = specific**
- **Total IgE = specific**

Selection criteria

Laboratory tests :

- Flow cytometry = not specific
- Peripheral blood cytology = not specific
- Marrow cytology (morphology, eosinophilia, blast cellen) = not specific
- Marrow biopsy = not specific

Scoring system

Parameters :

1. Absolute eosinophil count > 5000/µL : **2 points**
2. Absolute eosinophil count > 1500/µL : **1 point**
3. Serum vitamin B12 level > 800 ng/L : **1 point**
4. Serum tryptase level > 12 µg/L : **1 point**
5. Total IgE < 20 kU/L : **1 point**

Scoring system

5 biochemical parameters :

Score 0 – 2 : NO
Score ≥ 3 – 6 : YES

Score system without tryptase (4 parameters) :

Score 0 – 2 : NO
Score ≥ 3 – 5 : YES

Results

SCORE SYSTEM patient number	Absolute eosinophil count > 5000/µL	Absolute eosinophil count > 1500/µL	Serum Vit B12 level > 800ng/L	Serum tryptase level > 12 µg/L	total IgE < 20 kU/L	Som biochemische parameters (5)	TEST : YES 3-6 NO 0-2	SCORE SYSTEM without Tryptase	Absolute eosinophil count > 5000/µL	Absolute eosinophil count > 1500/µL	Serum Vit B12 level > 800ng/L	total IgE < 20 kU/L	Som biochemische parameters (4) without TRYPTASE	TEST : YES 3-5 NO 0-2
1	2	1	1	1	1	6	yes		2	1	1	1	5	yes
2	2	1	1	1	1	6	yes		2	1	1	1	5	yes
3	2	1	1	0	0	4	yes		2	1	1	0	4	yes
4	0	1	0	0	0	1			0	1	0	0	1	
6	0	0	1	0	0	1	no		0	0	1	0	1	no
7	0	0	0	0	0	0	no		0	0	0	0	0	no
8	2	1	1	0	0	4	yes		2	1	1	0	4	yes
9	2	1	0	0	0	3	yes		2	1	0	0	3	yes
10	2	1	0	1	0	4	yes		2	1	0	0	3	yes

Results

SCORE SYSTEM patient number	Absolute eosinophil count > 5000/µL	Absolute eosinophil count > 1500/µL	Serum Vit B12 level > 800ng/L	Serum tryptase level > 12 µg/L	total IgE < 20 kU/L	Som biochemische parameters (5)	TEST : YES 3-6 NO 0-2	SCORE SYSTEM without Tryptase	Absolute eosinophil count > 5000/µL	Absolute eosinophil count > 1500/µL	Serum Vit B12 level > 800ng/L	total IgE < 20 kU/L	Som biochemische parameters (4) without TRYPTASE	TEST : YES 3-5 NO 0-2
1	2	1	1	1	1	6	yes		2	1	1	1	5	yes
2	2	1	1	1	1	6	yes		2	1	1	1	5	yes
11	0	0	0	0	1	1	no		0	0	0	1	1	no
12	2	1	1	0	0	4			2	1	1	0	4	
17	0	1	0	1	0	2			0	1	0	0	1	
19	0	1	0	1	1	3	yes		0	1	0	1	2	no
20	0	1	1	1	1	4	yes		0	1	1	1	3	yes
	Conclusion : 12/20 useful test = 60 %								Conclusion: 11/20 useful test = 55 %					

Discussion

Main objective of our study :

- Develop a non-invasive scoring system including clinical symptoms and/or laboratory tests

- Clinical signs did not differ between F/P+ and F/P- patients

- We choice to use 4 laboratory tests for our scoring system
  1. Absolute eosinophil count
  2. Serum vitamin B12 level
  3. Serum tryptase level
  4. Total IgE

Discussion

- Using this scoring system, we will reduce the molecular analysis for FIP1L1-PDGFRA rearrangement significantly, namely by 40-45%

- Price : Vitamine B12 (2€) + IgE (8 €) + Tryptase (15 €) = **25 € VERSUS** RT-PCR peripheral blood = **> 100 €**

- Difficult to validate this scoring system due to the lack of positive patients in UZ-Leuven

To do/actions : conclusion

1. Survey of testing in other centers? (3 other centers in Belgium)

2. Discuss need for validation of scoring system as means for selecting patients who need this analysis