

Syndromes d'Hyperéosinophilie Idiopathique (SHE) et Leucémie Chronique à Eosinophile (LCE)

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Préambule

**Les syndromes d'Hyperéosinophilies
idiopathiques (HES) et la leucémie chronique
à éosinophile (CEL)**

...

**des pathologies rares dont la prévalence est
indéterminée...**

Classification OMS des Leucoses myéloïdes, rationnel ?

The World Health Organization (WHO) classification of the myeloid neoplasms

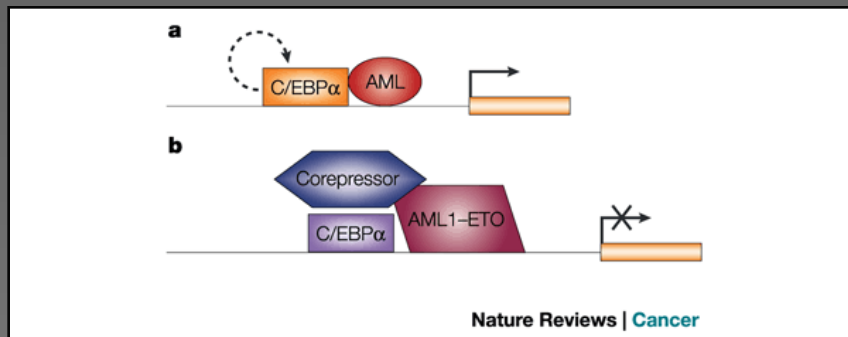
James W. Vardiman, Nancy Lee Harris, and Richard D. Brunning

Modèle multi-étapes de la leucémogénèse myéloïde

Translocations chromosomiques



Mutants dominants négatifs

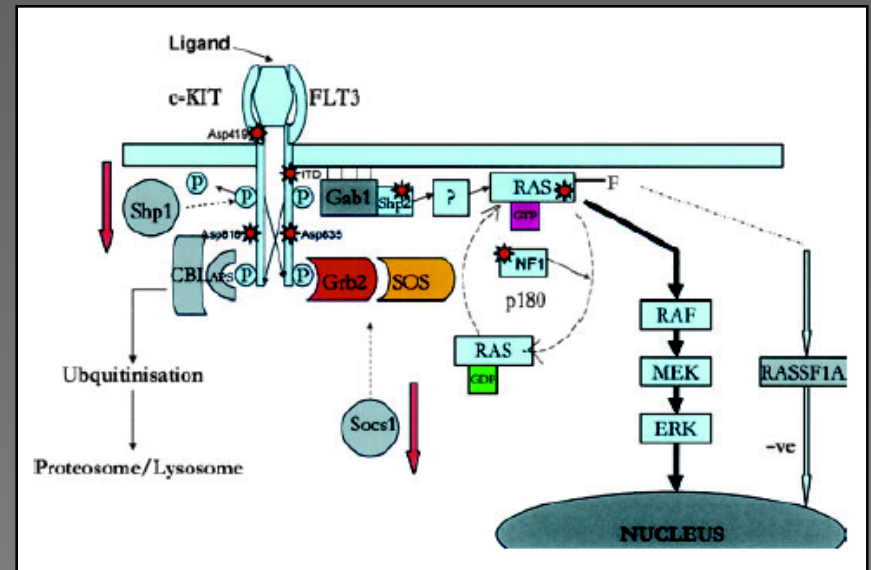


Bloquent la différenciation

Mutations RTKs



Activation voies de signalisation



Stimulent prolifération

Classification OMS des LMA

Acute myeloid leukemia with recurrent genetic abnormalities

Acute myeloid leukemia with t(8;21)(q22;q22), (*AML1/ETO*)

Acute myeloid leukemia with abnormal bone marrow eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22), (*CBF/MYH11*)

Acute promyelocytic leukemia with t(15;17)(q22;q12), (*PML/RAR*) and variants

Acute myeloid leukemia with 11q23 (*MLL*) abnormalities

Acute myeloid leukemia with multilineage dysplasia

Following MDS or MDS/MPD

Without antecedent MDS or MDS/MPD, but with dysplasia in at least 50% of cells in 2 or more myeloid lineages

Acute myeloid leukemia and myelodysplastic syndromes, therapy related

Alkylating agent/radiation-related type

Topoisomerase II inhibitor-related type (some may be lymphoid)

Others

Acute myeloid leukemia, not otherwise categorized

Classify as:

Acute myeloid leukemia, minimally differentiated

Acute myeloid leukemia without maturation

Acute myeloid leukemia with maturation

Acute myelomonocytic leukemia

Acute monoblastic/acute monocytic leukemia

Acute erythroid leukemia (erythroid/myeloid and pure erythroleukemia)

Acute megakaryoblastic leukemia

Acute basophilic leukemia

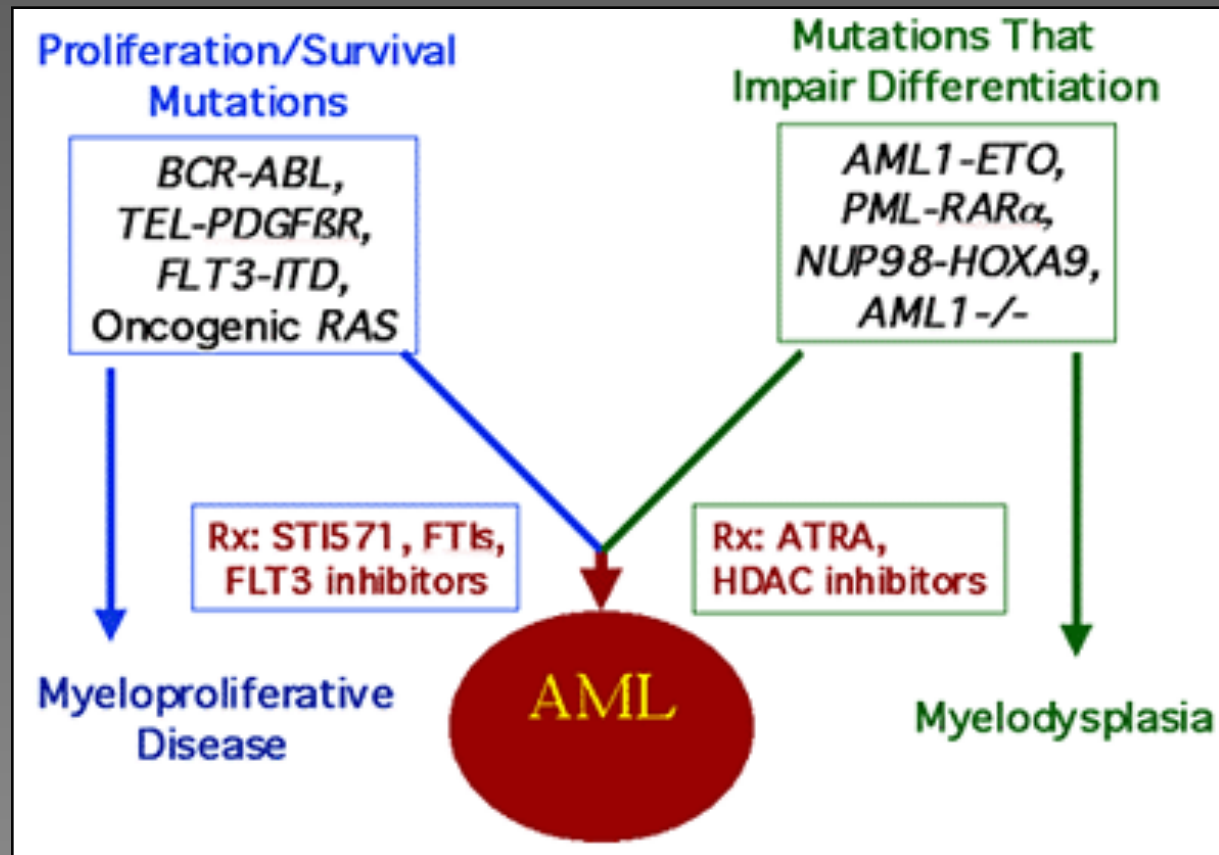
Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Des perspectives de thérapies nouvelles

Mutations de classe I

Mutations de classe II

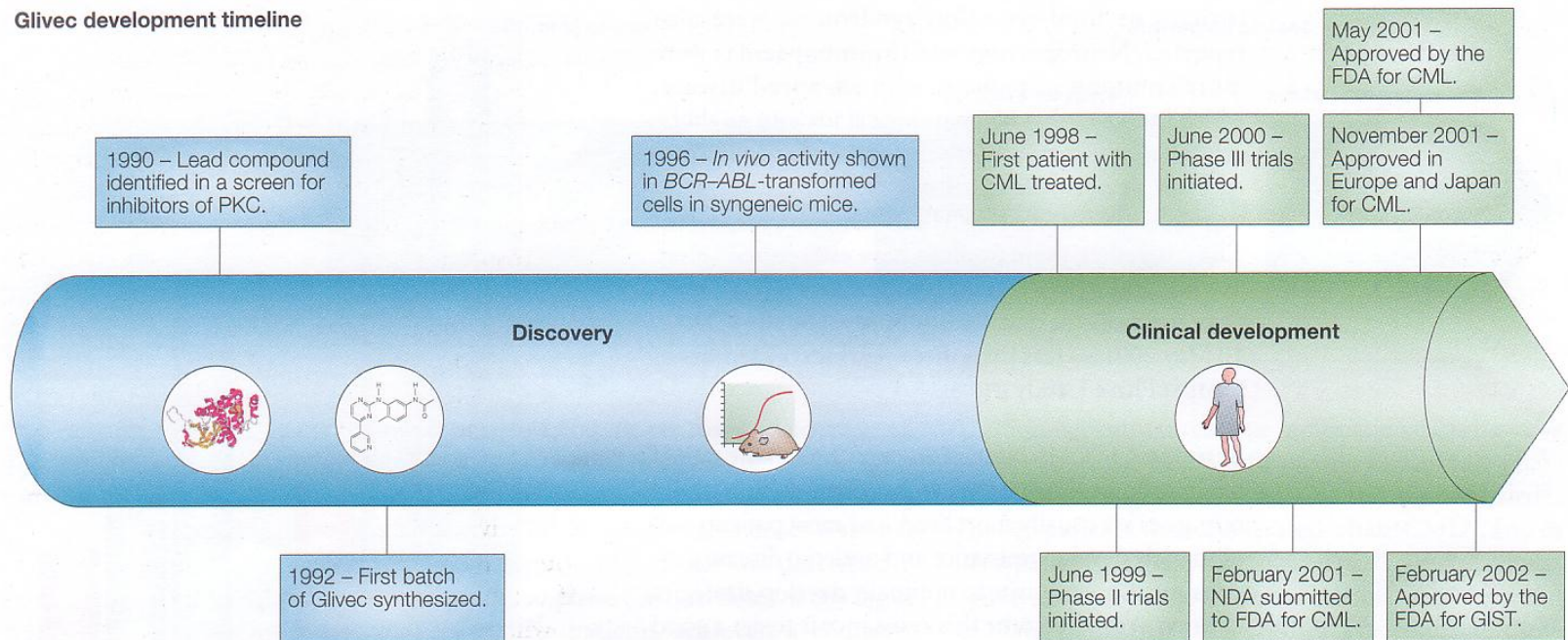


Classification OMS des SMPs

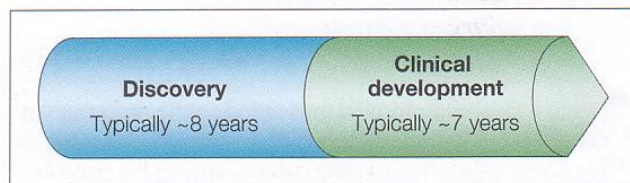
- ❑ Chronic myelogenous leukemia [Ph chromosome, t(9;22)(q34;q11), *BCR/ABL*positive]
- ❑ Chronic neutrophilic leukemia
- ❑ Chronic eosinophilic leukemia (and the hypereosinophilic syndrome)
- ❑ Polycythemia vera
- ❑ Chronic idiopathic myelofibrosis (with extramedullary hematopoiesis)
- ❑ Essential thrombocythemia
- ❑ Chronic myeloproliferative disease, unclassifiable

Vers une thérapie moléculaire ciblée des cancers: l'exemple du STI-571 (Imatinib – Glivec)

Glivec development timeline



Typical development timeline



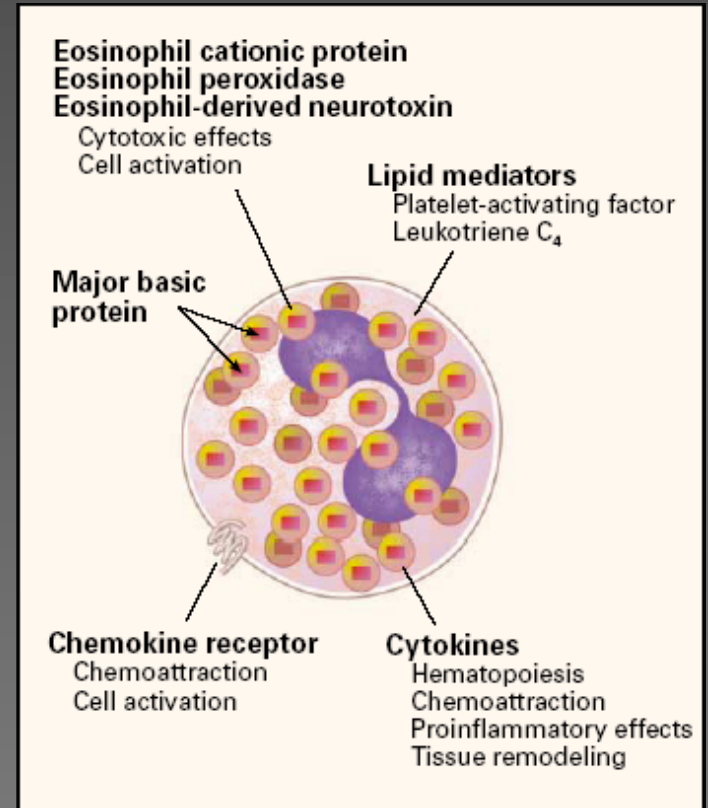
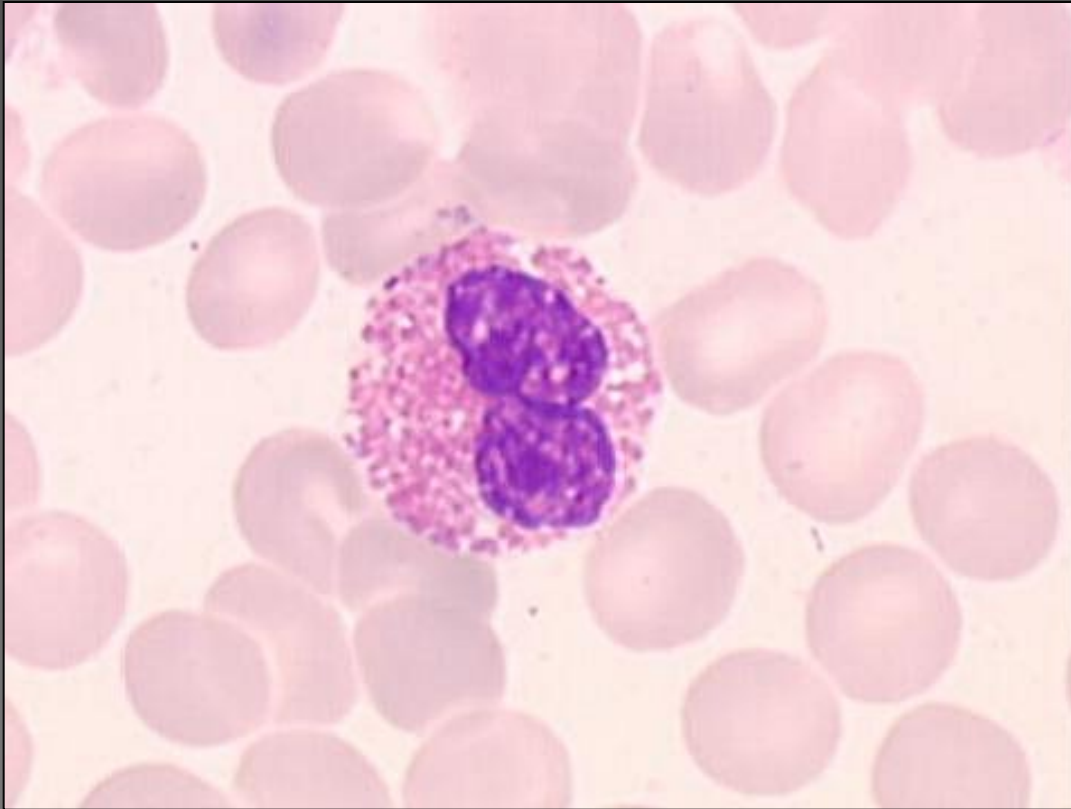
Les syndromes d'hyperéosinophilies

HES/CEL

- ❑ L'éosinophile et l'éosinopoïèse normale,
- ❑ L'hyperéosinophilie, un symptôme commun,
- ❑ Causes secondaires d'hyperéosinophilie,
- ❑ Hyperéosinophilies primaires,
- ❑ Actualités,
- ❑ Perspectives.

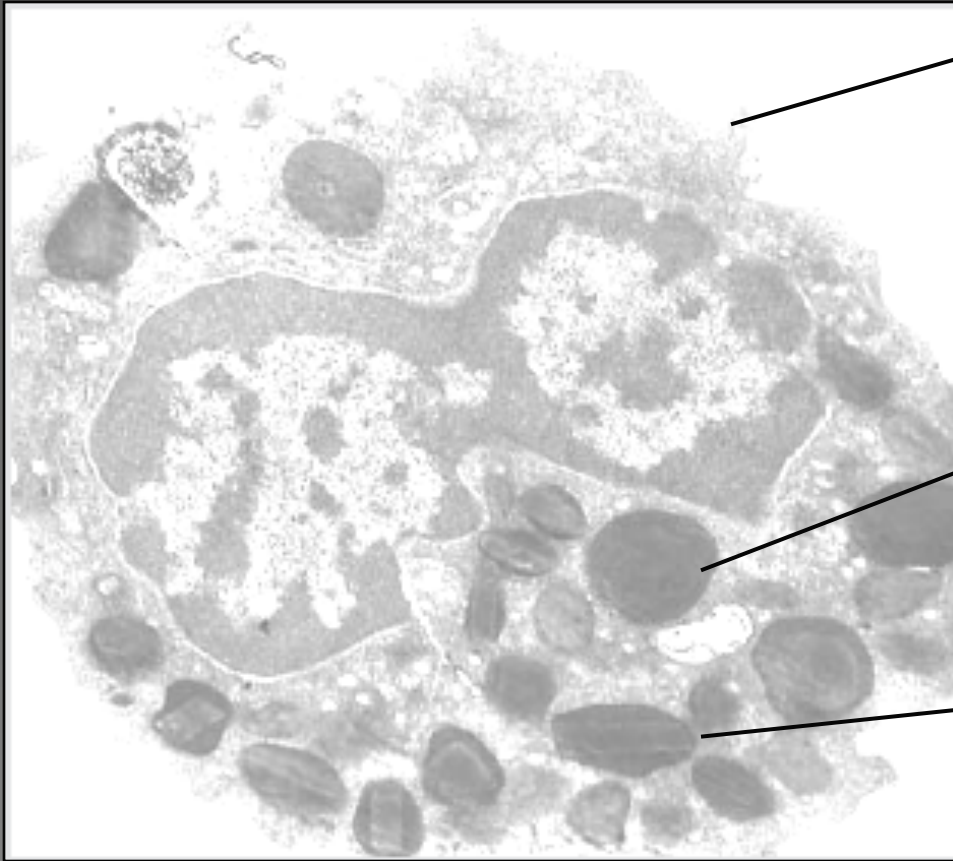
Le PN Eosinophile

PN éosinophile



- Paul Erlich 1879:
Lignée granulocytaire, noyau bilobé, granules
« éosinophiles ».

Granules I et II



Récepteurs membranaires:
IL-5, IL-3, GM-CSF, Ig, cplt

Granules primaires
(Charcot Leyden crystal (CLC))

Granules secondaires - spécifiques
(Major Basic Protein, ECP, EPO)

Valeurs normales

- ❑ 3-5% cell. médullaires (40% mature),
- ❑ Production: $0,2 \cdot 10^9/\text{kg}/\text{j}$; réserve médullaire: $0,1 \cdot 10^9/\text{kg}$
- ❑ Ratio m/sg: 5-7/1, tps transit m/sg: 3,5j,
- ❑ 1–3 % leucocytes périphériques,
- ❑ Compte Absolu d'Éosinophiles $< 0,5 \cdot 10^9/\text{l}$,
- ❑ Demi-vie sanguine: $\sim 18\text{h}$, tps transit sg/t: 26h

Fonctions

- ❑ Localisation peau, muqueuses digestives et respiratoires,
- ❑ Acteur de la réponse immune innée,
- ❑ Protection invasion par parasites (helminthes),
- ❑ Leucotriènes, radicaux libres et médiateurs pro-inflammatoires,
- ❑ Cytotoxicité cellulaire Ac dép. et complément dép., libération enzymes intracytoplasmiques (MBP),
- ❑ Rôles pro-inflammatoire et cytotoxique.

Eosinopoïèse normale

L'éosinopoïèse normale

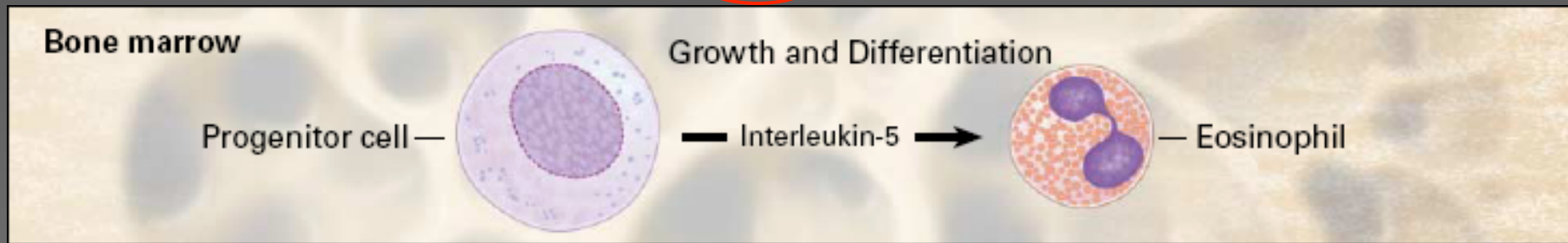
GM-CSF

IL-3

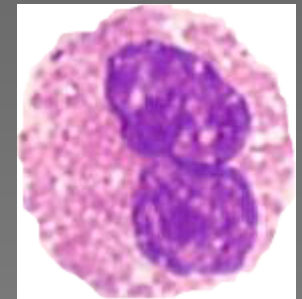
IL-5

Bone Marrow

Peripheral Blood



GEMM-CFU



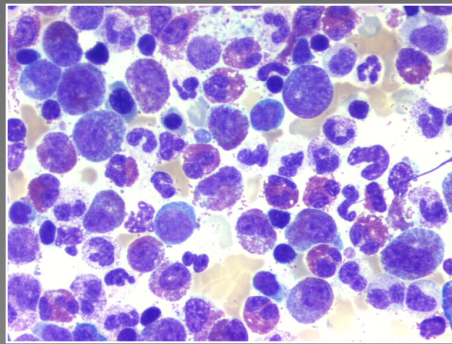
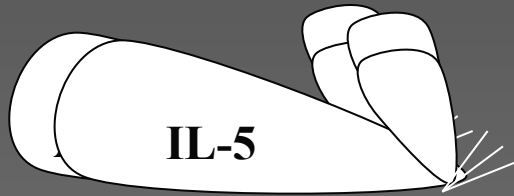
†



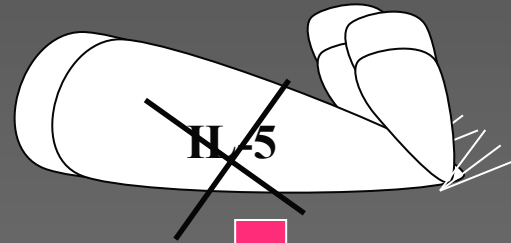
Tissues

IL-5 est "la" cytokine éosinotrope

Souris IL-5 transgéniques

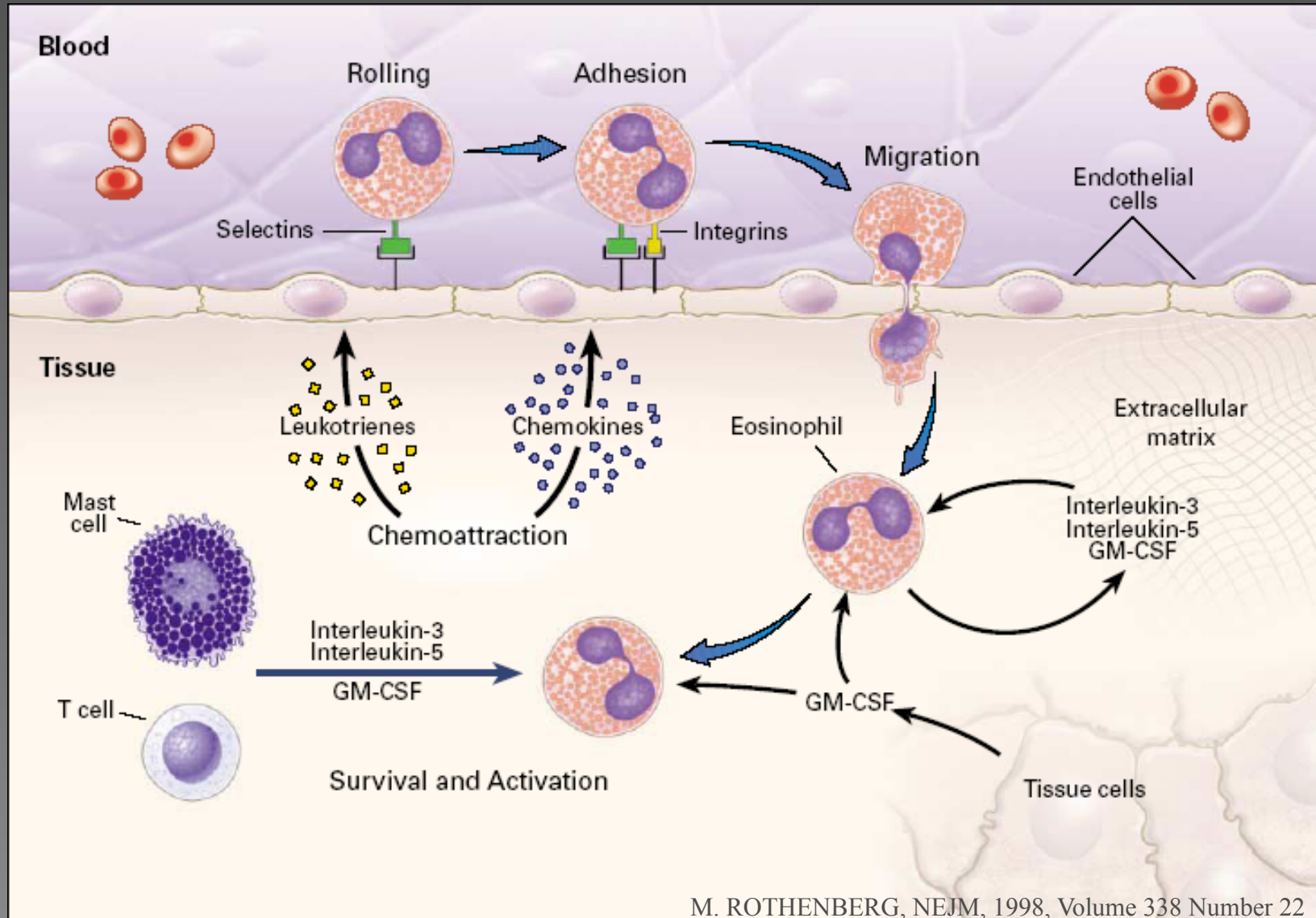


Souris IL-5 «K.O »

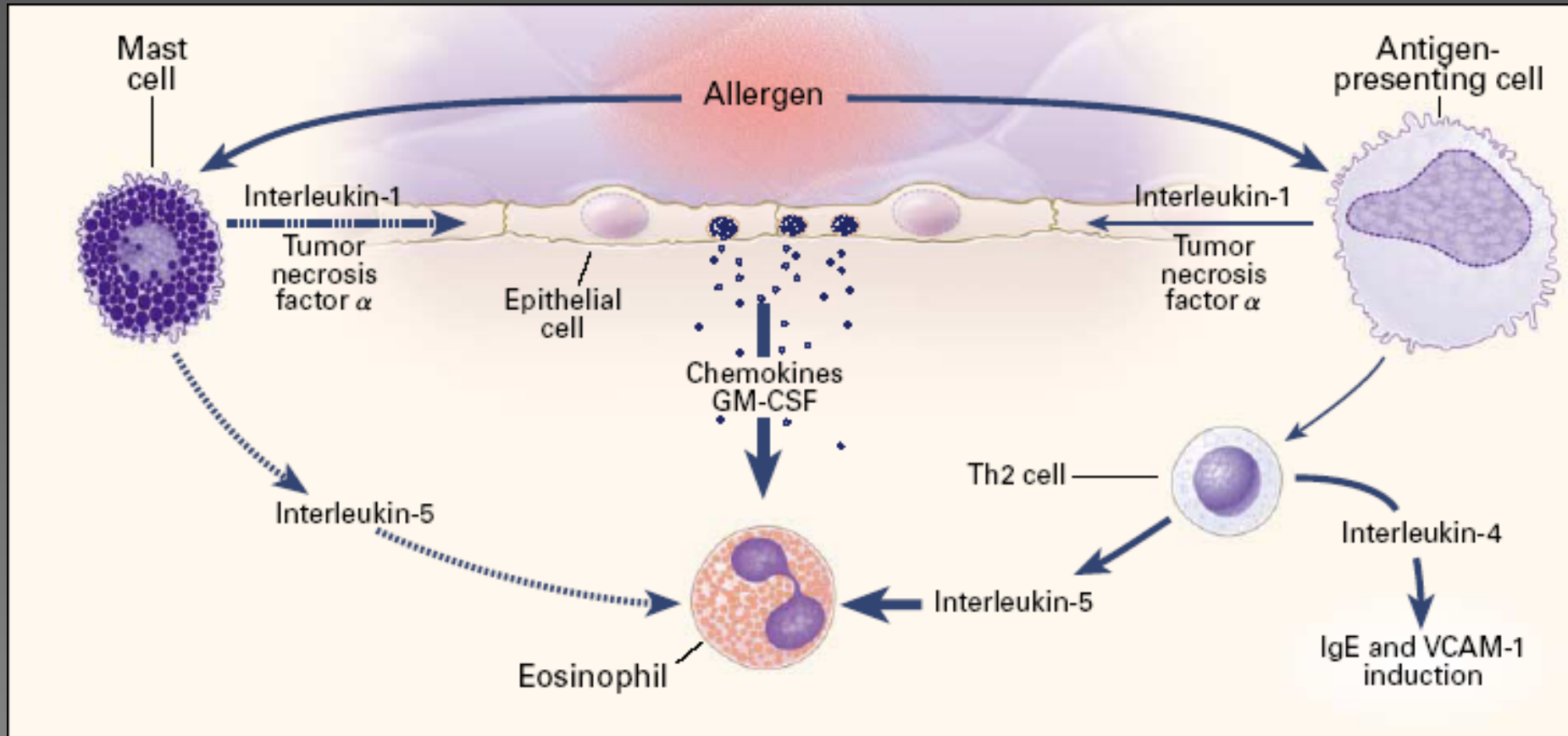


Niveau basal d'éosinophiles
médullaires N
MAIS
« anergie » aux stress
éosinophilotropes

Migration tissulaire



Activation tissulaire



L'hyperéosinophilie, un symptôme commun

L'éosinopoïèse pathologique



Classification opérationnelles des hyperéosinophilies

■ Gradation (*Wardlaw et al.*):

- Légère: $1.0 - 1.5 \times 10^9/l$
- Modérée: $1.5 - 5.0 \times 10^9/l$
- Sévère: $> 5.0 \times 10^9/l$

■ Cut-off hyperéosinophilies HEAD* vs atopies/asthme bronchique (*Kobayashi et al.*)

- $> 2,052 \times 10^9/l$,

* Chusid et al. et NIH, + drug atopy; angioedema épisodique; angéite granulomateuse allergique

L'hyperéosinophilie, un symptôme commun

TABLE 1. DISEASES ASSOCIATED WITH EOSINOPHILIA.

TYPE OF DISEASE	EOSINOPHILIA		EXAMPLES OF CAUSES
	PERIPHERAL BLOOD	TISSUE	
Infectious	Present	Present or absent	Infections with especially invasive helminths
Respiratory	Present or absent	Present	Eosinophilic pneumonitis, asthma
Gastrointestinal	Present or absent	Present	Inflammatory bowel dis- ease, eosinophilic gastro- enteritis, allergic colitis
Allergic	Present or absent	Present	Allergic rhinoconjunctivitis, asthma, eczema
Systemic	Present	Present	Idiopathic hypereosinophil- ic syndrome, vasculitis
Iatrogenic	Present	Present or absent	Drug reaction, cytokine in- fusions (e.g. granulo- cyte-macrophage colo- ny-stimulating factor)
Malignant	Present or absent	Present or absent	Lymphoma, colonic carci- noma

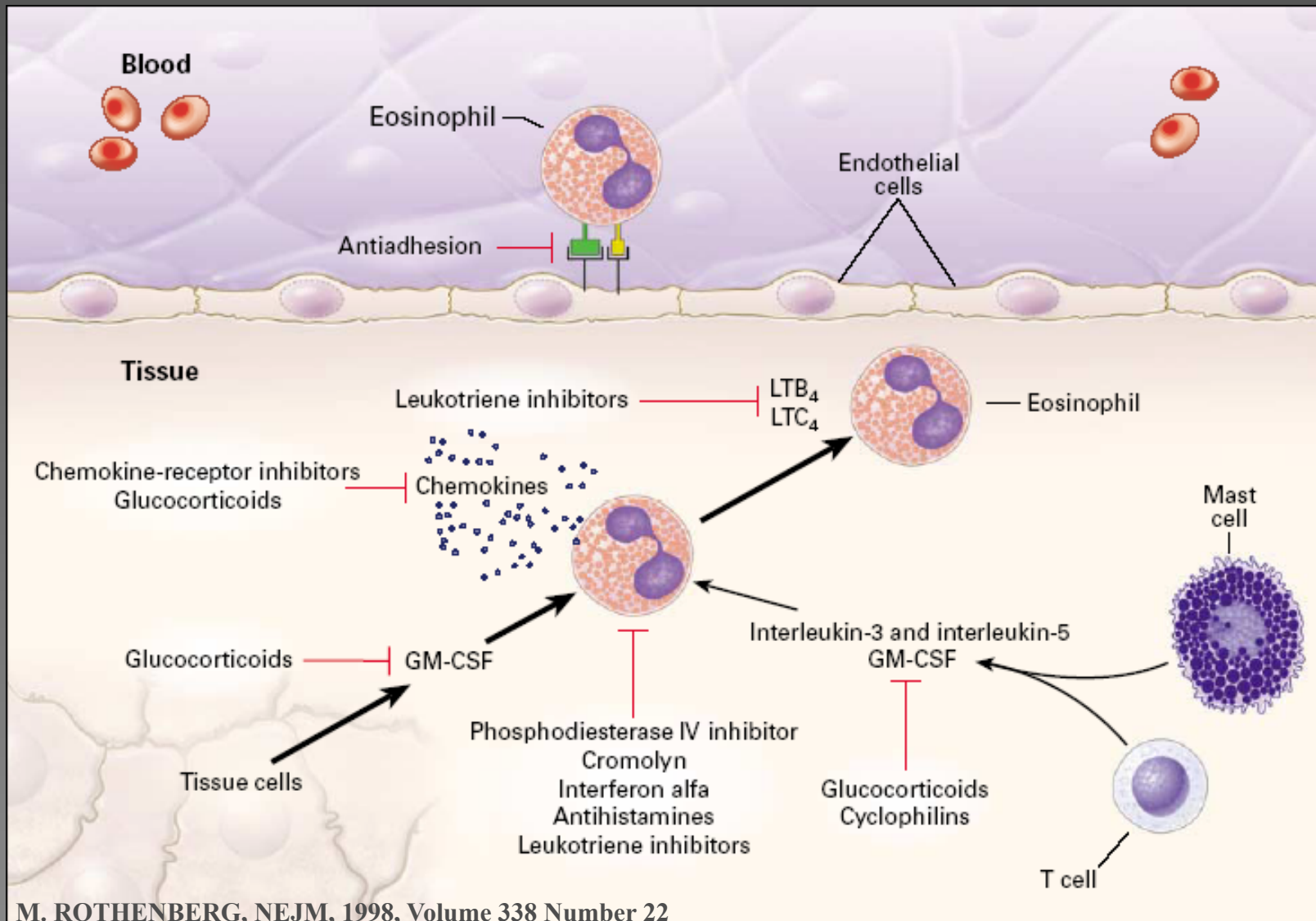
« End-organ damage »: morbidité

Table 2. Organ Involvement in Hypereosinophilic Syndrome

Organ System	Cumulative Frequency from 3 studies (%) ^{4, 19, 20}	Examples of Organ-Specific Manifestations
Hematologic	100	Leukocytosis with eosinophilia; neutrophilia, basophilia, myeloid immaturity, immature and/or dysplastic eosinophils; anemia, thrombocytopenia or thrombocytosis, increased marrow blasts, myelofibrosis. ^{19,21}
Cardiovascular	58	Cardiomyopathy, ^{28,29} constrictive pericarditis, ^{30,31} endomyocarditis, ^{32,33} mural thrombi, ^{27,34} valvular dysfunction, ^{27,35,36} endomyocardial fibrosis, ^{37,38} myocardial infarction ³⁹
Dermatologic	56	Angioedema, ⁴⁰ urticaria, ⁴⁰ papules/nodules, ⁴⁰ plaques, ⁴¹ aquagenic pruritis, ⁴² erythroderma, ⁴³ mucosal ulcers, ⁴⁴ vesico-bullous lesions, ⁴⁵ microthrombi, ^{46,47} vasculitis, ⁴⁸ Wells' syndrome ⁴⁹
Neurologic	54	Thromboembolism, ⁵⁰ peripheral neuropathy, ^{50,51} encephalopathy, ^{50,52} dementia, ^{53,54} epilepsy, ⁵⁵ cerebellar disease, ⁵⁶ eosinophilic meningitis ⁵⁷
Pulmonary	49	Pulmonary infiltrates, ^{9,58} effusions, ^{9,59} fibrosis, ⁴ emboli, ⁶⁰ nodules/focal ground glass attenuation, ⁶¹ acute respiratory distress syndrome (ARDS) ⁶²
Splenic	43	Hypersplenism, infarct ⁶³
Liver/Gallbladder	30	Hepatomegaly, ⁶⁴ focal or diffuse hepatic lesions on imaging, ⁶⁴ chronic active hepatitis, ⁶⁵ hepatic necrosis, ⁶⁶ Budd-Chiari syndrome, ⁶⁷ sclerosing cholangitis, ⁶⁸ cholecystitis, ⁶⁹ cholestasis ⁷⁰
Ocular	23	Microthrombi, ⁷¹⁻⁷³ choroidal infarcts, ⁷² retinal arteritis, ⁷³ episcleritis, ⁷⁴ keratoconjunctivitis sicca, ⁷⁴ Adie's syndrome (pupillotonia) ⁷⁵
Gastrointestinal	23	Ascites, ⁷⁶ diarrhea, ⁷⁷ gastritis, ⁷⁸ colitis, ^{69,78} pancreatitis ⁷⁷
Musculoskeletal	N/A	Arthritis, ^{79,80} effusions, ⁸⁰ bursitis, ⁸¹ synovitis, ⁸² Raynaud's phenomena, ⁸³ digital necrosis, ⁸⁴ polymyositis/myopathy ^{85,86}
Renal	N/A	Acute renal failure with Charcot-Leyden crystalluria, ⁸⁷ nephrotic syndrome, ⁸⁸ immunotactoid glomerulopathy, ⁸⁹ crescentic glomerulonephritis ⁹⁰

Modified from Weller PF and Bubley GJ. *Blood*. 1994; 83: 2760, and Brito-Babapulle F. *Blood Reviews*. 1997; 11: 139. N/A: not available

Cibles thérapeutiques



Mise au point des hyperéosinophilies: Démarche diagnostique

- Exclure les causes secondaires/réactionnelles
- Préciser le $\Delta\Delta$:
 - Primaires
 - Idiopathiques « vraies »

Hyperéosinophilies « réactionnelles »

- Secondaires et / ou réactionnelles:
 - Réponse physiologique production cytokines éosinotropes, chémoattractants
 - USA/EU: réactions allergiques, (hypersensibilité type I), asthme,
 - PVD: parasitoses,
 - Dysimmunitaires et connectivites,
 - Néoplasies: I ou II.

Syndromes d'Hyperéosinophilies « Idiopathiques » (HES)

- Primaires:
 - Désordres hématologiques perturbant l'éosinopoïèse,
 - Variant myéloïde
 - Variant lymphoïde
 - Production ectopique d'IL-5 par clone lymphocytaire Th2

Syndromes d' Hyperéosinophilies « Idiopathiques » (HES)

- Idiopathiques:
 - Physiopathologie mal / inconnue...
= Formes idiopathiques vraies

Causes secondaires
« communes »
d'hyperéosinophilie

Hyperéosinophilies réactionnelles

« secondaires »

Table 1. Reactive Causes of Eosinophilia

Allergic/hypersensitivity diseases

Asthma, rhinitis, drug reactions, allergic bronchopulmonary aspergillosis, allergic gastroenteritis

Infections

Parasitic

Strongyloidiasis, *Toxocara canis*, *Trichinella spiralis*, visceral larva migrans, filariasis, Schistosomiasis, *Ancylostoma duodenale*, *Fasciola hepatica*, *Echinococcus*, *Toxoplasma*, other parasitic diseases

Bacterial/Mycobacterial

Fungal (coccidioidomycosis, cryptococcus)

Viral (HIV, HSV, HTLV-II)

Rickettsial

Connective tissue diseases

Churg-Strauss syndrome, Wegener's granulomatosis, rheumatoid arthritis, polyarteritis nodosa, systemic lupus erythematosus, scleroderma, eosinophilic fasciitis / myositis

Pulmonary diseases

Bronchiectasis, cystic fibrosis, Loeffler's syndrome, eosinophilic granuloma of the lung

Cardiac diseases

Tropical endocardial fibrosis, eosinophilic endomyocardial fibrosis or myocarditis

Skin diseases

Atopic dermatitis, urticaria, eczema, bullous pemphigoid, dermatitis herpetiformis, episodic angioedema with eosinophilia (Gleich syndrome)

Gastrointestinal diseases

Eosinophilic gastroenteritis, celiac disease

Malignancies

Hodgkin's and Non-Hodgkin's lymphoma, acute lymphoblastic leukemia, Langerhans cell histiocytosis, angiolymphoid hyperplasia with eosinophilia (Kimura's disease), angioimmunoblastic lymphadenopathy, solid tumors (e.g. renal, lung, breast, vascular neoplasms, female tract cancers)

Immune system diseases / abnormalities

Wiskott-Aldrich syndrome, hyper-IgE (Job's) syndrome, hyper-IgM syndrome, IgA deficiency

Metabolic abnormalities

Adrenal insufficiency

Other

IL-2 therapy, L-tryptophan ingestion, toxic oil syndrome, renal graft rejection

Mise au point d'une HE (1)

- Anamnèse (**histoire familiale**, voyages, atopies...)
- Examen clinique (organomégalies, signes cutanés...)
- FHL, vit B12, tryptase sérique, IgE
- Sérologies connectivites
- Sérologies « infectieuses » virales, recherches de parasites (à répéter si négatives)
- Examen des selles / parasites ou oeufs (à répéter si négatives)
- Aspiration duodénale (Strongyloides)
- Traitement antiparasitaire d'essais ?

Mise au point d'une HE (1)

- ECG, Rx thorax
- Bilan imagerie (tumeur profonde, atteinte parenchymateuse...)
- Cytologie (%age éosinophiles, dysplasie, éosinophiles, blastose)
- histologie médullaire (fibrose ?, IHC tryptase, mastocytes ?)
- Typage lymphocytaire / Th2
- Cytogénétique (translocation balancée acquise, délétion)
- FISH (Δ PDGFRA, PDGFRB, 8p11...)
- Génétique moléculaire ($\Delta\Delta$, mutation activatrice de kinase, suivi traitement)

Difficultés diagnostiques:

- Difficultés diagn. / parasitoses torpides,
- Etats nosologiques intriqués (asthme + cause I ou II),
- Atteintes d'organe isolé vs maladie système avec atteinte organe (poumon, tube digestif, ...)
- I vs II pas tjs clair



Répétitions des tests avant diagnostic « HE I »

Hyperéosinophilies « primaires »:
DD idiopathiques vs variants
myéloïde et lymphoïde

Classification OMS des « CMPDs » : 2 entités associées avec HE

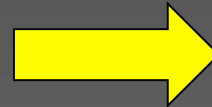
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- ❑ Essential thrombocythemia
- ❑ Chronic myeloproliferative disease, unclassifiable

Syndrome d'hyperéosinophilie idiopathique (IHES)

- Éosinophilie $> 1,5 \times 10^9/l$ ($1,5 \times 10^3/\mu l$),
 - Installation depuis > 6 mois,
- Dommages tissulaires (cœur, SNC, pulmonaire),
- Exclusion des causes connues d'éosinophilies secondaires.

Critères OMS ΔΔ: IHES vs CEL

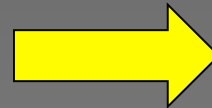
❑ Clonalité



« CEL »

- ❑ myélémie,
- ❑ blastose 5-20%,
- ❑ anomalie caryotypique

❑ « Pas » de clonalité



« IHES »

- ❑ Anomalies typage lymphocytaire (L-HES),
- ❑ Pas d'anomalies reconnues

Diagnostic différentiel, (I)HES

Préciser le DD (I)HES :

- **Variants myéloïde:**

- Hyperéosinophilie, tryptase sérique ↗ (SMP),

- **Variants lymphoïde:**

- Hyperéosinophilie secondaire / sécrétion pathologique de cytokines (i.e IL-5, clone Th2)



Numération et morphologie sanguine, examen médullaire, Typage lymphocytaire, cytogénétique et biologie moléculaire

Variants lymphoïde clinique

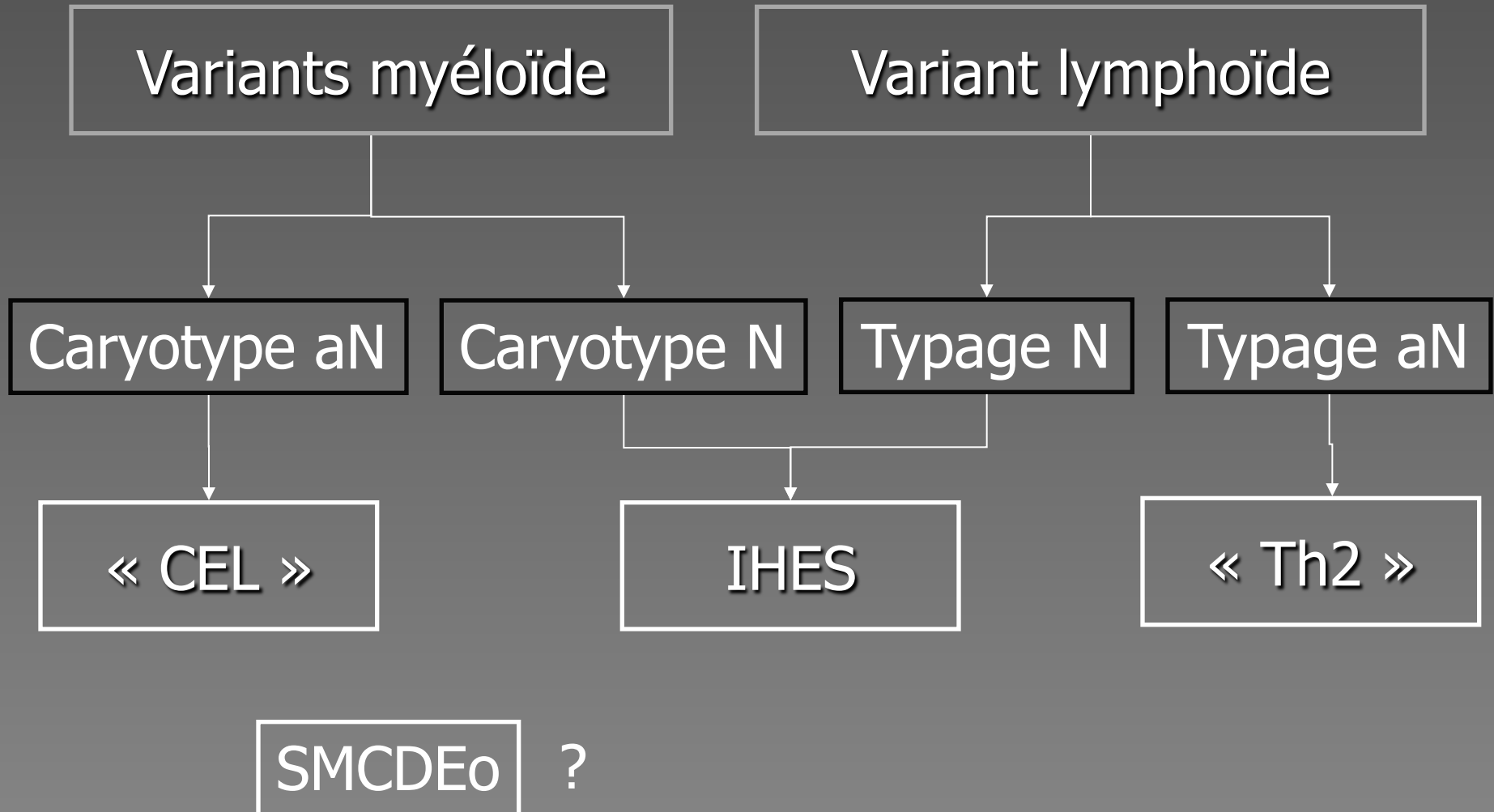
- Atteintes cutanées, IgE,
- aN lymphocytaires « clonale »,
- Clone « Th2 » (CD3-CD4+CD8-; CD4-CD3+; CD3+CD4-CD8-)
- Phénotype « activé », CD25+, DR+
- Mauvais pronostic: évolution vers LNH-T

HES, variants lymphoïde

Diagnostic

- Examen clinique (peau),
- Dosage IL-5 et CD25s,
- Phénotypage,
- Biologie moléculaire, TCR γ et β (pas indispensable)
- Profil cytokines

En résumé « I » HES

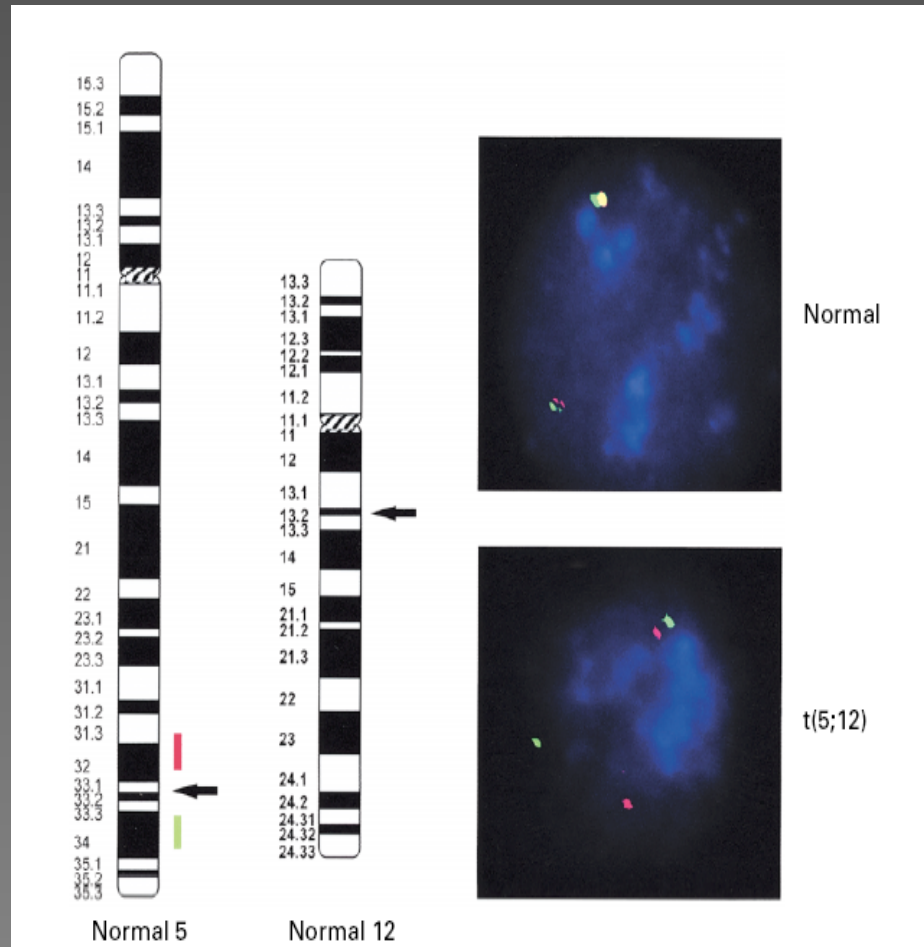


Des anomalies cytogénétiques
rares permettent d'affirmer la
clonalité des SMPs atypiques
avec HE

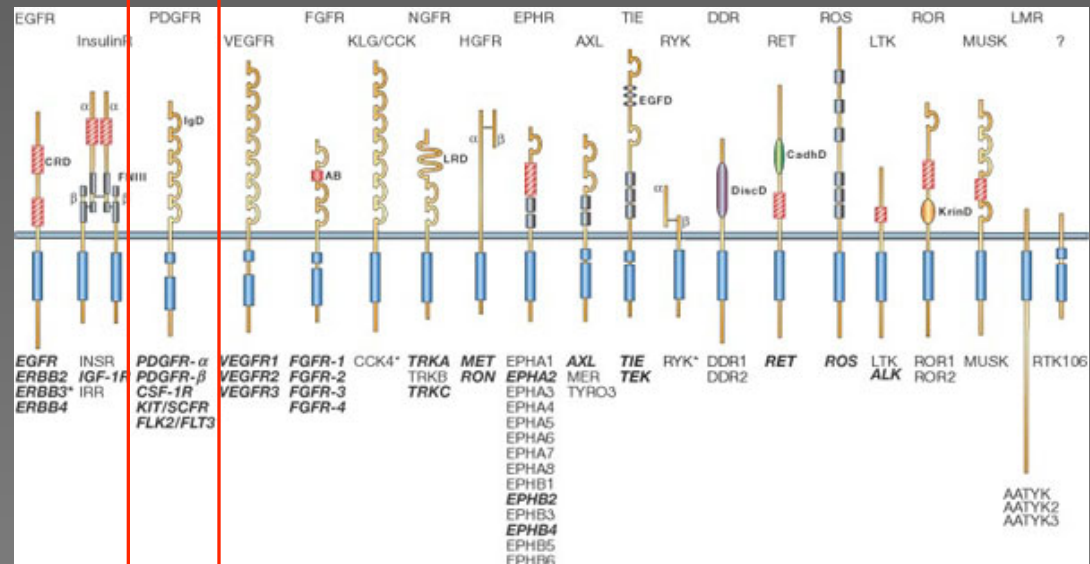
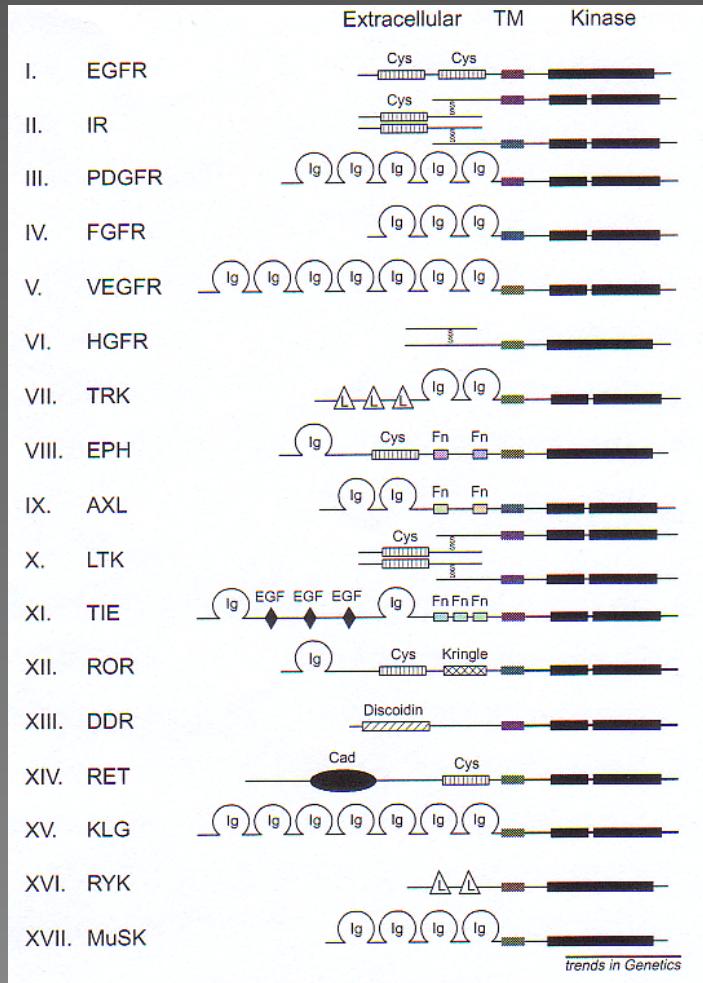
I. Anomalies du PDGFRB

Syndrome	Cytogenetic abnormality	Molecular genetic abnormality
Chronic myelomonocytic leukaemia	t(5;12)(q33;p13)	<i>TEL-PDGFR</i> fusion
with eosinophilia	t(5;10)(q33;q21)	<i>H4/D10S170-PDGFR</i> fusion [25]
CMML with eosinophilia/T-lineage lymphoblastic lymphoma/rarely B-lineage lymphoblastic leukaemia/lymphoma and frequent evolution to acute myeloid leukaemia	t(6;8)(q27;p12) t(8;9)(p11;q32-34) t(8;13)(p11;q12) t(8;17)(p11;q25)*	<i>FOP-FGFR1</i> fusion <i>FAN-FGFR1</i> fusion <i>ZNF198-FGFR1</i> fusion <i>FGFR1</i> rearranged [26]

t(5;12)(q32;q33)/ETV6-PDGFRB



PDGFRB, un membre de la classe III des récepteurs à activité « tyrosine kinase »



PDGFR α , PDGFR β , c-KIT, FLT3

Induction of myeloproliferative disease in mice by tyrosine kinase fusion oncogenes does not require granulocyte-macrophage colony-stimulating factor or interleukin-3

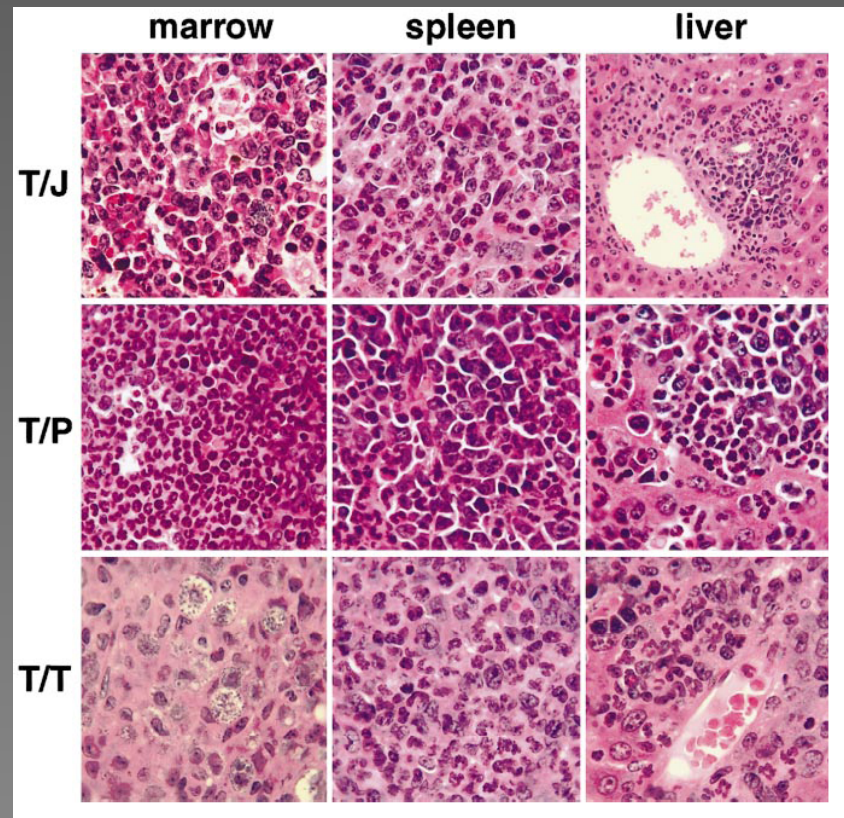
Michael H. Tomasson, Ifor R. Williams, Shaoguang Li, Jeffrey Kutok, Danielle Cain, Silke Gillessen, Glenn Dranoff, Richard A. Van Etten, and D. Gary Gilliland

NH2

ETV6

PDGFRB

COOH



D'autres mutations du PDGFRB

ETV6

Myomegalin

Rabaptin 5

HIP1

H4/D10S170

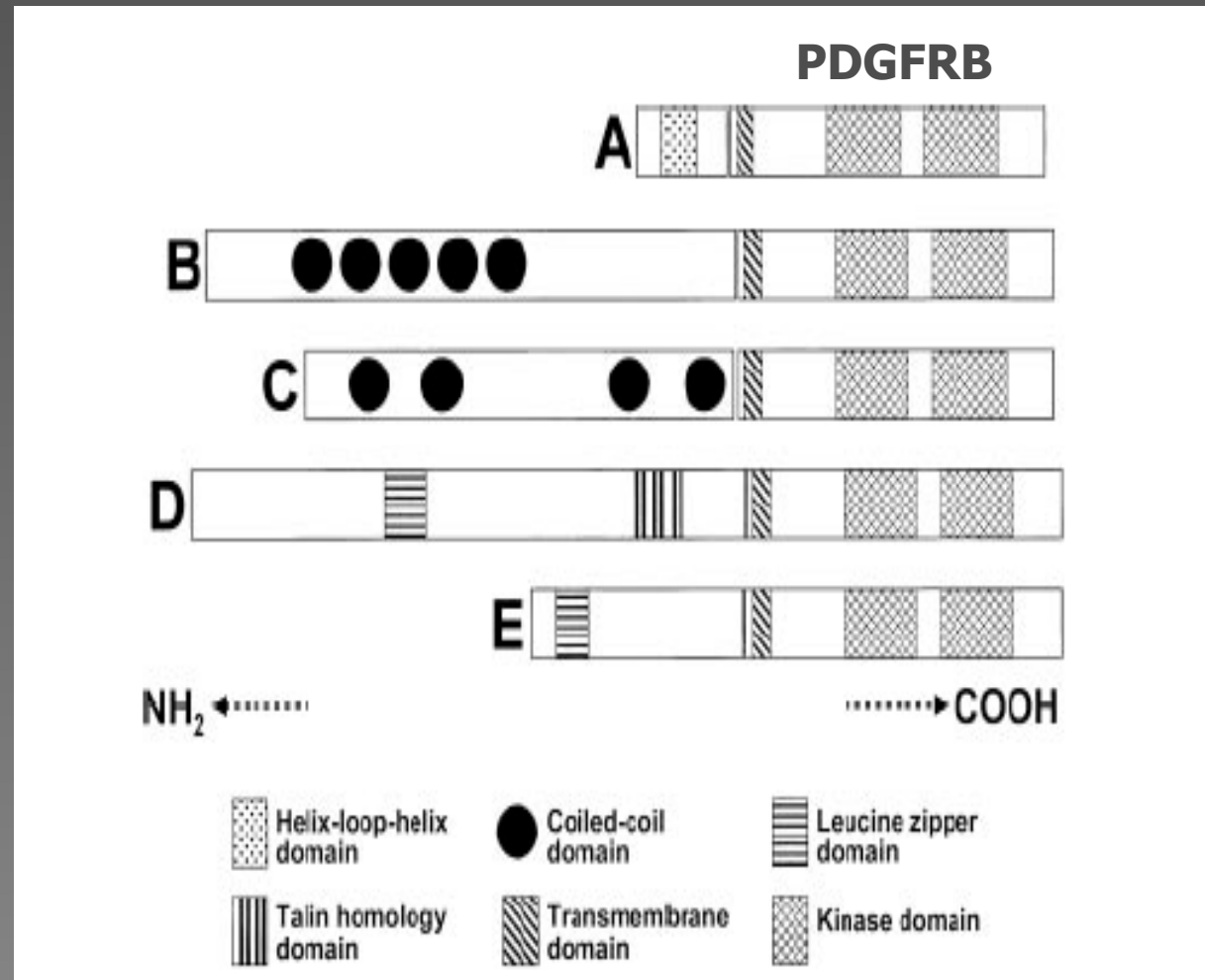


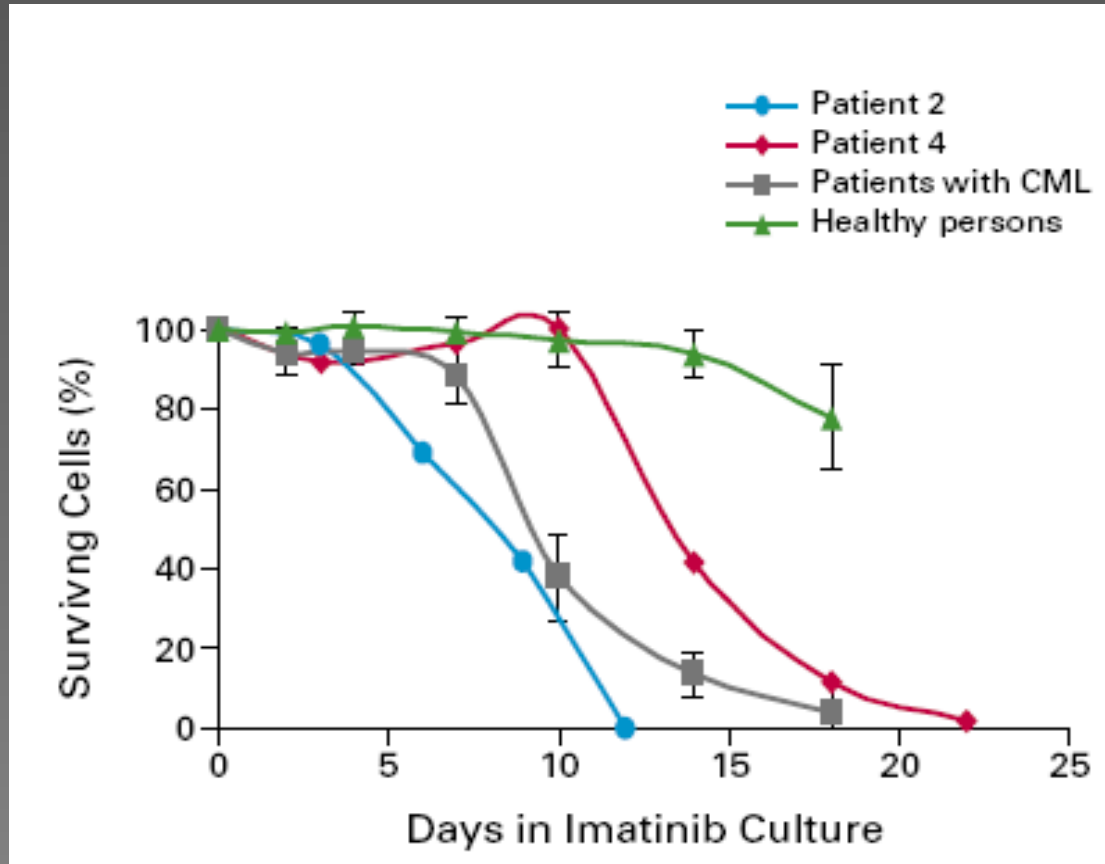
Illustration des essais cliniques dans les SMPs Ph-

IMATINIB MESYLATE IN CHRONIC MYELOPROLIFERATIVE DISEASES

RESPONSE TO IMATINIB MESYLATE IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISEASES WITH REARRANGEMENTS OF THE PLATELET-DERIVED GROWTH FACTOR RECEPTOR BETA

JANE F. APPERLEY, M.D., MARTINE GARDEMBAS, M.D., JUNIA V. MELO, M.D., ROBIN RUSSELL-JONES, M.D.,
BARBARA J. BAIN, M.D., E. JOANNA BAXTER, PH.D., ANDREW CHASE, PH.D., JUDITH M. CHESSELLS, M.D.,
MARIE COLOMBAT, PH.D., CLAIRE E. DEARDEN, M.D., SASA DIMITRIJEVIC, PH.D., FRANÇOIS-X. MAHON, M.D.,
DAVID MARIN, M.D., ZARIANA NIKOLOVA, M.D., EDUARDO OLAVARRIA, M.D., SANDRA SILBERMAN, M.D.,
BEATE SCHULTHEIS, M.D., NICHOLAS C.P. CROSS, PH.D., AND JOHN M. GOLDMAN, D.M.

Evaluation in vitro de la sensibilité à l' Imatinib.



ETV6-PDGFRB est inhibée par l'imatinib

TABLE 1. CLINICAL AND HEMATOLOGIC CHARACTERISTICS OF FOUR PATIENTS WITH *PDGFRB*-POSITIVE LEUKEMIAS.*

CHARACTERISTIC	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4
Sex	Male	Male	Male	Male
Age at diagnosis (yr)	32	50	68	6
At beginning of imatinib treatment				
Age (yr)	36	51	69	20
Splenomegaly	Yes	Yes	No	No
Hematologic characteristics				
Hemoglobin (g/dl)	10.1	12.3	12.5	9.7
White-cell count (per mm ³)	80,000	52,000	41,000	9,300
Eosinophil count (per mm ³)	7,820	4,800	1,640	4,100
Platelet count (per mm ³)	74,000	190,000	192,000	506,000
Bone marrow cytogenetics	100% 46,XY t(5;12)(q33;p13)	100% 46,XY t(5;12)(q33;p13)	100% 46,XY t(5;12)(q33;p13)	100% 46,XY t(5;12)(q33;q13)
Results on RT-PCR				
Single-step	+	+	+	NA
Heminested	+	+	+	NA
Most recent studies				
Duration of imatinib therapy (mo)	9	12	9	12
Current dose of imatinib (mg/day)	400	400	400	800
Hematologic characteristics				
Hemoglobin (g/dl)	14.5	13.7	14.6	14.3
White-cell count (per mm ³)	5,600	5,000	5,500	8,500
Eosinophil count (per mm ³)	100	200	100	700
Platelet count (per mm ³)	176,000	251,000	245,000	249,000
Bone marrow cytogenetics	100% 46,XY	100% 46,XY	100% 46,XY	100% 46,XY
Fluorescence in situ hybridization	NP	NP	NP	>95% 2 fused signals
Results on RT-PCR				
Single-step	—	—	—	NA
Heminested	+	—	+	NA

*RT-PCR denotes reverse-transcriptase polymerase chain reaction, NA not applicable, and NP not performed. A plus sign indicates a positive result, and a minus sign a negative result.



Apperley J, N Engl J Med, Vol. 347, No. 7 August 15, 2002

Figure 1. Patient 4 at the Beginning of Imatinib Therapy (Panel A) and after Eight Months of Therapy (Panel B).

II. Le syndrome 8p11

Cytogenetic Abnormality	Tyrosine Kinase Fusion Protein	Chronic Myeloproliferative Disorder
t(9;22)(q34;q11)	BCR-ABL	CML or acute lymphoblastic leukemia
t(8;22)(p11;q11)	BCR-FGFR1	BCR-ABL-negative CML
t(4;22)(q12;q11)	BCR-PDGFR A	Atypical CML
t(8;13)(p11;q12)	ZNF198-FGFR1	8p Myeloproliferative syndrome
t(6;8)(q27;p11)	FOP-FGFR1	8p Myeloproliferative syndrome
t(8;9)(p12;q33)	CEP110-FGFR1	8p Myeloproliferative syndrome
t(8;19)(p12;q13)	HERV-K-FGFR1	8p Myeloproliferative syndrome
t(5;12)(q33;p13)	TEL-PDGFRB	Chronic myelomonocytic leukemia or atypical CML
t(5;7)(q33;q11)	HIP1-PDGFRB	Chronic myelomonocytic leukemia or atypical CML
t(5;17)(q33;p13)	RAB5-PDGFRB	Chronic myelomonocytic leukemia or atypical CML
t(5;10)(q33;q21)	H4-PDGFRB	Chronic myelomonocytic leukemia or atypical CML
t(9;12)(q34;p13)	TEL-ABL	Atypical CML or BCR-ABL-negative CML
t(9;12)(p24;p13)	TEL-JAK2	Atypical CML or BCR-ABL-negative CML
t(9;22)(p24;q11)	BCR-JAK2	Atypical CML or BCR-ABL-negative CML
del(4)(q12)	FIP1L1-PDGFR A	Hypereosinophilic syndrome



Syndomes 8p11-12

- Maladie rare (60 cas décrits)
- Histoire naturelle Tri-phasique
- Phase chronique SMP avec HE f
- Accélération, cell. Myéloïdes immatures,
- Accutisation ss forme de LNH lymphoblastique B/T
- Atteinte lignées myéloïdes et lymphoïdes
- Anomalie clonale cell. souche multipotente
- T(X;8)(X;p11), 9 partenaires décrits, protéines de fusions X-FGFR1
- Pas sensible au glivec

Une thématique commune :
mutation de gène encodant des
protéines à activité Tyrosine
kinase

PDGFR β , PDGFR α , ABL, FGFR1

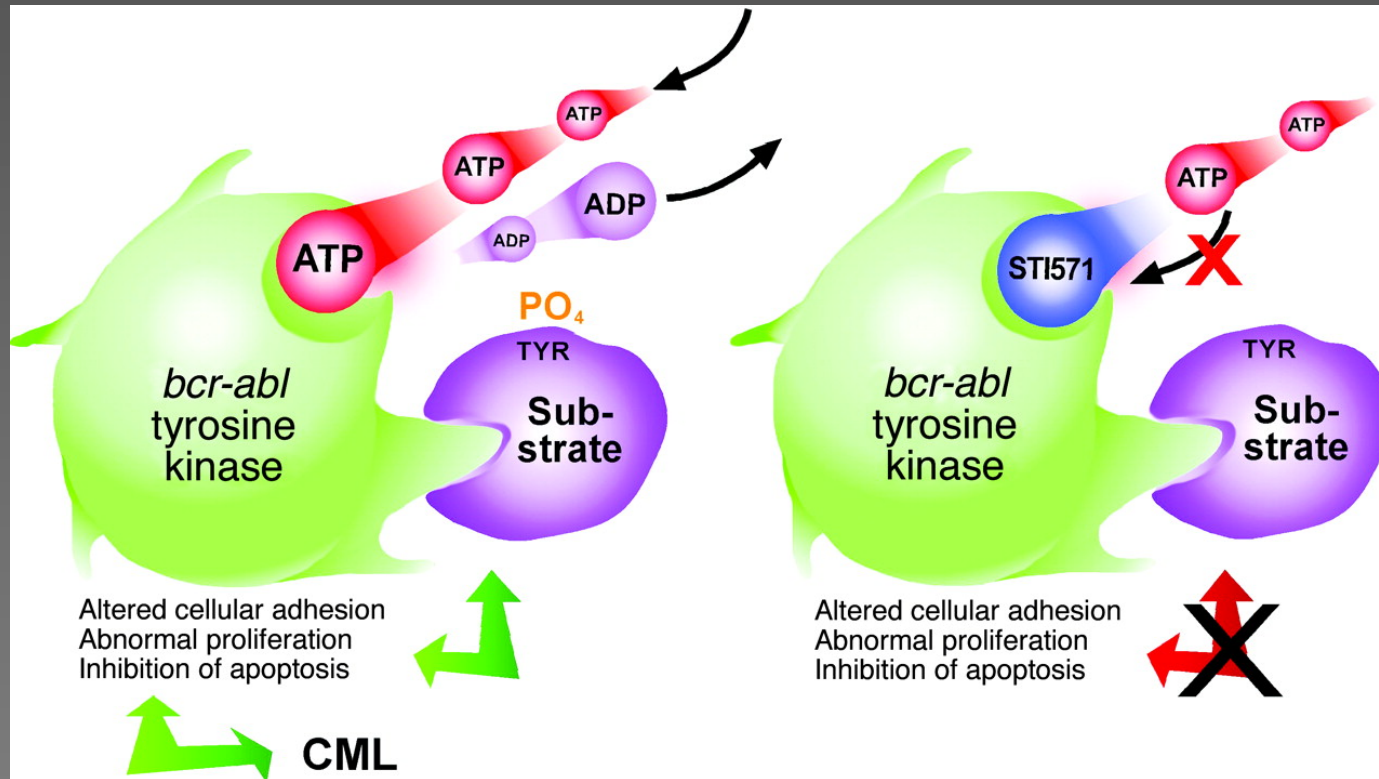
Imatinib therapy for Phi neg CMPDs

Review article

Imatinib targets other than *bcr/abl* and their clinical relevance in myeloid disorders

Animesh Pardhanani and Ayalew Tefferi

Schematic representation of the mechanism of action of STI571



Mauro, M. J. et al. *Oncologist* 2001;6:233-238

Imatinib Is Highly Selective for Multiple Kinases

<u>Kinases Inhibited</u>		<u>Kinases Not Inhibited</u>	
v-Abl	0.1–0.3	Flt-3	>10
p210Bcr-Abl	0.25	c-Fms, v-Fms	>10
p185Bcr-Abl	0.25	EGF receptor	>100
TEL-Abl	0.35	c-erbB2	>100
PDGF-R	0.1	Insulin receptor	>100
TEL-PDGF-R	0.15	IGF-1 receptor	>100
c-Kit	0.1	v-Src	>10
		JAK-2	>100

PDGF-R = platelet-derived growth factor receptor; EGF = epidermal growth factor; IGF-I = insulin-like growth factor I.

Druker BJ et al. *Nat Med.* 1996;2:561-566.

Tyrosine kinase constitutivement activée dans les SMP sensibles au Glivec®

Table 1. Partial list of constitutively active tyrosine kinases in *bcr/abl*-negative chronic myeloid disorders that are predicted or documented to be imatinib sensitive

Fusion	Translocation	Deletion	Mutation	Disease association	Imatinib response*	Study
PDGFRB						
ETV6/PDGFRB	t(5;12)(q33;p13)	—	—	CMML	Yes	Golub et al ¹⁰
CEV14/PDGFRB	t(5;14)(q33;q32)	—	—	AML	—	Abe et al ³⁶
HIP1/PDGFRB	t(5;7)(q33;q11)	—	—	CMML	—	Ross et al ²³
H4 (D10S170)/PDGFRB	t(5;10)(q33;q21)	—	—	uCMPD	Yes	Kulkarni et al ³⁴ , Schwaller et al ³⁵
RAB5/PDGFRB	t(5;17)(q33;p13)	—	—	CMML	—	Magnusson et al ³³
PDE4DIP/PDGFRB	t(1;5)(q23;q33)	—	—	uCMPD	Yes	Wilkinson et al ¹⁸
PDGFRA						
BCR/PDGFRB	t(4;22)(q12;q11)	—	—	uCMPD	Yes	Trempat et al ³⁷ , Baxter et al ³⁸
F1P1L1/PDGFRB	—	4q12‡	—	SM-eos/CEL	Yes	Cools et al ¹¹ , Pardanani et al ¹⁴
c-kit						
—	—	—	D816V/F/Y§	MCD	No	Ma et al ⁸⁷
—	—	—	V560G	—	—	Furitsu et al ⁵¹
—	—	—	E839K	—	—	Longley et al ²⁸
—	—	—	D820G	—	—	Pignon et al ²⁹
Abl						
ETV6/ABL	Complex†	—	—	uCMPD	Yes	Van Limbergen et al ³⁰ , Andreasson et al ³¹
ET6/ARG	t(1;12)(q25;p13)	—	—	AML	Yes	Nishimura et al ³²

aCML indicates atypical chronic myeloid leukemia; uCMPD, unclassified chronic myeloproliferative disorder (some of these patients are described as having atypical CML); SM-eos, eosinophilia-associated systemic mastocytosis; MCD, mast cell disease; ETV6, ets variant gene 6 (TEL oncogene); CEV14, clonal evolution-related gene on chromosome 14; HIP1, Huntington interacting protein 1; RAB5, rabaptin 5; BCR, breakpoint cluster region; D10S170, DNA segment on chromosome 10 (unique) 170; PDE4DIP, phosphodiesterase 4D interacting protein (myomegalin); FIP1L1, FIP1-like 1; and —, not applicable.

*Clinical response to imatinib therapy documented (where tested).

†All patients have complex chromosomal rearrangements that require at least 3 chromosomal breaks.

‡Deletion is submicroscopic.

§Imatinib resistant.

Imatinib et SMPa (1)

Table 3. Summary of representative studies that describe use of imatinib in the treatment of CMML or atypical CMPD*

Study	Clinical diagnosis	No. patients	Cytogenetic findings	Molecular findings	Response	Imatinib dose, mg/d
Magnusson et al ^{80*}	CMML	1	t(5;17)(q33;p13)	<i>Rab5/PDGFRB</i>	CR	400
Pitini et al ^{82†}	CMML	1	t(5;12)(q33;p13)	<i>ETV6/PDGFRB</i>	CR	400
Cortes et al ⁹⁷	CMML	3	2 normal 1 trisomy 21	NA	3 NR	400
Wilkinson et al ^{18*}	aCMPD	1	t(1;5)(q23;q33)	<i>PDE4DIP/PDGFRB</i>	CR	NA
Trempat et al ^{37*}	aCMPD	1	t(4;22)(q12;q11)	<i>BCR/PDGFRB</i>	CHR	400
Garcia et al ^{67*}	aCMPD	1	t(5;10)(q33;q22)	<i>H4(D10S170)/PDGFRB</i>	CR	400
Demetri et al ^{68*‡}	aCMPD	1	Complex abnormalities	<i>ETV6/ABL</i>	CHR	600
Cortes ⁹⁷	aCMPD	7	NA	NA	1 MHR	400

CHR indicates complete hematologic response (normalization of blood counts); MHR, major hematologic response (increase in hemoglobin concentration from 95 to 137 g/L [9.5 to 13.7/dL]); D10S170, DNA segment on chromosome 10 (unique 170). Remaining abbreviations are explained in Tables 1 and 2.

*Data supported the presence of imatinib-responsive molecular target before the start of therapy.

†Some patients were described as having atypical CML based on a CML phenotype without Philadelphia chromosome or *bcr/abl* fusion and association with dysplasia. Does not include patients with prominent peripheral eosinophilia.

‡Patient was treated in chronic phase of atypical CML after receiving induction therapy for blast crisis.

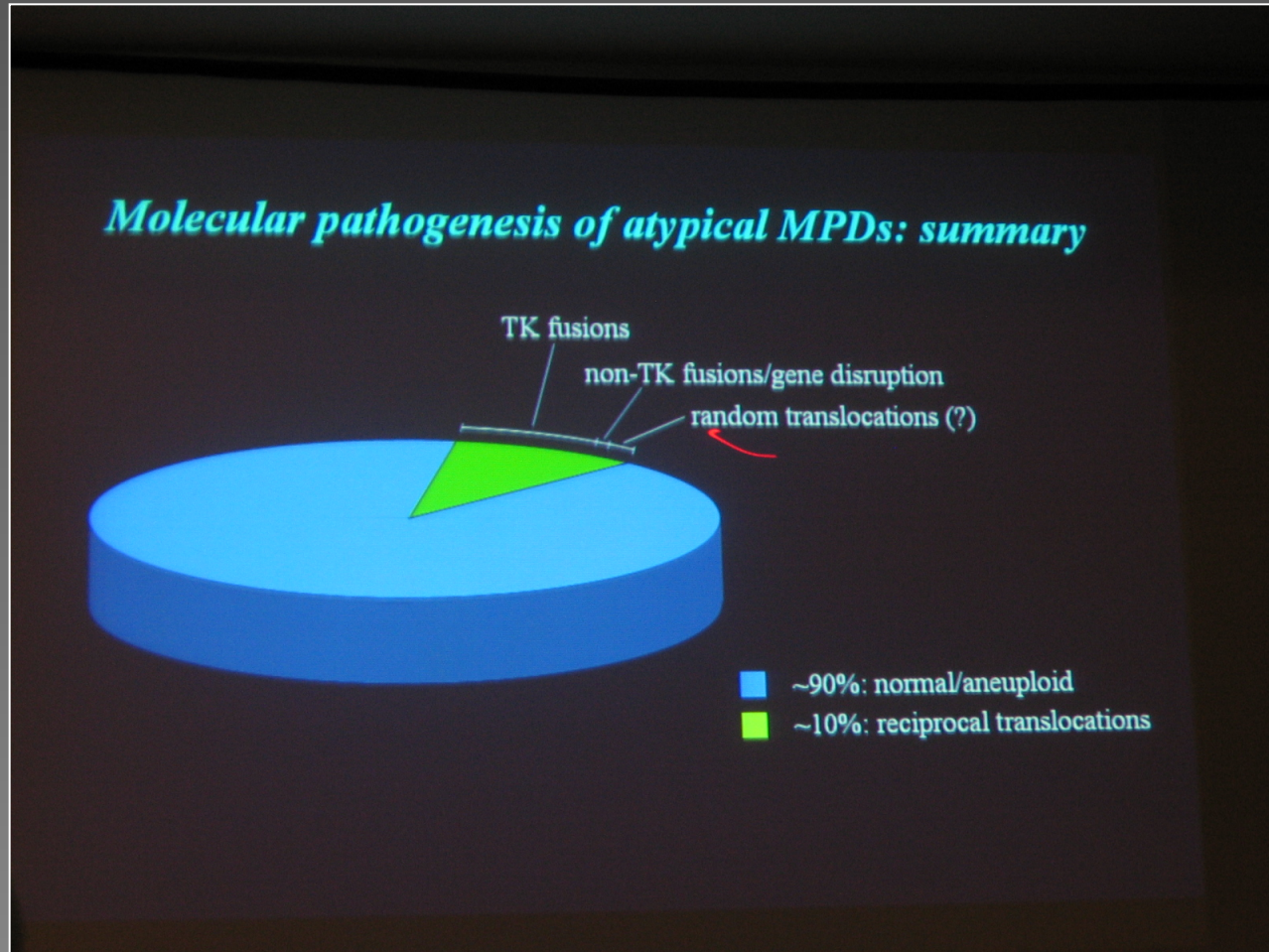
Imatinib et HES/SMCDEo (2)

Table 2. Summary of representative studies (series consisting of 4 patients or more) that describe use of imatinib in the treatment of primary eosinophilic disorders

Study	Clinical diagnosis	No. patients	Cytogenetic findings	c-kit or PDGFR mutations	Clinical response	Molecular or cytogenetic response	Imatinib dose, mg/d
Gleich et al ⁷⁰	HES	5	3 normal 2 ND	Negative for c-kit D816V (n = 4)	4 CR 1 NR	NA	100
Pardanani et al ⁷³	HES/CMPD-eos	7	6 normal 1 trisomy 8	Negative for c-kit and PDGFRB mutations (n = 6)	3 CR 1 PR 3 NR	Patient with +8 did not respond	100-400 100-400
Cortes et al ⁷²	HES	9	All normal	ND	4 CR 1 PR 4 NR	NA	100-400
Apperley et al ¹⁶	CMPD-eos	4	t(5;12)(q33;p13) in all 4 patients	ETV6/PDGFRB (n = 3)	4 CR	4 CR	400
Cools et al ¹¹	HES, AML	11	9 normal 1 t(1;4)(q44;q12) 1 trisomy 8 and 19	FIP1L1/PDGFR in 5 of 10 tested, including the 2 patients with abnormal cytogenetics	9 CR 2 NR	ND	100-400
Klion et al ⁴⁵	MHES	6	All normal	FIP1L1/PDGFR in all 6 patients	6 CR	ND	400
Pardanani et al ¹⁴	SM-eos	5	All normal	FIP1L1/PDGFR (n = 3) c-kit D816V (n = 2)	3 CR 2 NR	3 CR	100-400
Klion et al ¹⁷	MHES	7	All normal	FIP1L1/PDGFR in all 7 patients	7 CR	5 CR (n = 6)	300-400
Vandenberghe et al ⁴⁶	CEL	4	All normal	FIP1L1/PDGFR in all 4 patients	4 CR	2 CR	100
Salem et al ⁷⁹	HES	6	5 normal 1 trisomy 8	Negative for ETV6/PDGFRB	6 CR	NA	100

CR indicates complete response; NR, no response; PR, partial response; MHES, myeloproliferative variant of HES; eos-CMPD, eosinophilia associated chronic myeloproliferative disorder; D816V, aspartate 816 to valine mutation; ND, not done or not reported; NA, not applicable. Remaining abbreviations are explained in Table 1.

Anomalies rares et rencontrées dans une minorité de cas de SMP atypiques avec hyperéosinophilie



Actualités

Activation constitutive de protéines à activité « TK » dans les CMPDs, un mécanisme général d'activation ?

The NEW ENGLAND JOURNAL of MEDICINE

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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

Jan Cools, Ph.D., Daniel J. DeAngelo, M.D., Ph.D., Jason Gotlib, M.D., Elizabeth H. Stover, M.Phil., Robert D. Legare, M.D., Jorge Cortes, M.D., Jeffrey Kutok, M.D., Ph.D., Jennifer Clark, M.D., Ilene Galinsky, R.N., James D. Griffin, M.D., Nicholas C.P. Cross, Ph.D., Ayalew Tefferi, M.D., James Malone, M.D., Rafeul Alam, M.D., Ph.D., Stanley L. Schrier, M.D., Janet Schmid, M.D., Michal Rose, M.D., Peter Vandenberghe, M.D., Ph.D., Gregor Verhoef, M.D., Ph.D., Marc Boogaerts, M.D., Ph.D., Iwona Wlodarska, Ph.D., Hagop Kantarjian, M.D., Peter Marynen, Ph.D., Steven E. Coutre, M.D., Richard Stone, M.D., and D. Gary Gilliland, M.D., Ph.D.

Etude Cools and Co, NEJM March 2003

- Cas rapportés de réponses aux STI-571 chez patients avec SMPs atypiques, LMMC et IHES,
- Hypothèse cause sous jacente = activation constitutionnelle kinase type ABL, PDGFRA/B ou kit,
- Sélection cohorte de patients IHES traités par imatinib (**n = 11 de 16**) et identification base moléculaire de la réponse.

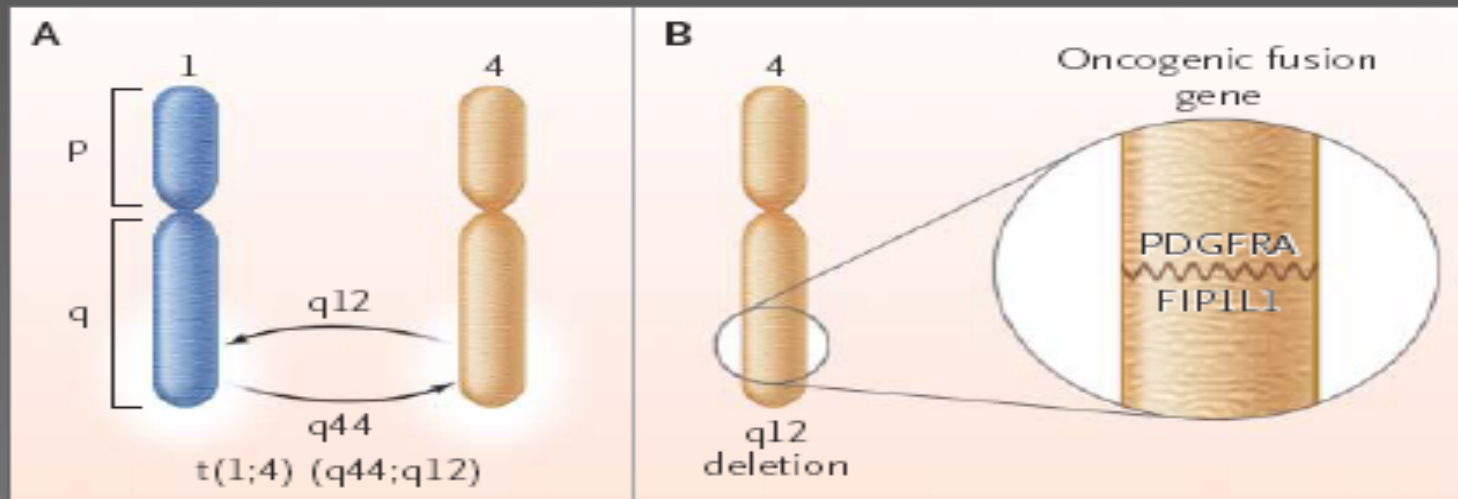
Descriptif:

- Age médian: 46 ans,
- Sex ratio: 9 H / 2 F,
- Thérapies préalables: corticostéroïdes, hydroxyurée, IFN α , CT, cyclosporine, RT,
- Atteintes organiques: fibrose endomyocardique, cardiomyopathie restrictive, atteinte GI, pulmonaire, SNC et paraspinal, cutanée, hépatosplénomégalie et thrombose,
- Éosinophilie moyenne: 14500 / mm³
- Caryotype: 9 N, 1 t(1;4)(q44;q12), 1 tris 8 -19 -add2q-del6q (LMA),
- BCR-ABL négatifs (K et FISH),
- Imatinib: 100 – 400 mg p.o

Résultats

- **Rémission cytologique complète (normalisation éosinophilie):**
 - **10 /11** (médiane 4 semaines) dont
 - 9 durant plus de trois mois et 1 transitoire,
- Durée médiane de réponse: 7 mois [3-15]

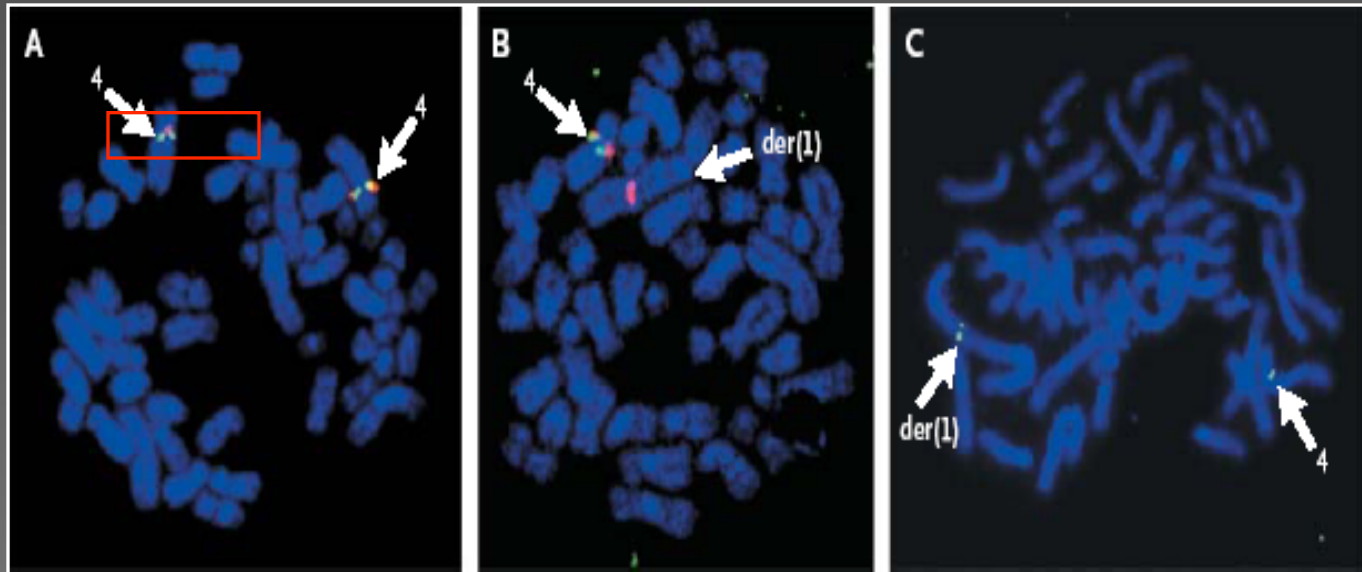
Identification des bases moléculaires de la réponse au traitement



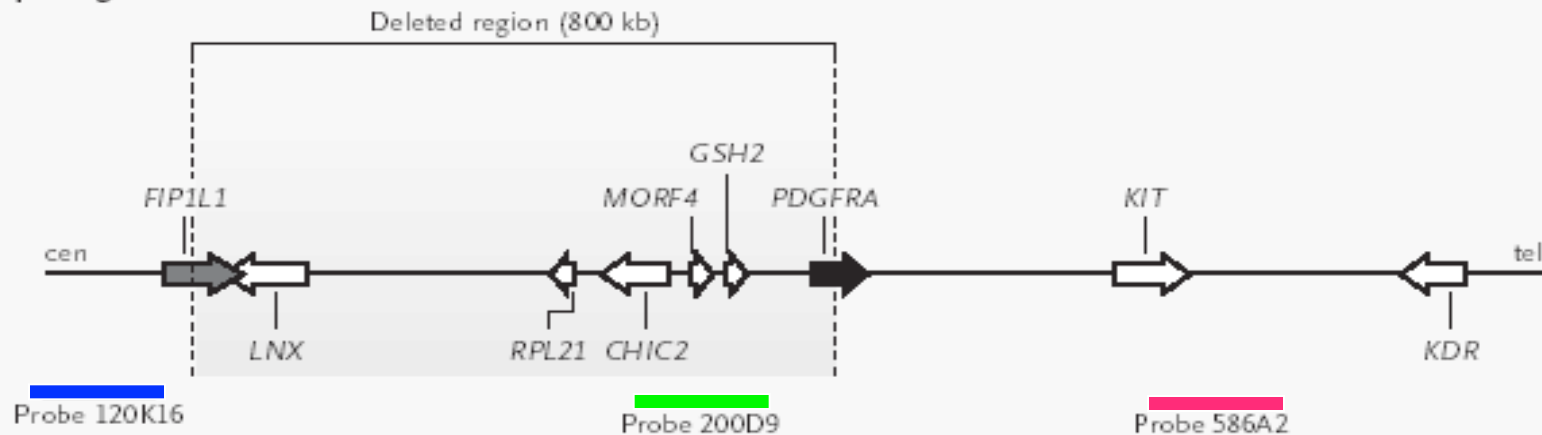
The Chromosomal Aberration in the Hypereosinophilic Syndrome.

The $t(1;4)(q44;q12)$ translocation (Panel A) found in one patient with the disease provided the clue to the oncogenic lesion. This translocation was not found in any other patient with the hypereosinophilic syndrome, whereas the molecular lesion shown in Panel B was present in 9 of 16 patients. In this lesion, deletion of a region in the long arm of chromosome 4 (q12) was repaired by the joining of pieces of two genes, *PDGFRA* and *FIP1L1*. The product of the fusion gene is a constitutively active tyrosine kinase.

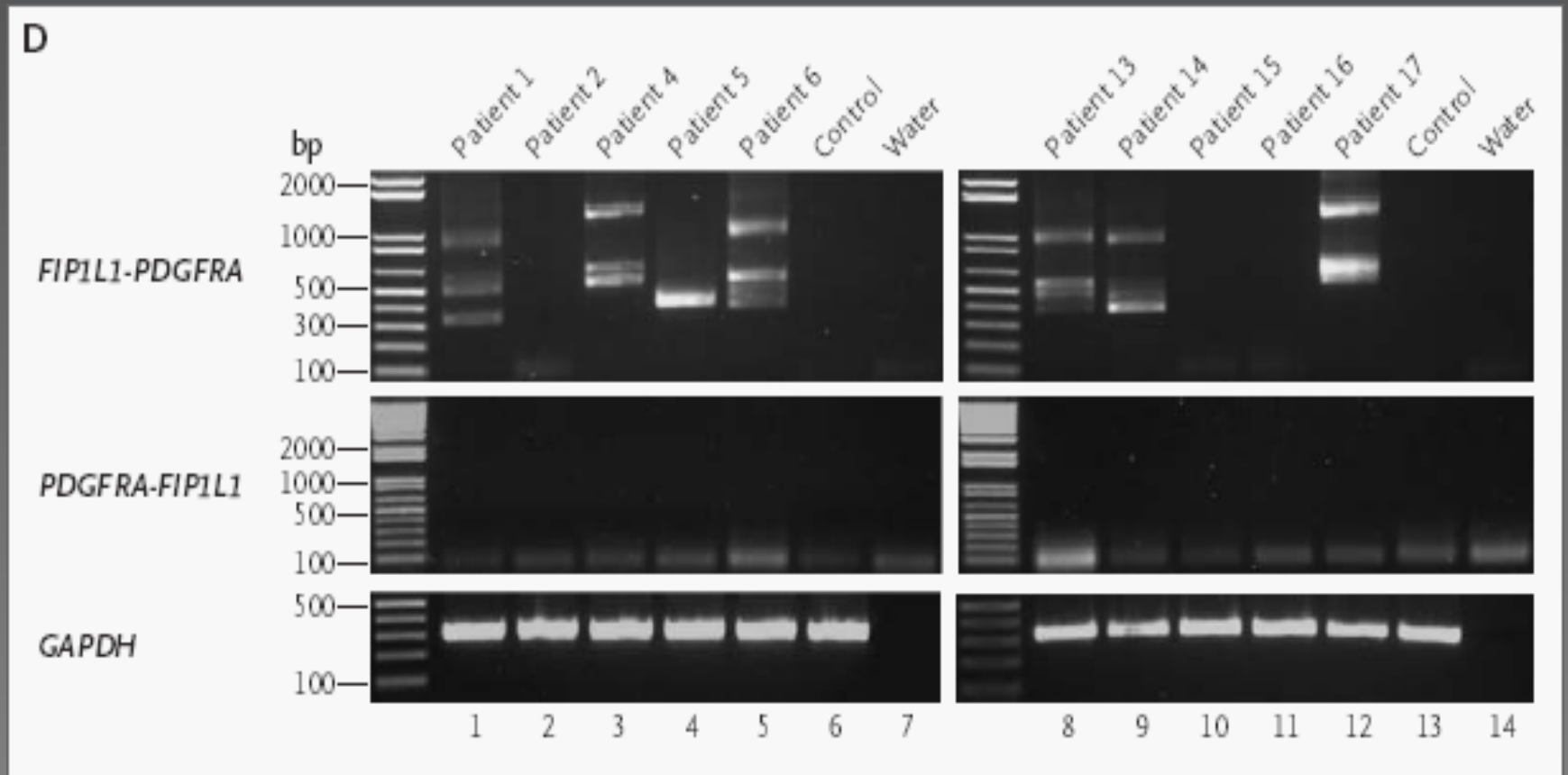
FISH del 4q12: délétion CHIC2



A 4q12 Region



Clonage du transcrit chimérique FIP1L1-PDGFRA



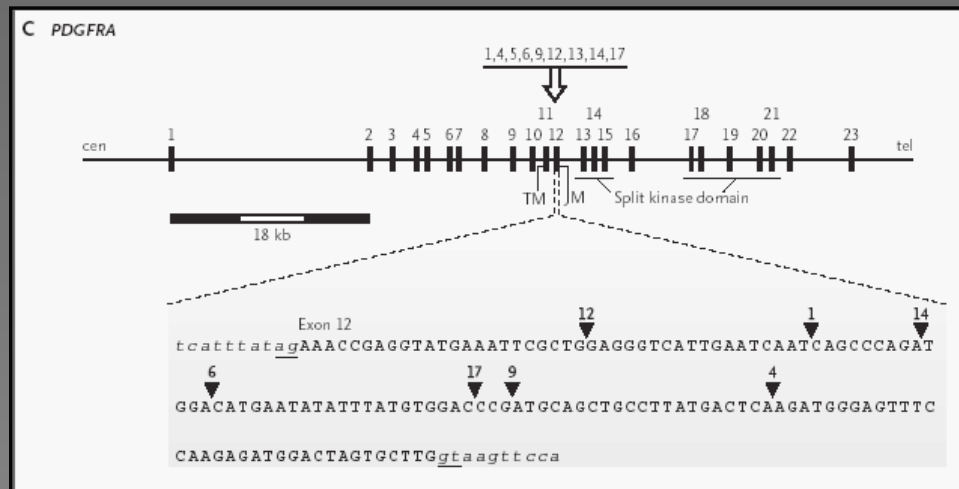
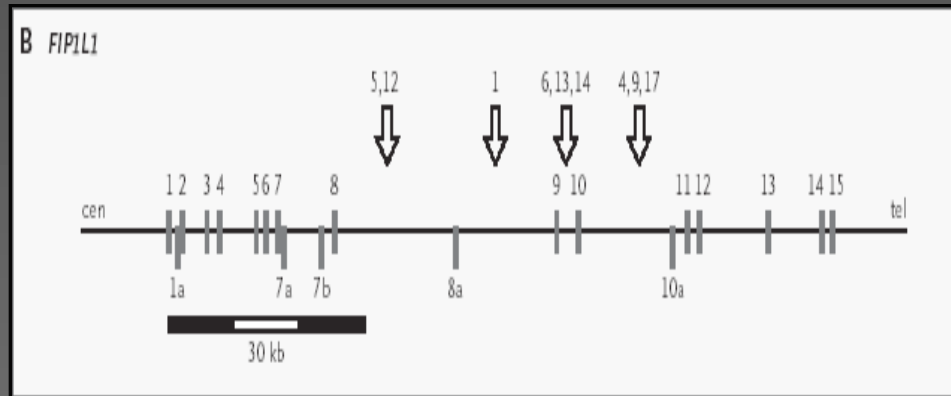
Identification du transcrit de fusion chez des patients avec caryotype N !

Clonage des points de cassure dans l'exon 12 du gène PDGFRA

B PDGFRA Exon 12 Break Points

		12	1	14	6	17	9	4
		▼	▼	▼	▼	▼	▼	▼
		GAAATTGCTGGAGGGTCATTGAATCAATCAGCCCAGATGGACATGAATATATTTATGTGGACCCGATGCAGCTGCCTTATGACTCAAGATGGGAGTTTCCAAGA						
Patient 1	Intron 8a	agcaaatgcttctgttttaattcttaatgca	CAGCCC	GGATGGACATGAATATATTTATGTGGACCCGATGCAGCTGCCTTATGACTCAAGATGGGAGTTTCCAAGA				
		agcaaatgcttctgttttaattcttaatgcacattaaagacatttctgttgaatgatgtcc						
Patient 4	Intron 10	tcaaaaaattatattcttgcgaagagattgattaatatttcagctctggctctctctgtataccggttttcctttt	ag	gagactgctac				AGATGGGAGTTTCCAAGA
		tcaaaaaattatattcttgcgaagagattgattaatatttcagctctggctctctctgtataccggttttcctttt	ag	gagactgctactgggtgcctgggtatttat				
Patient 6	Intron 9	ataagttatttaatttgttataatgatttgttaatttatgat	CATGAATATATTTATGTGGACCCGATGC	CAGCTGCCTTATGACTCAAGATGGGAGTTTCCAAGA				
		ataagttatttaatttgttataatgatttgttaatttatgataatctttttcctttggcaaccctgagtaa						
Patient 9	Intron 10	aatttaaatgtattttaatgttgcccttttaacatttagacttctaattgtttttaaaagtaaagtcc	ATGCAGCTGCCTTATGACTCAAGATGGGAGTTTCCAAGA					
		aatttaaatgtattttaatgttgcccttttaacatttagacttctaattgtttttaaaagtaaagtc	caaaaatgatgatgagataagtatagtaatgat					
Patient 12	Intron 8	aaaattttaaacGAGGGTCATTGAATCAATCAGCCC	GGATGGACATGAATATATTTATGTGGACCCGATGCAGCTGCCTTATGACTCAAGATGGGAGTTTCCAAGA					
		aaaattttaaacttacaagaattgcacaaaaaaagtacaaat						
Patient 14	Intron 9	cttgttaaatgatctttatgaagtactttttctgtgt	TGGACATGAATATATTTATGTGGACCCGATGC	CAGCTGCCTTATGACTCAAGATGGGAGTTTCCAAGA				
		cttgttaaatgatctttatgaagtactttttctgtgtggagtgcttgtattaattactttttatta						
Patient 17	Intron 10	taagcgagattgtgcagtaattgtctttctgtgcctggcttatttcacttag	cacagtgctcct	CCGATGCAGCTGCCTTATGACTCAAGATGGGAGTTTCCAAGA				
		taagcgagattgtgcagtaattgtctttctgtgcctggcttatttcacttag	cacagtgctcctccaggttcatgcatgttgtc	caaaatgaca				

Points de cassure génomique FIP1L1 et PDGFRA



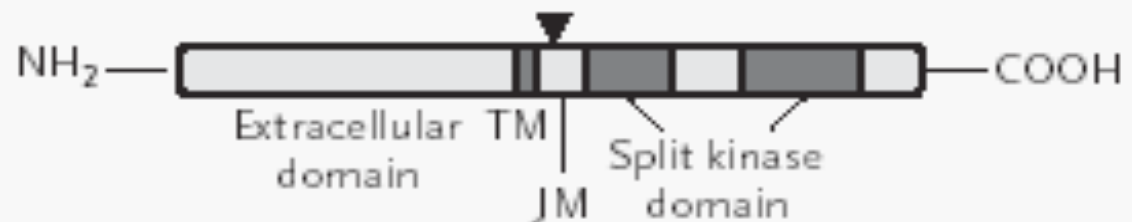
Création d'une protéine chimérique

A

FIP1L1



PDGFR α



FIP1L1-PDGFR α

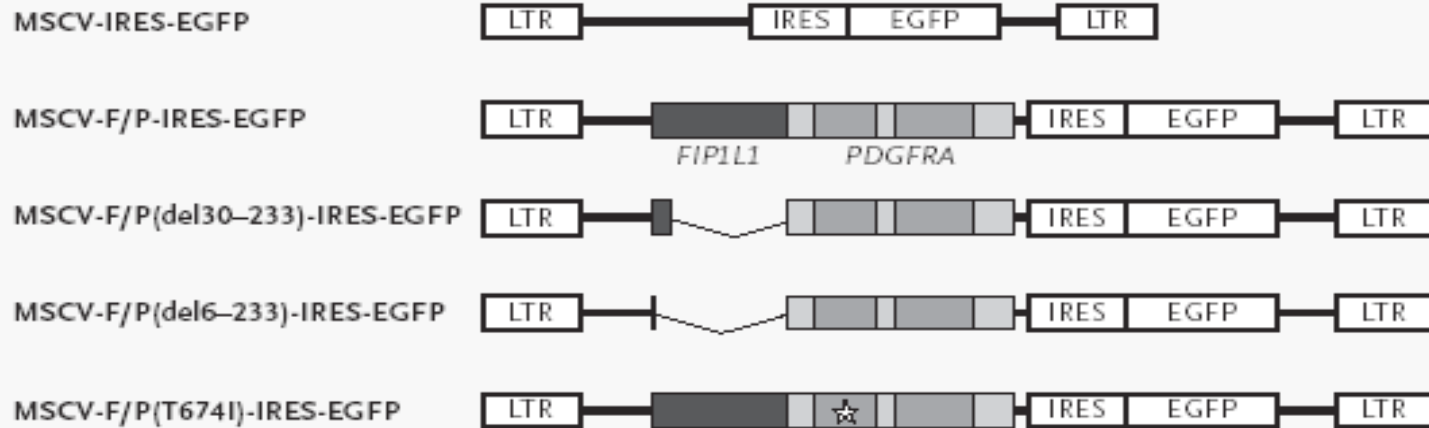


FIP1L1-PDGFR α T674I

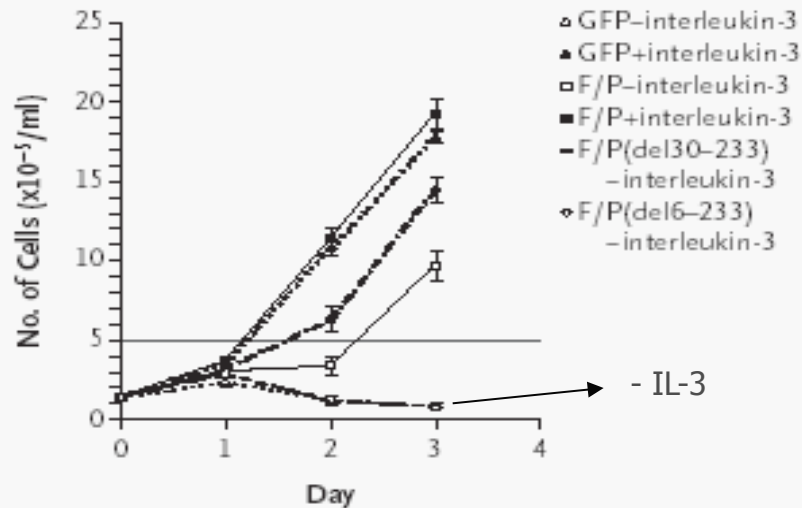


FIP1L1-PDGFR α est doté d'un pouvoir transformant *in vitro* (lignée IL-3 dép.)

A



B

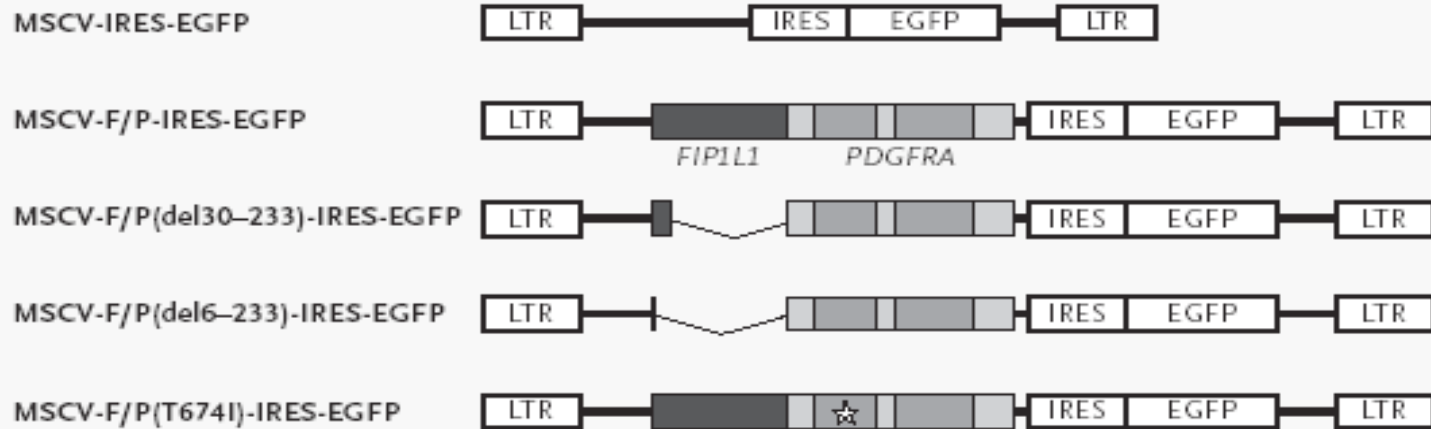


F/P confère l'indépendance à l'IL-3

FIP1L1 ne semble pas nécessaire

L'imatinib inhibe *in vitro* le pouvoir transformant

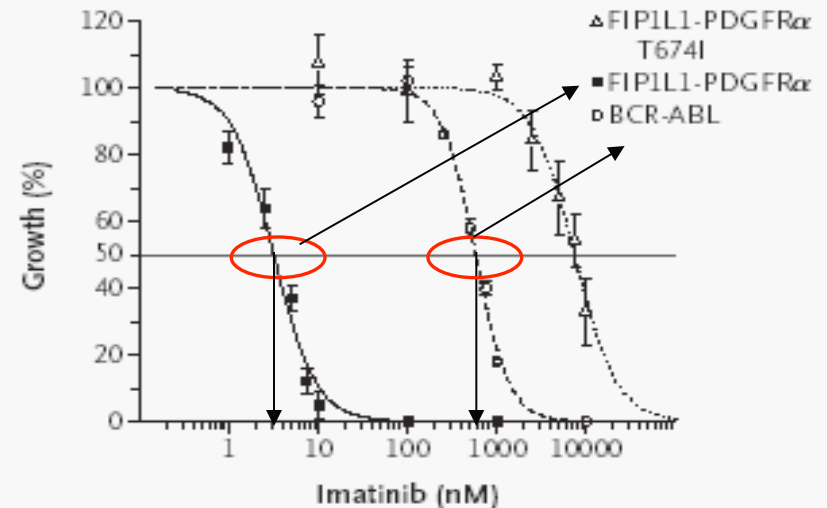
A



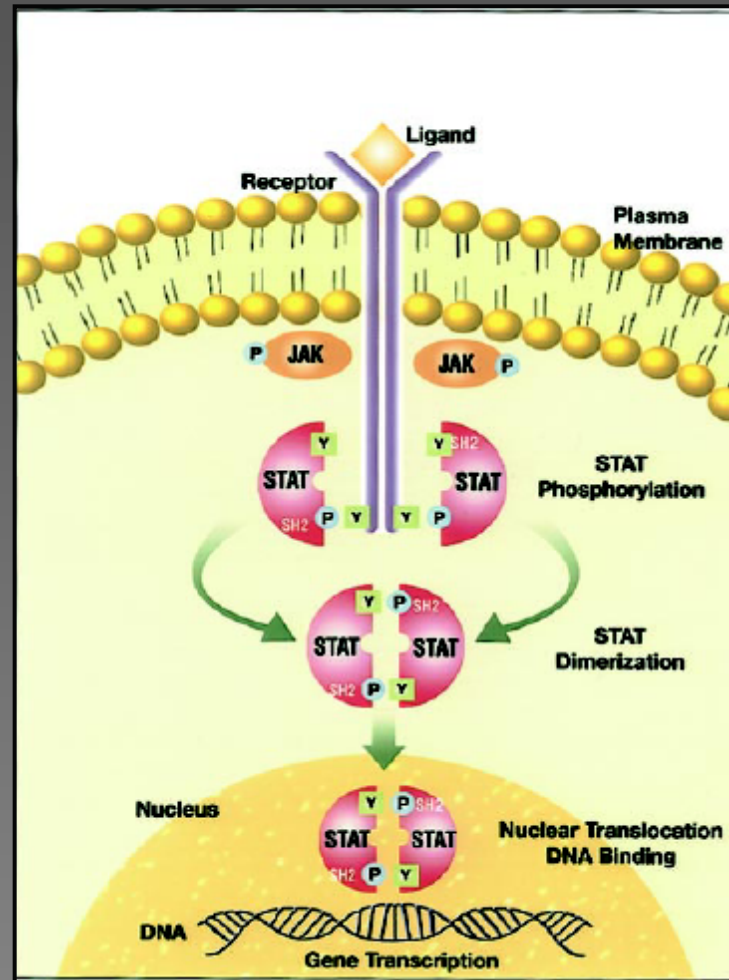
L'imatinib inhibe l'effet transformant

La mutation T674I confère la
Résistance au Glivec

C



Activation constitutive de la voie de signalisation des « STATs »



FIP1L1-PDGFR α $\rightarrow$ STAT5a $\rightarrow$ P-STAT5a

FIP1L1-PDGFR α présente une activité « TK » constitutive, phosphoryle STAT5 et est inhibée par l'imatinib

- Phosphorylation IL-3 indép. STAT5
 - – / Imatinib 5 nM
- Phosphorylation tyrosine IL-3 indép.
 - – / Imatinib 5 nM
- T674I confère résistance

Conclusions:

- Identification d'un gène de fusion FIP1L1-PDGFRΑ suite délétion interstitielle 4q12,
- Encode une protéine à activité Tyrosine kinase constitutionnelle dotée de propriétés transformantes des cellules souches hématopoïétique,
- FIP1L1-PDGFRΑ est inhibé par le STI-571, I.C 50 = 3,2 nM,
- Détection rétrospective du transcrit chez 9/16 patients avec IHES,
- Parmi 9 répondeurs au Gleevec, 5 sont + pour le transcrit FIP1L1/PDGFR1,
- Rechute chez un patient associée à l'apparition d'une mutation T674I du gène PDGFRΑ

Confirmations: des résultats de l'Imatinib / 0 les HES

Table 3. Published Reports of Imatinib in HES, CEL, and SM with Eosinophilia

Author (Year) [Reference]	No. Patients Treated with Imatinib	Disease	Responses†	Comments
Schaller and Burkland* (2001) [169]	1	HES	CR	Initial report; rapid hematologic remission on imatinib 100 mg/d
Gleich <i>et al.</i> * (2002) [170]	5	HES	4 CR	IL-5 levels normal in responders
Ault <i>et al.</i> * (2002) [167]	1	HES	CR	Resolution of 70% eosinophilia in 18 days on imatinib 100 mg/d
Pardanani <i>et al.</i> * (2003) [171]	7	HES, Eos-CMD	3 CR, 1 PR	IL-5 levels elevated in responders
Cortes <i>et al.</i> * (2003) [172]	9	HES	4 CR	3 responses at imatinib 100 mg/d; 1 response at 400 mg/d
Cools <i>et al.</i> (2003) [3]	11	HES/CEL	9 CR	<i>FIP1L1-PDGFR</i> A fusion present in 5/9 responders
Klion <i>et al.</i> (2003) [175]	7	HES-MPD	7 CR	Molecular remission in 5 of 6 patients tested for <i>FIP1L1-PDGFR</i> A after 1-12 months of imatinib
Pardanani <i>et al.</i> (2003) [176]	6	SM with eosinophilia	3 CR	Responders had <i>FIP1L1-PDGFR</i> A fusion and no D816V <i>KIT</i> mutation
n=47			C.R=32	

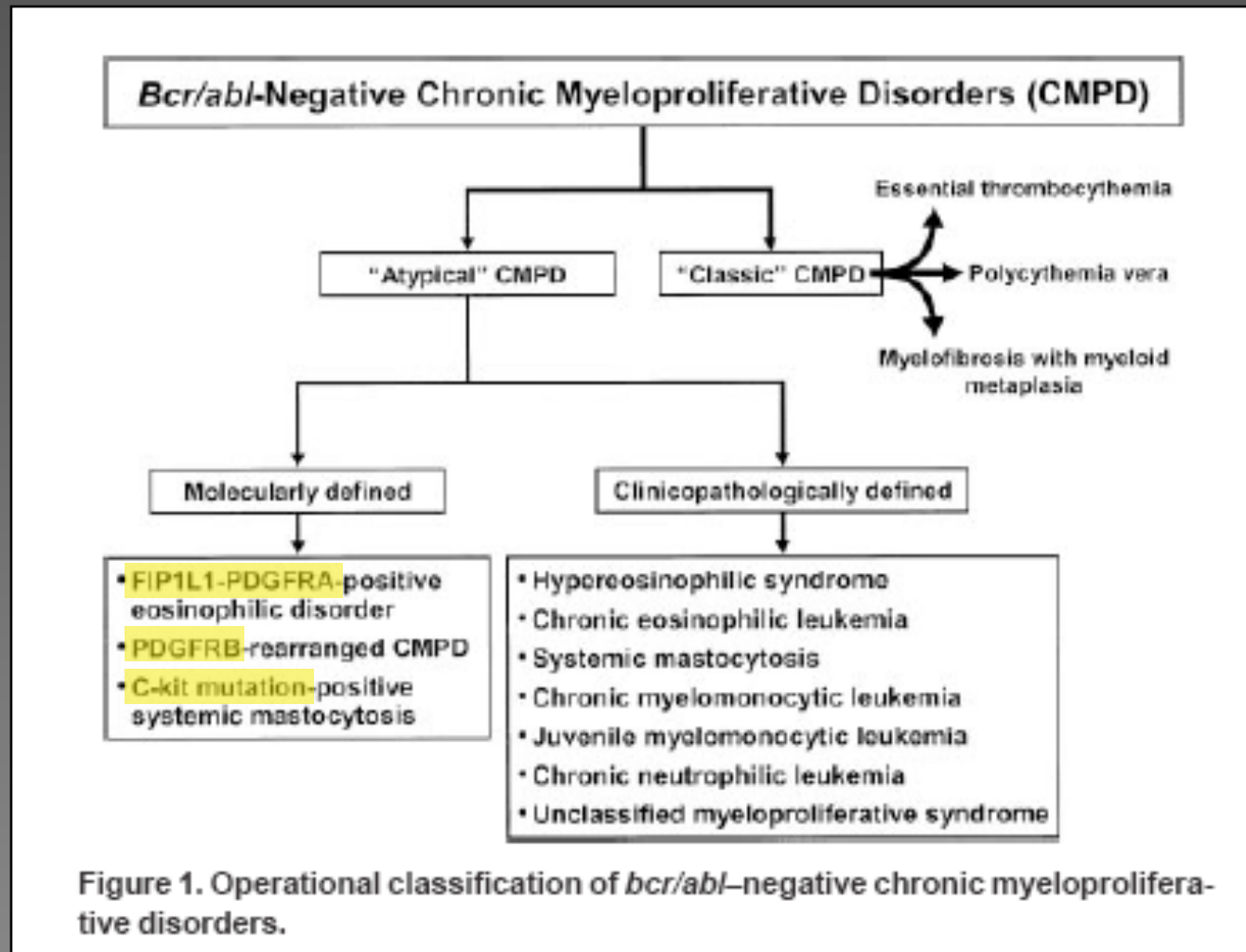
†Refer to individual studies for response criteria

**FIP1L1-PDGFR*A fusion not assessed

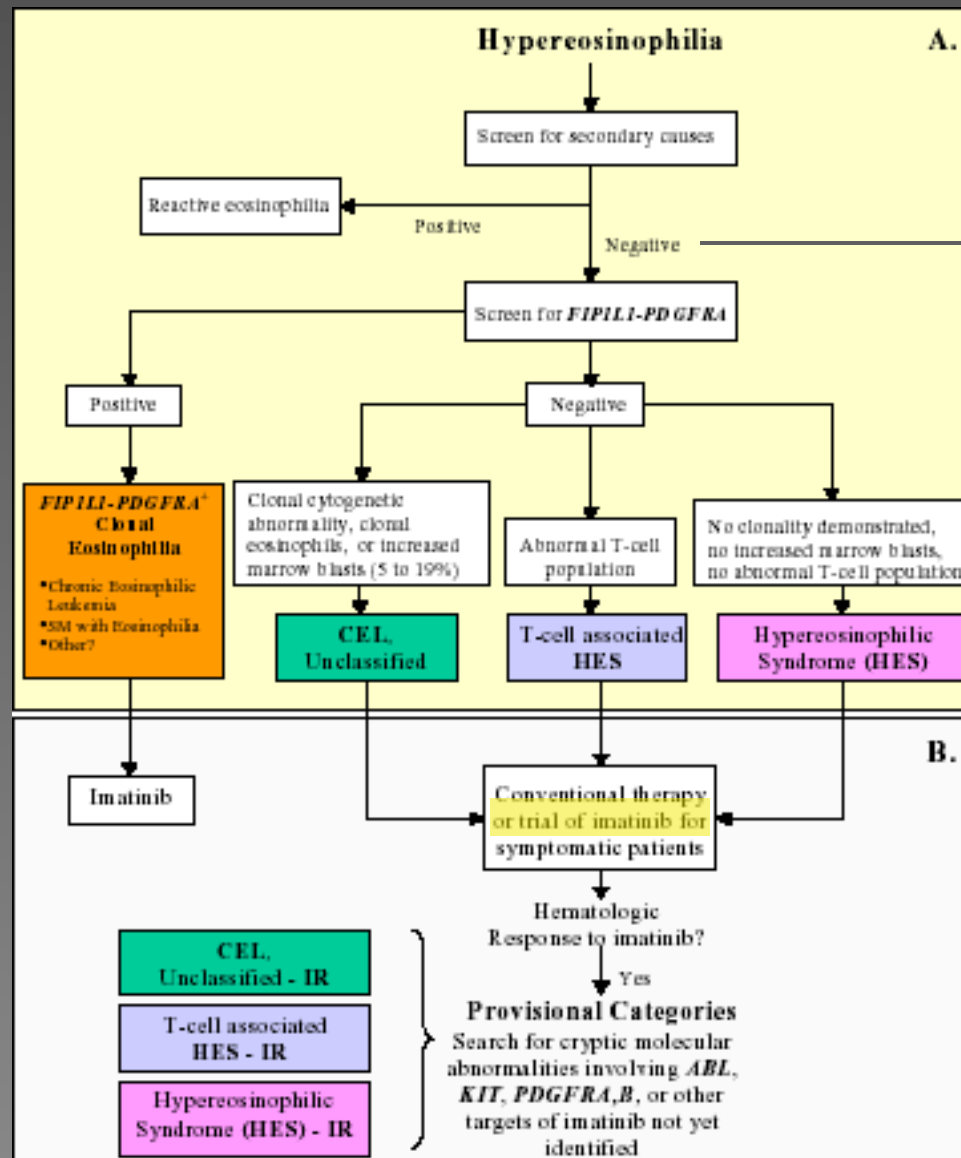
SM= systemic mastocytosis; Eos-CMD= eosinophilia-associated chronic myeloproliferative disorder;

HES-MPD= myeloproliferative variant of HES; CR= complete hematologic remission; PR= partial hematologic remission

Classification opérationnelle des SMP Phi-



Stratégie diagnostique



Organomégalie
Vit B12
Tryptase sérique
Histologie SMP

Questions en cours (1)

- Biologiques :
 - Origine de la cell. mutée ?
 - CEL vs SMCDEo, une ou deux pathologies ?
 - Mécanisme responsable activation constitutive du PDGFRA ?
 - Autres gènes encodant des protéines « TK » mutés ?
 - Lien mutations PDGFRA et éosinophilie ?
 - Voies de signalisation activées ?

Questions en cours (2)

■ Thérapeutiques:

- Dosage imatinib optimum ?
- Guérison possible ?
- Place de la greffe ?

■ Diagnostiques:

- Tests D pour HES/CEL F/P - ?
- Tests prédictifs réponse à l' imatinib ?
- DD SMCDEo vs CEL ?

Origine de la cellule souche leucémique ?

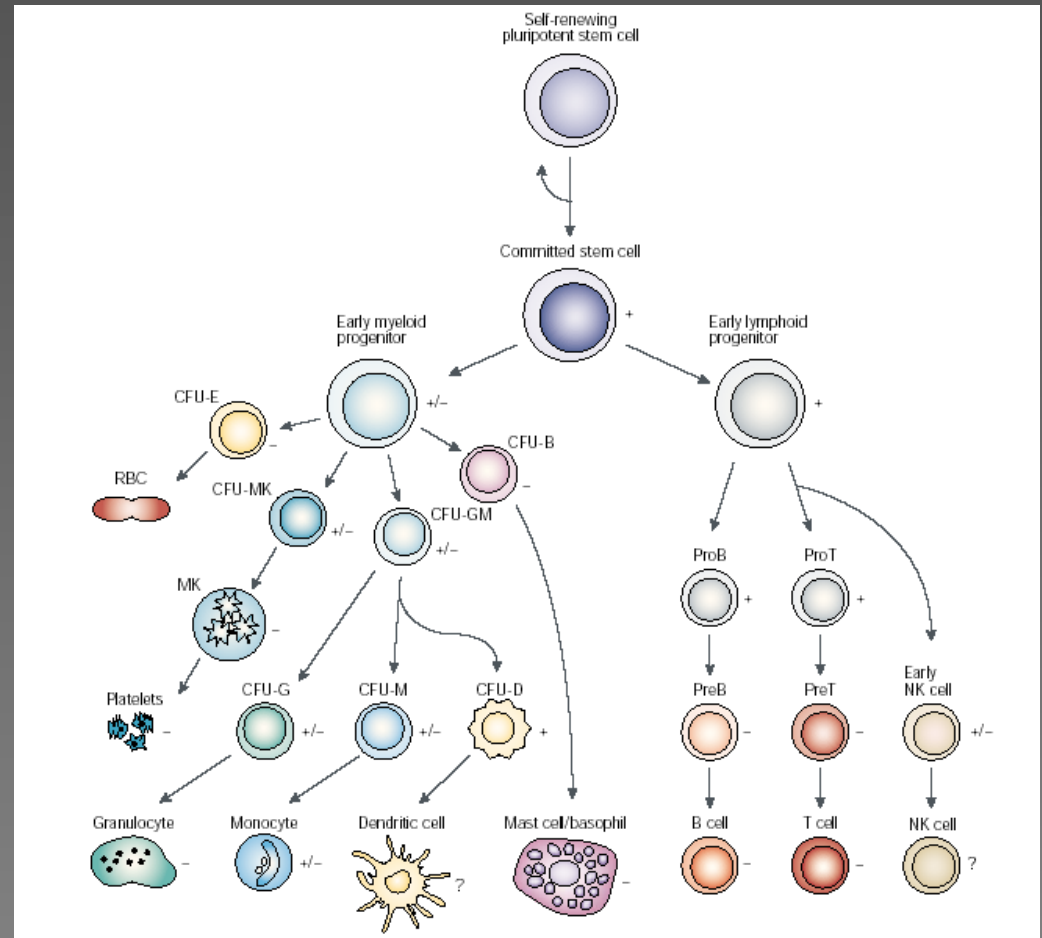
8p11 syndr.



CEF F/P+ ?



CEF F/P+ ?



Origine de la cell. souche leucémique ?

Table 1. Detection of either c-kit D816V or FIP1L1-PDGFR α mutation in 3 informative patients with systemic mast cell disease associated with eosinophilia.

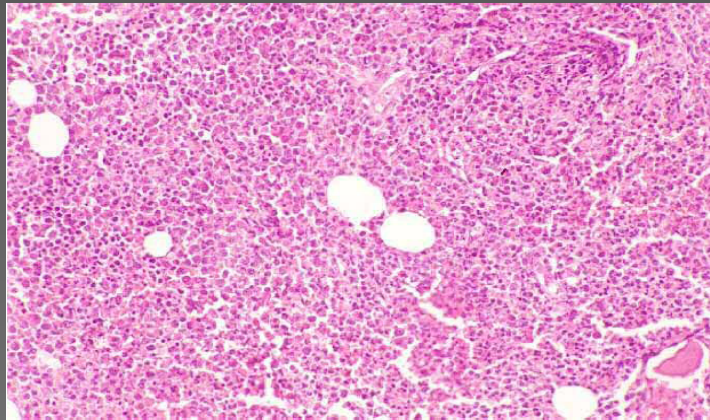
<i>pt</i>	<i>Mutation</i>	<i>Eosinophil</i>	<i>Neutrophil</i>	<i>CD34⁺</i>	<i>CD3⁺</i>	<i>CD19⁺</i>
1	CHIC2-	22.5%	6%	27%	8.5%	3.5%
2	D816V	+	+	+	—	—
3	CHIC2-	79%	16%	8%	1%	3%

Percentages represent the proportion of purified cell populations with deletion of the CHIC2 locus, a surrogate for FIP1L1-PDGFR α . CD34, myeloid progenitor cells; CD3, T lymphocyte; CD19, B lymphocyte. The median abnormal signal percentage in 25 controls was 0% (range 0-2%).

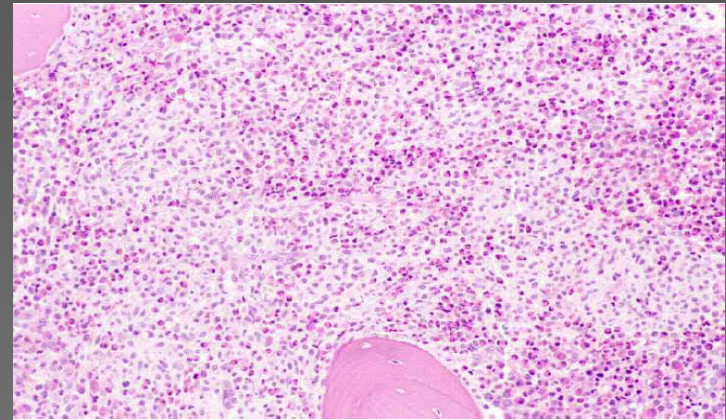
FIP1L1-PDGFR α + CMPDs

How many diseases ?

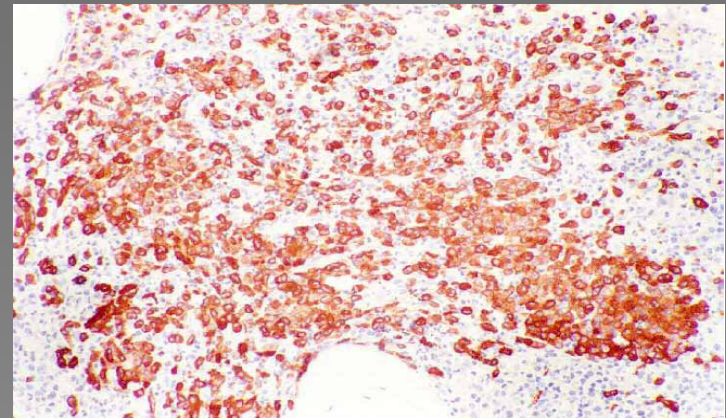
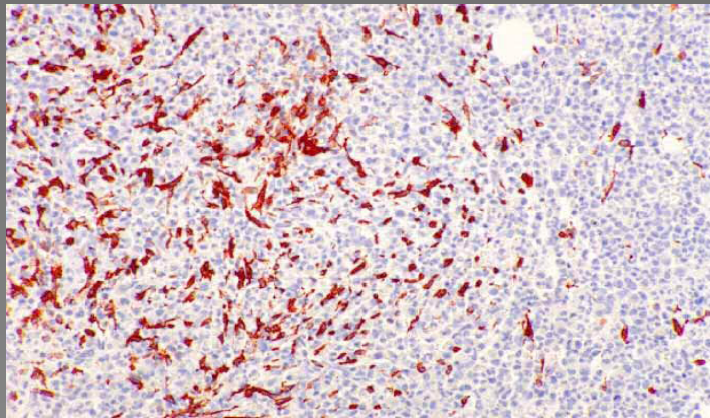
FIP1L1-PDGFR α +



FIP1L1-PDGFR α -



(H&E)-stained



Mastocytose systémique (MS), critères diagnostics OMS

■ Critère majeur:

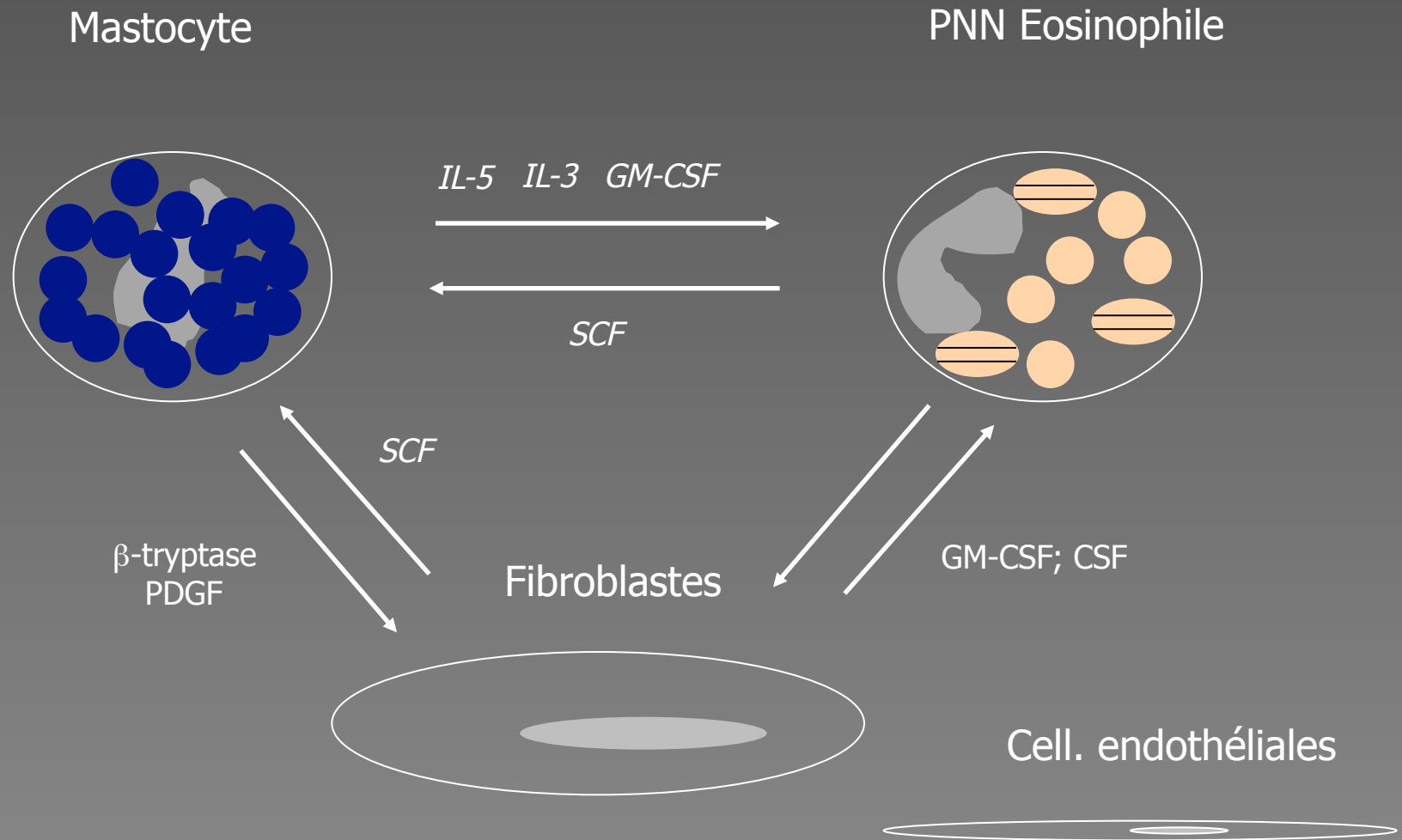
- Infiltrats denses multifocaux médullaires ou d'un autre organe atteint, par des cellules mastocytaires (>15 cell agrégées)

■ Critères mineurs:

- a. >25% du total des cell. mastocytaires sont atypiques sur frottis médullaire ou sont en « fuseau » dans les infiltrats viscéraux
- b. mutation ponctuelle du codon 816 du gène c-kit
- c. cell. mastocytaires médullaires, périphériques ou autre organe extra cutané expriment le CD2 et/ou le CD25
- Concentration basale en tryptase sérique > 20 ng/ml (non valable en présence d'une autre atteinte myéloïde)

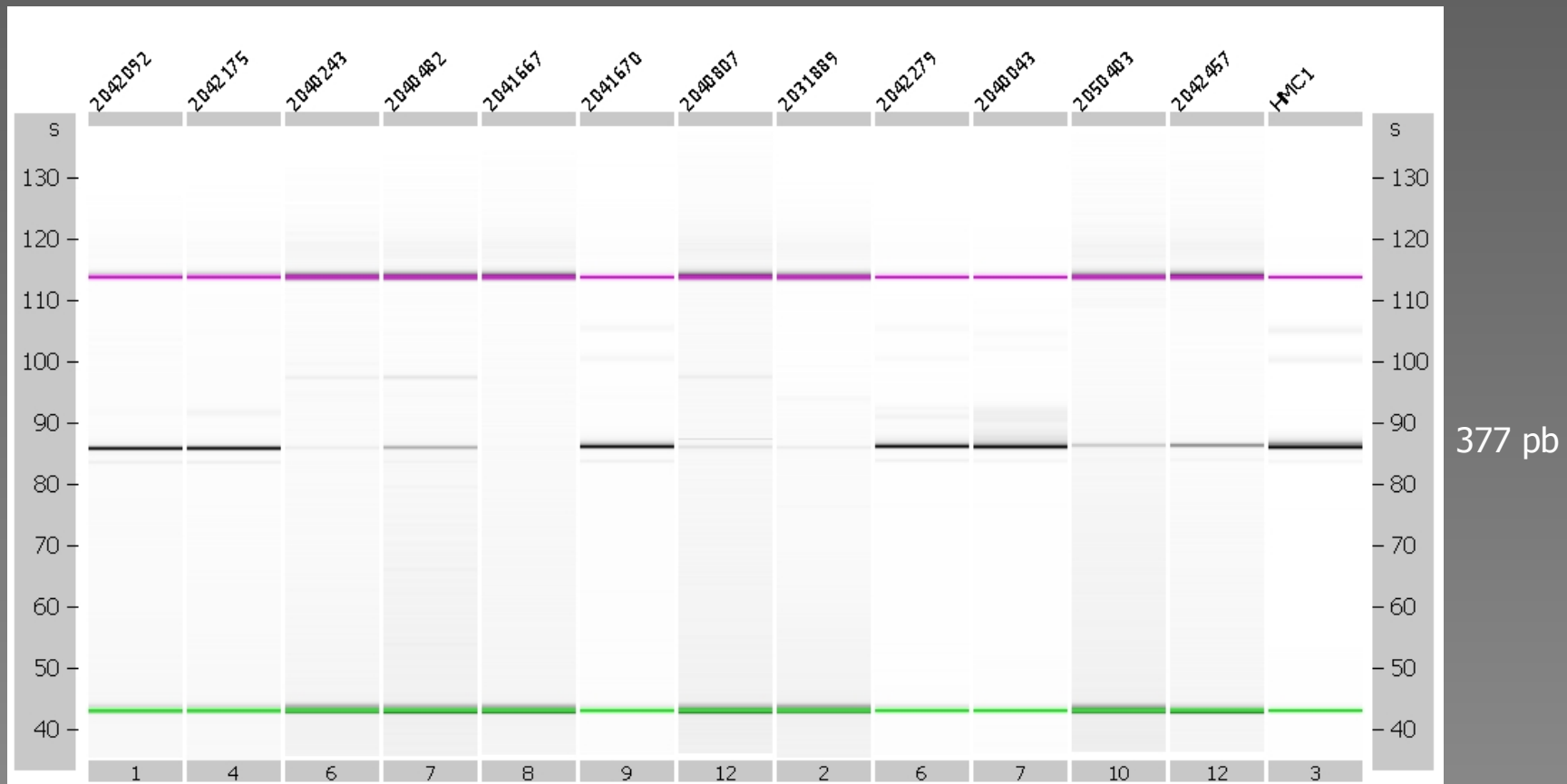
Un critère majeur et un mineur ou trois mineurs remplis pour Δ

Interrelations Eosinophiles et Mastocytes

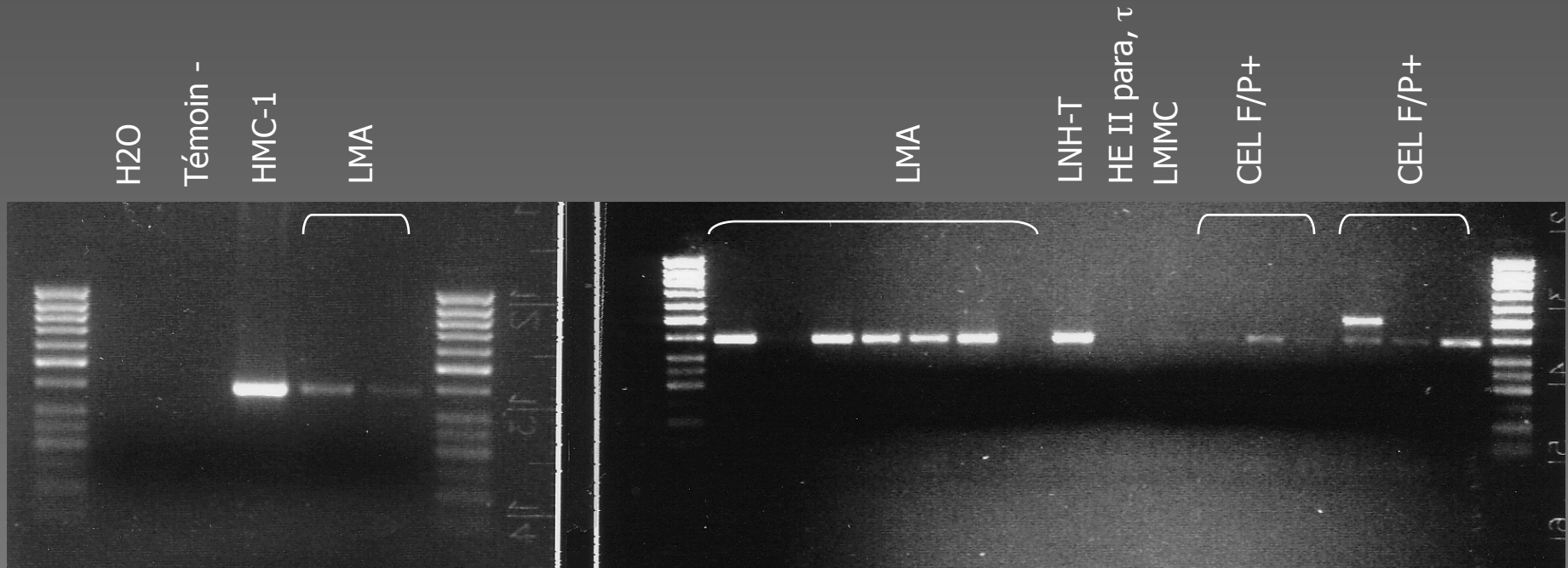


Expression aberrante du gène de la Tryptase ($\alpha+\beta$)

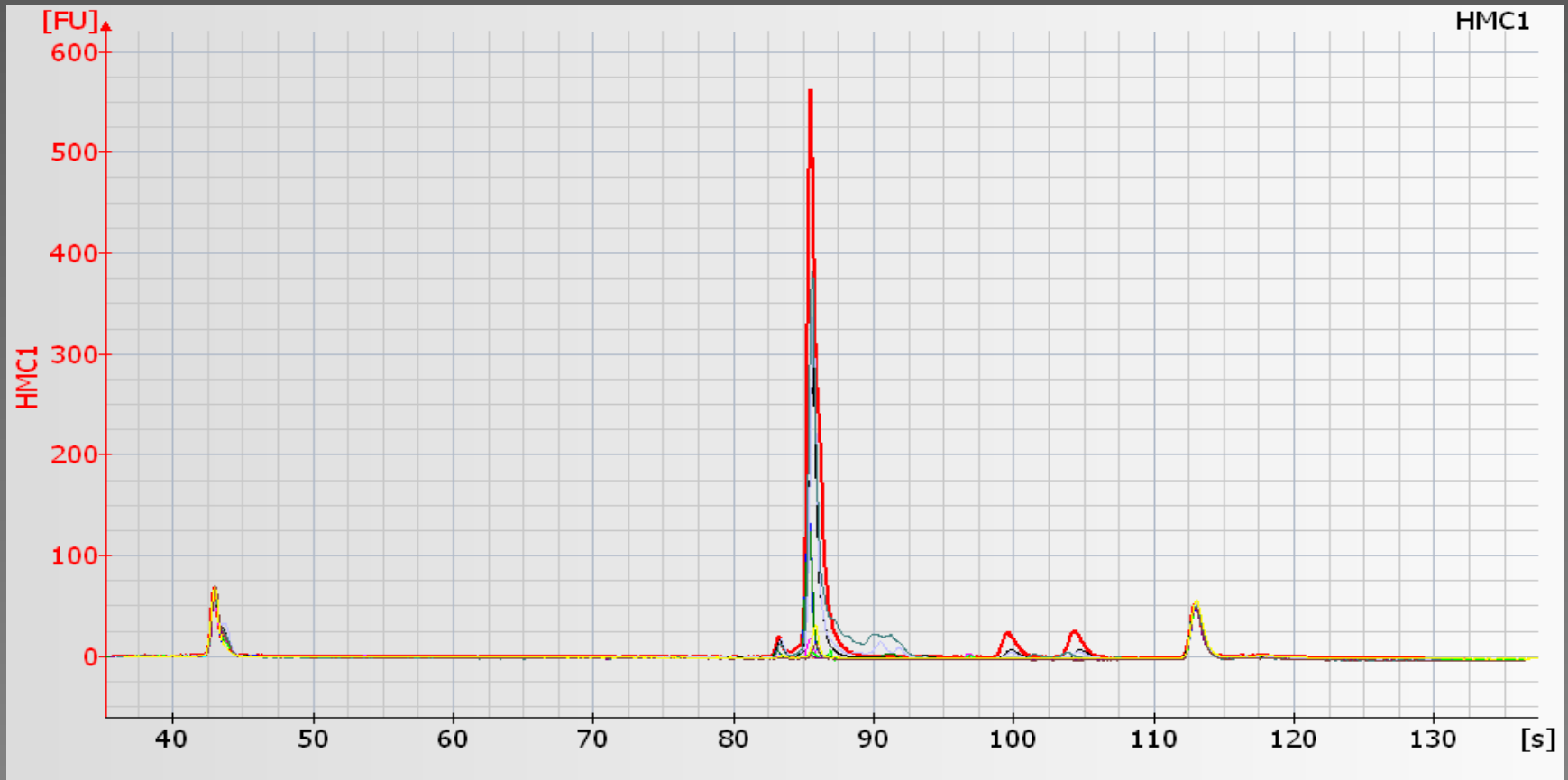
SM
 CEL F/P+
 CEL t(5;12)
 CEL F/P-
 Colite eos
 HE II para
 LAM M4
 HE II X ?



Expression aberrante du gène de la Tryptase ($\alpha+\beta$)



Expression aberrante du gène de la Tryptase ($\alpha+\beta$)



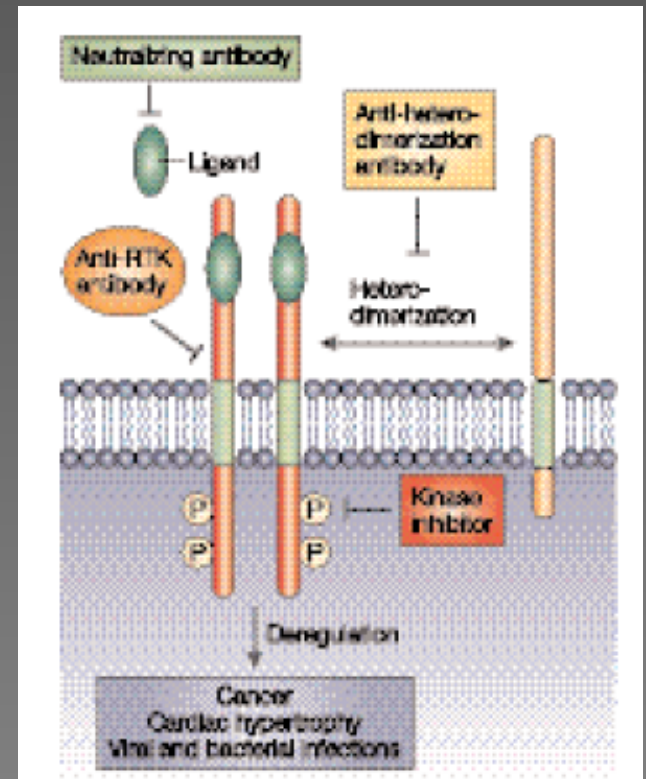
Perspectives

Des connaissances nouvelles

- Physiopathologies des SMPs avec éosinophilie (8p11, CEL, LMMC, CEL)
- Mécanismes généraux de leucémogénèse , le rôle des mutations de gènes encodant des protéines à activité « Tk »

De nouvelles approches thérapeutiques

- Imatinib mésylate,
- Autres inhibiteurs de « Tk »,
- Stratégies anti IL-5.



De nouveaux inhibiteurs de « Tk » ?

Table 2 | **Cancer therapies targeted to receptor tyrosine kinases**

Names	Targets	Status	Description	Company
Trastuzumab, Herceptin	HER2	Approved for metastatic breast cancer	Humanized anti-HER2 IgG1κ	Genentech
Imatinib, Glivec, STI571	BCR-ABL, KIT, PDGFR	Approved for CML and GIST	2-Phenylaminopyrimidine	Novartis
Gefitinib, Iressa, ZD1839	EGFR	Approved for NSCLC	Quinazoline	AstraZeneca
Cetuximab, Erbitux	EGFR	Approved for colorectal cancer	Chimeric anti-EGFR IgG1	ImClone/Merck
Bevacizumab, Avastin	VEGF	Approved for colorectal cancer	Humanized anti-VEGF (rhu mAb-VEGF)	Genentech
OSI-774, Tarceva	EGFR	Clinical development	Quinazoline	Genentech/ Roche/OSI
CI-1033	EGFR, HER2	Clinical development	4-Anilinoquinazoline, irreversible inhibitor	Pfizer
EKB-569	EGFR, HER2	Clinical development	4-Anilinoquinoline-3-carbonitrile, irreversible inhibitor	Wyeth
CDP860	PDGFR	Clinical development	Anti-PDGFRβ-receptor antibody fragment	Celltech
Pertuzumab, Omnitarg, 2C4	HER2	Clinical development	Humanized anti-HER2 (heterodimerization inhibitor)	Genentech
SU6668	VEGFR2, PDGFR, FGFR	Clinical development	Indoline-2-one	Sugen/Pfizer
SU11248	VEGFR2, KIT, PDGFR, FLT3	Clinical development	Indoline-2-one	Sugen/Pfizer
ZD6474	VEGFR2	Clinical development	Quinazoline	AstraZeneca
PTK-787/ZK222584	VEGFR1/2, PDGFR	Clinical development	Anilinophthalazine	Novartis/Schering
AG013736	VEGFR2, PDGFR	Clinical development	–	Pfizer
CP549, 632	VEGFR2, FGFR1, TIE2	Clinical development	–	Pfizer
PKC-412, midostaurin	PKC, VEGFR2, PDGFR, FLT3, KIT	Clinical development	N-Benzoylstaurosporine	Novartis
CEP-701	FLT3, TRK kinases	Clinical development	Indolocarbazole alkaloid	Cephalon
MLN-518, CT53518	PDGFR, KIT, FLT3	Clinical development	Quinazoline	Millennium

CML, chronic myelogenous leukaemia; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FLT, FMS-related tyrosine kinase; GIST, gastrointestinal stromal tumour; HER, human EGFR-related; Ig, immunoglobulin; NSCLC, non-small-cell lung carcinoma; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

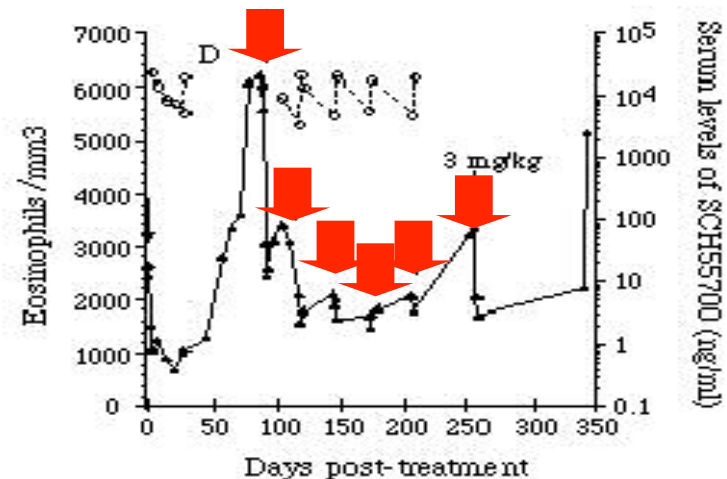
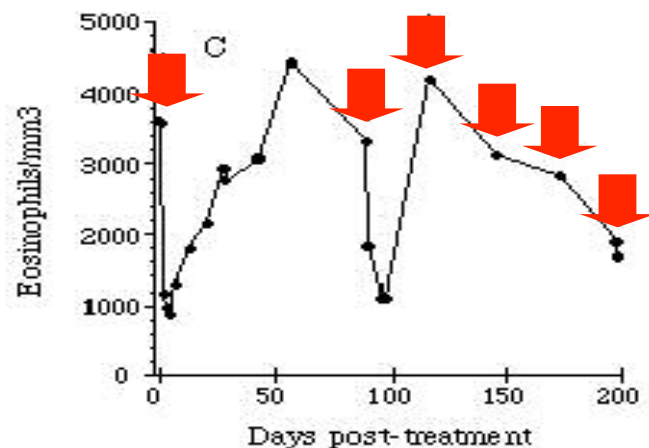
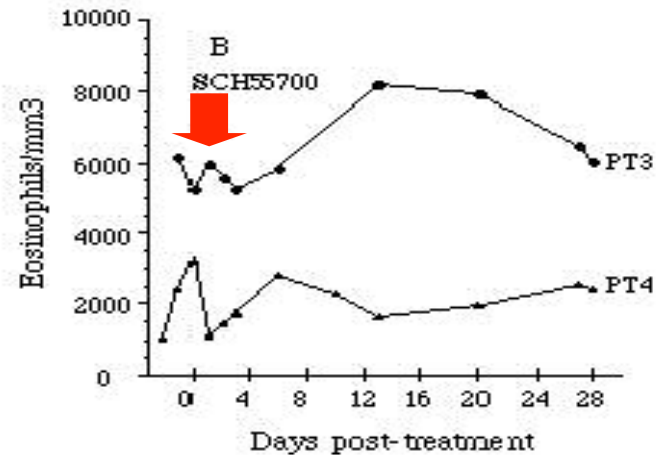
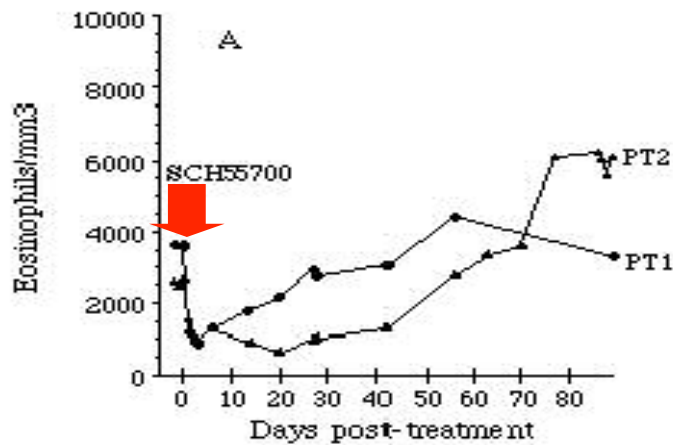
De nouveaux inhibiteurs de « Tk » ?

Table 2 | **Tyrosine-kinase inhibitors in early clinical trials**

Compound	Company	Route inhibition	Receptor	Side effects	Responses	Clinical development
CEP-701	Cephalon	Oral	FLT3 (wild-type and mutant)	Nausea, fatigue and neutropaenia	1 of 8 patients: <5% blasts in bone marrow	Phase II trial in adult AML*; accrual in progress
SU5416	Sugen	IV	FLT3 (wild-type and mutant), KIT, FMS and PDGFR	Headaches, dyspnoea, infusion reaction and thrombosis	3 of 55 patients: partial remission with 6–25% bone-marrow blasts	Phase II trial in adult AML and high-grade MDS
SU11248	Sugen	Oral	FLT3 (wild-type and mutant), KIT, FMS and PDGFR	Fatigue	32 patients, 13 of 16 evaluable patients: >50% reduction in peripheral blasts	Phase I trial in adult AML; accrual in progress
MLN518	Millenium	Oral	FLT3 (wild-type and mutant), KIT and PDGFR	Not reported yet	8 patients, 2 of 3 evaluable patients: 50% reduction in marrow blasts	Phase I trial in adult AML and high-grade MDS; accrual in progress
PKC412	Novartis	Oral	FLT3 (wild-type and mutant), PKC, VEGFR and PDGFR	Nausea; two deaths of uncertain aetiology; pulmonary toxicity; lethargy	1 of 8 patients: >50% reduction in marrow blasts	Phase II trial in adult AML
L-000021649	Merck	Oral	FLT3 (wild-type and mutant) and VEGFR	Not reported yet	Not reported yet	Phase I trial in paediatric AML and ALL: starting accrual

* Patients with AML had to have either an *FLT3* internal tandem duplication or a mutation in the tyrosine-kinase domain. ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; FLT3, FMS-like tyrosine kinase 3; IV, intravenous; MDS, myelodysplasia; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; VEGFR, vascular endothelial growth factor receptor.

Approche anti IL-5 SCH55700



SCH55700

Nouvelle approche anti IL-5

Patient	Sex	Age	Clinical Manifestations	Therapy*		Baseline eosinophil count (eos/mm ³)***	F/P mutation**	Serum IL-5 level (ng/ml)			Bone marrow biopsy cellularity (%)			Granulocytic precursors in bone marrow aspirate (%)		
				Prior to study	During study			Pre	Post (3d)	Post (1m)	Pre	Post (1m)	Post (last dose)	Pre	Post (1m)	Post (last dose)
1	M	52	Mucosal ulcerations, dermatitis, splenomegaly	P, HU, IFN	HU	3630	Y	2	<1.95	3.3	70	50	30	58	68	76
2	F	32	Angioedema, dermatitis, myoedema, arthralgias, myalgias	P, HU, IFN	P	2620	N	2	<1.95	6.1	55	60	60	80	70	78
3	M	38	Mucosal ulcerations, dermatitis, anemia, thrombocytopenia, splenomegaly	P, HU, IFN	P, HU IFN	6133	Y	<1.95	<1.95	<1.95	95	95	NA	ND	41	NA
4	F	47	Pericarditis, pleuritis, urticaria, cerebrovascular accident, digital ulcerations	P, HU, IFN, Imat	HU	3209	N	223	14.5	24.4	40	40	NA	61	34	NA

Une base moléculaire communes aux SMPs ?

Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis

Ross L. Levine,^{1,2,11} Martha Wadleigh,^{2,11} Jan Cools,⁶ Benjamin L. Ebert,^{2,8} Gerlinde Wernig,¹ Brian J.P. Huntly,¹ Titus J. Boggon,⁴ Iwona Wlodarska,⁶ Jennifer J. Clark,¹ Sandra Moore,¹ Jennifer Adelsperger,¹ Sumin Koo,¹ Jeffrey C. Lee,⁸ Stacey Gabriel,⁸ Thomas Mercher,¹ Alan D'Andrea,³ Stefan Fröhling,¹ Konstanze Döhner,⁷ Peter Marynen,⁶ Peter Vandenberghe,⁶ Ruben A. Mesa,⁹ Ayalew Tefferi,⁹ James D. Griffin,² Michael J. Eck,⁴ William R. Sellers,^{2,8} Matthew Meyerson,^{2,8} Todd R. Golub,^{5,8,10} Stephanie J. Lee,^{2,*} and D. Gary Gilliland^{1,2,10,*}

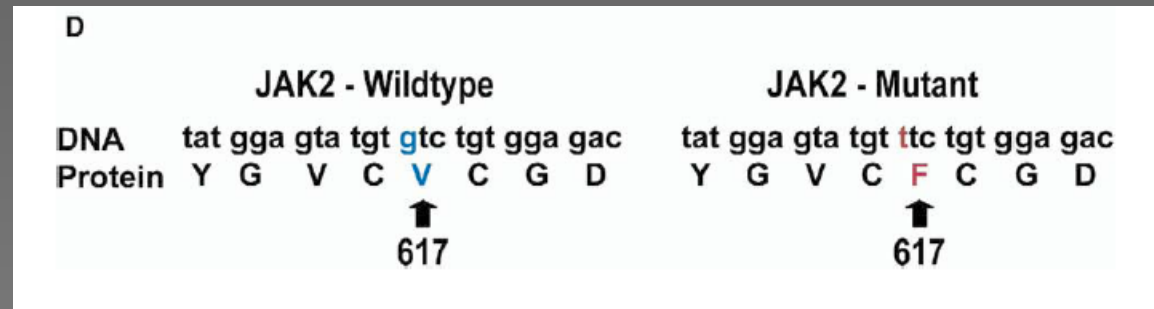


Table 2. Mutational status by disease

Mutation	Total (n = 345 ¹)	PV (n = 164 ²)	ET (n = 115 ³)	MMM (n = 46)
None	151 (44)	43 (26)	78 (68)	30 (65)
Heterozygous	126 (36)	80 (49)	34 (29)	12 (26)
Homozygous	48 (14)	41 (25)	3 (3)	4 (9)
Unknown	20 (6)			

Number and (percent) are indicated.

¹Two subjects have MPD, not otherwise specified.

²7 additional patients were not evaluable for *JAK2V617F* mutations.

³11 additional patients were not evaluable for *JAK2V617F* mutations.

Une base moléculaire communes aux SMPs ?

letters to nature

.....

A unique clonal *JAK2* mutation leading to constitutive signalling causes polycythaemia vera

**Chloé James^{1*}, Valérie Ugo^{1,2,3*}, Jean-Pierre Le Couédic^{1*},
Judith Staerk⁴, François Delhommeau^{1,3}, Catherine Lacout¹,
Loïc Garçon¹, Hana Raslova¹, Roland Berger⁵,
Annelise Bennaceur-Griscelli^{1,6}, Jean Luc Villeval¹,
Stefan N. Constantinescu⁴, Nicole Casadevall^{1,3} & William Vainchenker^{1,7}**

Une base moléculaire communes aux SMPs ?

The NEW ENGLAND JOURNAL *of* MEDICINE

ORIGINAL ARTICLE

A Gain-of-Function Mutation of JAK2 in Myeloproliferative Disorders

Robert Kralovics, Ph.D., Francesco Passamonti, M.D., Andreas S. Buser, M.D.,
Soon-Siong Teo, B.S., Ralph Tiedt, Ph.D., Jakob R. Passweg, M.D.,
Andre Tichelli, M.D., Mario Cazzola, M.D., and Radek C. Skoda, M.D.

Conclusions

- Les SMP avec hyperéosinophilies sont des pathologies rares,
- L'étude de leur physiopathologie à permis de mieux comprendre les mécanismes sous-jacents,
- Cette approche s'étend actuellement à l'ensembles des SMP,
- Une base moléculaire à la classification OMS des SMP voit le jour,
- De nouvelles options thérapeutiques apparaissent.

Notre expérience

Présentations cliniques:

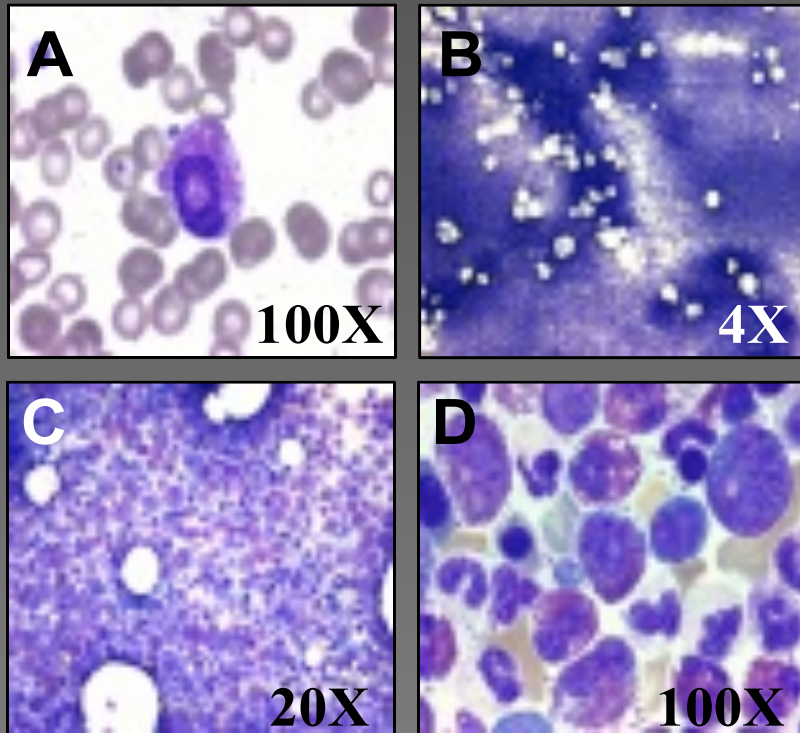
Table 1: Patient characteristics.

Cases	patient # 1	patient # 2
Age (year) / sex	39 / Male	73 / Male
Dominant signs	Thoracic and back pain	Lab discovery
Splenomegaly	No	No
Cardiac involvement	Mitral disease	No
Other organ involvement	Pulmonary	No
RBC, X 10 ¹² /L	3,58	2,75
WBC, X 10 ⁹ /L	15,08	15,3
Platelet, X 10 ⁹ /L	197	293
Absolute Eosinophil Count	8900	6000
Relative Eosinophil Count (%)	59	39
Serum Vit B12 (pg/mL)	> 2000	841
Serum IgE (UI/ml)	14,1	47,9
Serum tryptase (µg/L)	10,2 (=)	9,5 (=)
Serum IL-5 (pg/mL)	Not Done	Not Done
Central blastosis (%)	< 5	2
Bone Marrow eosinophils (%)	40	19
Bone Marrow mast cell (%)	Not Done	Not Done
Karyotype	46, XY [23]	46, XY [21]
Therapy before imatinib mesylate	Corticosteroids	No
BCR-ABL fusion gene transcript (RT-PCR)	-	-
TCR gene rearrangement (PCR)	Oligoclonal	Oligoclonal
FIP1L1-PDGFRα fusion gene (RT-PCR)	Present	Present
CHIC2 deletion / FISH (% abnormal nuclei)	70	24

(=) indicates normal value

Cytologie

Case #1



Case #2

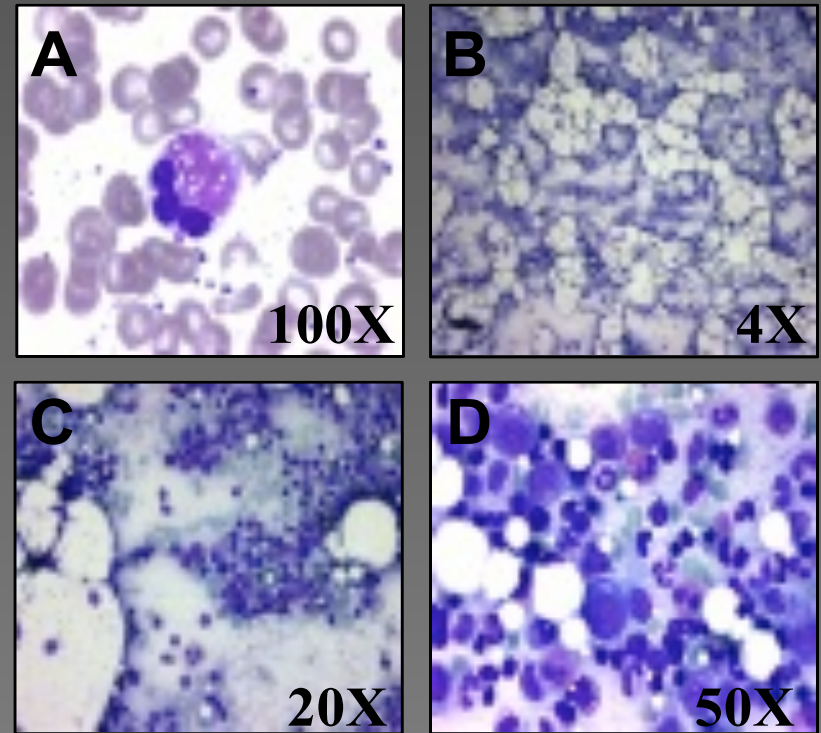
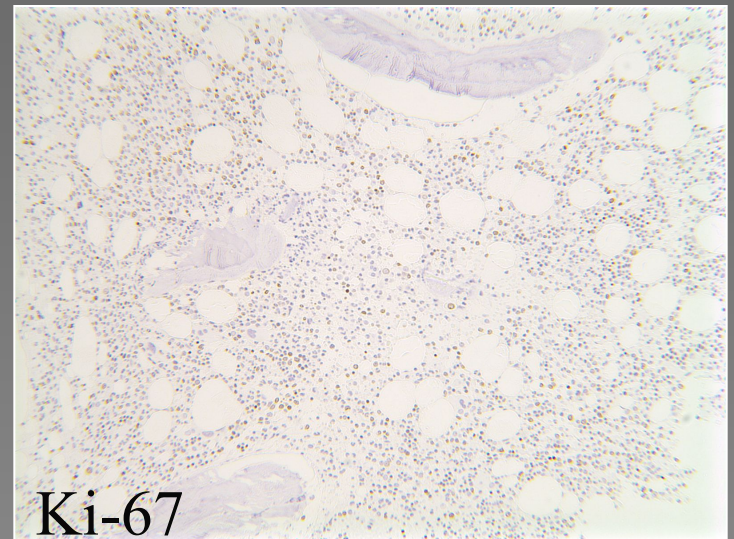
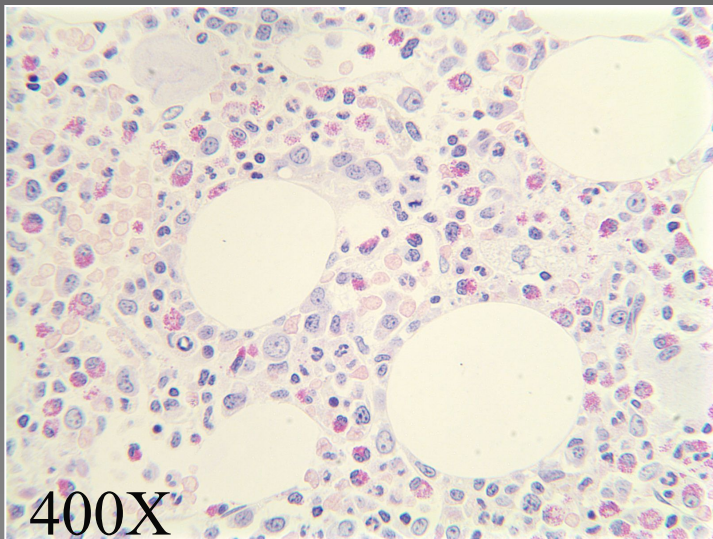
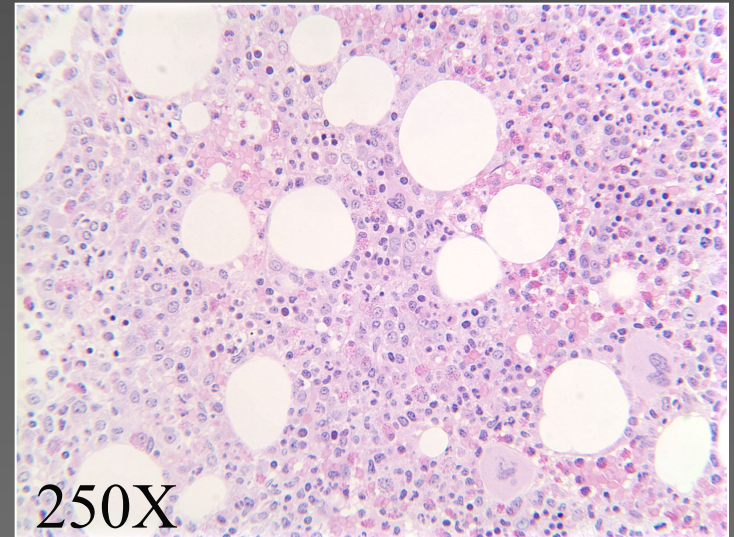
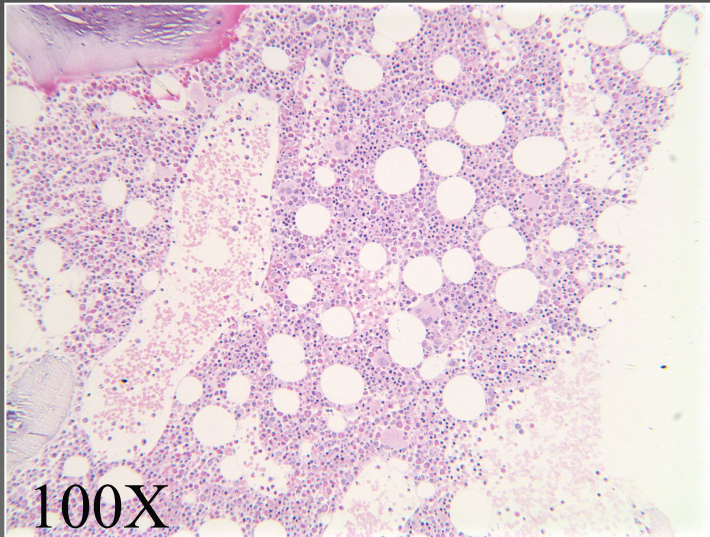
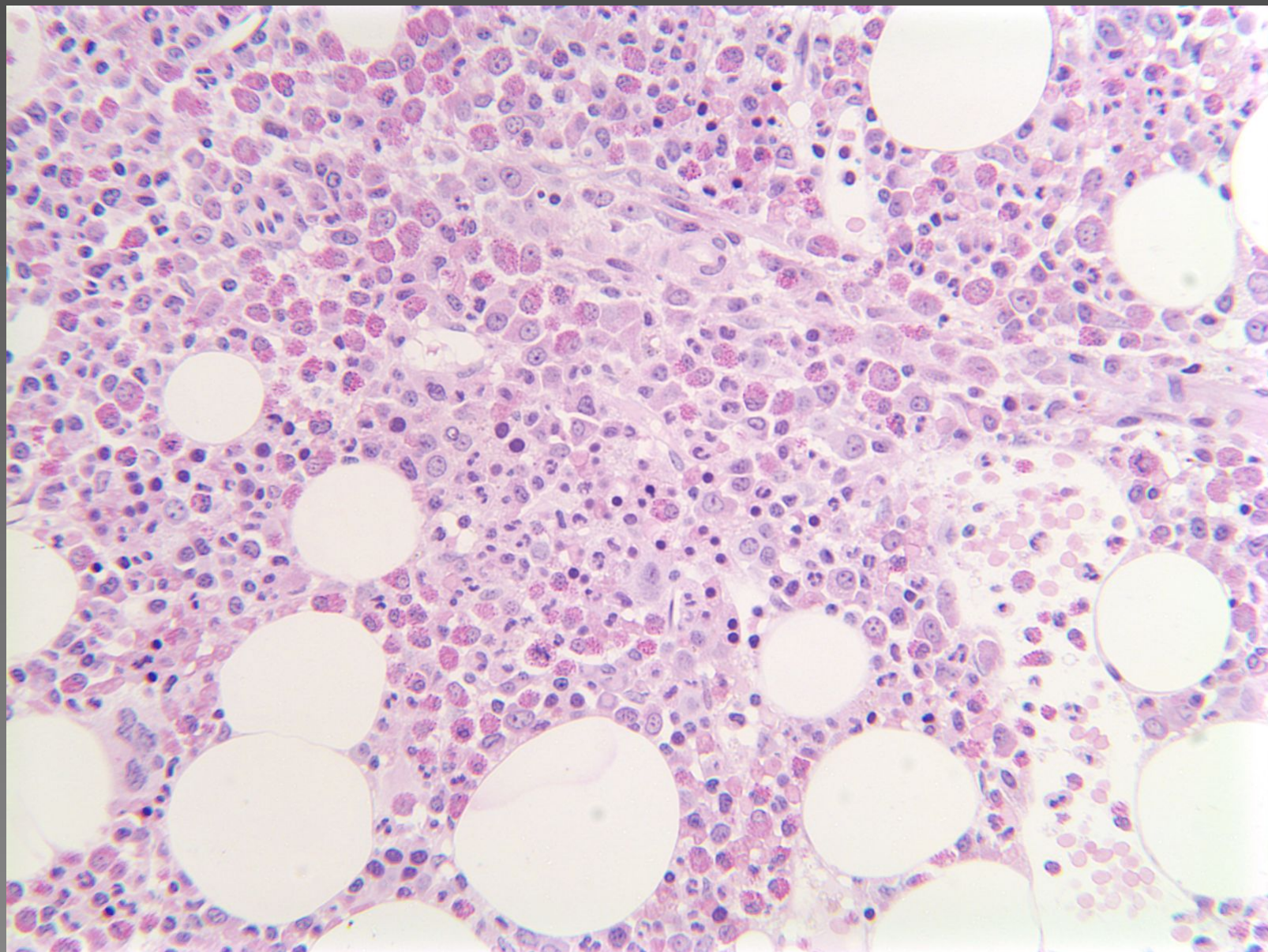


Fig. 1: PB and BM features.

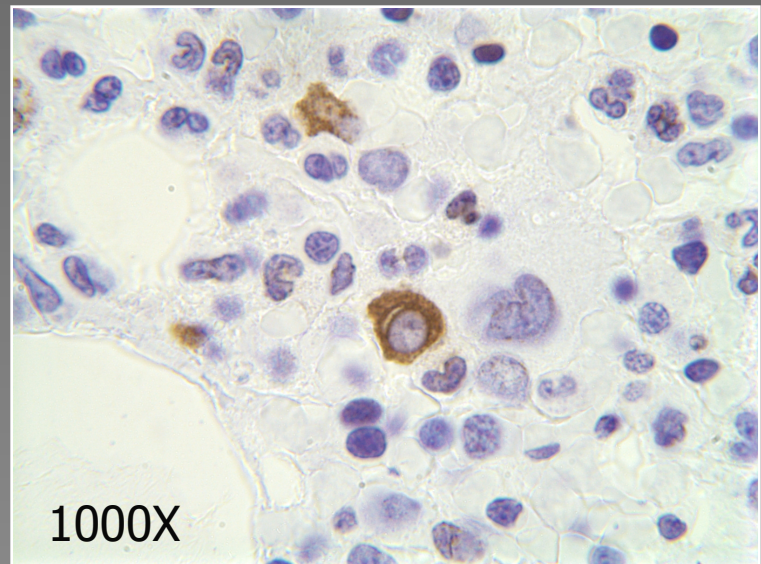
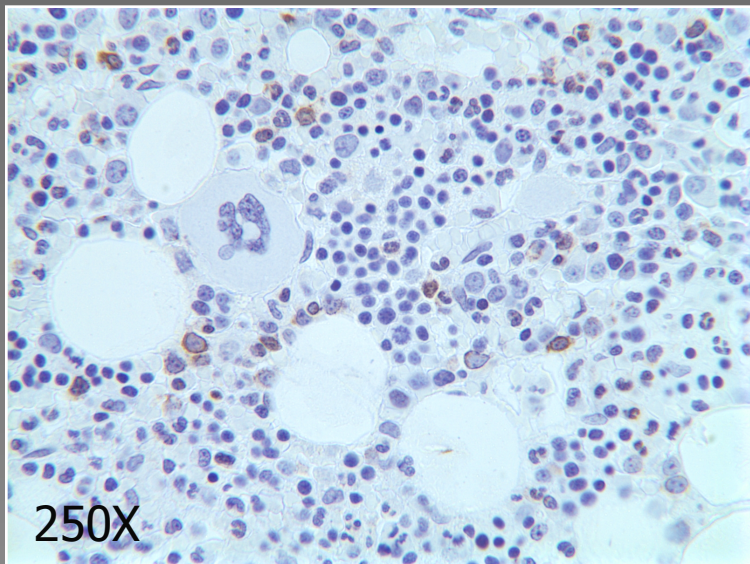
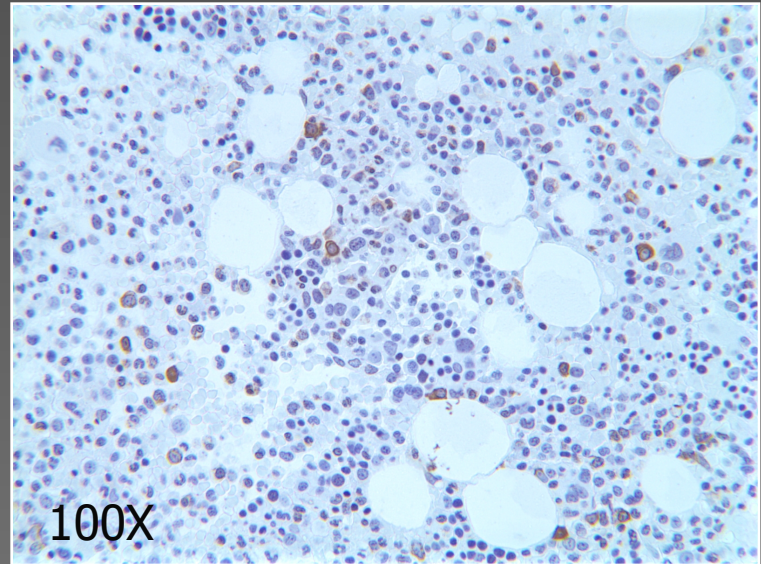
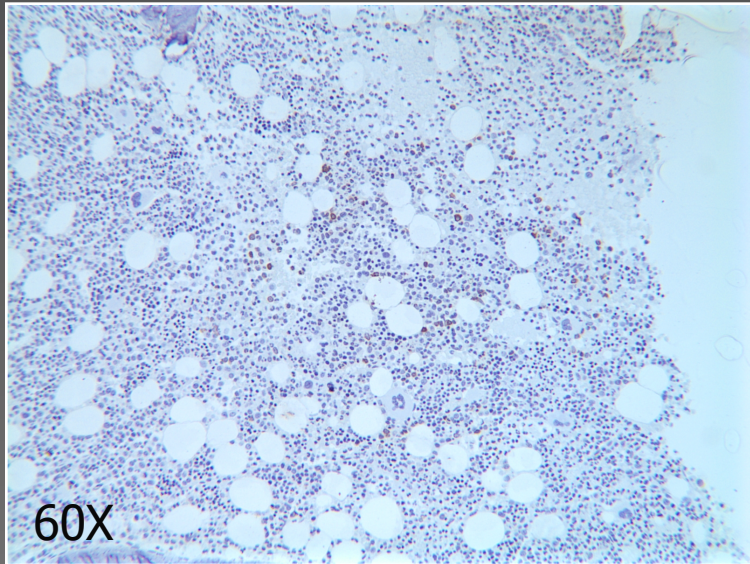
Panels A show peripheral blood dysmorphic eosinophils with ring nucleus and hypoplastic granulations (MGG, original magnification 100X). Panels B, C and D illustrate bone marrows with marked granulocytic hyperplasia and increased numbers of eosinophils precursors (MGG, original magnification specified).

Histologie médullaire, cas #1

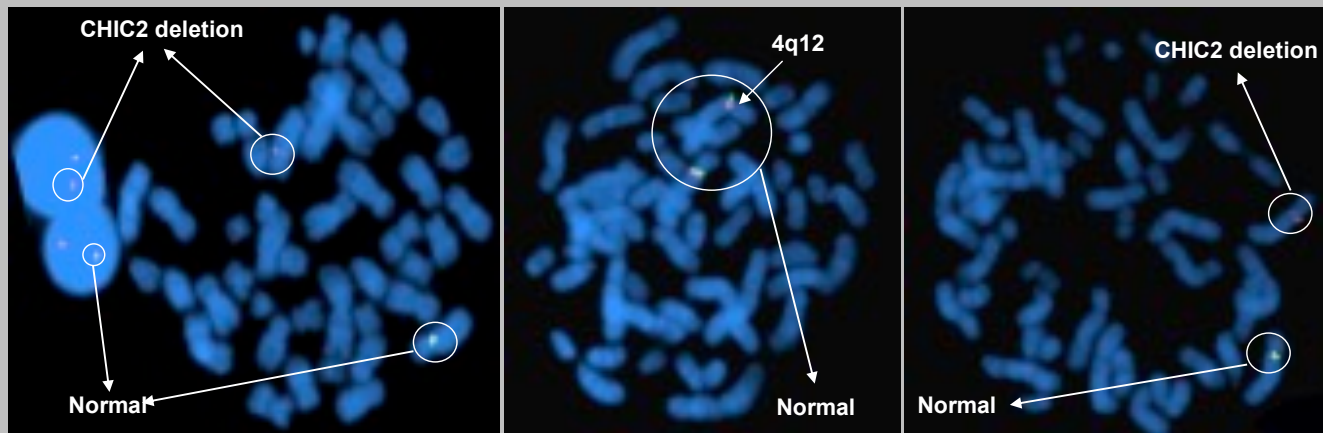
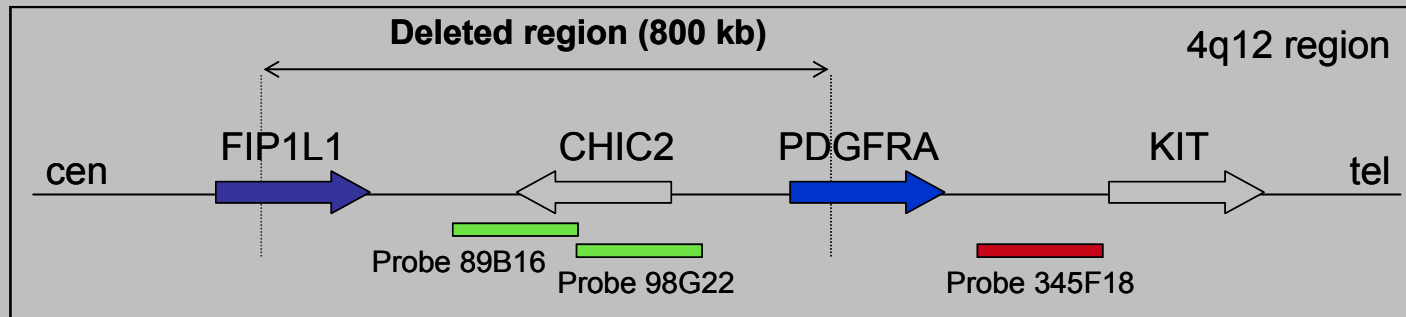




IHC, tryptase CEL vs SMCDEo ?

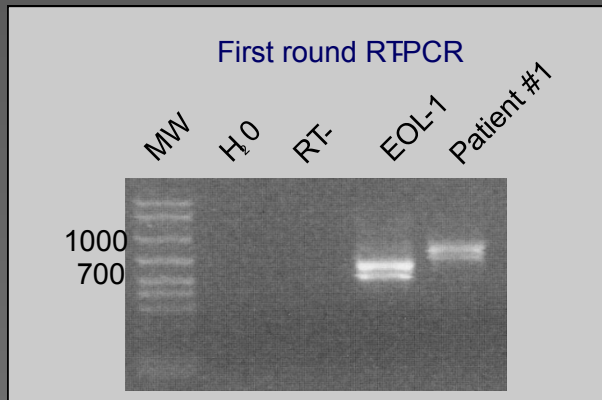


Recherche del 4q12;q12 par stratégie de FISH

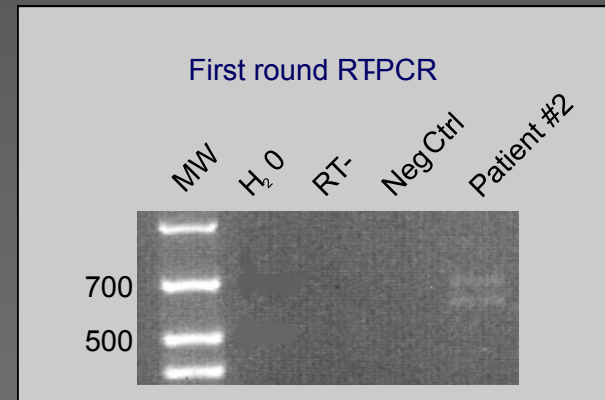


Transcrit de fusion FIP1L1-PDGFR

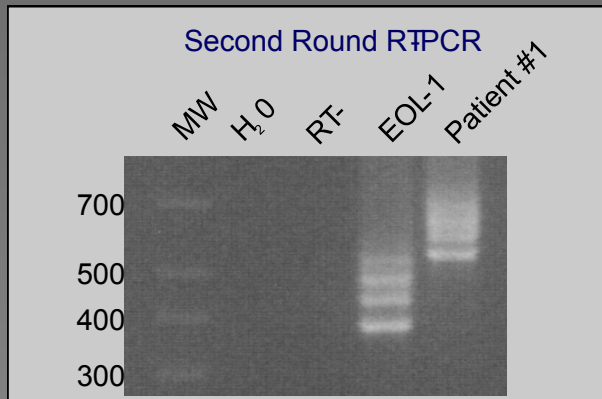
Patient # 1



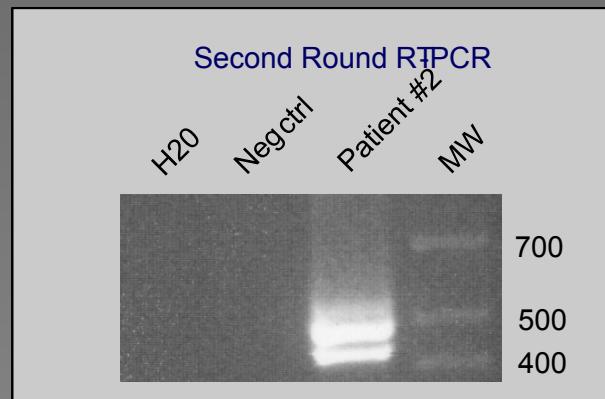
Patient # 2



Second Round RT-PCR



Second Round RT-PCR



Séquençage des transcrits

Case #1

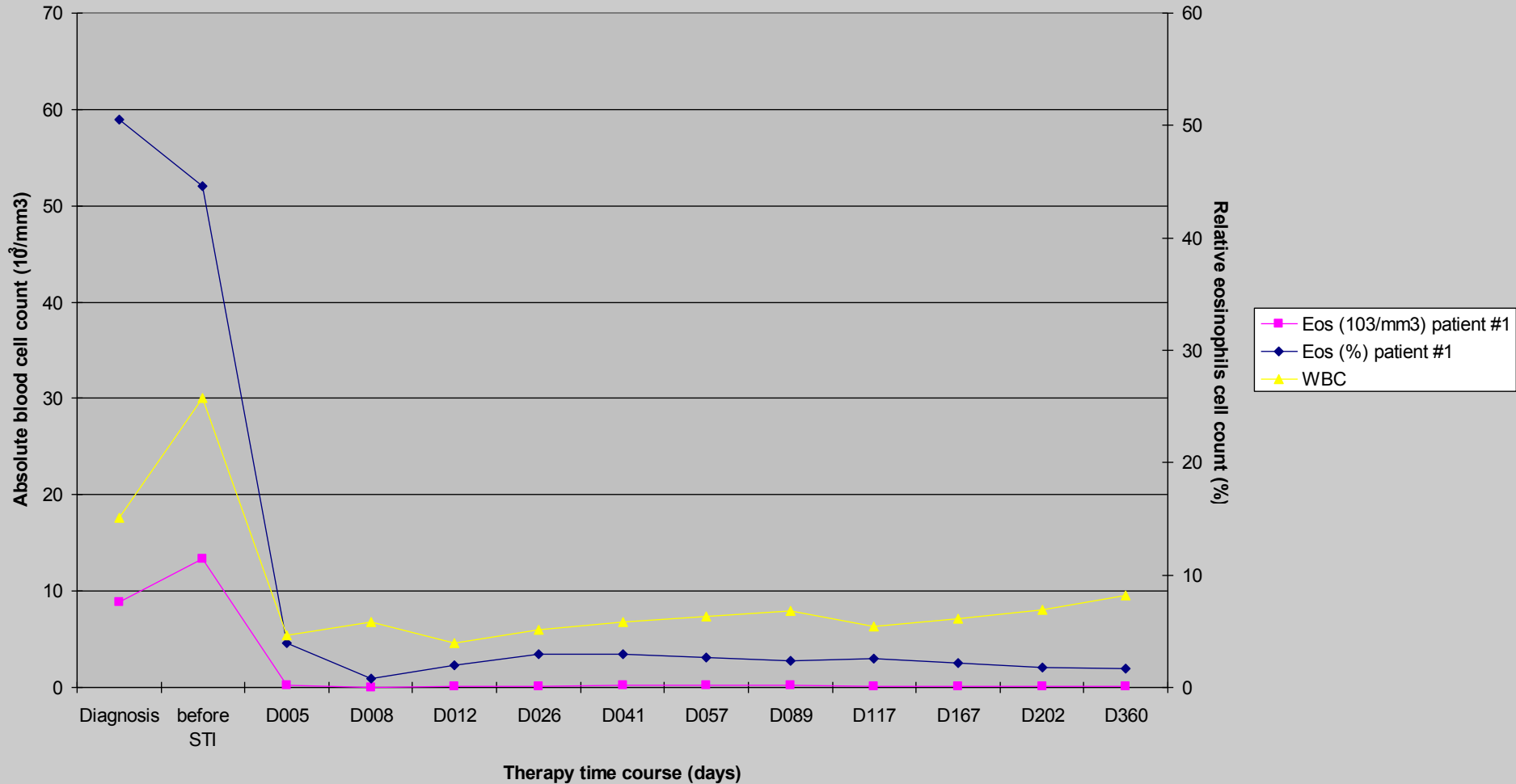
FIP1L1 exon 13	FIP1L1 intron 13	PDGFRA exon 12
5'-AGCACTGCTCCACCTCTGATTCCACCACCG	GGCTGGAGTACAGTGGCACTATC	TTATGACTCAAGATGGGAGTTTCCAAGA-3'
*S*T*A*P*P*L*I*P*P*P*	*G*W*S*T*V*A*L*S	*Y*D*S*R*W*E*F*P*R*

Case #2

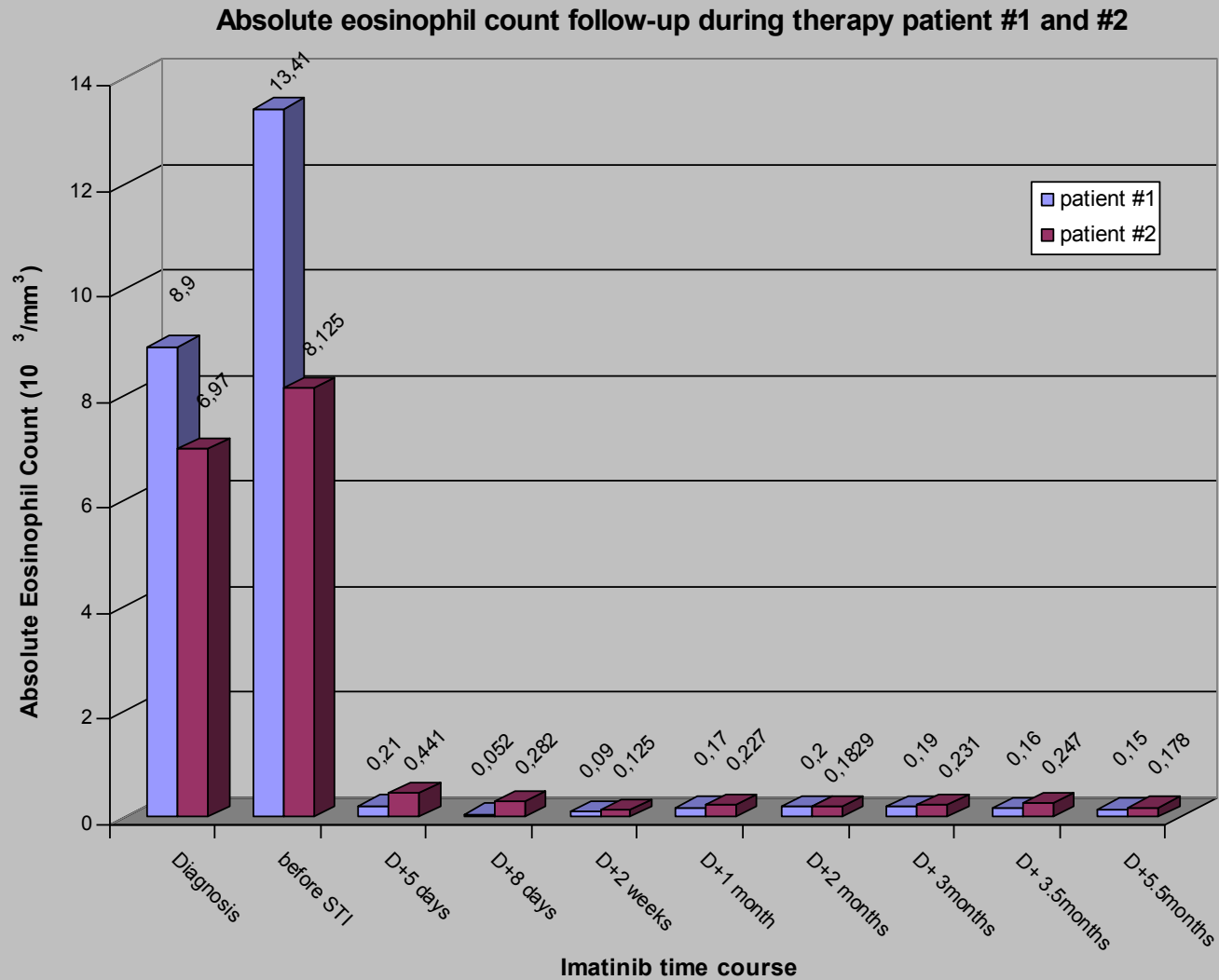
FIP1L1 exon 11	PDGFRA exon 12
5'-TATGGGAGGGCCGAATCACCTGATCTAA	GCCC G GATGGACATGAATATATTTATGTGGACCCGATGCAGCTGCCTTAT -3'
*Y*G*R*A*E*S*P*D*L*	*S*P*D*G*H*E*Y*I*Y*V*D*P*M*Q*L*P*Y*

Réponse cytologique, #1

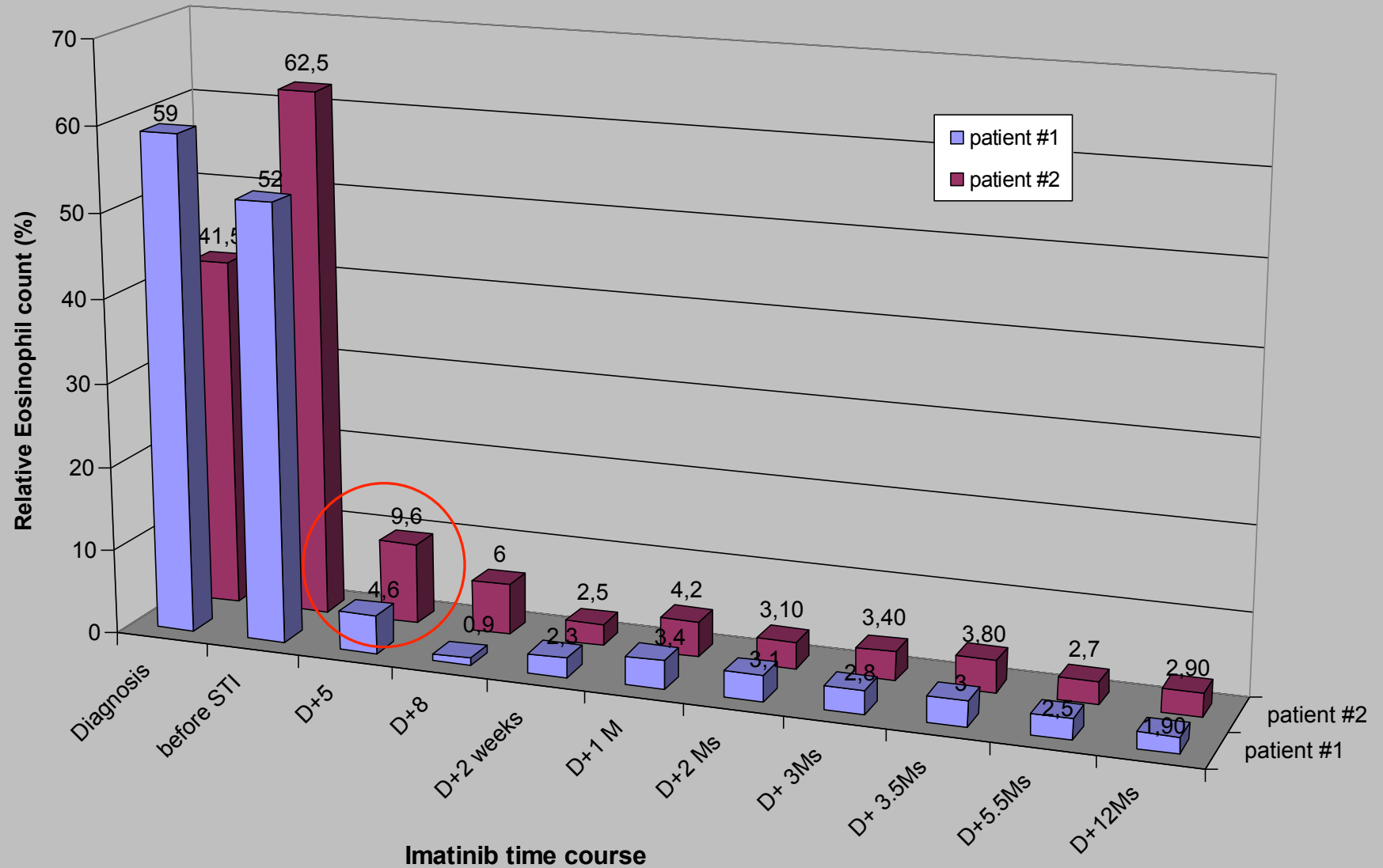
Imatinib therapy patient #1



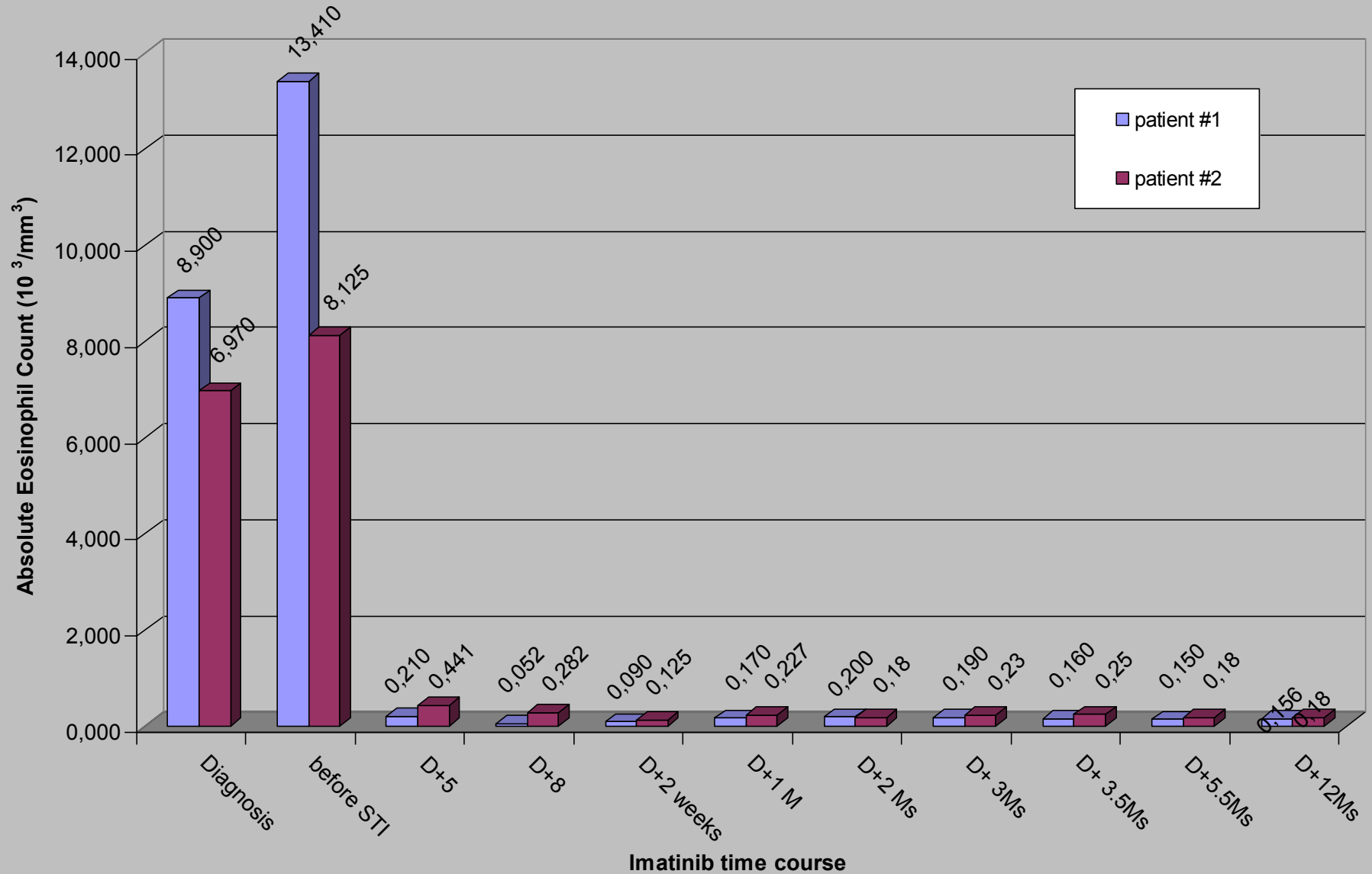
Réponse cytologique, #1 et 2



Relative eosinophil count follow-up during therapy patient #1 and #2



Eosinophilie absolue sous imatinib



Construction d'un système de RT-PCR quantitative

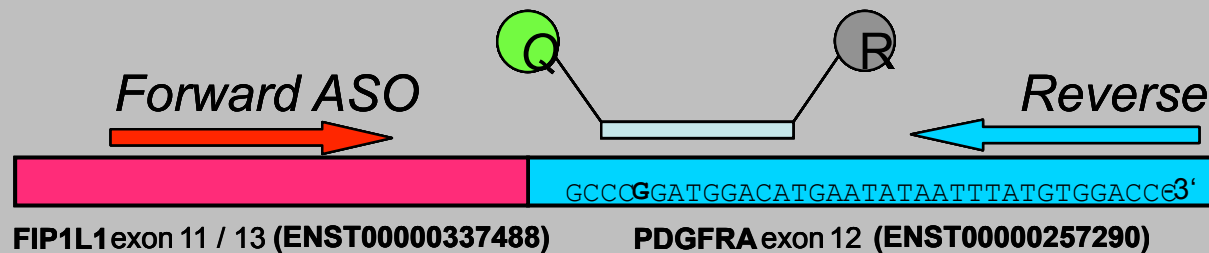
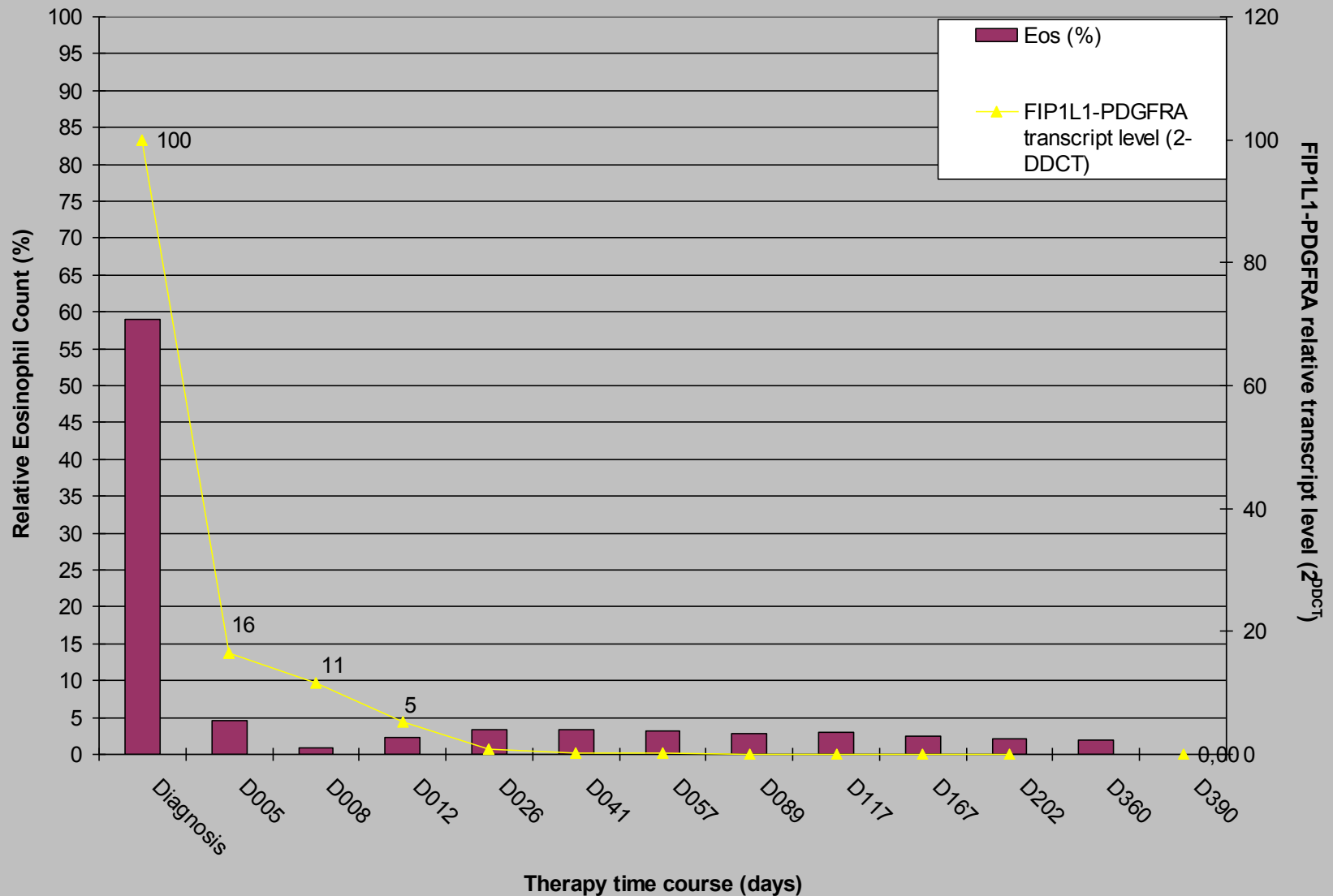


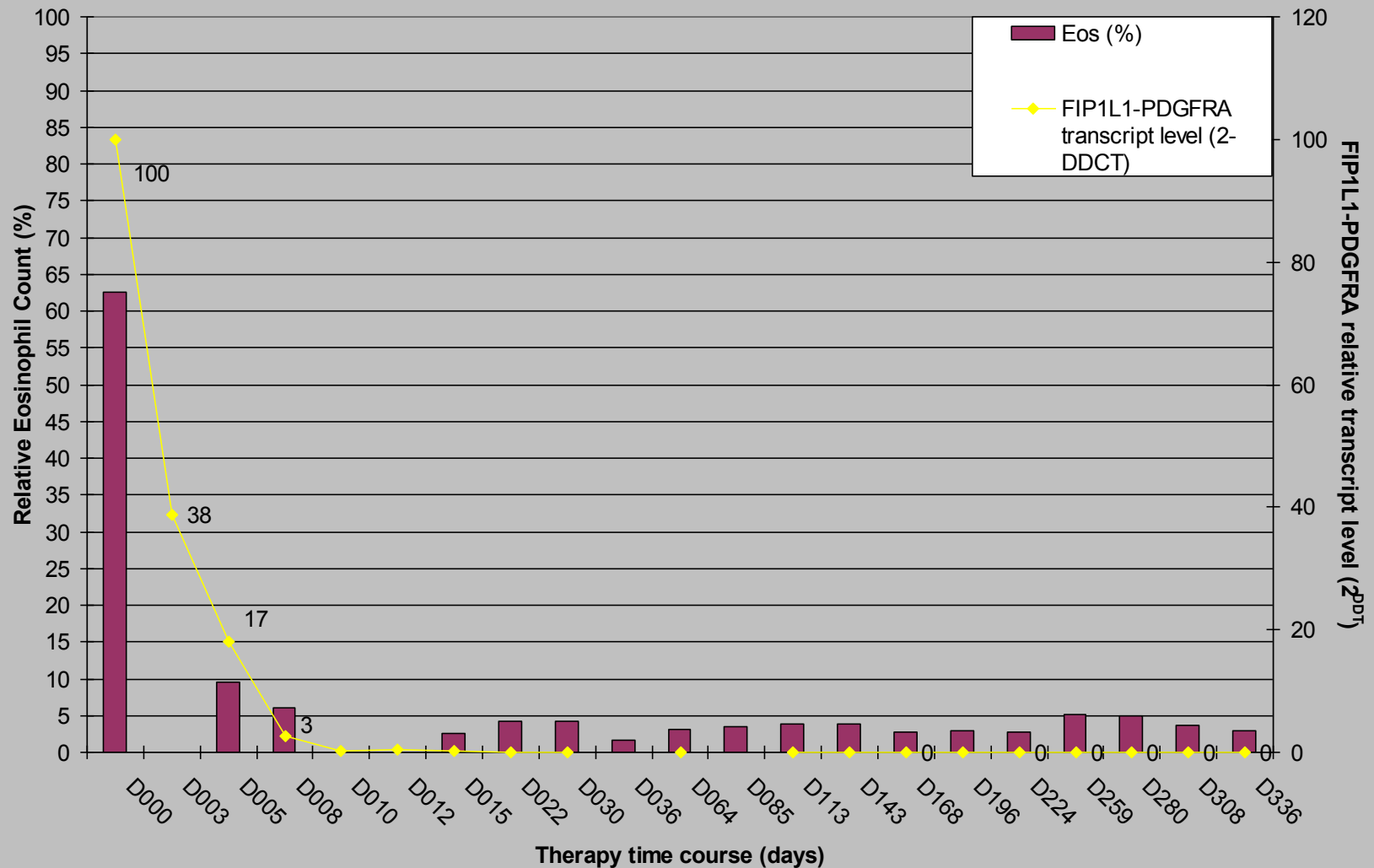
Fig.5: FIP1L1-PDGFRα RQ RT-PCR TaqMan® Assay.

The strategy we applied allow us to use a common probe and reverse primer. Forward ASO confer specificity.

Monitoring de la réponse au glivec, #1



Monitoring de la réponse au glivec, #2



Réponse cytologique, #1 et 2

Table 2. Imatinib mesylate treatment response evaluated on blood and bone marrow

Cases	Patient # 1		Patient # 2	
Imatinib mesylate starting date (dd/mm/yyyy)	10/03/2004		07/06/2004	
Imatinib mesylate induction / maintenance dose (mg/d)	400 / 100		100 / 100	
Response status	CR ^{\$}		CR ^{\$}	
Time to achieve C.R on PB (< 0.5 10 ⁹ /L)(days)	6 days		4 days	
Follow-up (months)	6,5		4	
Therapy status on blood	Diagnosis	After therapy*	Diagnosis	After therapy*
Hgb, g/dL	11,50	13,50	10,40	11,40
Ht, (%)	35,20	41,20	31,60	34,00
WBC, X 10 ⁹ /L	15,80	6,10	12,70	5,80
ANC, X 10 ⁹ /L	1,81	3,03	7,58	3,20
ALC, X 10 ⁹ /L	3,80	2,50	2,22	1,90
AMC, X 10 ⁹ /L	0,45	0,26	0,41	0,44
AEC, X 10⁹/L	8,90	0,15	2,37	0,23
ABC, X 10 ⁹ /L	0,00	0,03	0,51	0,03
Platelet, X 10 ⁹ /L	194	216	314	259
FIP1L1-PDGFR fusion gene (RT-PCR, +/-)	+	-	+	-
FIP1L1-PDGFR fusion gene (RQ RT-PCR, %)	100	< LOD	100	< LOD
Therapy status on bone marrow	Diagnosis	After therapy*	Diagnosis	After therapy*
BM eosinophils (%)	40	< 2	19	< 3
FIP1L1-PDGFR fusion gene, RT-PCR (+/-)	+	-	+	-
FIP1L1-PDGFR fusion gene, RQ RT-PCR (%)	100	< LOD	100	< LOD
CHIC2 deletion, FISH (% abnormal nuclei)	70	< cut-off [#]	24	< cut-off [#]

WBC indicates white blood cell count; ANC, absolute neutrophil count; ALC absolute lymphocyte count; AMC, absolute monocyte count; AEC, absolute eosinophil count; ABC, absolute basophile count; Ht, hematocrit; PB, peripheral blood; CR, complete hematological remission; CCR, complete cytogenetic remission; LOD, limit of detection; LOQ, limit of quantification.

[#] cut-off value was evaluated from normal bone marrow, secondary hypereosinophilia and CCR patients around 5%.

^{\$} CR, complete haematological remission is defined as an absolute peripheral eosinophils count < 0.5 10⁹/L,

* Data at the time of first bone marrow evaluation, 24/08/04 for case #1 and 25/09/04 for case #2.

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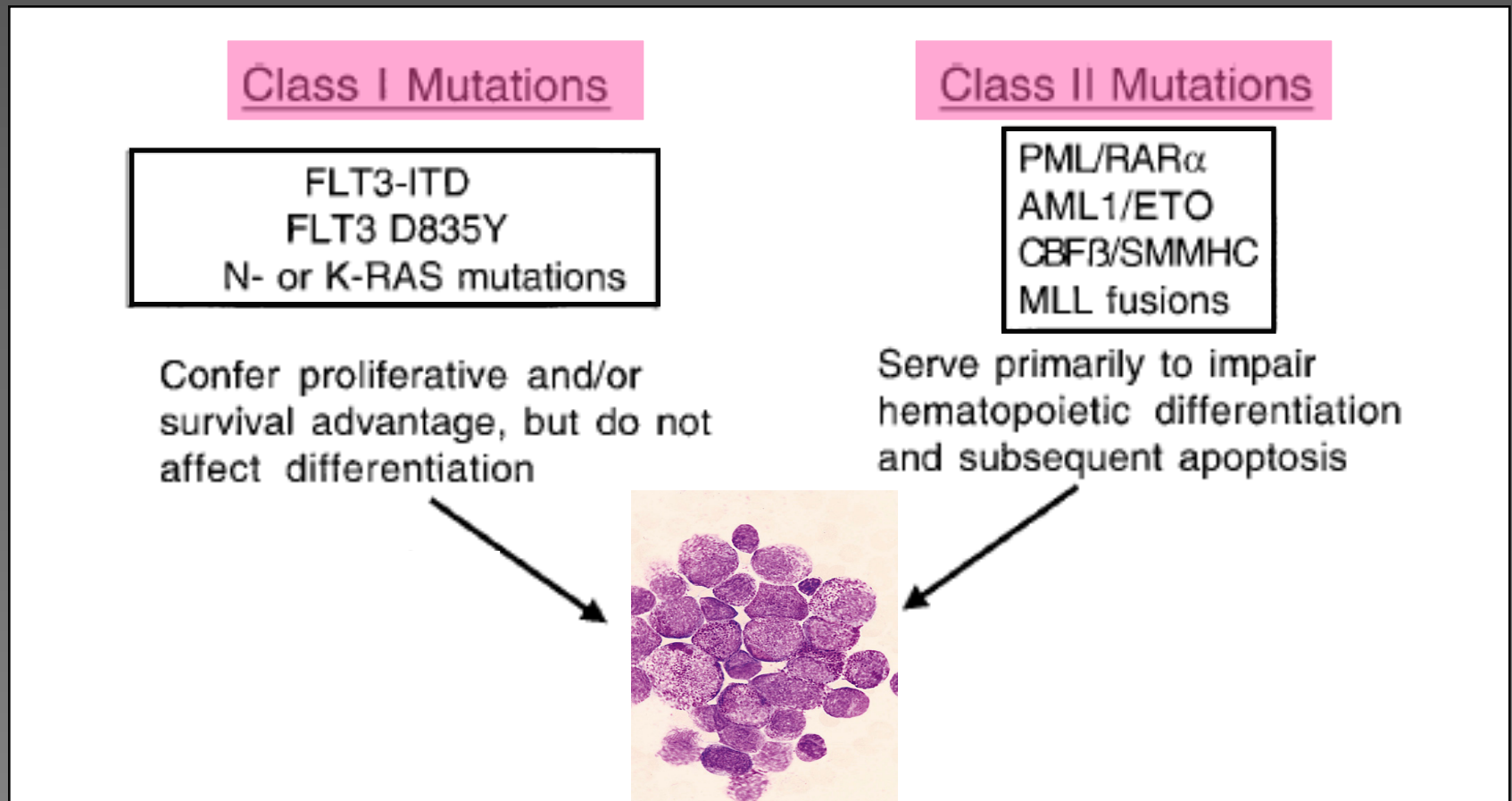
Bibliographie

■ Eosinoipoïèse

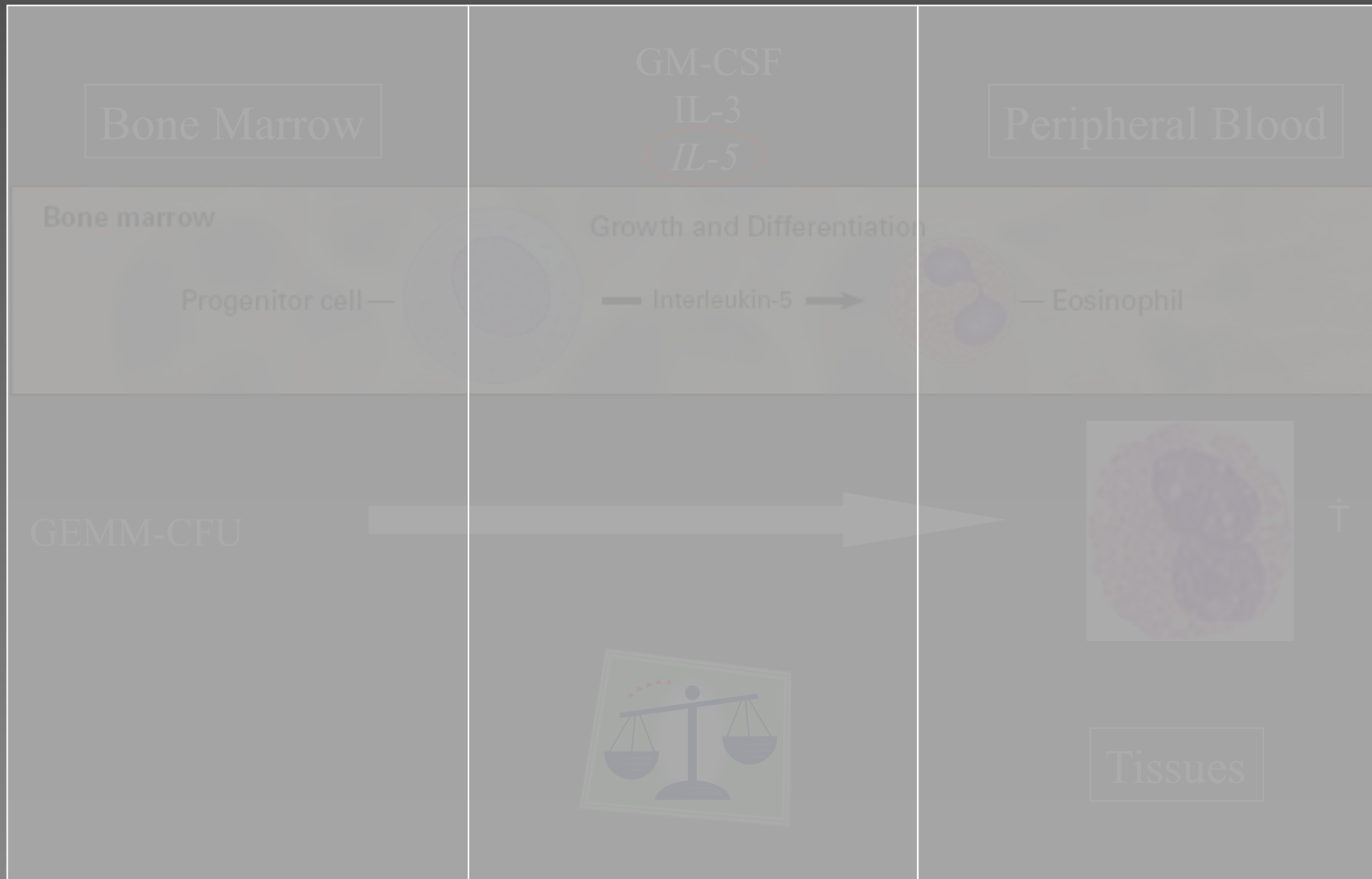
- S. J. Ackerman and J.H. Butterfield, « Eosinophilia, Eosinophil-Associated Diseases and the Hypereosinophilic Syndrome » in Host Defense and Its Disorders, part V
- M. E. Rothenberg, « Eosinophilia », NEJM, May 28, 1998
- S. Kobayashi et al. « Incidence of peripheral blood eosinophilia and the threshold eosinophile count for indicating hypereosinophilia-associated diseases, Allergy 2002;57:950-956.

Stock images

Modèle multi-étapes de la leucémogenèse myéloïde



Hyperéosinophilies



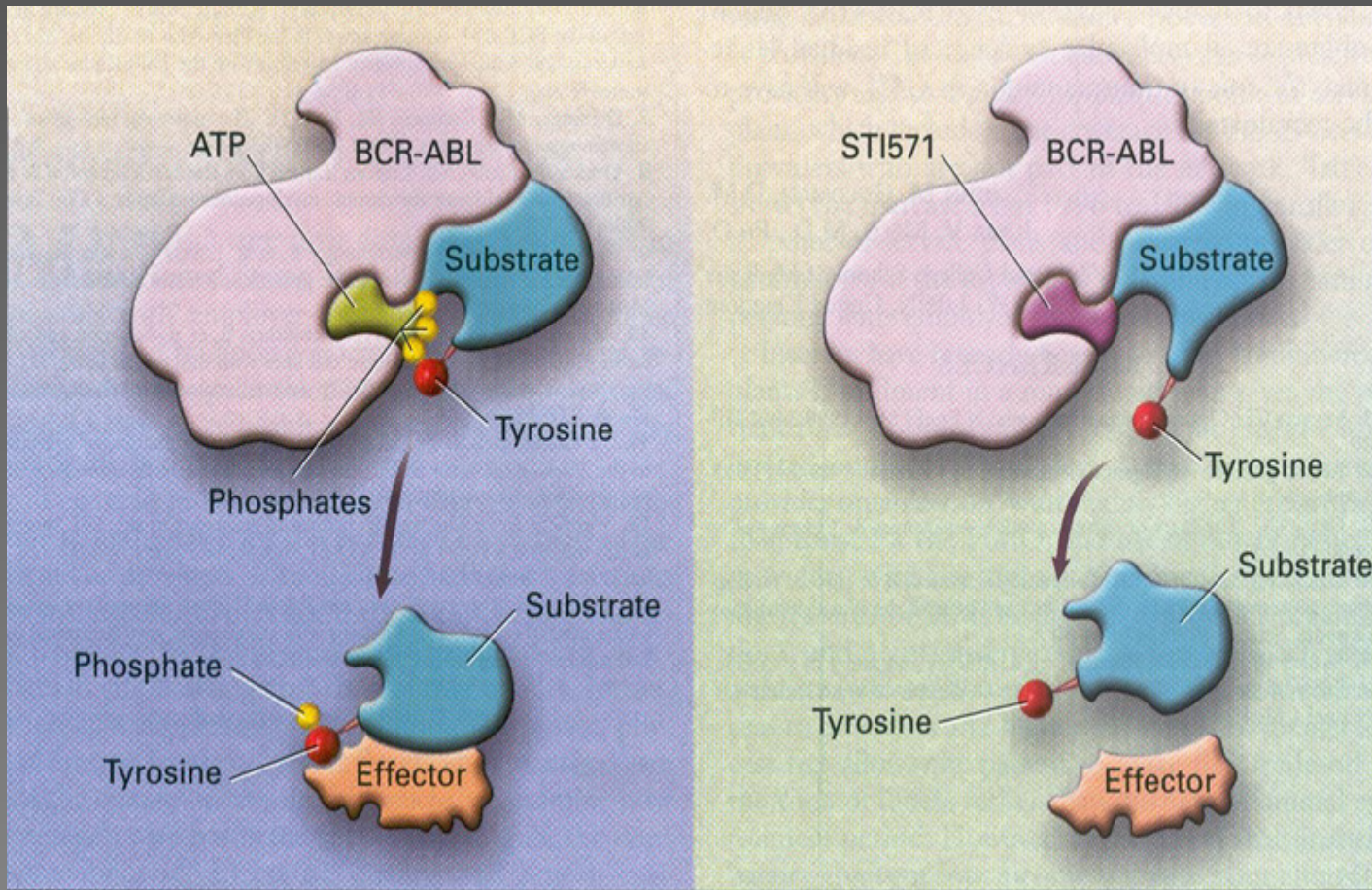
Classification alternative:

- Clonalité:
 - Variant myéloïde, CEL
- Pas de clonalité:
 - Clone Th2: « variant lymphoïde »,
 - Forme idiopathique vraie: « HES »

Le syndrome « 8p11-12 » ou « Pluripotent Stem Cell Syndrome », anomalies de FGFR1

Syndrome	Cytogenetic abnormality	Molecular genetic abnormality
CMML with eosinophilia/T-lineage lymphoblastic lymphoma/rarely B-lineage lymphoblastic leukaemia/lymphoma and frequent evolution to acute myeloid leukaemia	t(6;8)(q27;p12) t(8;9)(p11;q32-34) t(8;13)(p11;q12) t(8;17)(p11;q25)*	<i>FOP-FGFR1</i> fusion <i>FAN-FGFR1</i> fusion <i>ZNF198-FGFR1</i> fusion <i>FGFR1</i> rearranged [26]

Mechanism of Action of Imatinib

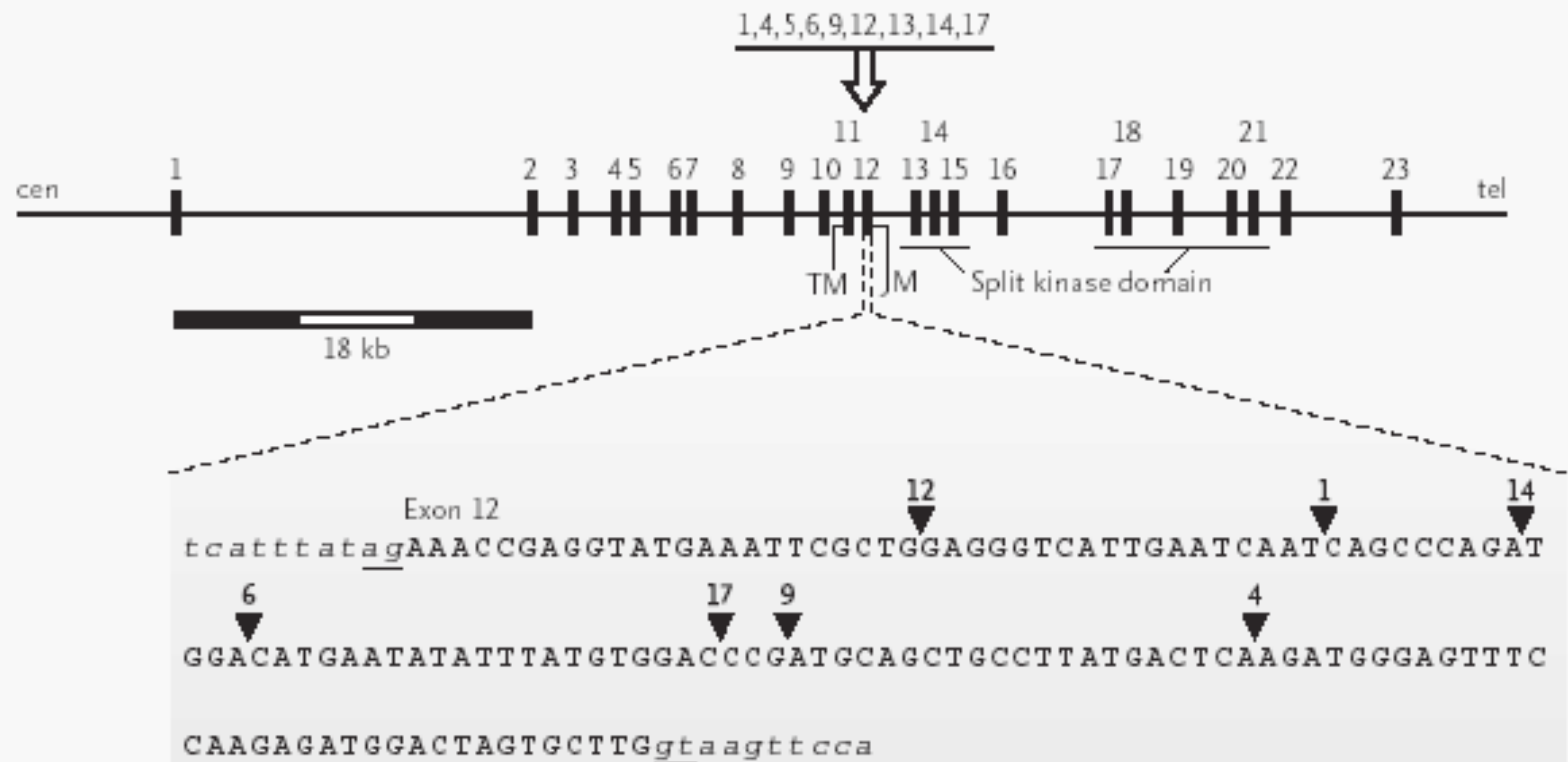


Classification opérationnelle des hyperéosinophilies

Persistent eosinophilia ($\geq 1.5 \times 10^9/L$) for ≥ 6 months associated with evidence of end-organ disease	
Non-idiopathic	
<u>Clonal eosinophilia:</u> 1) identification of a cytogenetic or molecular abnormality*, or 2) bone marrow morphologic evidence of a myeloid disorder**	<u>Reactive eosinophilia:</u> parasites, atopy, drug reaction, connective tissue disease, non-myeloid malignancy (eg. Hodgkin's lymphoma) etc.
Idiopathic	
Hypereosinophilic syndrome	

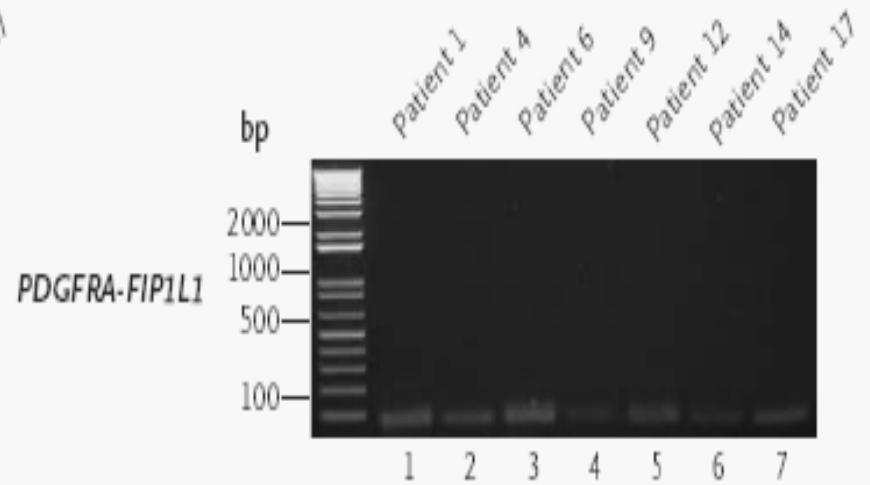
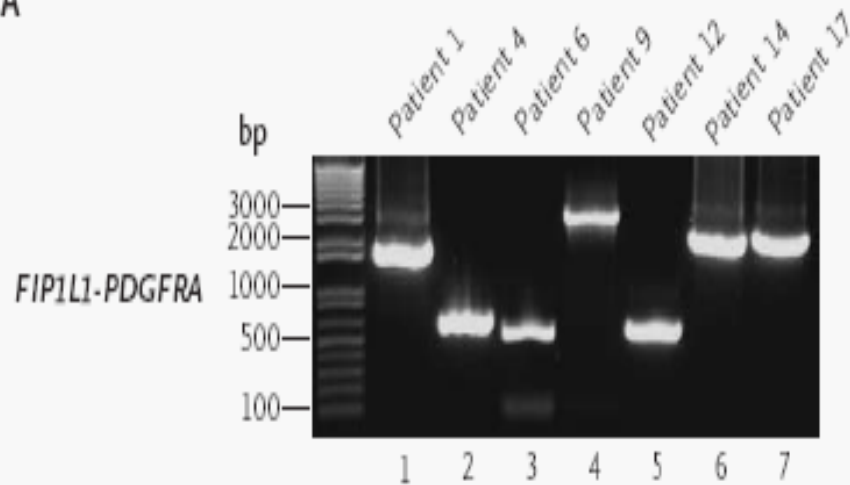
Points de cassure génomique PDGFRA

C PDGFRA



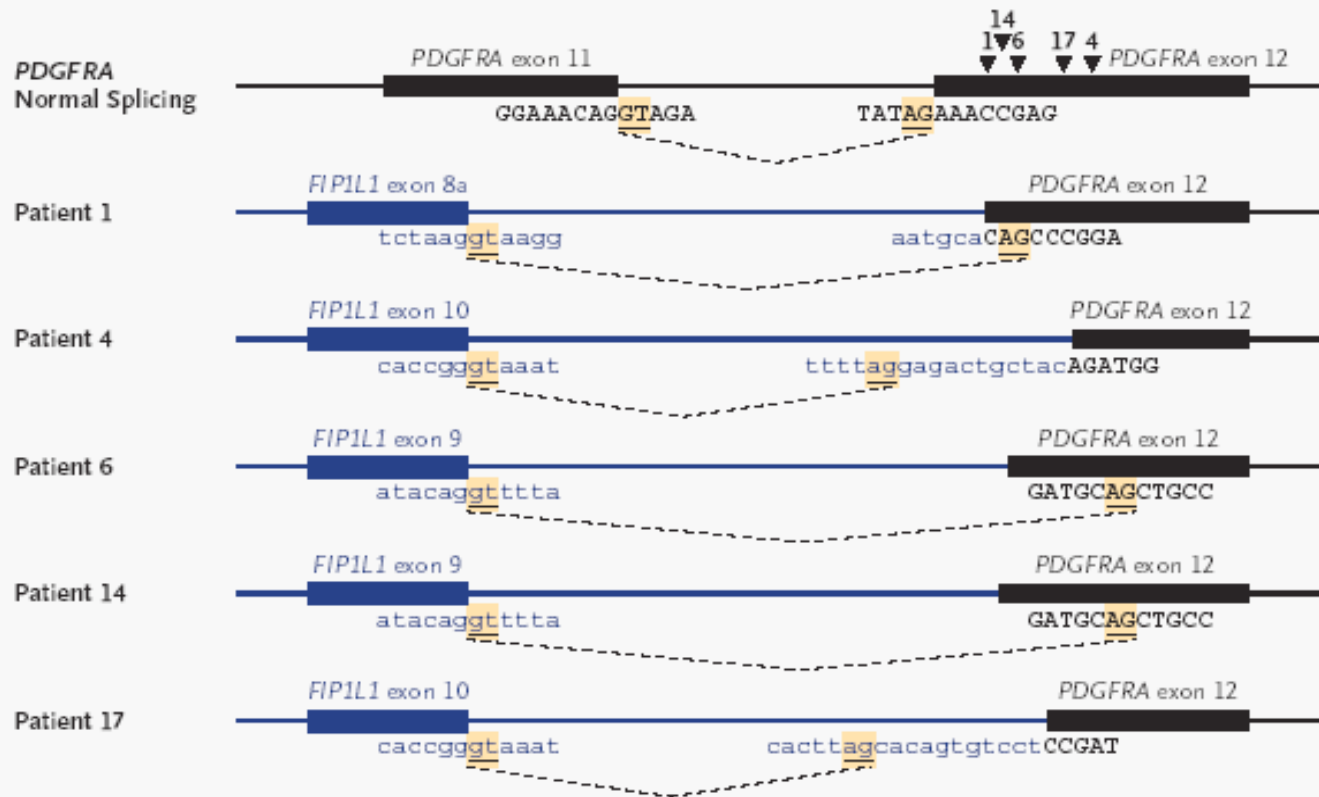
PCR génomiques FIP1L1-PDGFR α

A

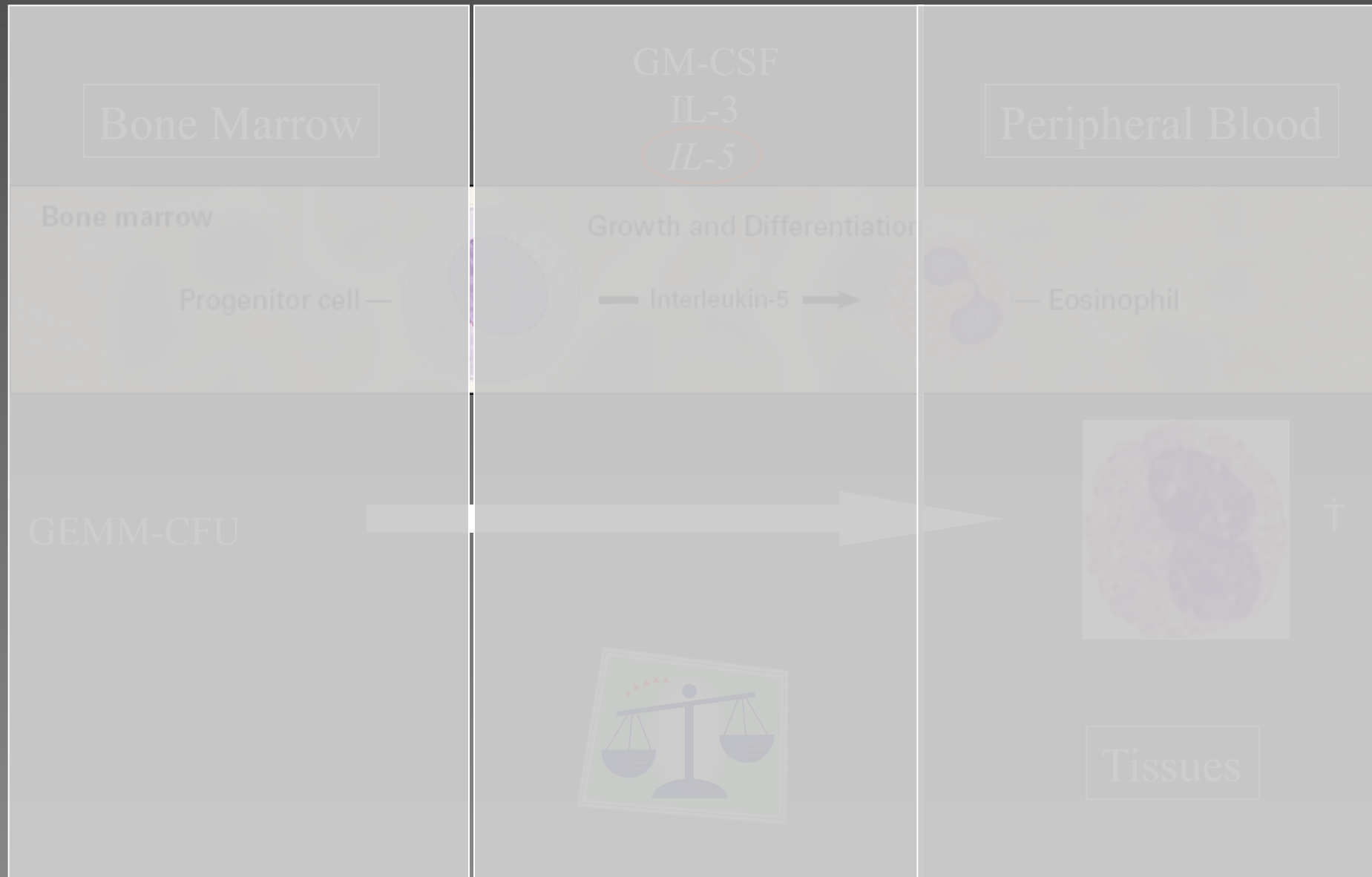


Sites d'épissage alternatifs

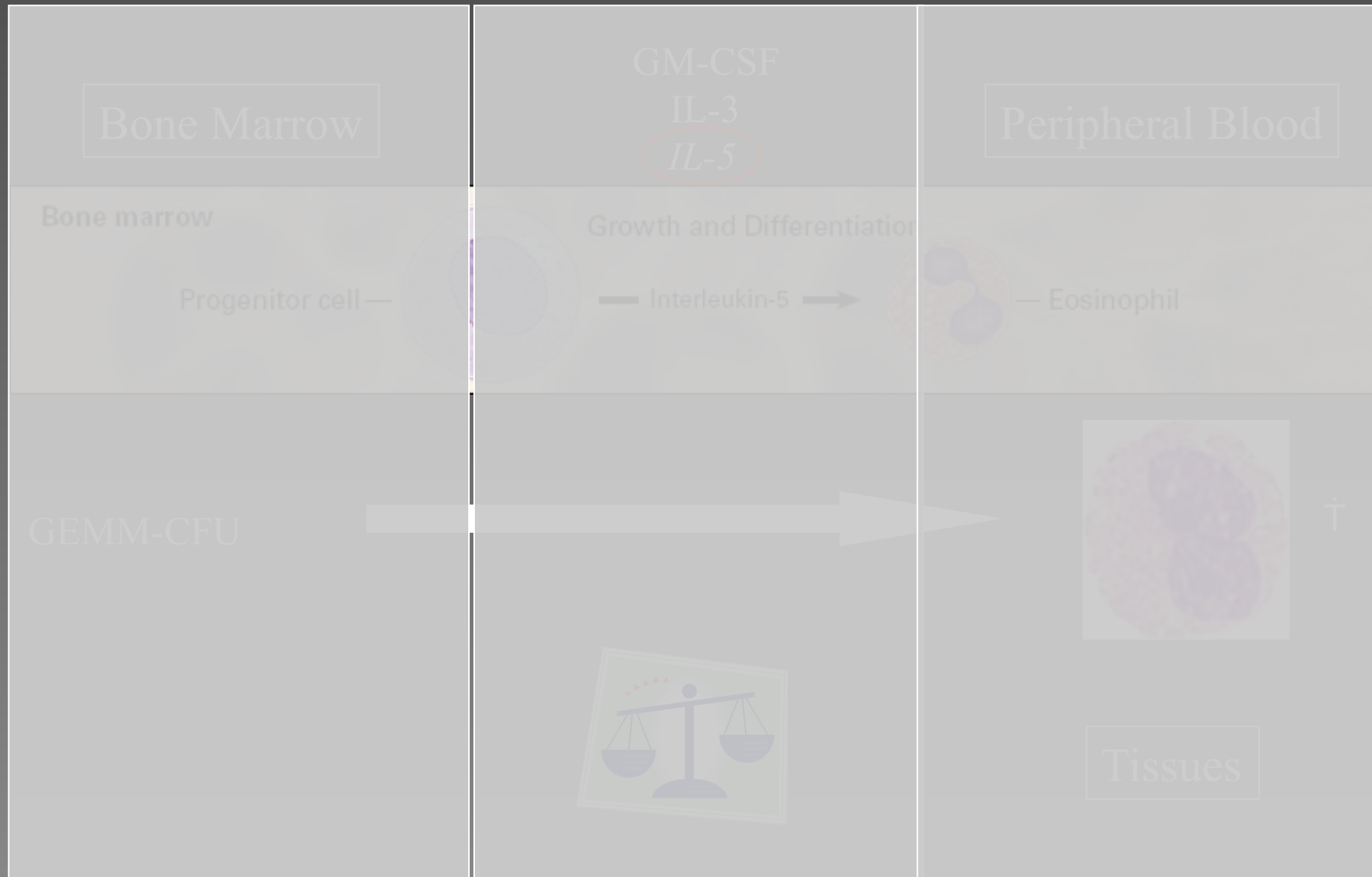
C



L'éosinopoïèse normale

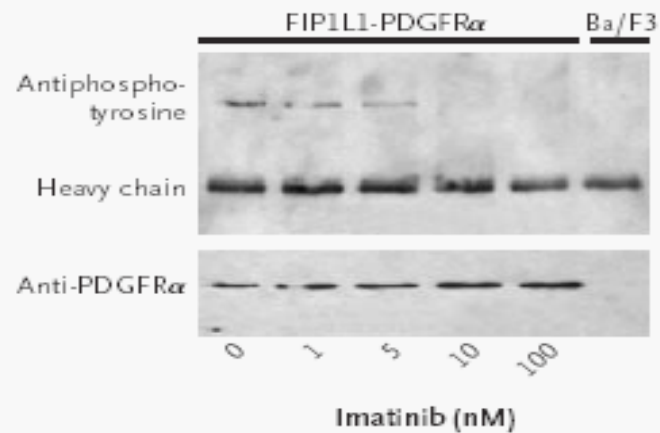


L'éosinopoïèse normale

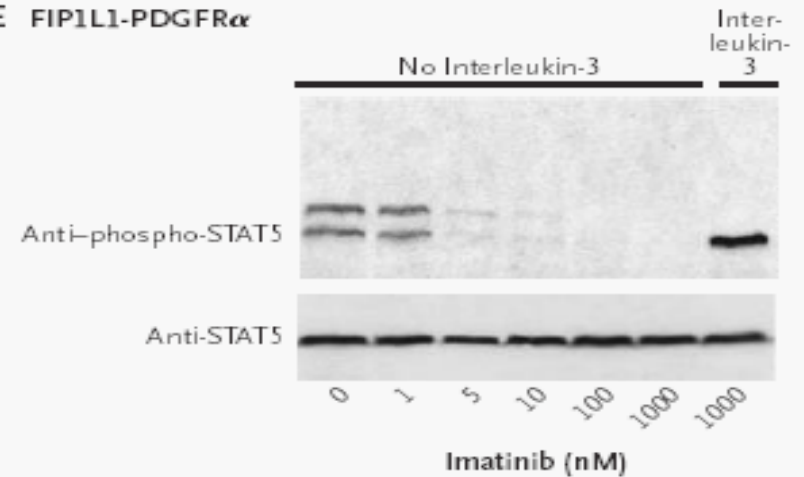


Activité constitutive TK de FIP1L1-PDGFR α

D FIP1L1-PDGFR α



E FIP1L1-PDGFR α



Clinical and molecular features of *FIP1L1-PDFGRA* (+) chronic eosinophilic leukemias

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- N= 17,
- Critères WHO / IHES ou CEL,
- RT-PCR et FISH,
- FIP1L1-PDGFR α + (8), FIP1L1-PDGFR α - (3), IHES (6),
- Sex ratio: 13 H / 4 F, age moyen: 47.7 ans,
- Clinique:
 - Splénomégalie (5),
 - Vit B12 sérique augm. (8 FIP1L1-PDGFR α +) / N IHES,
 - Moelle: image de SMP

Clinical and molecular features of FIP1L1-PDGFR1(+) chronic hyperéosinophilic leukemia

- FIP1L1-PDGFR1+, mauvaise réponse corticostéroïdes, Hydroxyurée, IFNα
- Prob. élevée endomyocardite (4), DRD (4),
- C.R / imatinib 4/4 FIP1L1-PDGFR1+,
- C.M.R 2/3
- Ccls:
 - FIP1L1-PDGFR1(+) CEL = entité clinico-biologique,
 - Réponse moléculaire sous Imatinib

Evolution hématologique

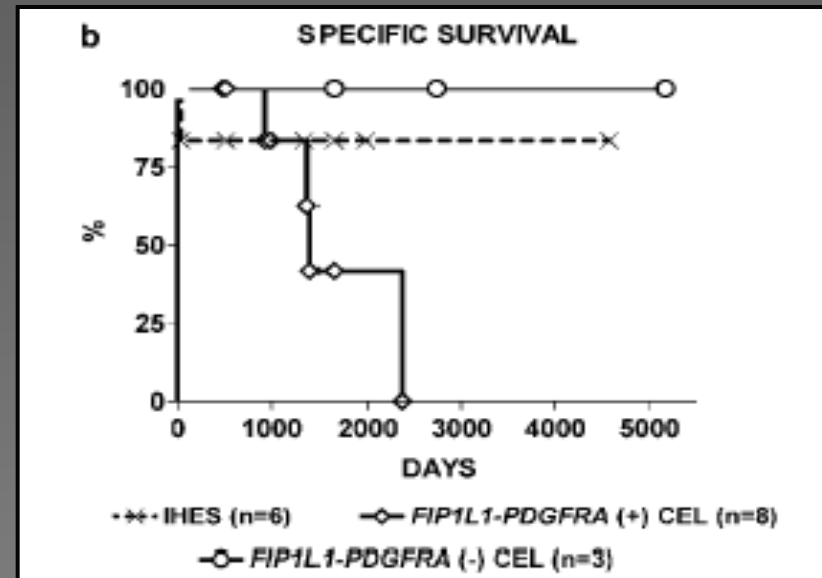
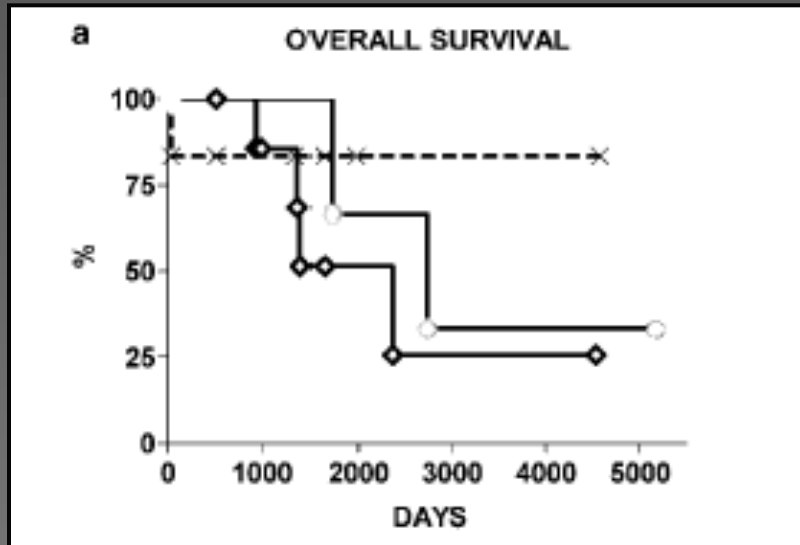
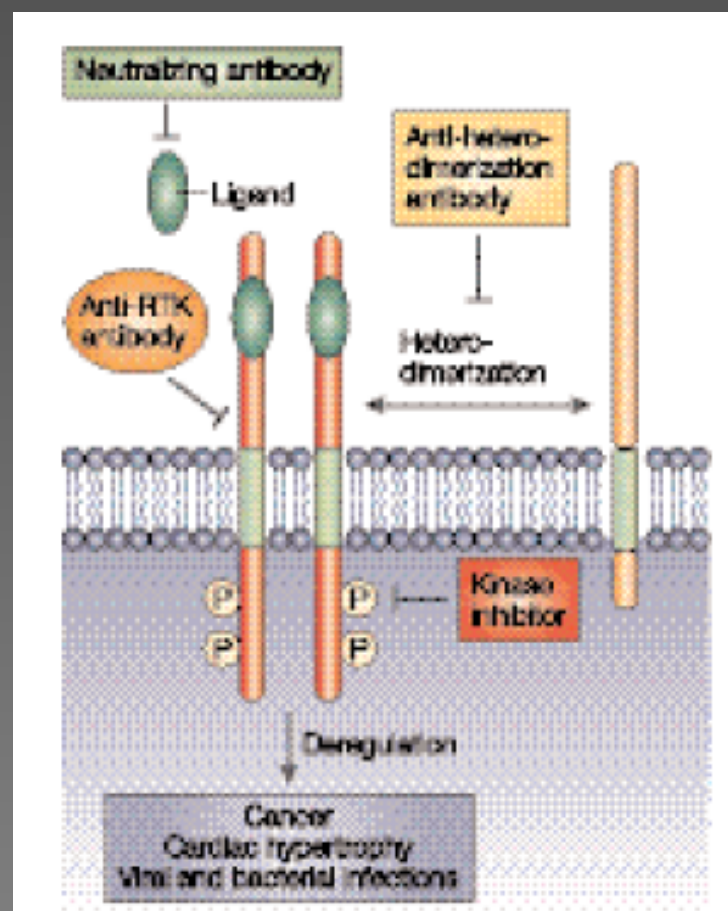


Table 1. Frequency of the *JAK2* V617F Mutation.

Group	Total No. of Subjects	<i>JAK2</i> Genotype in Granulocytes		
		G/G (wild type)	G/T (heterozygous)	T/T (homozygous)
		<i>number of subjects (percent)</i>		
Patients with myeloproliferative disorders	244	127 (52)	74 (30)	43 (18)
With 9pLOH	51	0	8 (16)	43 (84)
Without 9pLOH	193	127 (66)	66 (34)	0
Patients with polycythemia vera	128	45 (35)	48 (38)	35 (27)
With 9pLOH	43	0	8 (19)	35 (81)
Without 9pLOH	85	45 (53)	40 (47)	0
Patients with essential thrombocythemia	93	72 (77)	18 (19)	3 (3)
With 9pLOH	3	0	0	3 (100)
Without 9pLOH	90	72 (80)	18 (20)	0
Patients with idiopathic myelofibrosis	23	10 (43)	8 (35)	5 (22)
With 9pLOH	5	0	0	5 (100)
Without 9pLOH	18	10 (56)	8 (44)	0
Patients with CML*	9	9 (100)	0	0
Patients with secondary erythrocytosis	11	11 (100)	0	0
Healthy controls	71	71 (100)	0	0



Variants de « splicing » avec respect du cadre de lecture.

E

	<i>FIP1L1</i>		<i>PDGFRA</i>
	Exon 8a		Exon 12
Patient 1	atatgggagggccgaatcacctgatctaag		CCCGGATGGACATGAATATATTTATGTGGA
	Y G R A E S P D L S		P D G H E Y I Y V D
	Exon 10	Intron 10	Exon 12
Patient 4	cctctgattccaccaccgg	gagactgctac	AGATGGGAGTTTCCAAGAGATGGACTAGTG
	P L I P P P	G D C Y	R W E F P R D G L V
	Exon 8		Exon 12
Patient 5	tttgttcaagactgggcttccaccgagcag		AGATGGACATGAATATATTTATGTGGACCC
	L F K T G L P P S R		D G H E Y I Y V D P
	Exon 9		Exon 12
Patient 6	cgacgggcaaatagagaacagcaacatacag		CTGCCTTATGACTCAAGATGGGAGTTTCCA
	R R A N A N S N I Q		L P Y D S R W E F P
	Exon 9		Exon 12
Patient 13	cgacgggcaaatagagaacagcaacatacag		CTGCCTTATGACTCAAGATGGGAGTTTCCA
	R R A N A N S N I Q		L P Y D S R W E F P
	Exon 9		Exon 12
Patient 14	cgacgggcaaatagagaacagcaacatacag		CTGCCTTATGACTCAAGATGGGAGTTTCCA
	R R A N A N S N I Q		L P Y D S R W E F P
	Exon 10	Intron 10	Exon 12
Patient 17	cctctgattccaccaccgg	cacagtgtcct	CCGATGCAGCTGCCTTATGACTCAAGATGG
	P L I P P P	A Q C P	P M Q L P Y D S R W