ADVANCES IN THE TREATMENT OF GENETIC DISEASES
FROM GENE SEARCH TO DRUG DISCOVERY AND CLINICAL TRIALS

Department of Genetics and IMAGINE Institute
Hospital Necker-Enfants Malades
University Paris-Descartes, France

« The past explains our present and enlights our future » *Tocqueville*

LEARNING OBJECTIVES

To point, but not to blame:

- the ignorance of what is presently available and working
- the fascination for futuristic prospects (*gene therapy, stem cells*)
- the trend to preconceived ideas and « *a prioris* » among scientists
- our inability to communicate clearly and honestly
- the oversimplification of information presented by the media

To outline:

- what is already possible: *"render under Caesar what is Caesar` s«*
- that causality is what it is all about
- that gene identification puts us on the right track
- that replacement of a gene is not the universal panacea
- that current treatments are changing quality of life/life expectancy

The options are no longer to either recover or die but to live with a chronic disease

Until recently, what was feasible and efficient owed virtually nothing to gene identification

"render under Caesar what is Caesar' s"

- **Dietary management** ***(early 70ies)***
- **Vitamin-responsive metabolic diseases**
- **Organ transplantations** ***(early 80ies)***
- **Protein/drug engineering** ***(early 90ies)***
- **Enzyme therapy** ***(early 20ies)***
- **Conventional pharmacology**

ADVANCES IN TREATMENT OF GENETIC DISEASES

- **Dietary management** ***(early 70ies)***

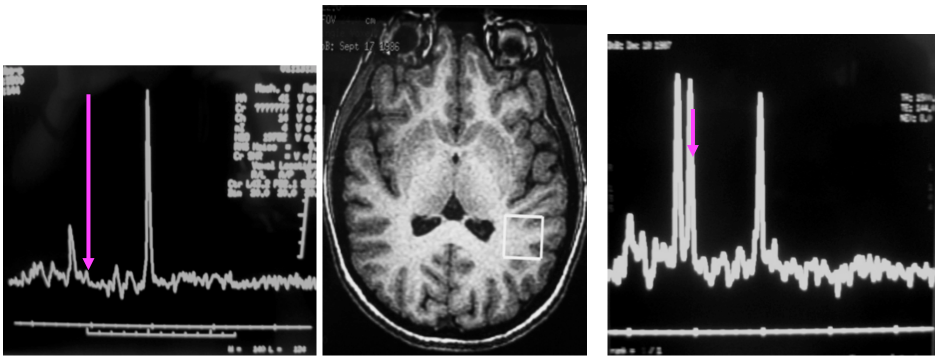
Low protein diet	***PKU, MSUD, hyperammonemias***
Low fat diet:	***Hypercholesterolemias Refsum***
Ketogenic diet:	***OXPHOS deficiency***
High glucose diet:	***Fatty acid oxidation disorders***
Pancreatic extracts:	***Cystic Fibrosis, Pearson***
High mannose diet:	***CDG1b (PMI deficiency)***

- Vitamin responsive metabolic diseases
- Organ transplantations
- Protein/drug engineering
- Enzyme therapy
- Gene therapy
- Conventional pharmacology

ADVANCES IN TREATMENT OF GENETIC DISEASES

- dietary management
- **vitamin/cofactor/substrate responsive metabolic diseases**
  - biotine (B8) responsive carboxylase deficiency
  - pyridoxine (B6) responsive homocystinuria
  - cobalamine (B12) responsive organic aciduria
  - tocopherol (E) responsive pseudo-Friedreich ataxia
  - carnitine responsive lipid myopathy / cardiomyopathy
  - quinone (CoQ_{10}) responsive ataxia / OXPHOS deficiency
  - creatine : mental retardation and autistic syndromes
- organ transplantation
- protein/drug engineering
- enzyme therapy
- gene therapy
- conventional pharmacology

CREATINE DEFICIENCY IN MR AND AUTISTIC SYNDROMES



- **psychomotor retardation, autistic features, seizures**
- **three disease genes** (AGAT, GAMT, CT)
- **diagnosis: NMR spectroscopy**
- **treatment** (AGAT, GAMT): **creatine** (1mg/kg/d), **arginine, ornithine**
- **improvement of epilepsy, cognitive functions, dystonia**

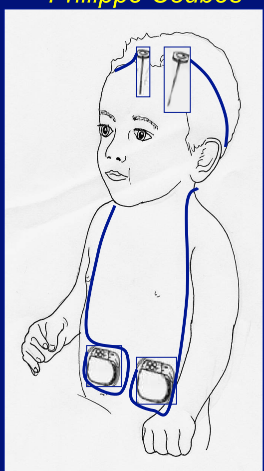
ALL MR/ASD CHILDREN DESERVE INVESTIGATIONS ! (dg:25%)

ADVANCES IN TREATMENT OF GENETIC DISEASES

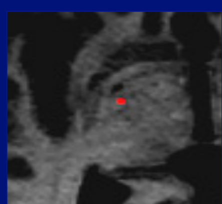
- dietary management
- vitamin responsive metabolic diseases
- **Organ transplantation/neo-organes** *(early 80ies)*
  - **kidney :** ***PKD, nephronophtisis, Alport, OXPHOS***
  - **liver :** ***α1AT, biliary atresia, metabolic diseases, OXPHOS***
  - **heart :** ***CMO, malformations, OXPHOS deficiency***
  - **bone marrow :** ***SCID, storage diseases***
  - **deep brain electrostimulation:** ***torsion dystonia (DYT1)***
- protein/drug engineering
- enzyme therapy
- gene therapy
- conventional pharmacology

DEEP BRAIN STIMULATION IN GENETIC DYSTONIAS

Philippe Coubes



- **bilateral implantation of electrodes by NMR stereotaxy under general anesthesia**
- **Medtronic^{R} Quadripolar**
- **target : the postero-ventral nucleus of the Globus Pallidum**
- **torsion dystonia, Pentothenate kinase deficiency, Huntington chorea, OXPHOS**



ADVANCES IN TREATMENT OF GENETIC DISEASES

- dietary management
- vitamin-responsive metabolic diseases
- organ transplantations
- **Protein/drug engineering** *(early 90ies)*
  - **Factor VIII:** ***hemophilia***
  - **Insuline:** ***diabetes mellitus***
  - **GH:** ***growth failure***
  - **Steroids:** ***congenital adrenal hyperplasia***
  - **G-MCSF:** ***agranulocytosis, Pearson***
- enzyme therapy
- gene therapy
- conventional pharmacology

ADVANCES IN TREATMENT OF GENETIC DISEASES

- dietary management
- vitamin-responsive metabolic diseases
- organ transplantation
- protein/drug engineering
- **Enzyme therapy** *(early 20ies, 250K€/yr)*
  - ***Fabry disease***
  - ***Gaucher disease***
  - ***Pompe disease***
  - ***Hurler, Hunter, Maroteaux-Lamy disease***
- gene therapy
- conventional pharmacology

ADVANCES IN TREATMENT OF GENETIC DISEASES

- dietary management
- vitamin responsive metabolic diseases
- organ transplantations
- protein/drug engineering
- enzyme therapy
- gene therapy
- **Conventional pharmacology**

to rectify protein folding: vaptans, *Diabetes Insipidus*

to re-express a fetal gene: Hydroxyurea, *Sickle cell anemia*

to clear/chelate a toxic: benzoate, *IVA*, cysteamine, *Cystinosis*

to lock a pathway: NTBC, *Tyrosinemia 1*

to activate a pathway: fibrates, colchicine, *Familial Mediterranean Fever*

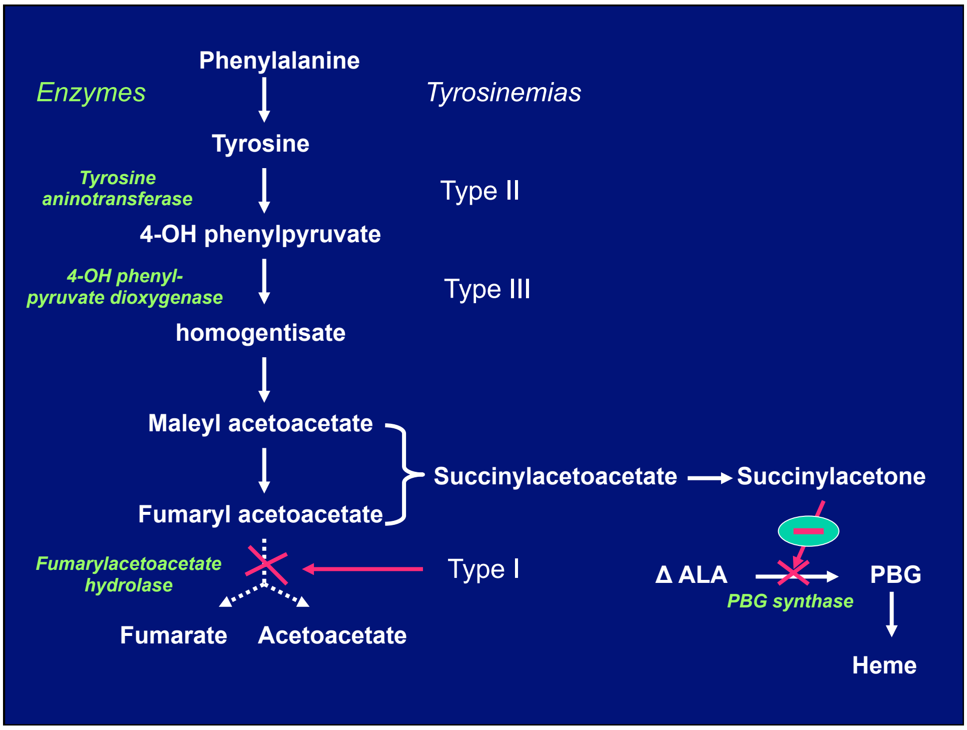
to inhibit a function: bisphosphonates, *Osteogenesis imperfecta*

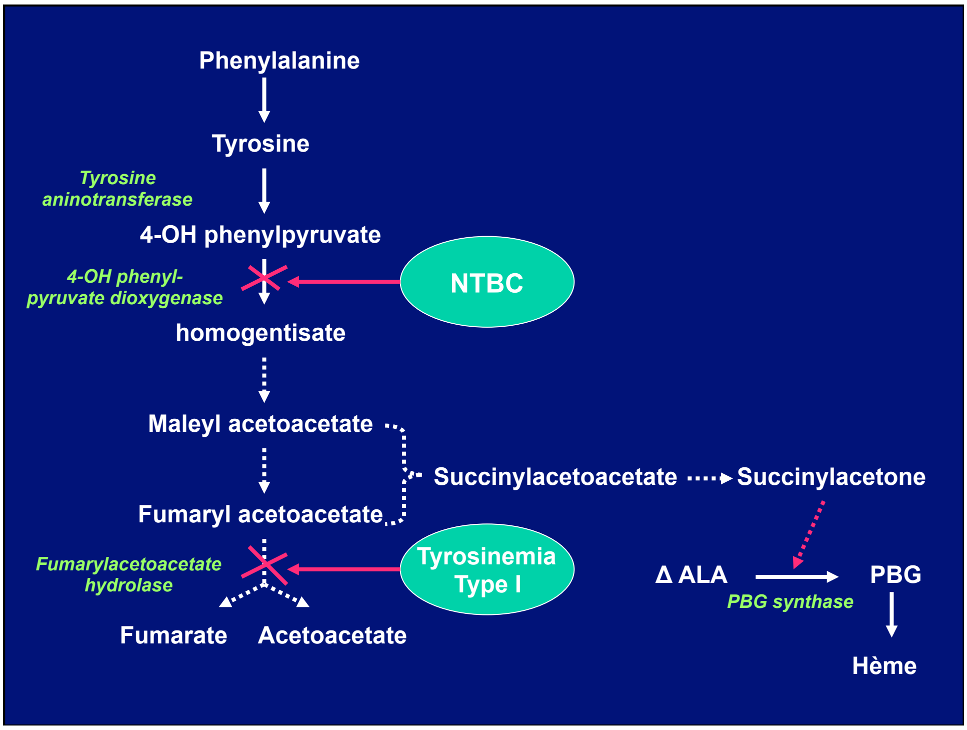
to replace a function: chenodeoxycholic acid, *bile salt synthesis disorders*

TO LOCK A PATHWAY ? NTBC IN TYROSINEMIA TYPE 1

- **Autosomal recessive condition (1/100.000, 1/2.000 in Quebec)**
- **fumarylacetoacetate hydrolase (FAAH, 15q23-q25)**
- **liver failure, carcinoma, tubulopathy, porphyria-like syndrome (succinyl-acetone)**
- **fatal outcome : liver failure 70%, carcinoma 17%**
- **Enormous efforts on gene therapy**

- **NTBC-responsive forms : 90%**


Phenylalanine
Enzymes
Tyrosinemias
Tyrosine
Tyrosine aninotransferase
Type II
4-OH phenylpyruvate
4-OH phenyl-pyruvate dioxygenase
Type III
homogentisate
Maleyl acetoacetate
Succinylacetoacetate
Succinylacetone
Fumaryl acetoacetate
Fumarylacetoacetate hydrolase
Type I
Δ ALA
PBG
PBG synthase
Fumarate
Acetoacetate
Heme


Phenylalanine
Tyrosine
Tyrosine aninotransferase
4-OH phenylpyruvate
NTBC
4-OH phenyl-pyruvate dioxygenase
homogentisate
Maleyl acetoacetate
Succinylacetoacetate
Succinylacetone
Fumaryl acetoacetate
Fumarylacetoacetate hydrolase
Tyrosinemia Type I
Δ ALA
PBG
PBG synthase
Fumarate
Acetoacetate
Hème

ADVANCES IN TREATMENT OF GENETIC DISEASES

- Until recently, advances in the treatment of genetic diseases have owed little to gene identification and to gene therapy
- They owed to biochemical elucidation of disease mechanism by the previous generation (i.e. NTBC, vaptans..)
- Discoveries were occasionally fortuitous … thanks to careful non-scientist, yet talented GPs (i.e hydroxyurea, colchicine)

One does not suffer from a mutation, but
from its functional consequences !!
To address consequences is fair enough !

ADVANCES IN TREATMENT OF GENETIC DISEASES

Things are changing with NGS and exome sequencing !!!

- Gene identification now puts us on the right track
- Gene identification points to the target/pathway
- Gene identification inspires elegant/efficient cures
- Gene is indeed the cause, yet gene replacement is not the unique riposte !
- Gene therapy is not, and will never become a panacea

The Imagine Institute, Necker-Enfants Malades Hospital

Daytime view

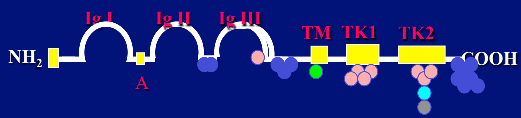
ADVANCES IN TREATMENT OF GENETIC DISEASES

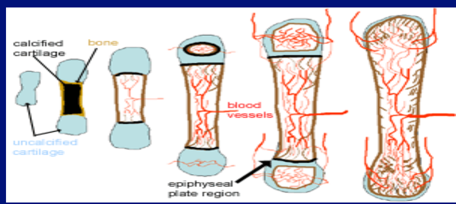
Things are gradually changing !!!

- Achondroplasia (*FGFR3 signaling and CNP analogue*)
- Acromicric/geleophysic dysplasia, Marfan (*TGFβ and Ab*)
- Freidreich ataxia (*iron sulphur clusters and iron chelators*)
- Neonatal Diabetes Mellitus *(K channel and sulphonylurea)*
- Generalized pustulous Psoriasis (*IL36-Ra and anti IL-1)*
- Mycobacterial infections *(M.Tuberculosis-TB and IGNγ)*
- Somatic gain-of-function mutations (*mastocytose and TK-I*)
- Metastastic cancers (*personalized genomics*)

ACHONDROPLASIA AND FGFR3 SIGNALING

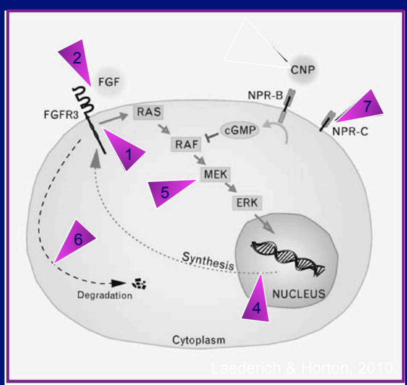
- Achondroplasia is the most frequent cause of dwarfism (1/15.000)
- *de novo* mutations of FGFR3, a tyrosine kinase receptor
- constitutive activation of a key regulator of endochondral ossification





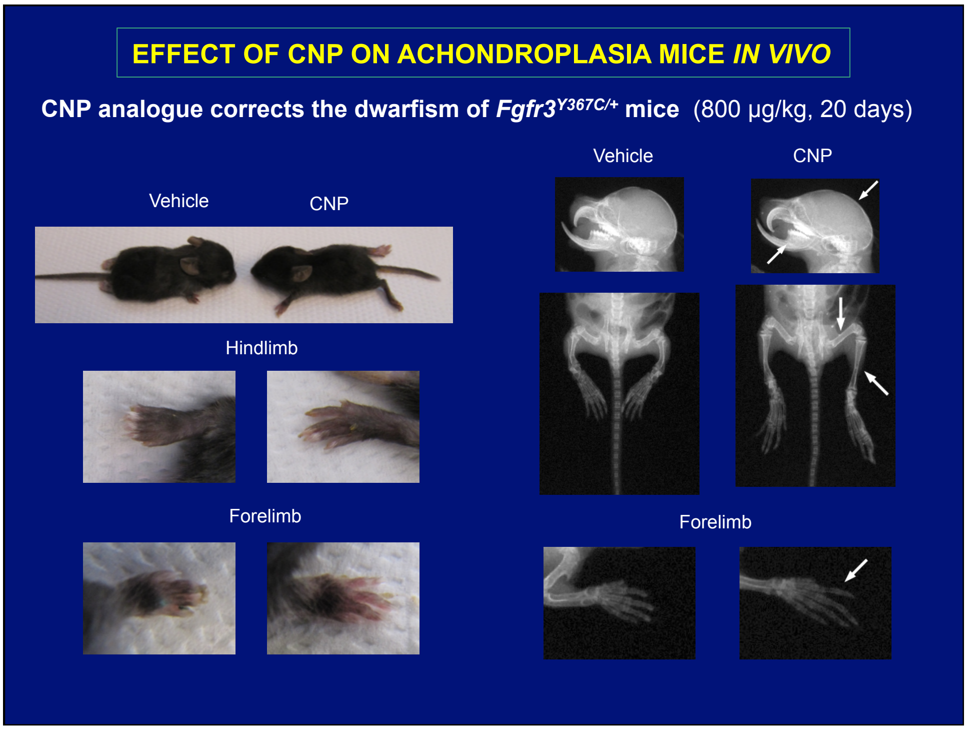
POTENTIAL CURATIVE TARGETS IN ACHONDROPLASIA

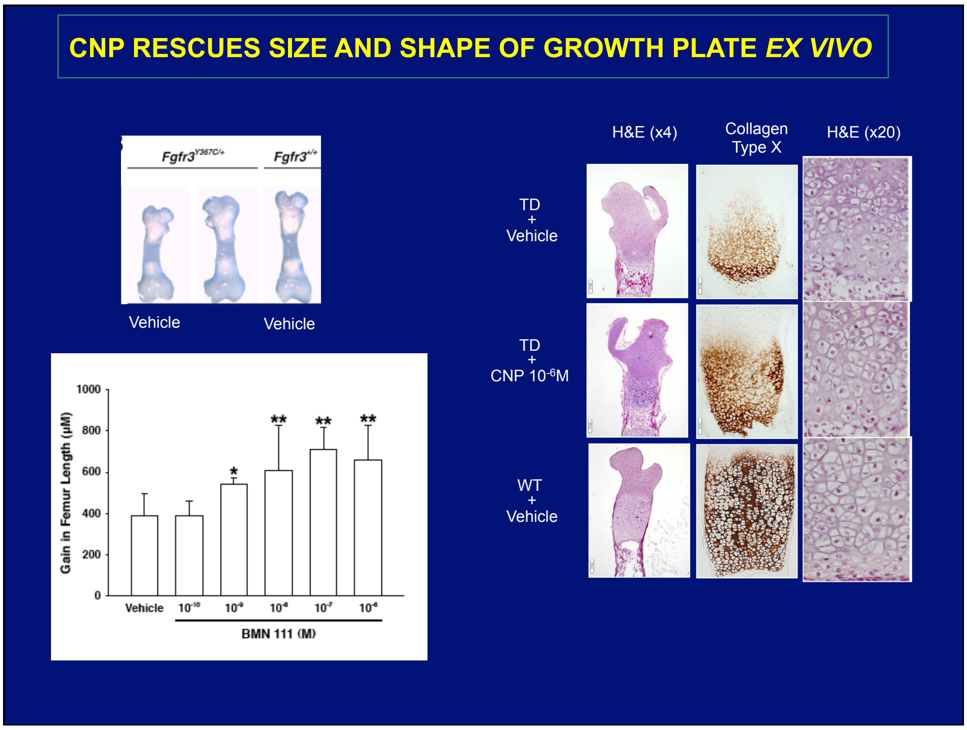
Laurence Legeai-Mallet



- FGFR3 TK INHIBITORS ?
- ANTI-FGFR3 ANTIBODIES?
- FGFR3 RNAi ?
- BLOCKADE OF DOWNSTREAM PATHWAY ?

  C-type natriuretic peptide

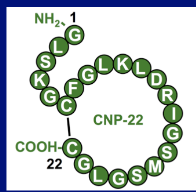

EFFECT OF CNP ON ACHONDROPLASIA MICE IN VIVO
CNP analogue corrects the dwarfism of Fgfr3Y367C/+ mice (800 µg/kg, 20 days)
Vehicle
CNP
Hindlimb
Forelimb
Vehicle
CNP
Forelimb


CNP RESCUES SIZE AND SHAPE OF GROWTH PLATE EX VIVO
Fgfr3Y367C/+
Fgfr3+/+
Vehicle
Vehicle
Gain in Femur Length (µM)
1000
800
600
400
200
0
Vehicle
10-10
10-9
10-8
10-7
10-6
BMN 111 (M)
H&E (x4)
Collagen Type X
H&E (x20)
TD + Vehicle
TD + CNP 10-6M
WT + Vehicle

CLINICAL TRIAL WITH C-type NATRIURETIC PEPTIDE (CNP)

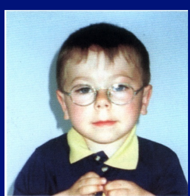
- CNP binds to its receptor (NPR-B) and inhibits the MAP kinase pathway
- engine analogue (39 aa) for systemic and growth plate delivery
- circulating half-life sufficient for once daily SC injection
- Phase 1 in healthy volonteers completed
- Phase 2 in Western Europe : january 2013

BIOMARIN



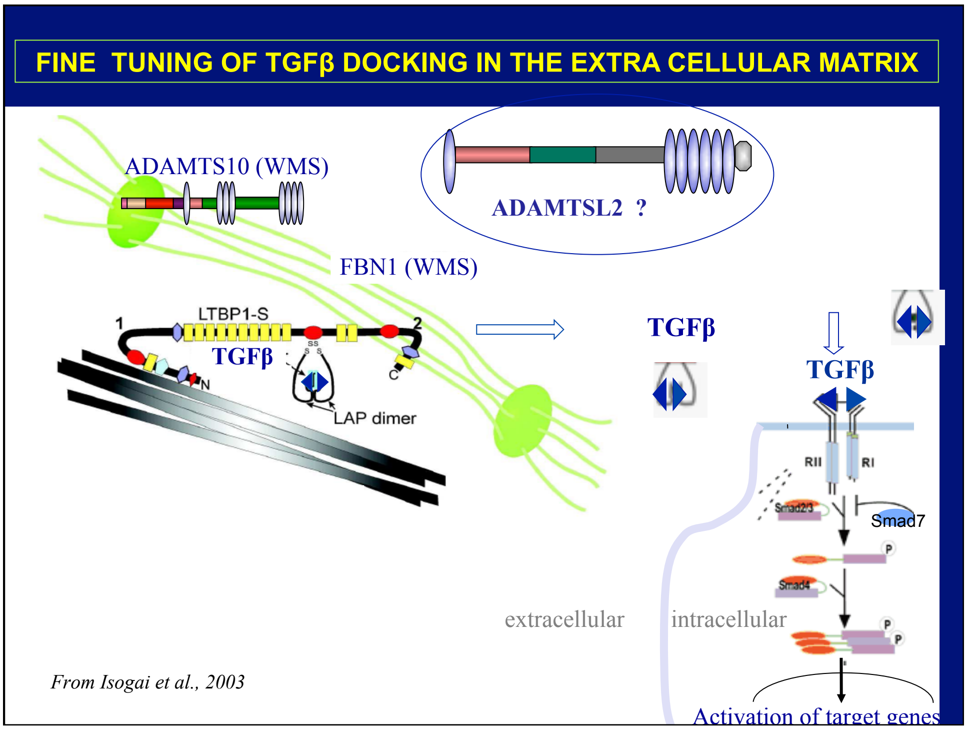
THE ACROMELIC DYSPLASIA GROUP

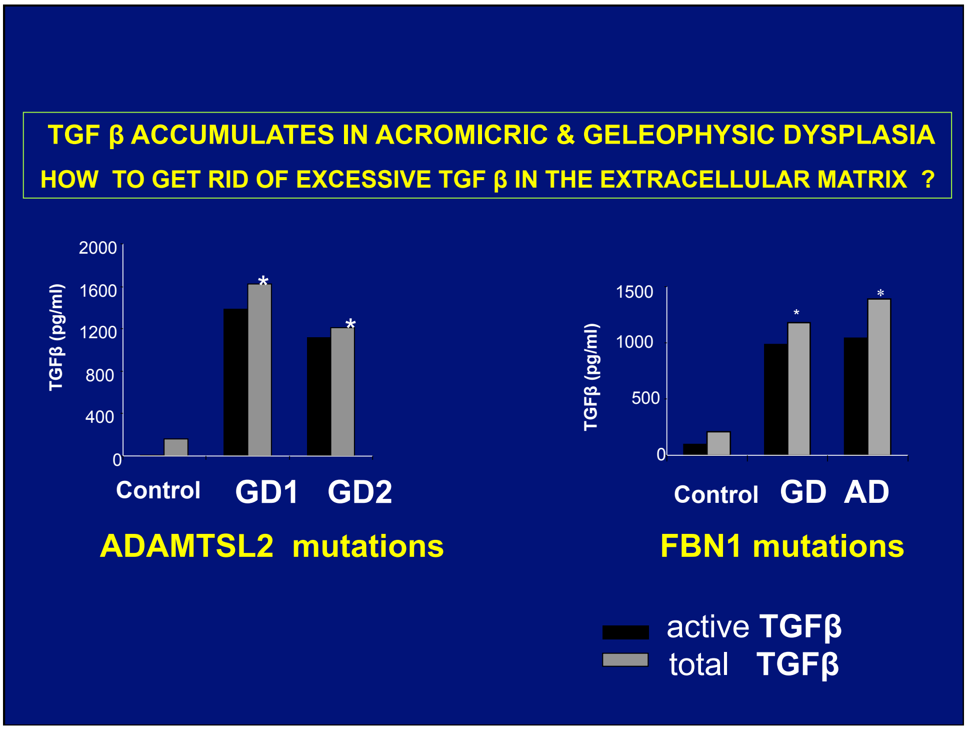
Valérie Cormier-Daire



Short Stature
< - 3 SD
Brachydactyly
Stiff joint
Thick skin
Muscular build

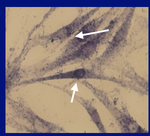
Geleophysic	Acromicric	Weill-Marchesani	Myhre
↓	↓	↓	↓
ADAMTSL2	*FBN1*	*ADAMTS 10*	*SMAD4*


FINE TUNING OF TGFβ DOCKING IN THE EXTRA CELLULAR MATRIX
ADAMTS10 (WMS)
ADAMTSL2 ?
FBN1 (WMS)
LTBP1-S
TGFβ
LAP dimer
TGFβ
TGFβ
RII
RI
Smad7
extracellular
intracellular
From Isogai et al., 2003
Activation of target genes


TGF β ACCUMULATES IN ACROMICRIC & GELEOPHYSIC DYSPLASIA
HOW TO GET RID OF EXCESSIVE TGF β IN THE EXTRACELLULAR MATRIX ?
TGFβ (pg/ml)
2000
1600
1200
800
400
0
Control GD1 GD2
ADAMTSL2 mutations
TGFβ (pg/ml)
1500
1000
500
0
Control GD AD
FBN1 mutations
active TGFβ
total TGFβ

TARGETING TGFB SIGNALING IN GELEOPHYSIC DYSPLASIA

- **Anti TGFβ Antibody ?**
- **Inhibitor of TGFβ signaling ?** *angiotensin II-receptor blockers, Losartan*



GD fibroblasts

↓

Analysis of the extracellular matrix

Mouse model

↓

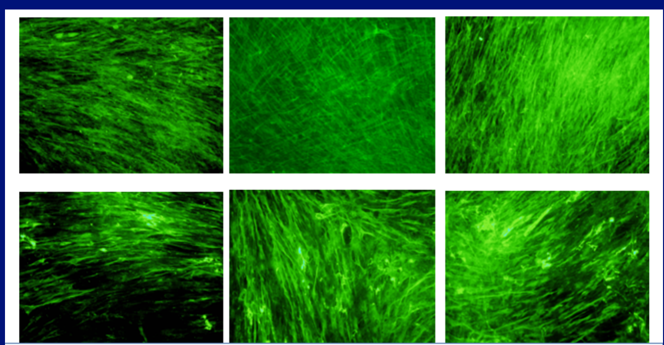
Lung, Heart

ANTI-TGF β ANTIBODIES IN GELEOPHYSIC DYSPLASIA FIBROBLASTS

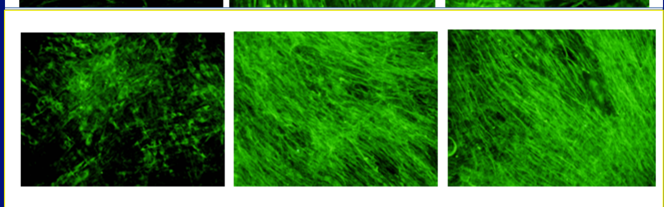
	No treatment	10µg/ml	20µg/ml

Control

Control



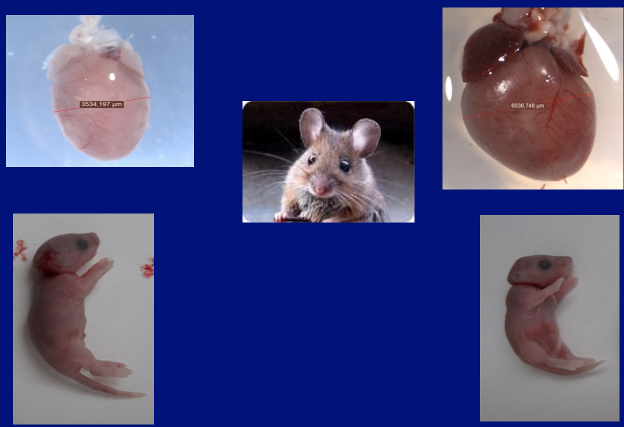
GD



AN ANIMAL MODEL OF ACROMICRIC & GELEOPHYSIC DYSPLASIA

ADAMTSL2 KO mice display heart involvement, early death and growth failure

WT — *Cre-CMV Adamtsl2* $^{f/f}$



MIMICKING mTOR INHIBITION IN TUBEROUS SCLEROSIS

- **Hamartine and Tuberine normally dimerize and inhibit the mTOR pathway**
- **Either gene is inactivated in Tuberous Sclerosis (TS)**
- **The mTOR pathway is no longer inhibited**
- **Rapamycin, an antibiotic, mimicks the inhibitory effects of hamartine/tuberin dimers**

- **Rapamycin prevents epilepsy in a mouse model of TS (*Ann Neurol 2008*)**
- **Rapamycin controls renal angiomyolipomes and facial angiofibroma in TS** ***(Am J Kidney Diseases 2006, Br J Dermatol 2008)***

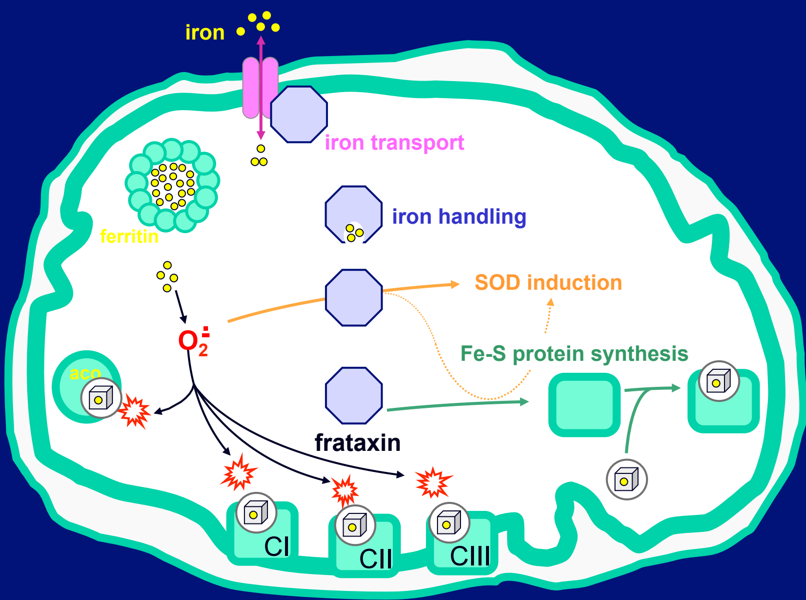
Agnès Rötig

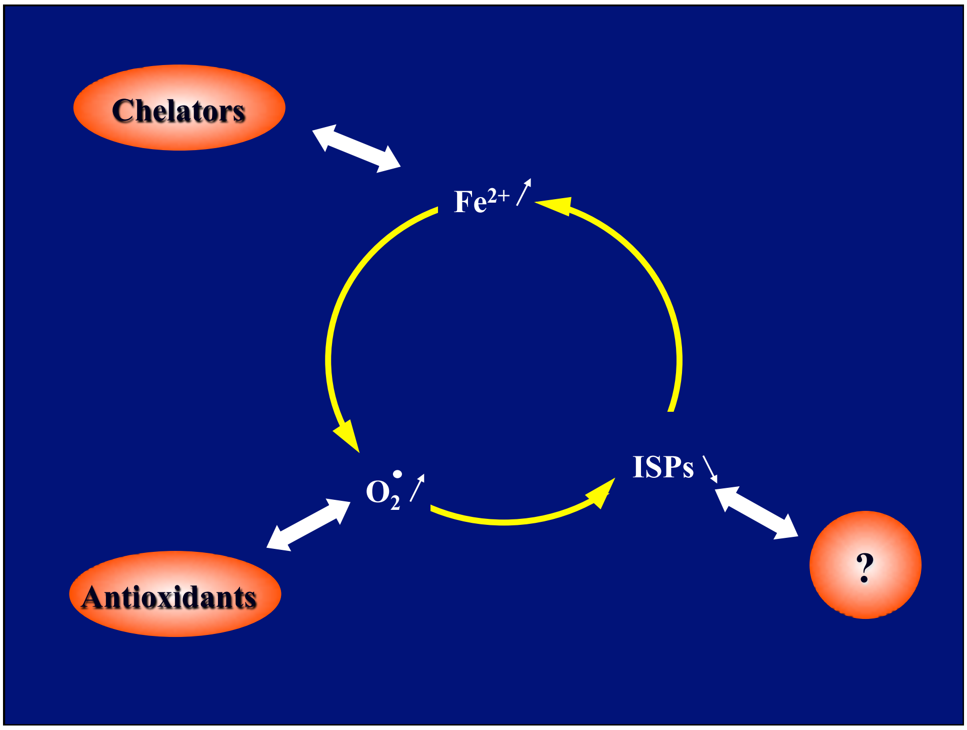
FRIEDREICH ATAXIA

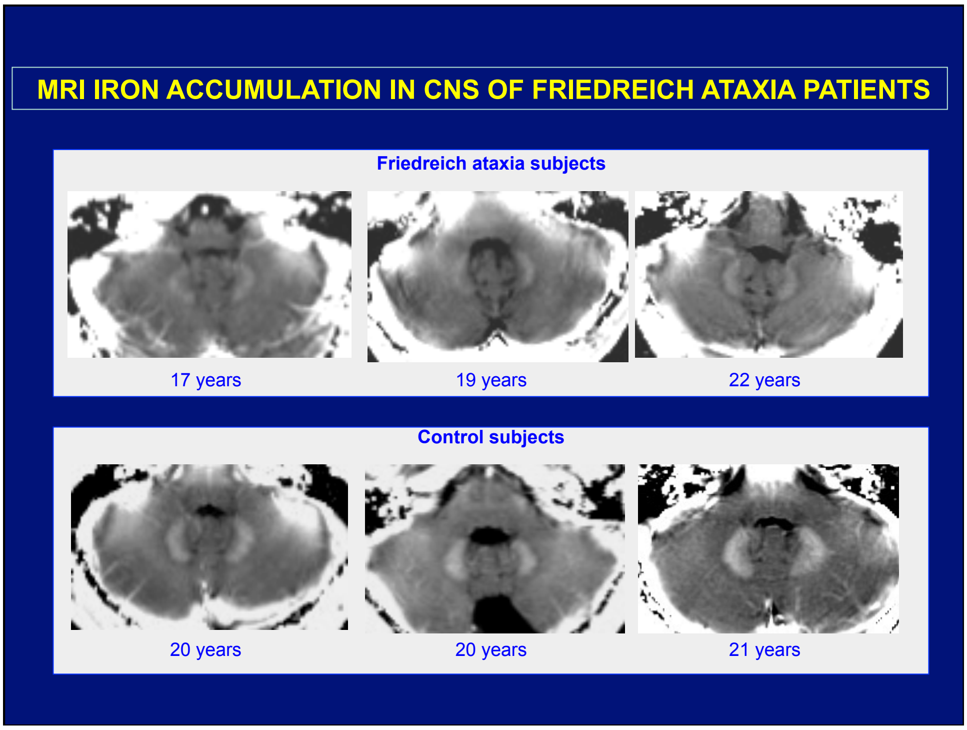
- frequent falls
- gait ataxia
- loss of gait
- cardiomyopathy
- Diabetes (10%)

- autosomal recessive
- incidence : 1/50,000
- gene : frataxin
- GAA expansion in intron 1

Possible functions of frataxin



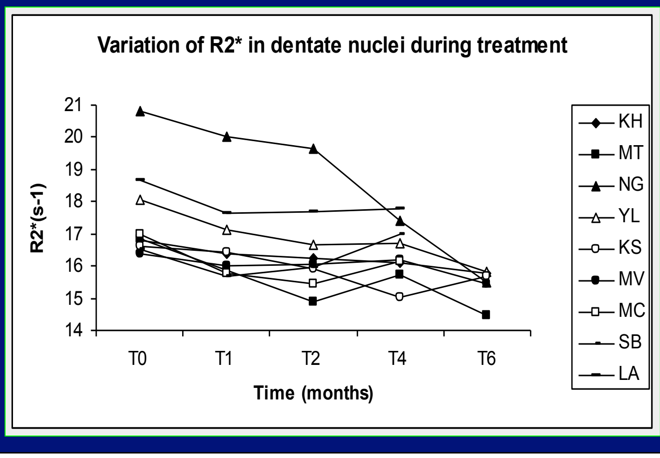

Chelators
Fe^{2+} ↗
$O_2^{\bullet -}$ ↗
ISPs ↘
?
Antioxidants


MRI IRON ACCUMULATION IN CNS OF FRIEDREICH ATAXIA PATIENTS
Friedreich ataxia subjects
17 years
19 years
22 years
Control subjects
20 years
20 years
21 years

BRAIN IRON CHELATION IN FRIEDREICH ATAXIA AND OTHER NBIA

- a brain-permeant chelator removes iron from dentate nuclei in FA
- benefits on gait and balance in the youngest (14yrs), still valid subjects
- unexpected clinical benefits in wheelchair-bound subjects
- coldness, foot pain, tremor, voice, incontinence
- ongoing multicentric randomized trial (*Apopharm, Toronto*)

Nathalie Boddaert



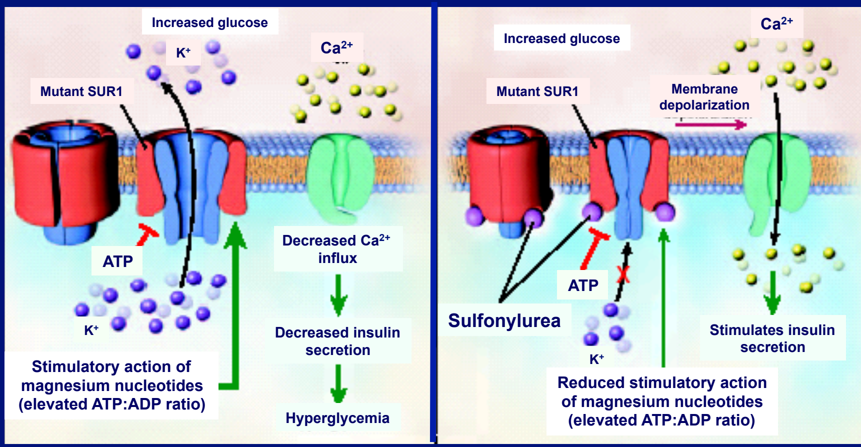
NEONATAL DIABETES MELLITUS AND K CHANNEL MUTATIONS

The Glidkir study, Hospital Necker-Enfants Malades

Michel Polak

- neonatal diabetes mellitus (DM): a life-long condition
- permanent and "transient" DM will need glucose lowering drugs
- DM + neurological issues (MR, epilepsy, hypotonia, dyspraxia)
- dominant SUR1 mutations account for a fraction of cases (~15%)
- treatable with oral hypoglycaemic agents

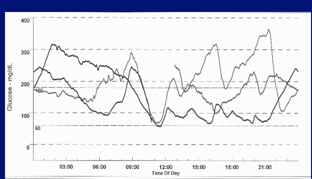
Mutant β Cell / Mutant β Cell in the Presence of Sulfonylurea



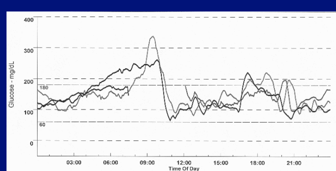
NEONATAL DIABETES MELLITUS AND K CHANNEL MUTATIONS

The Glidkir study, Hospital Necker-Enfants Malades

- 19 patients (2-19yrs) on glybenclamide, insulin discontinued
- results: excellent HbA1c control (6.4%), no side effects
- improvement of tonus, concentration, but not IQ
- future trial of French network : 88 subjects (*N Engl J Med 2006*)



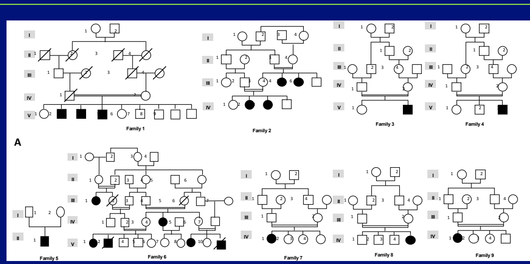
Insulin pomp



glybenclamide

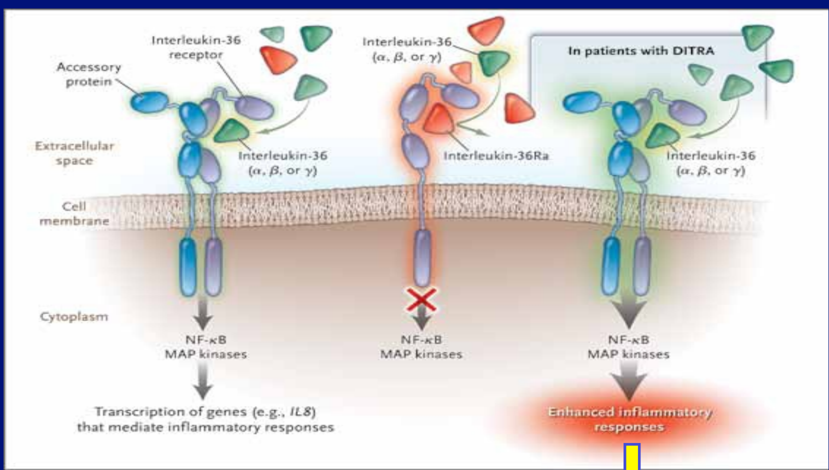
Asma Smahi

INTERLEUKINE 36-RECEPTOR ANTAGONIST DEFICIENCY IN GENERALISED PUSTULAR PSORIASIS



- repeated flares with sudden onset
- diffuse, erythematous skin-eruption, rapid coverage with pustules
- high fever (39- 41°C), elevated C-reactive protein levels, asthenia,

Marrakchi S, Guigue P et al, N Engl J Med. 2011

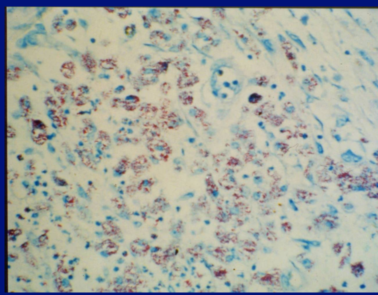


OVERPRODUCTION OF IL-1β

Successful treatment of GPP with the IL-1 receptor antagonist Anakinra
Viguier M et al. Ann Intern Med 2010

MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASES & TB

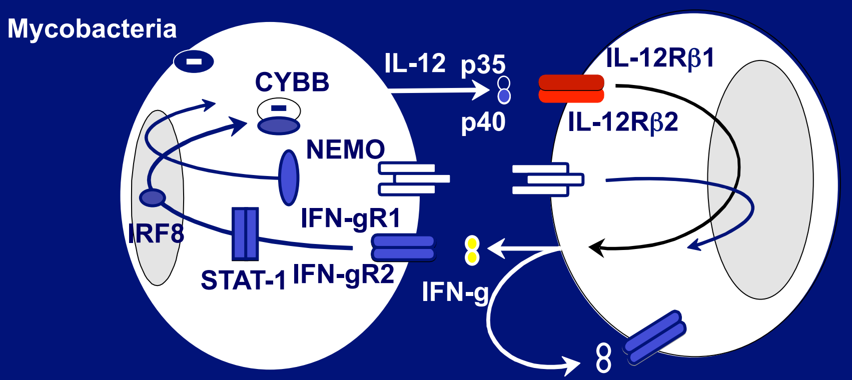
Jean-Laurent Casanova



Infections by BCG and environmental Mycobacteria

Otherwise healthy children - salmonellosis (~ 1/50,000)

TWO GENETIC CAUSES IN CHILDHOOD TB



Macrophage / Dendritic Cell NK / T Lymphocyte

MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASES

- Genetic defects of IFNγ synthesis cause mycobacterial infections (TB)

  → *IFNγ given to infected children (25-50ug/m2/day)*

- Genetic defects of cytokines IL17A and IL17F synthesis cause chronic cutaneomucous candidiasis

  → *should respond to recombinant G-CSF and GM-CSF*

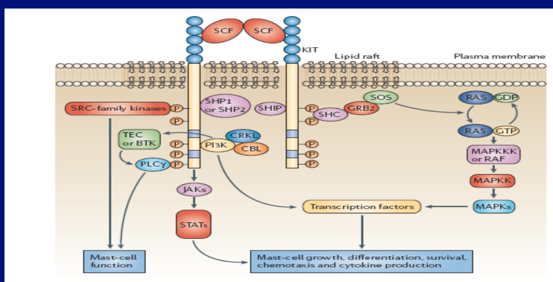
- Induction of IFNα/β via TLR3 causes Herpes encephalitis (HSV1)

  → *ongoing trial with recombinant IFNα in Herpes encephalitis*

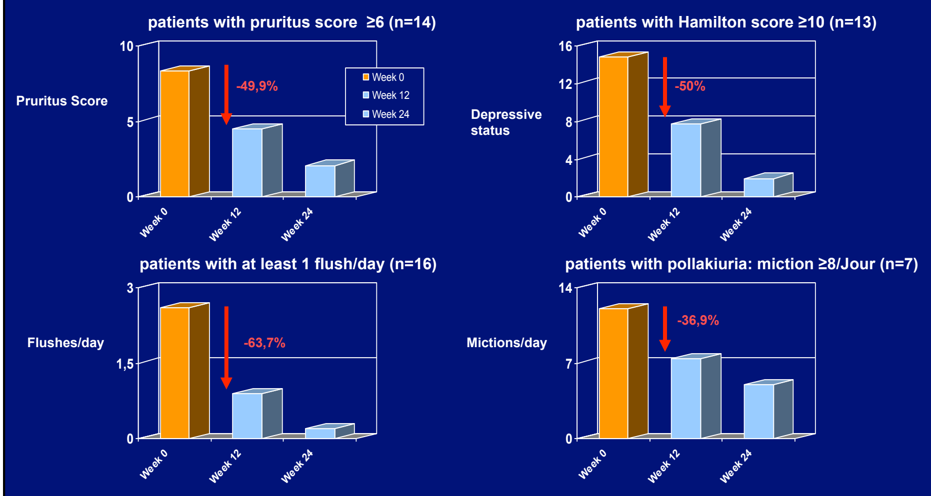
SOMATIC C-KIT MUTATIONS IN MASTOCYTOSIS

- a myeloproliferative disorder, cutaneous or systemic
- abnormal growth and accumulation of mast cells
- skin lesions, depression, memory loss, asthenia, pruritus
- muscle and joint pain, allergy, headache, dyspnea
- gain-of-function c-Kit mutations in a subset of BM cells

Olivier Hermine

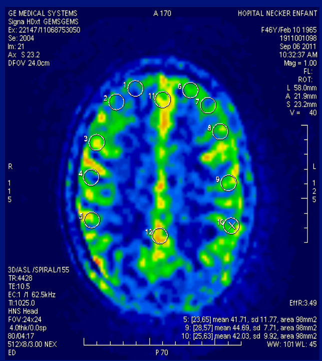


A C.KIT- SPECIFIC TYROSINE KINASE INHIBITOR, MASITINIB, IMPROVES COGNITIVE DYSFUNCTION IN MASTOCYTOSIS

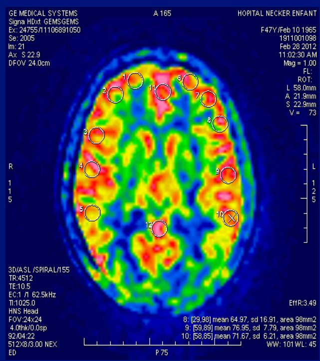


A C-KIT SPECIFIC TYROSINE KINASE INHIBITOR, MASITINIB, IMPROVES BRAIN PERFUSION IN MASTOCYTOSIS (ASL)

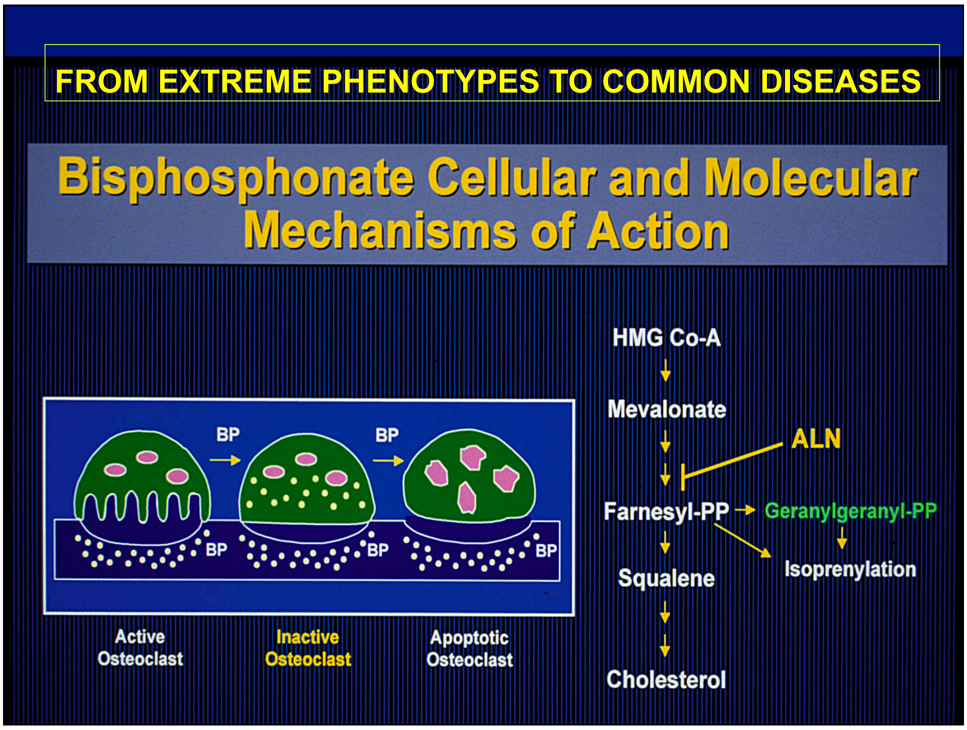
Nathalie Boddaert

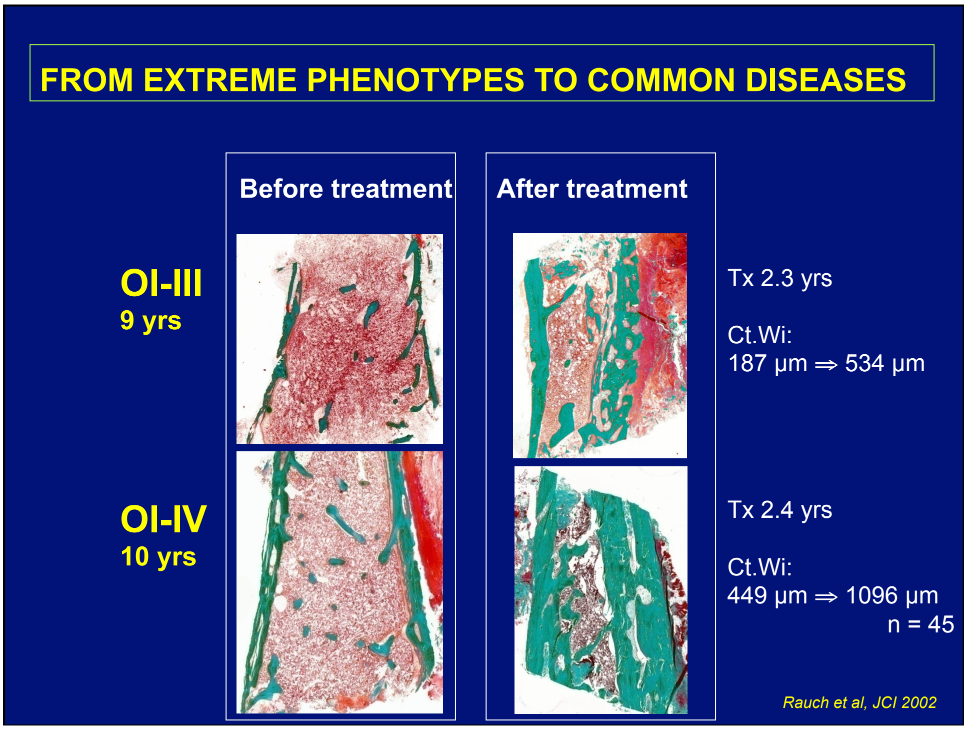


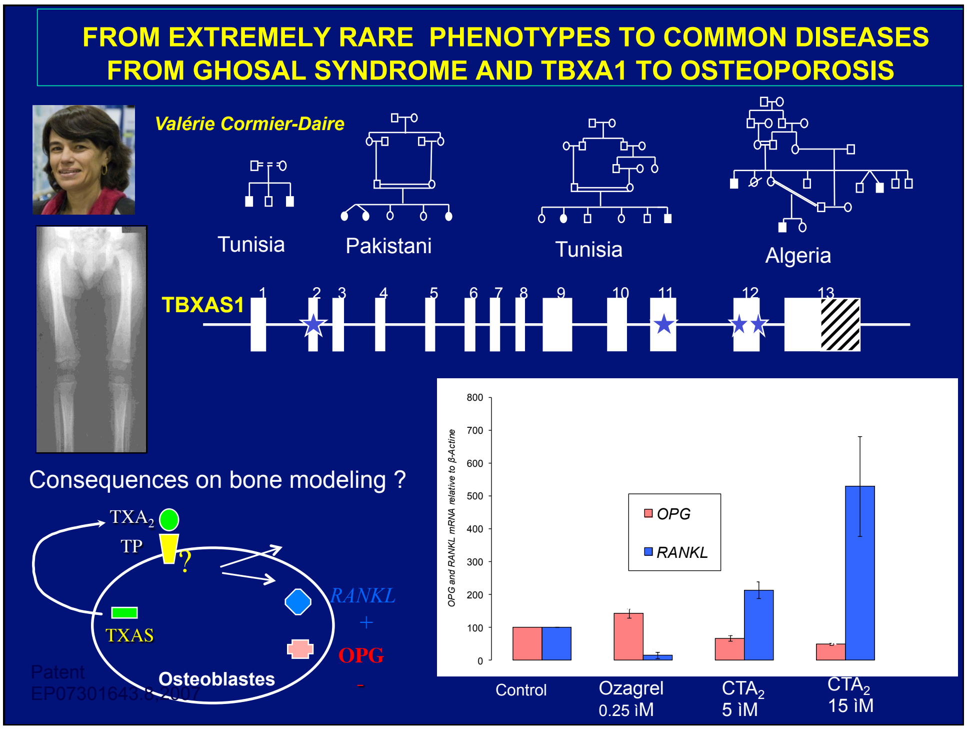
Before Masitinib



Six months after Masitinib


FROM EXTREME PHENOTYPES TO COMMON DISEASES
Bisphosphonate Cellular and Molecular Mechanisms of Action
BP
BP
BP
BP
BP
Active Osteoclast
Inactive Osteoclast
Apoptotic Osteoclast
HMG Co-A
Mevalonate
ALN
Farnesyl-PP
Geranylgeranyl-PP
Isoprenylation
Squalene
Cholesterol


FROM EXTREME PHENOTYPES TO COMMON DISEASES
Before treatment
After treatment
OI-III
9 yrs
Tx 2.3 yrs
Ct.Wi:
187 µm ⇒ 534 µm
OI-IV
10 yrs
Tx 2.4 yrs
Ct.Wi:
449 µm ⇒ 1096 µm
n = 45
Rauch et al, JCI 2002



PERSONALIZED GENOMICS IN TREATMENT OF METASTATIC CANCERS

- aim : to identify genomic signatures in an individual tumor and enable treatment by re-purposed or newly developed drugs
- tools : NGS and advanced bio-informatics
- . endpoint : tumor reduction (imaging) and clinical response in terms of survival and quality of life
- population: relapsed, refractory or newly diagnosed cases of metastatic disease, with no known curative therapy
- all patients/legal guardians must sign an IRB-approved form

Shah et al, Nature 2012

ADVANCES IN TREATMENT OF GENETIC DISEASES

Conclusion I

Many questions and concerns for genetics of the future !!!

- are clinical trials on very small series/single cases feasible?
- how to proceed from extremely rare to common diseases?
- how should academics and industry cooperate to maximize fertile interactions for fast drug discovery?
- yet, will our options still remain economically and ethically acceptable?
- how shall we preserve our values of frugality and solidarity?

ADVANCES IN TREATMENT OF GENETIC DISEASES

Conclusion II

Diagnosis and causality is what it is all about…

The challenge is not the Promethean dream to cure all diseases, but rather to identify what is possibly treatable…

One should beware of single thought, dogmatisms, and certainties. Science is pragmatism, not ideology….

«One cannot order a discovery…» Lavoisier

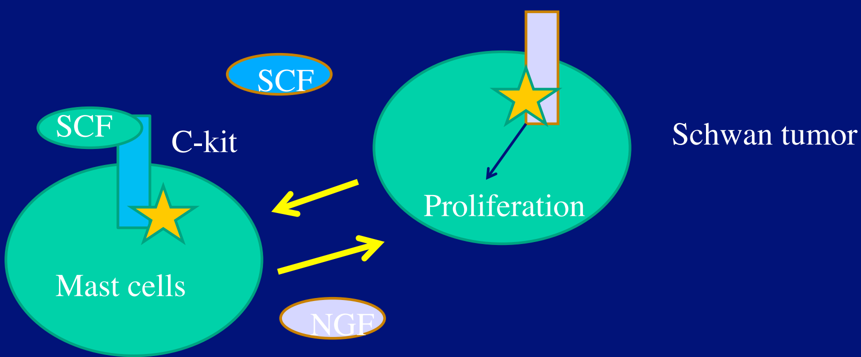
Let us learn from our past mistakes, keep our eyes open and not put all our eggs into the same basket …

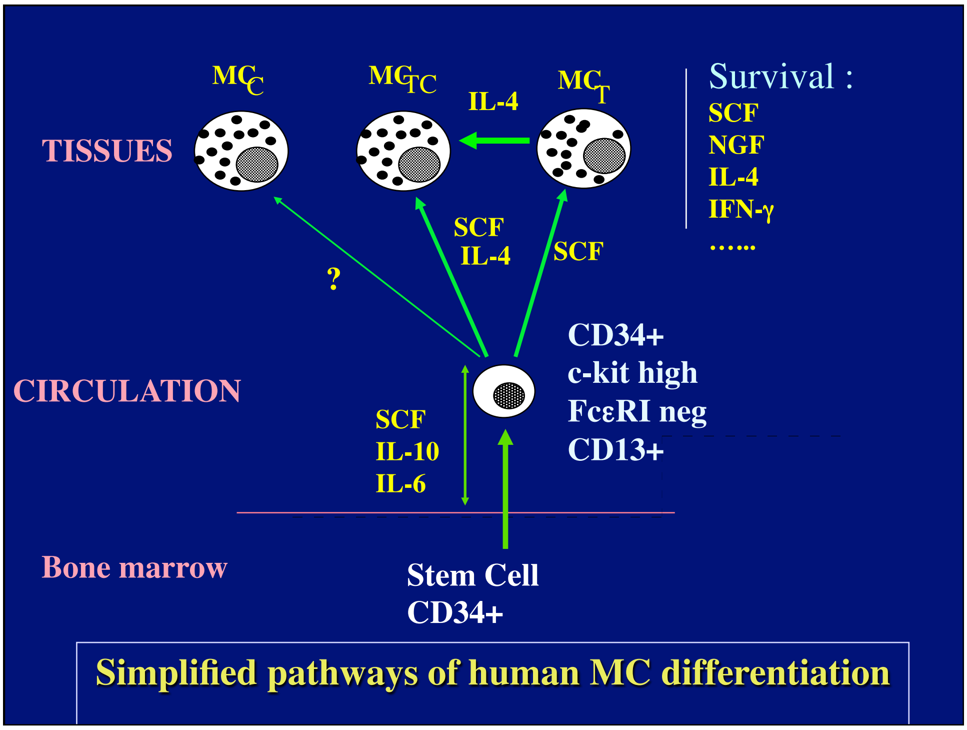
THANK YOU !!

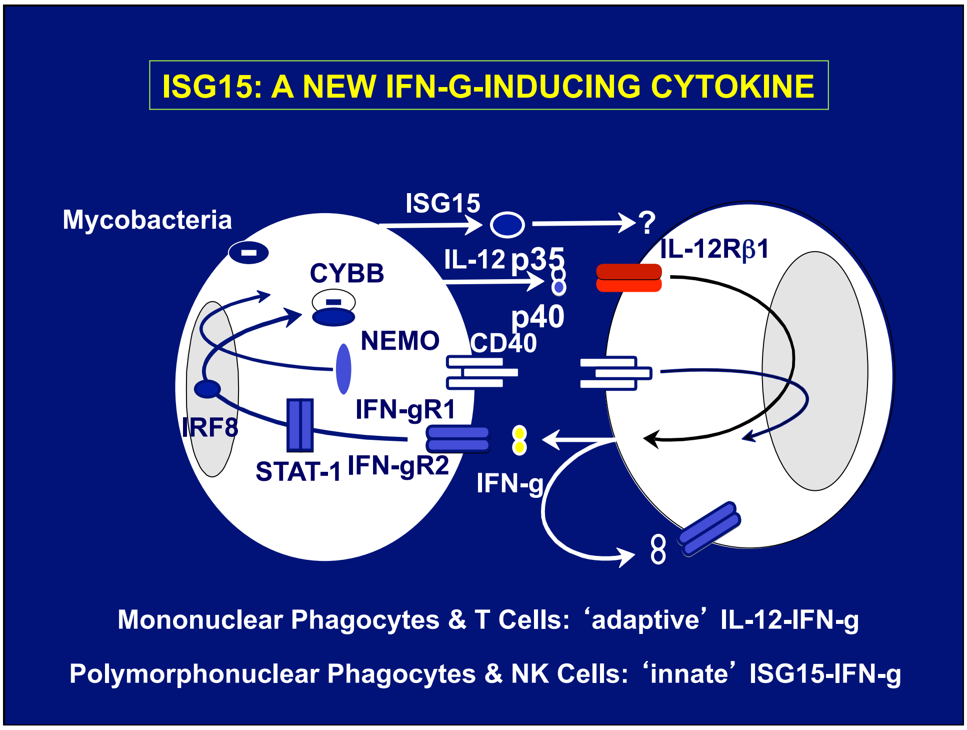
The Imagine Institute, Necker-Enfants Malades Hospital

Daytime view

Mast cells and Neurofibromatosis




MC_C
MC_TC
IL-4
MC_T
Survival :
SCF
NGF
IL-4
IFN-γ
……
TISSUES
SCF
IL-4
SCF
?
CD34+
c-kit high
FcεRI neg
CD13+
CIRCULATION
SCF
IL-10
IL-6
Bone marrow
Stem Cell
CD34+
Simplified pathways of human MC differentiation


ISG15: A NEW IFN-G-INDUCING CYTOKINE
Mycobacteria
ISG15
?
IL-12Rβ1
IL-12 p35
CYBB
p40
NEMO
CD40
IRF8
IFN-gR1
STAT-1
IFN-gR2
IFN-g
Mononuclear Phagocytes & T Cells: 'adaptive' IL-12-IFN-g
Polymorphonuclear Phagocytes & NK Cells: 'innate' ISG15-IFN-g

IDENTIFICATION OF ADAMTSL2 PARTNERS BY YEAST TWO-HYBRID

Latent TGFβ Binding Protein 1 (LTBP1) is a partner of ADAMTSL2