CENTER FOR RESEARCH IN FOP AND RELATED DISORDERS

THE UNIVERSITY OF PENNSYLVANIA

FOP ITALIA

Cerignola, Italy
March 20-21, 2009

FOP Mission

- Cause
- Cure

From Cause to Cure

- MUTATIONS
- MECHANISMS
- MODELS
- MEDICINES

From Cause to Cure

MUTATIONS

- Discovered the FOP gene
- Identified major clinical and molecular variants of FOP

From Cause to Cure

MECHANISMS

- Demonstrated that the mutant FOP receptor has leaky BMP signaling at rest and hyper-responsive BMP signaling when triggered by inflammatory signals in the cellular microenvironment

- Unveiled a key co-conspiratory protein,FKBP12, that binds less efficiently to the FOP fuse and permits leaky signaling in the absence of BMPs

From Cause to Cure

MECHANISMS

- Recognized that circulating monocytes and tissue macrophages are critical inflammatory triggers of FOP flare- ups

- Revealed progenitor cells of vascular origin that contribute to every stage of the FOP lesion

From Cause to Cure

MECHANISMS

- Modeled the structure of the mutant protein encoded by the FOP gene and identified a previously unrecognized and unstable switch enabled by the FOP mutation.

- Showed that hypoxia dramatically enhances BMP signaling in FOP cells

From Cause to Cure

MODELS

- Developed a Stem Cell Model for FOP using discarded baby teeth
- Rescued a lethal ACVR1 knockout in zebrafish with the mutant FOP gene and thereby demonstrated functional over-activity of the FOP gene in an animal model
- Developed a chimeric mouse model of FOP

From Cause to Cure

MEDICINES

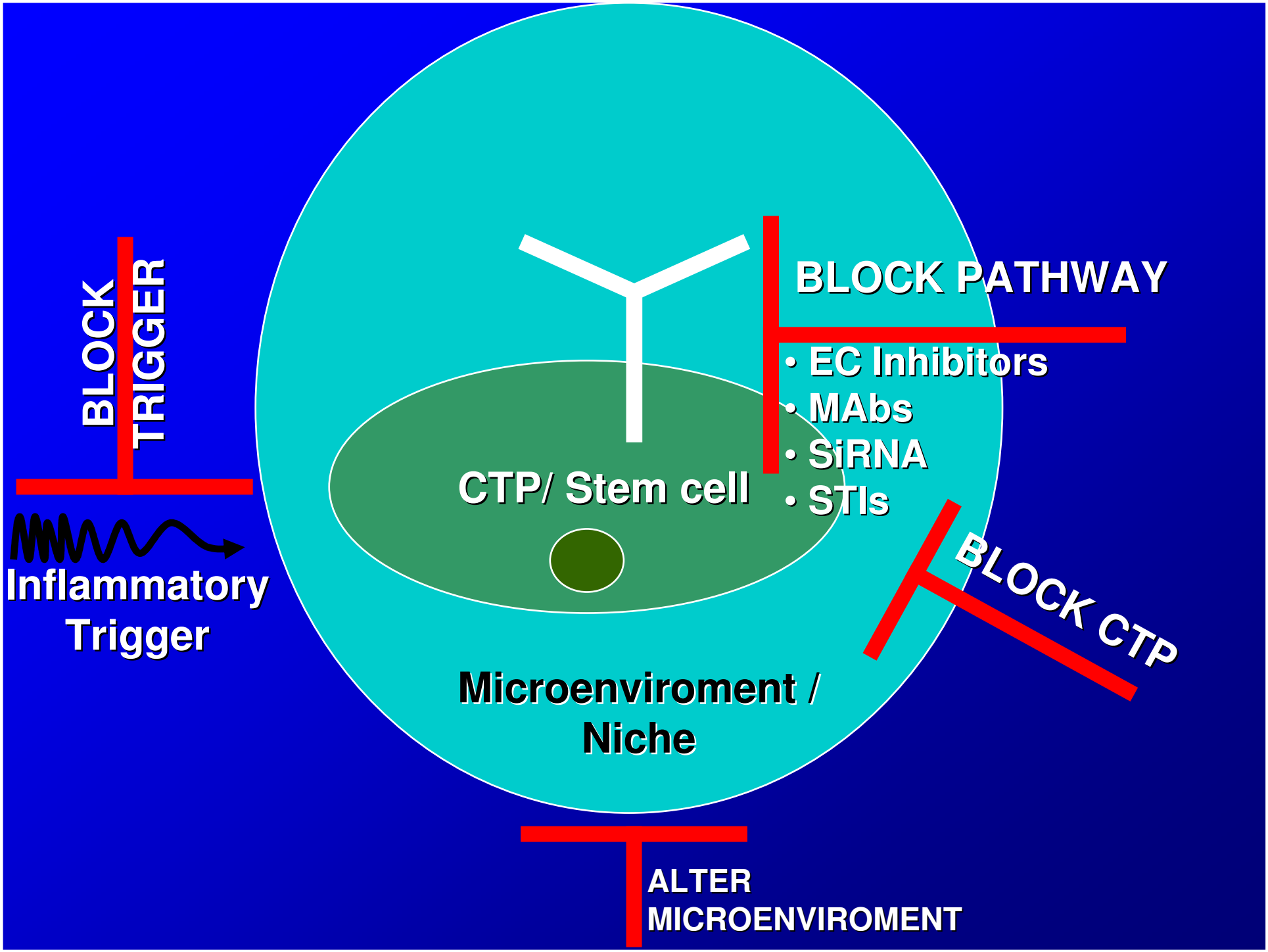
- Identified a class of compounds that inhibit FOP-like lesions in animal models and that may serve as a basis for future drug development

- Re-wrote the FOP Treatment Guidelines (www.ifopa.org)

April 14,1736

" There came a boy of healthy look,
and about 14 years of age,
to ask us at the hospital,
what should be done to cure him
of the many large swellings on his back,
which began 3 years since,
and have continued to grow as large
on many parts as a penny- loaf,
particularly on the left side.
They arise from all the vertebrae of the neck,
and reach down to the os sacrum.
They likewise arise from every rib of the body,
and joining together in all parts of his back,
as the ramifications of coral do,
they make, as it were, a fixed bony pair
of bodice. "

-John Freke, Ophthalmologist
St. Bartholomew's Hospital, London
Philos. Trans. Royal. Society, 1740


BLOCK
TRIGGER
Inflammatory
Trigger
BLOCK PATHWAY
• EC Inhibitors
• MAbs
• SiRNA
• STIs
CTP/ Stem cell
BLOCK CTP
Microenviroment /
Niche
ALTER
MICROENVIROMENT

1.

Hematopoietic Stem-Cell Contribution to Ectopic Skeletogenesis

By Frederick S. Kaplan, MD, David L. Glaser, MD, Eileen M. Shore, PhD, Robert J. Pignolo, MD, PhD, Meiqi Xu, BS, Yi Zhang, MD, PhD, David Senitzer, PhD, Stephen J. Forman, MD, and Stephen G. Emerson, MD, PhD

Investigation performed at the Center for Research in Fibrodysplasia Ossificans Progressiva and Related Disorders, Department of Orthopaedic Surgery, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, and the Division of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center, Duarte, California

2.

STEM CELLS®

TISSUE-SPECIFIC STEM CELLS

Dysregulation of Local Stem/Progenitor Cells as a Common Cellular Mechanism for Heterotopic Ossification

Lixin Kan[1*], Yijie Liu[1], Tammy L. McGuire[1], Diana M. Palila Berger[2], Rajeshwar B. Awatramani[1], Susan M. Dymecki[3], John A Kessler[1]

3.

nature medicine

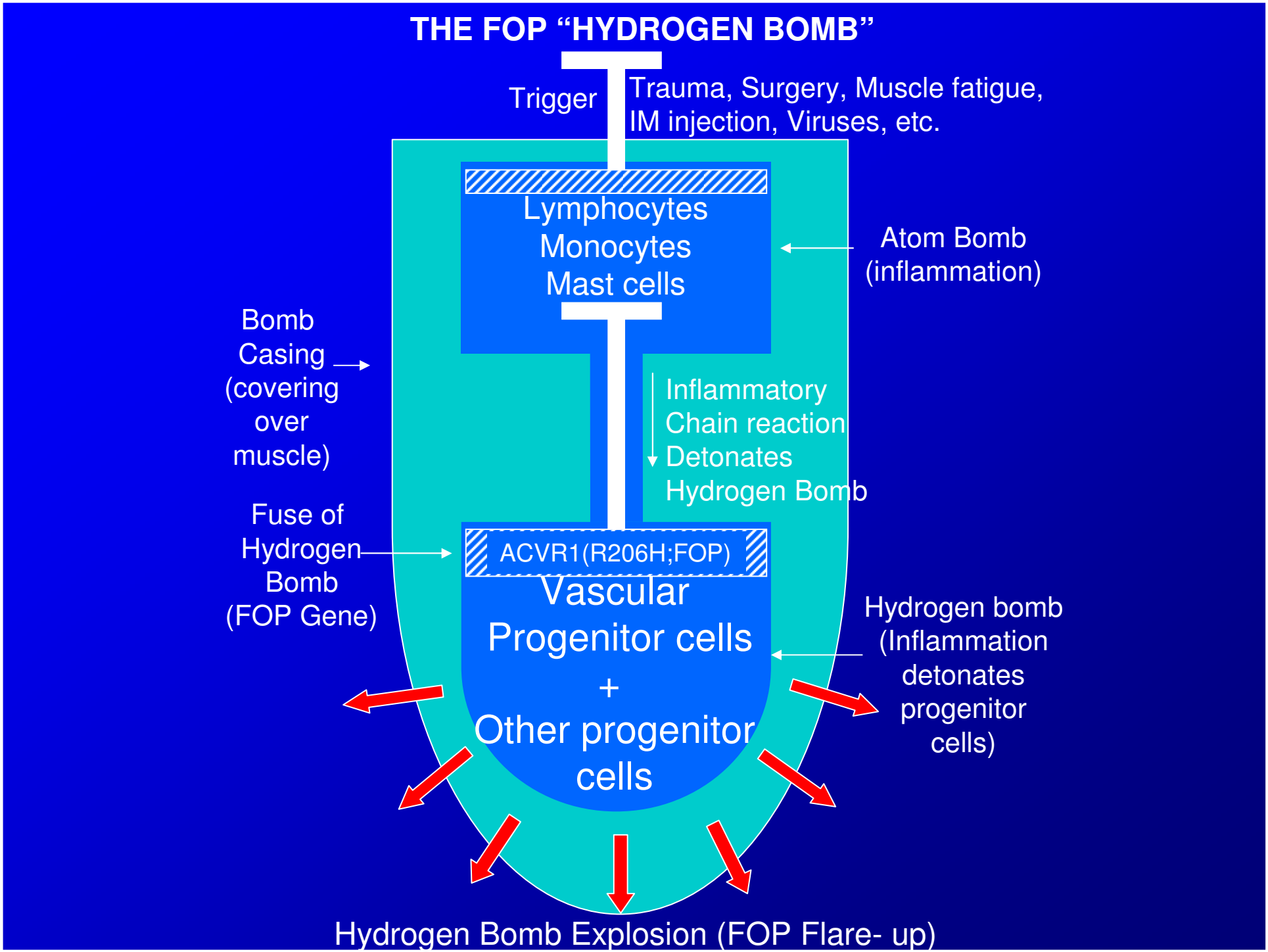
BMP type I receptor inhibition reduces heterotopic ossification

Paul B Yu[1,2], Donna Y Deng[1], Carol S Lai[1], Charles C Hong[3], Gregory D Cuny[4], Mary L Bouxsein[5], Deborah W Hong[1], Patrick M McManus[1], Takenobu Katagiri[6], Chetana Sachidanandan[1], Nobuhiro Kamiya[7], Tomokazu Fukuda[7], Yuji Mishina[7–9], Randall T Peterson[1,9] & Kenneth D Bloch[1,2]

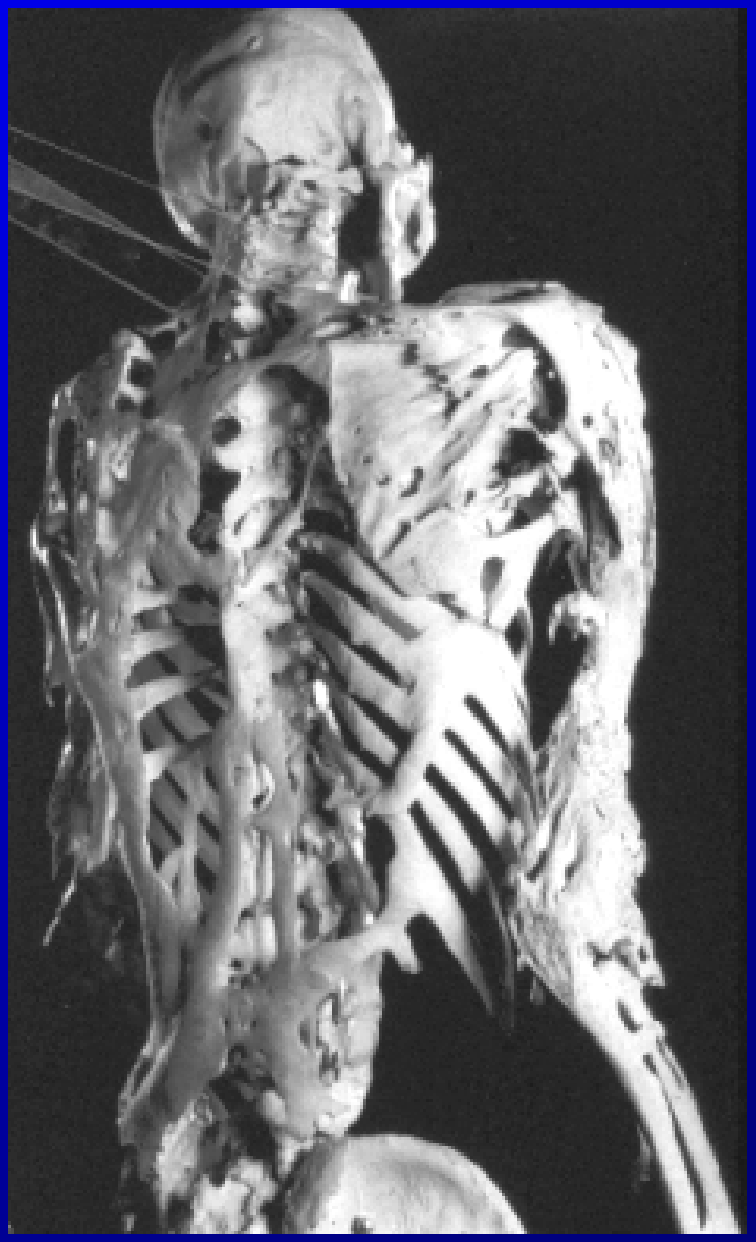
4.

Identification of Progenitor Cells That Contribute to Heterotopic Skeletogenesis

By Vitali Y. Lounev, PhD, Rageshree Ramachandran, MD, PhD, Michael N. Wosczyna, BS, Masakazu Yamamoto, PhD, Andrew D.A. Maidment, PhD, Eileen M. Shore, PhD, David L. Glaser, MD, David J. Goldhamer, PhD, and Frederick S. Kaplan, MD


THE FOP "HYDROGEN BOMB"
Trigger
Trauma, Surgery, Muscle fatigue, IM injection, Viruses, etc.
Lymphocytes
Monocytes
Mast cells
Atom Bomb (inflammation)
Bomb Casing (covering over muscle)
Inflammatory Chain reaction Detonates Hydrogen Bomb
Fuse of Hydrogen Bomb (FOP Gene)
ACVR1(R206H;FOP)
Vascular Progenitor cells
+
Other progenitor cells
Hydrogen bomb (Inflammation detonates progenitor cells)
Hydrogen Bomb Explosion (FOP Flare- up)

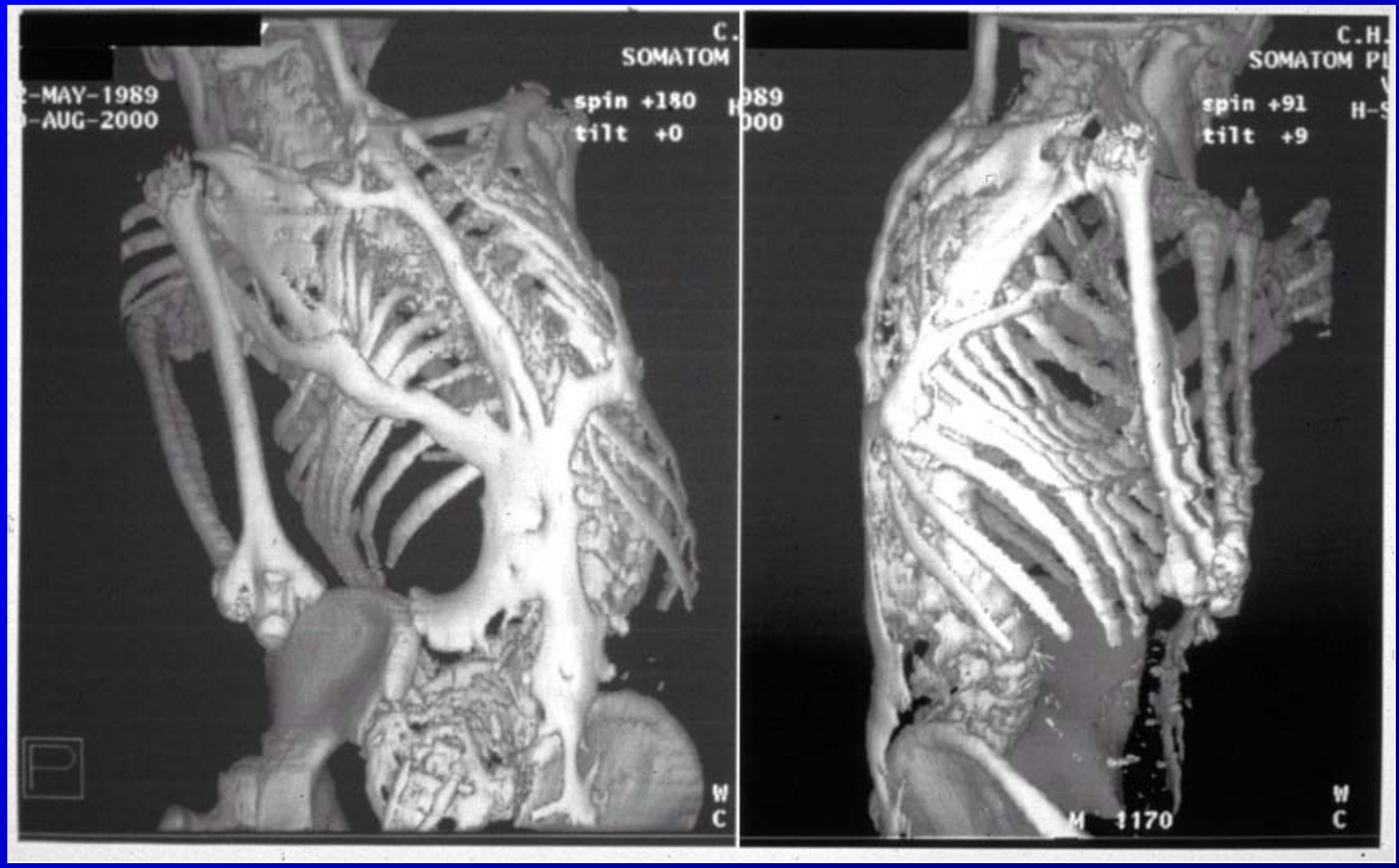
Cells That Build A Second Skeleton



Clinical Features of FOP

Early FOP Lesions

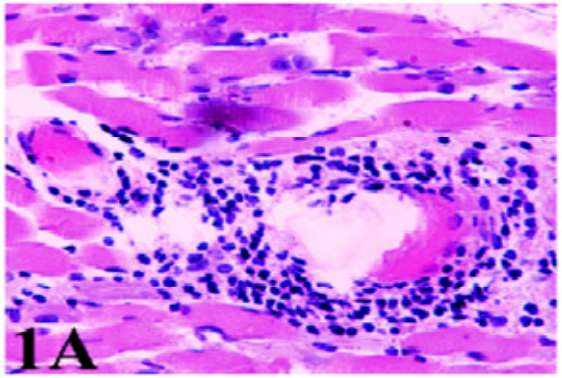
Progressive Heterotopic Skeletogenesis



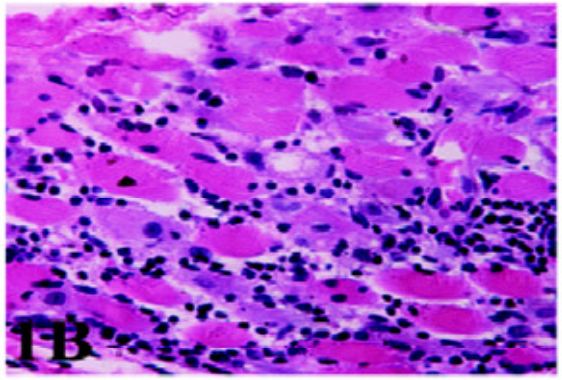
Catastrophic Misdiagnosis In FOP

FOP is a Metamorphosis

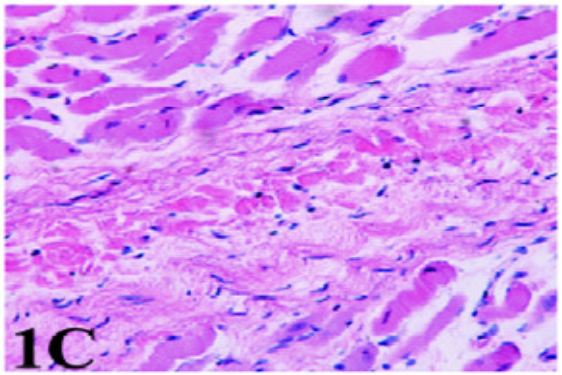
Perivascular Lymphocytic Infiltration



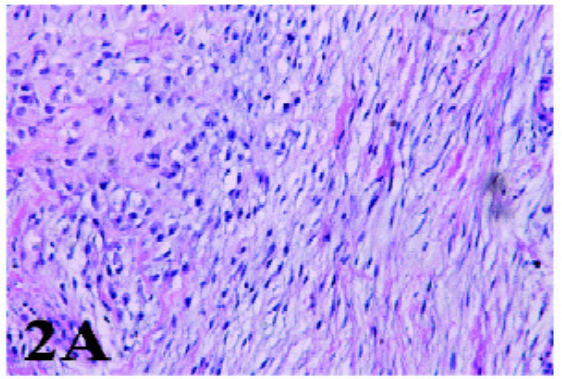
Intramuscular Lymphocytic Infiltration



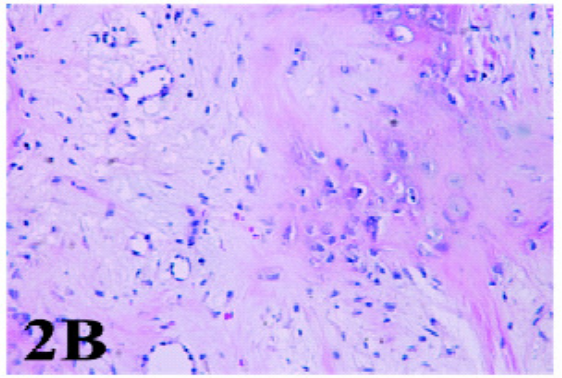
Muscle Degradation



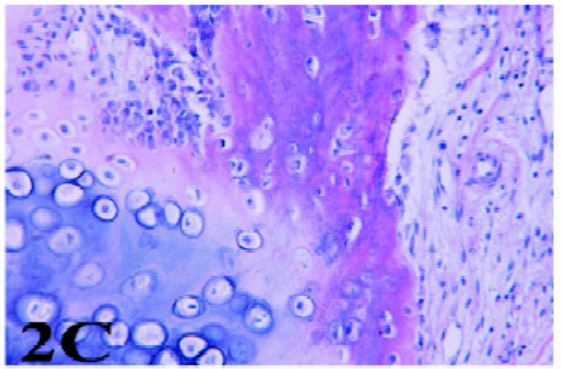
Fibro-Proliferation/ Angiogenesis



Chondrocyte Condensation



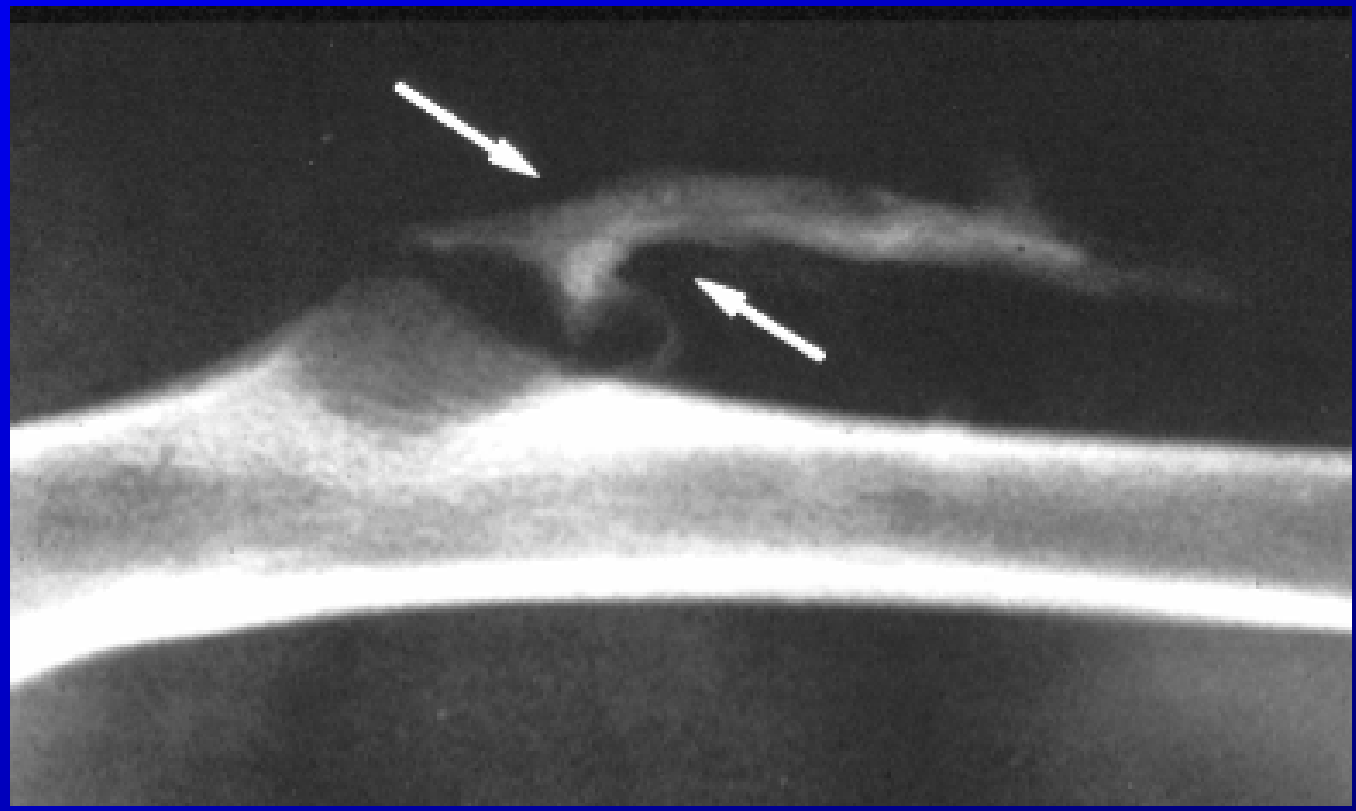
Endochondral Ossification



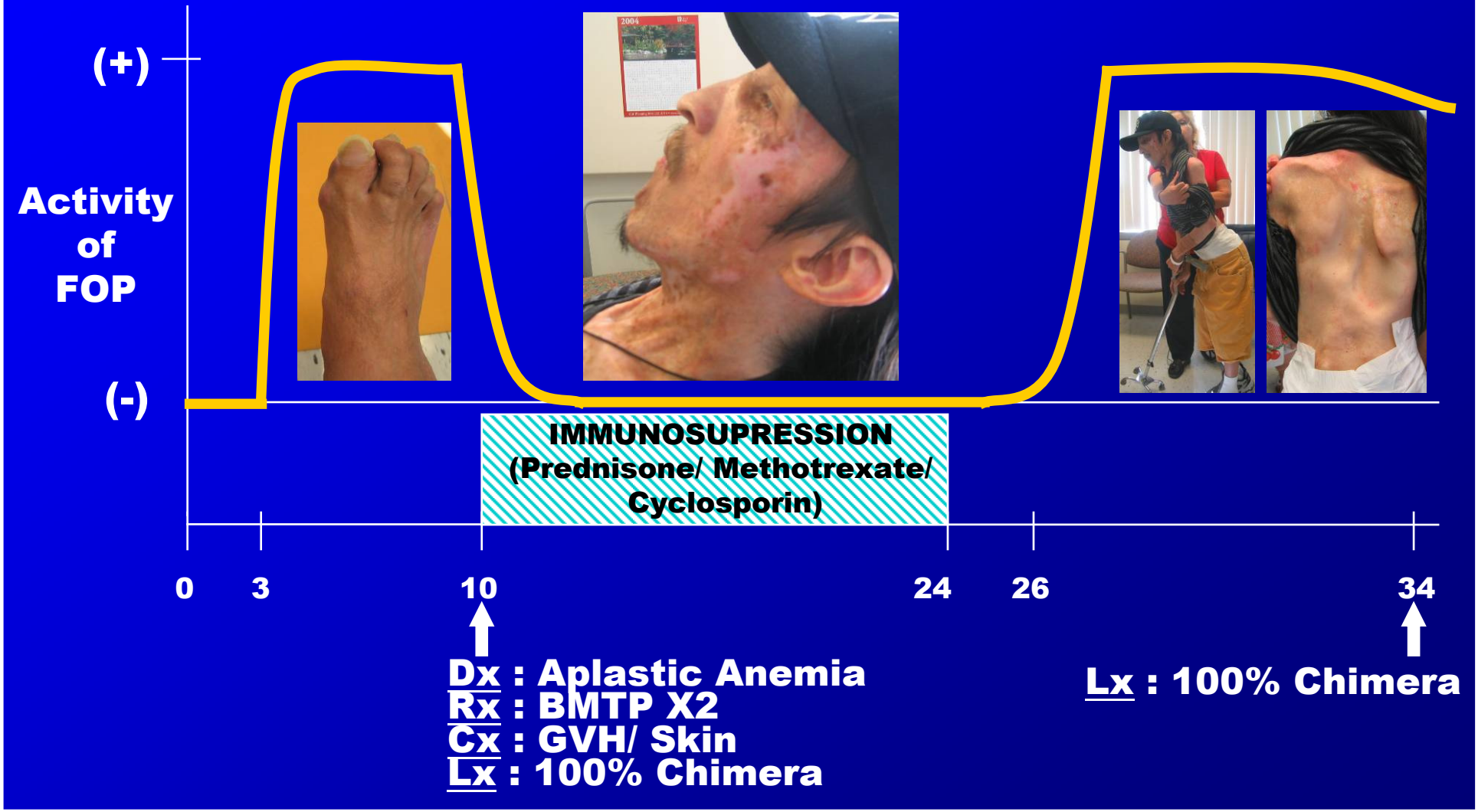
Permanent heterotopic ossification at the injection site after diphtheria-tetanus-pertussis immunizations in children who have fibrodysplasia ossificans progressiva

Thomas F. Lanchoney, BS, Randolph B. Cohen, MD, David M. Rocke, PhD, Michael A. Zasloff, MD, PhD, and Frederick S. Kaplan, MD

From the Departments of Orthopedic Surgery, Pediatrics, and Genetics, University of Pennsylvania School of Medicine, Philadelphia, and the Graduate School of Management, University of California, Davis



- **BMT Does NOT Cure FOP**
- **Even a Normal Immune System Can Trigger FOP in a Genetically Susceptible Host**



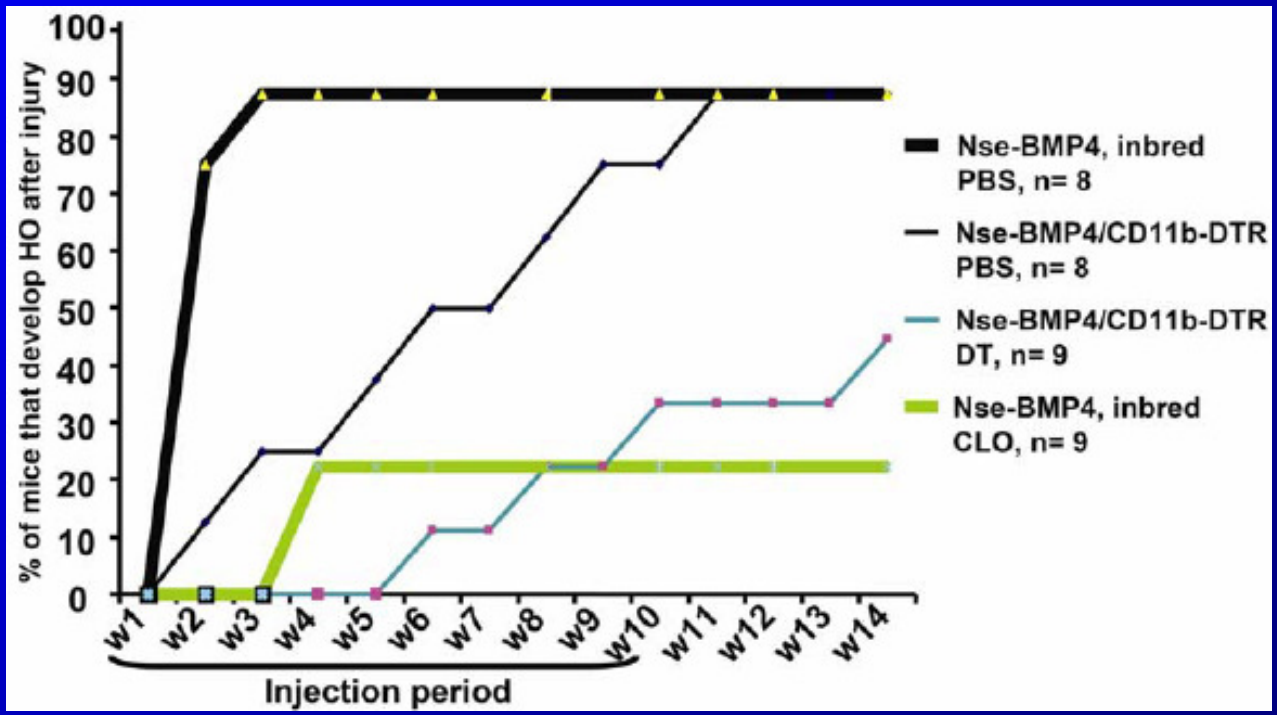
$E= MC^2$ for The Skeleton

Inflam

(Trigger)

FOP= ACVR1(c.617 G>A; R206H)

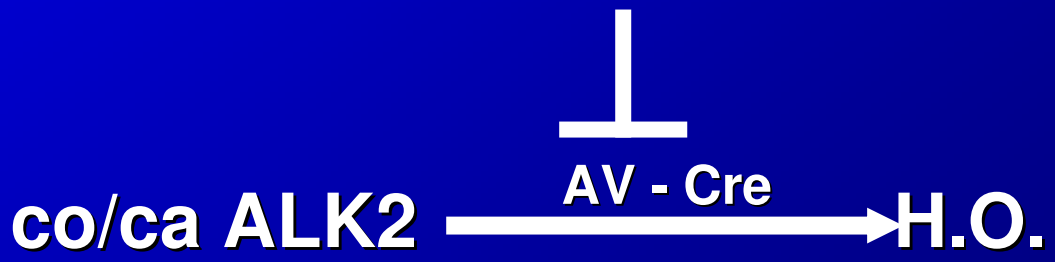
Macrophages Stimulate H.O. In NSE-BMP4 Mice



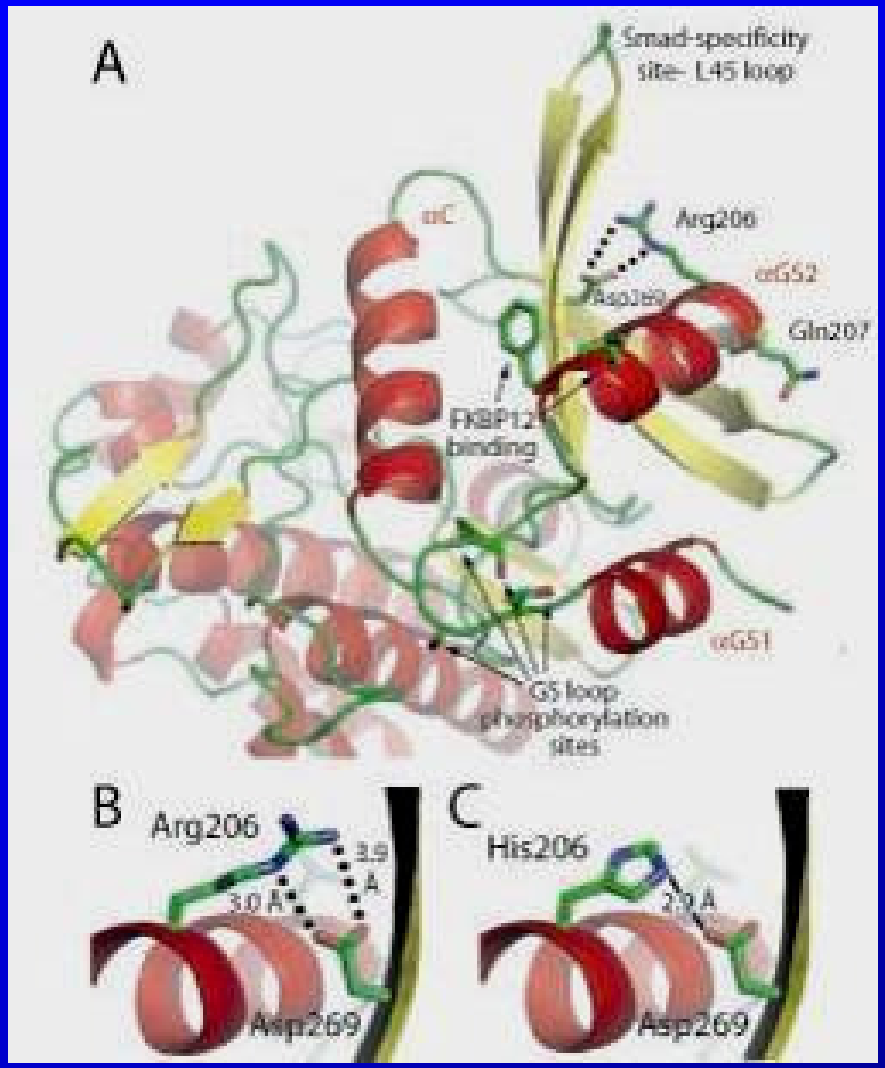
Inflammation Triggers H.O. in The Setting of Increased BMP Pathway Activity

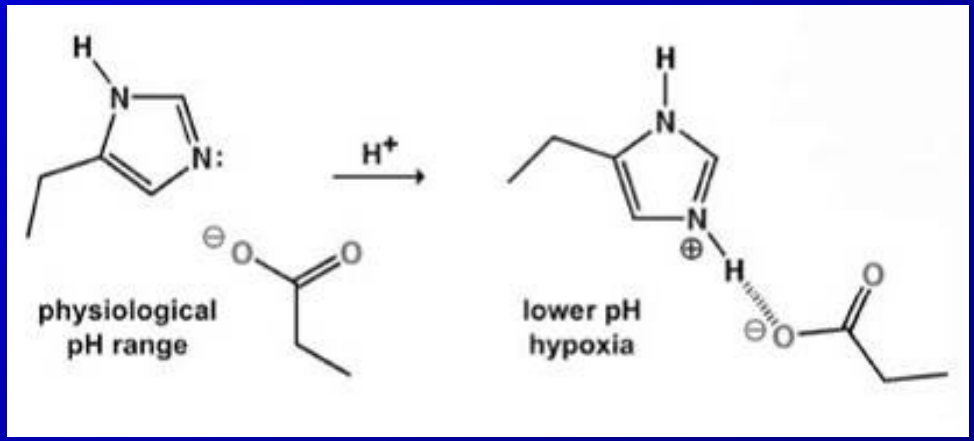
MØ – inhibitors (induction)
Lymphocyte inhibitors (propogation)

Corticosteroids;
Dm - derivatives

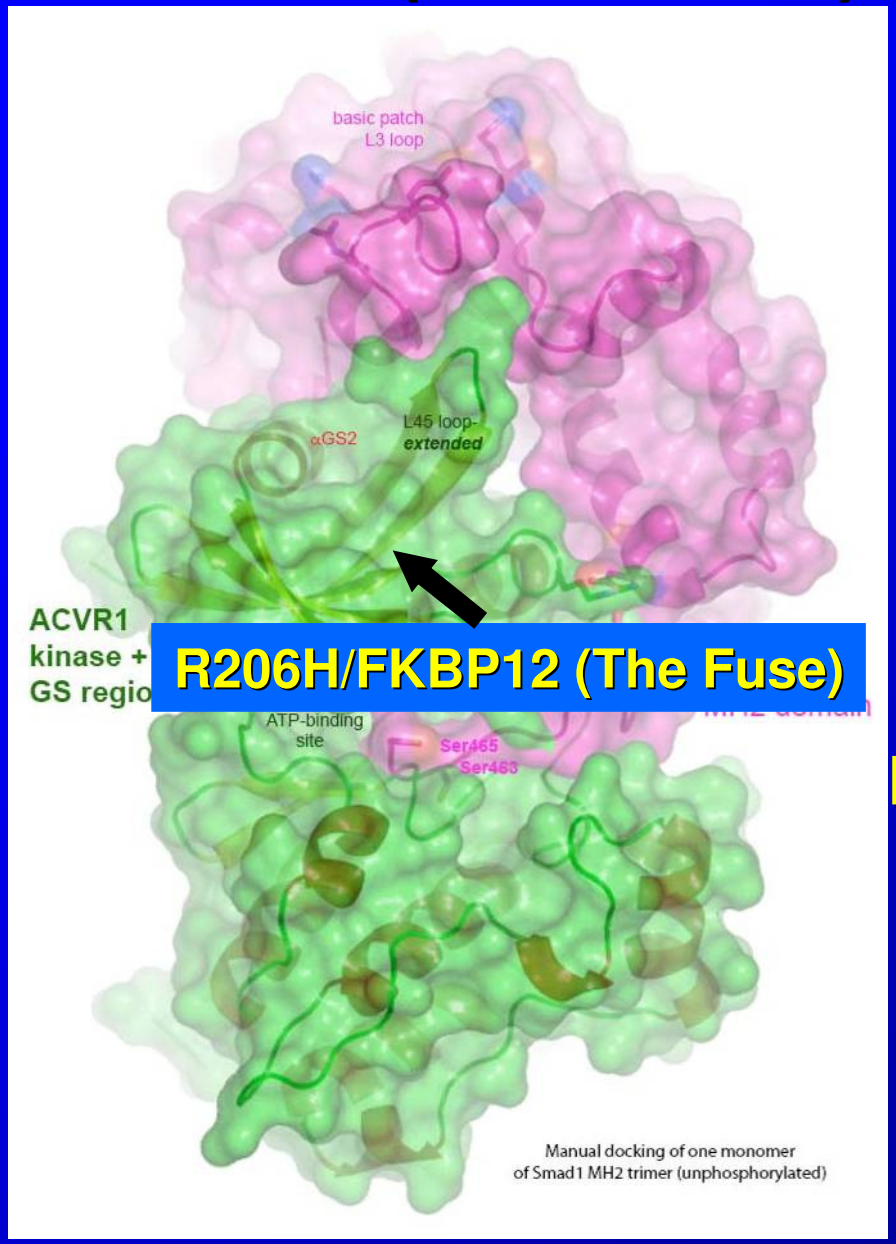


ACVR1 (R206H): A pH – Sensitive Switch?





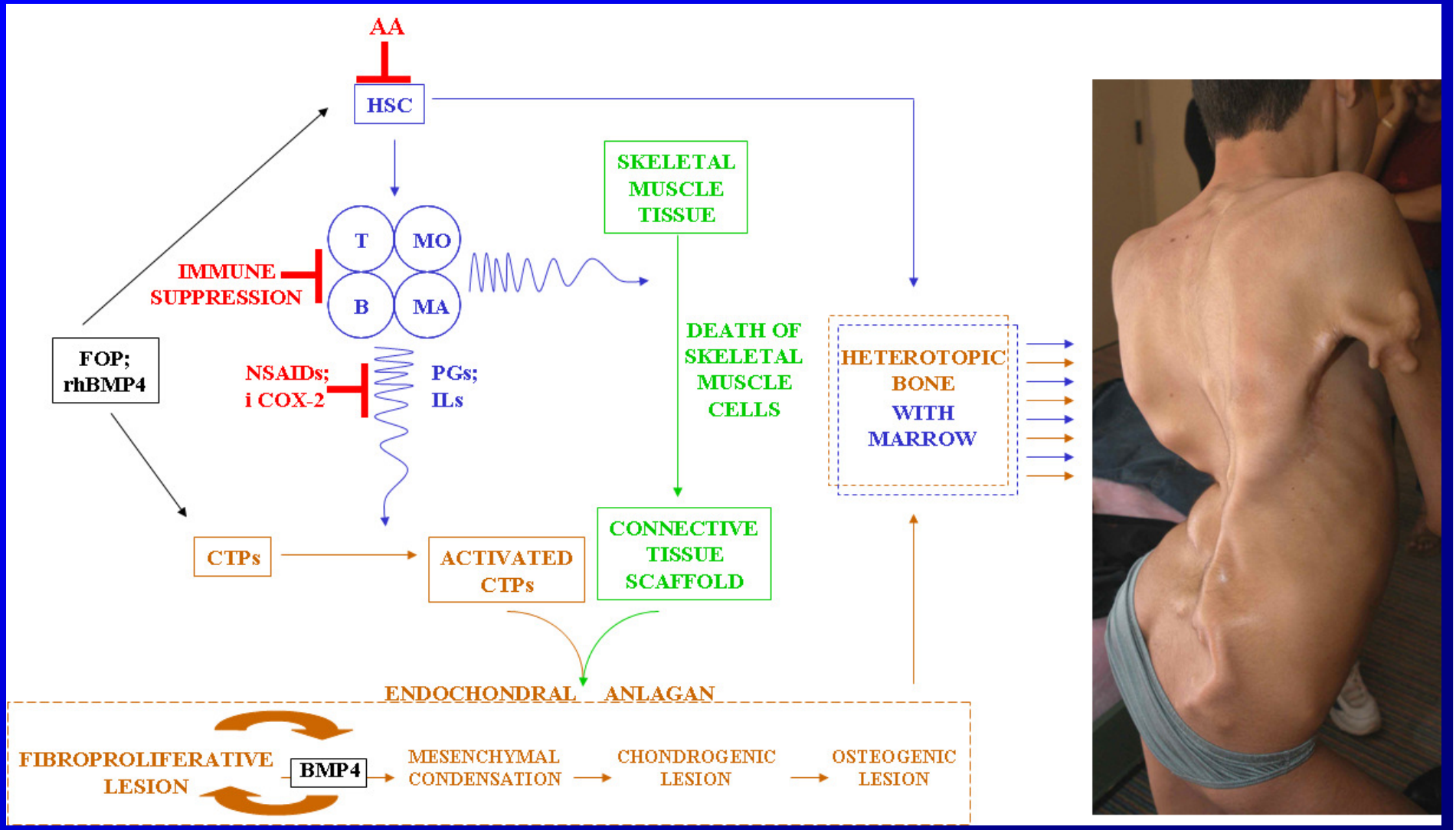
ACVR1 (The Bomb)



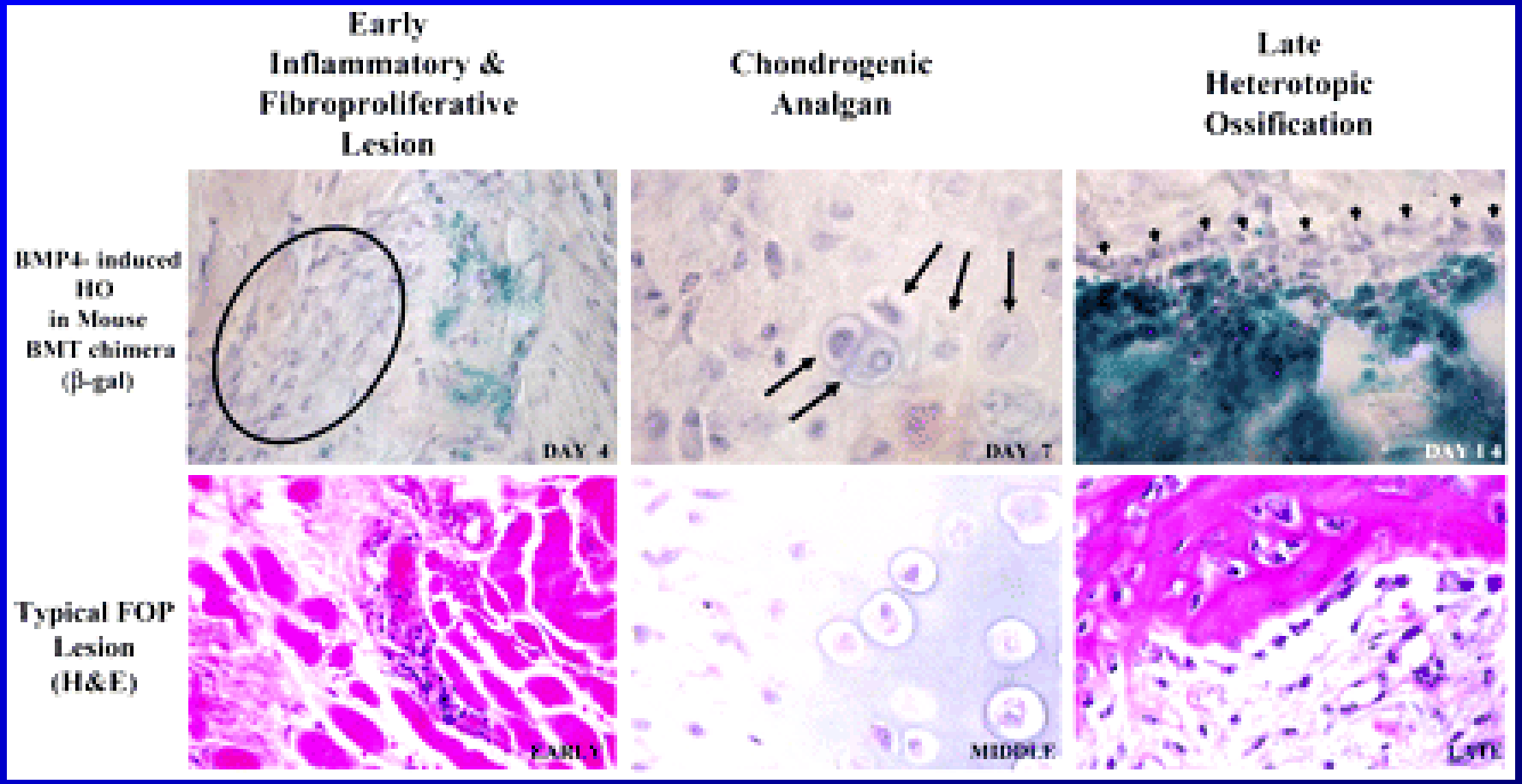
INFLAM (Trigger)

pH/ Hypoxia (Fuel)

FOP Is A Stem Cell Disease



Cells of Hematopoietic Origin Contribute to Early & Late Lesions But Not to the Heterotopic Anlagen



Schematic of Cre/ loxP lineage Tracing Methodology

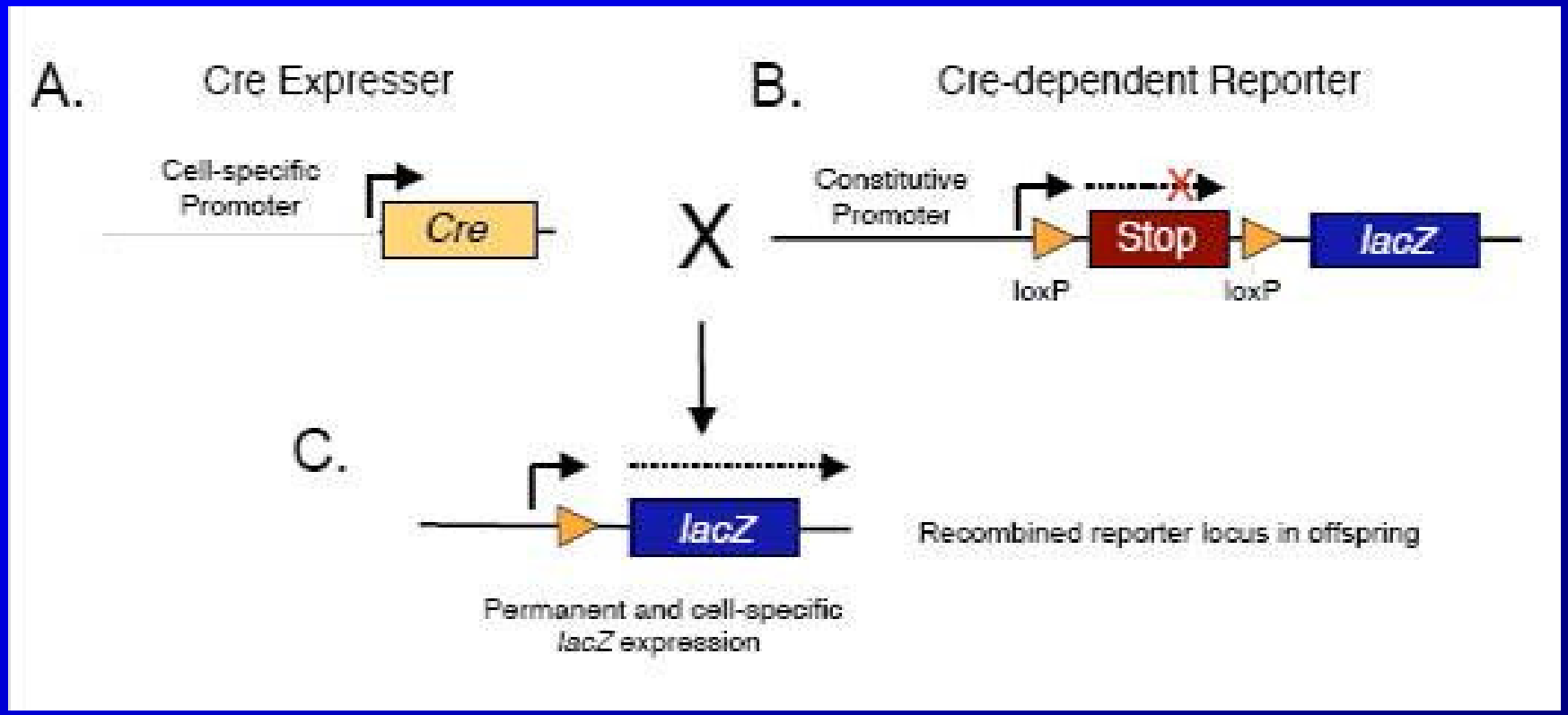
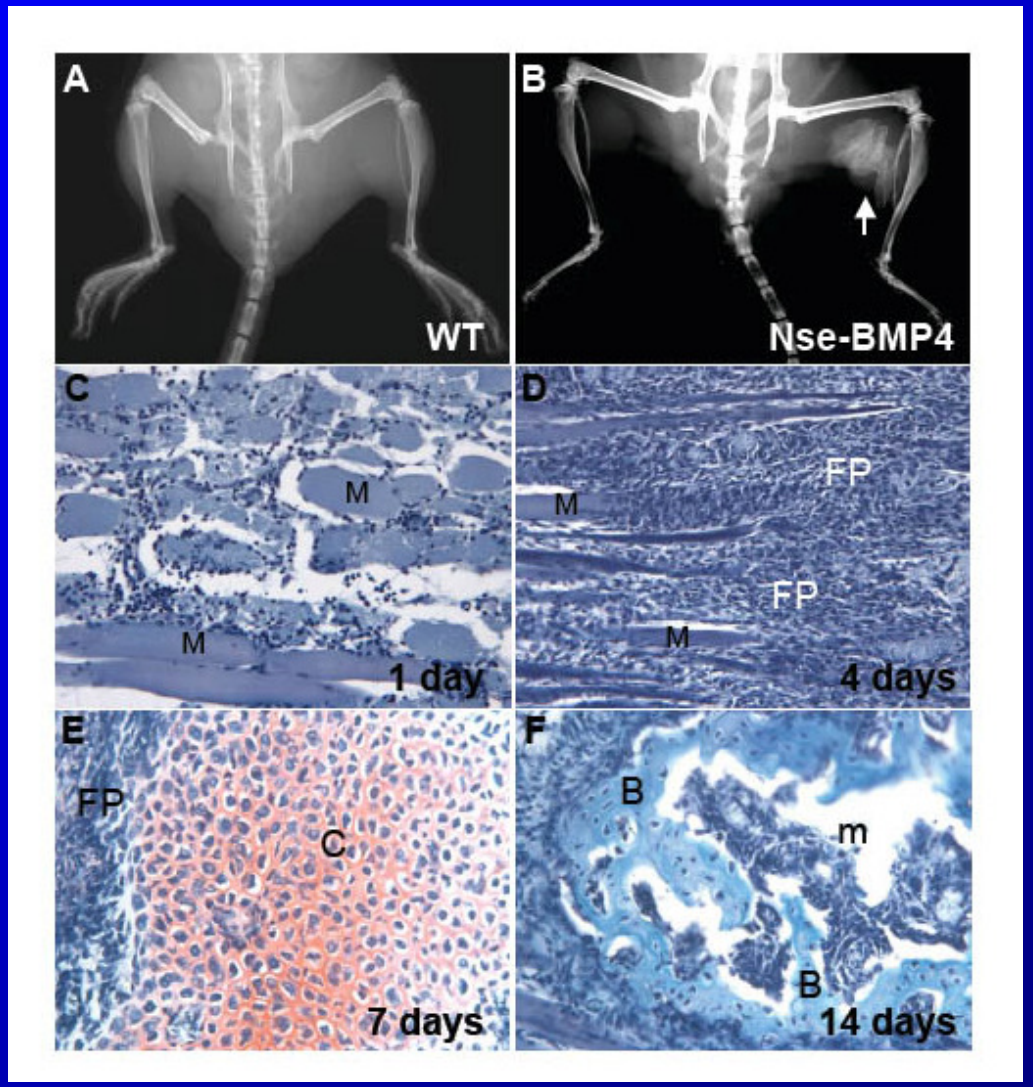


Table 1. Cell lineage contributions to the heterotopic endochondral anlagen[a,b]

		Heterotopic Skeletal Anlagen Stages		
Promoter	Cell Lineage	Fibroproliferative	Chondrogenic	Osteogenic
MyoD	Skeletal Muscle	<5	<1	ND[c]
SMMHC	Vascular smooth muscle	ND	ND	ND
Tie2	Endothelial	40-50	40-50	40-50

Cardiotoxin Injury of Skeletal Muscle Stimulates and Synchronizes Heterotopic Ossification in Nse- BMP4 Transgenic Mice

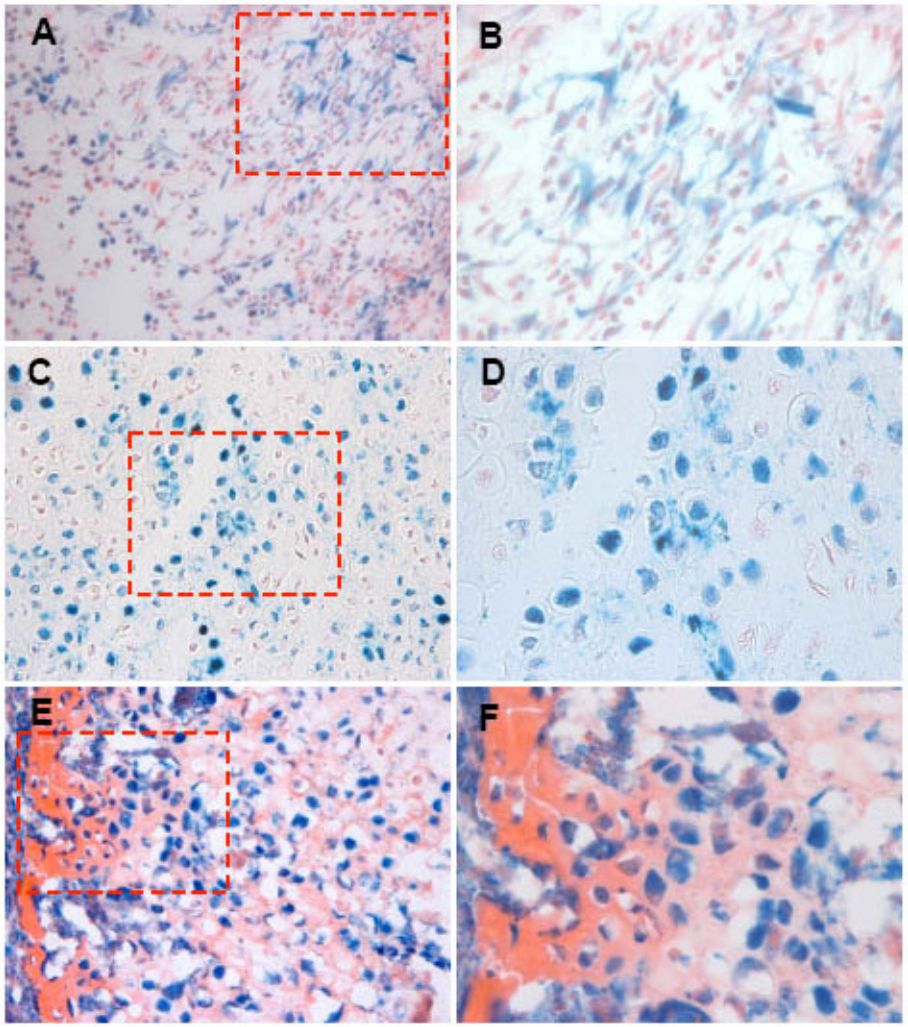


Tie2+ Cells Contribute to All Stages of Heterotopic Ossification After Cardiotoxin-Induced Muscle Injury in Tie2-Cre; R26R/NSE-BMP4 Trasgenic Mice

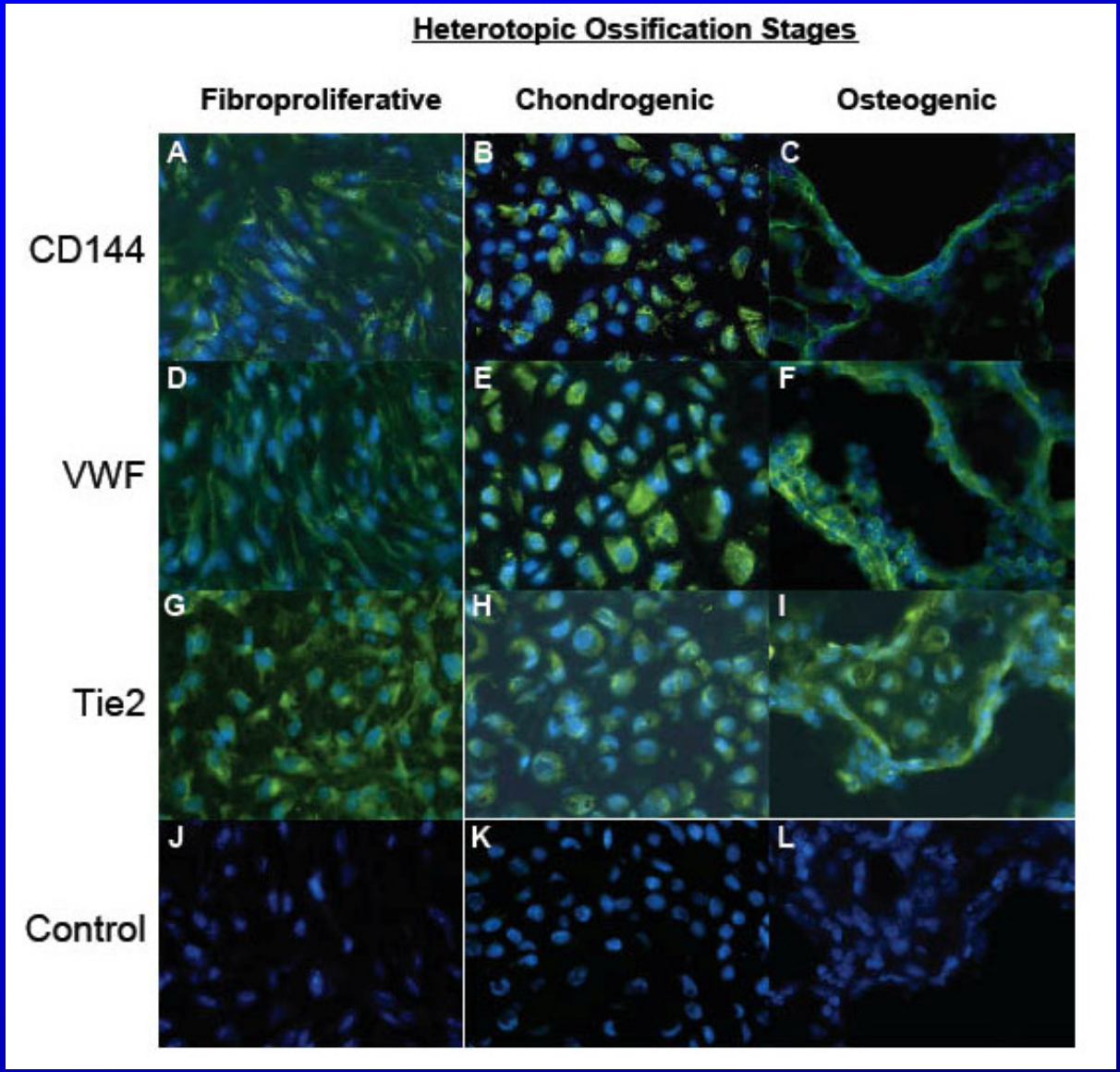
FP

CP

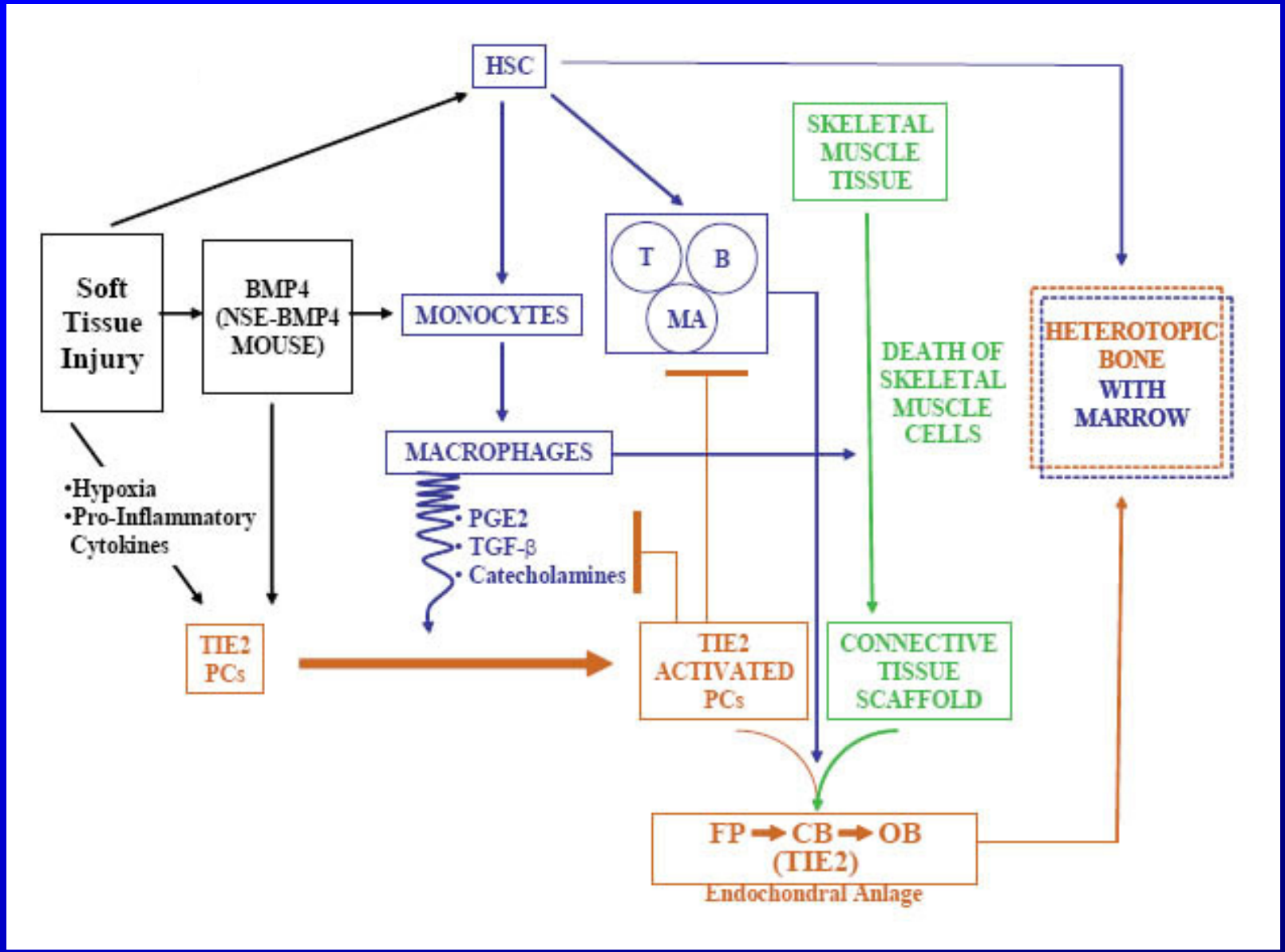
OP



Endothelial Markers Are Expressed At All Stages of The Endochondral Anlagen In BMP4 Associated Heterotopic Ossification



Working Model of BMP- Associated Heterotopic Ossification



Principles of Skeletal Metamorphosis in FOP

- Data from FOP patients and from *in vivo* animal models of FOP strongly suggest that inflammatory signals, in response to soft tissue injury, mobilize resident connective tissue progenitor cells of vascular origin that contribute to every stage in the development of the heterotopic anlagen.

- Inflammatory signals, in response to soft tissue injury, are sufficient to induce heterotopic ossification in a BMP-conducive environment.

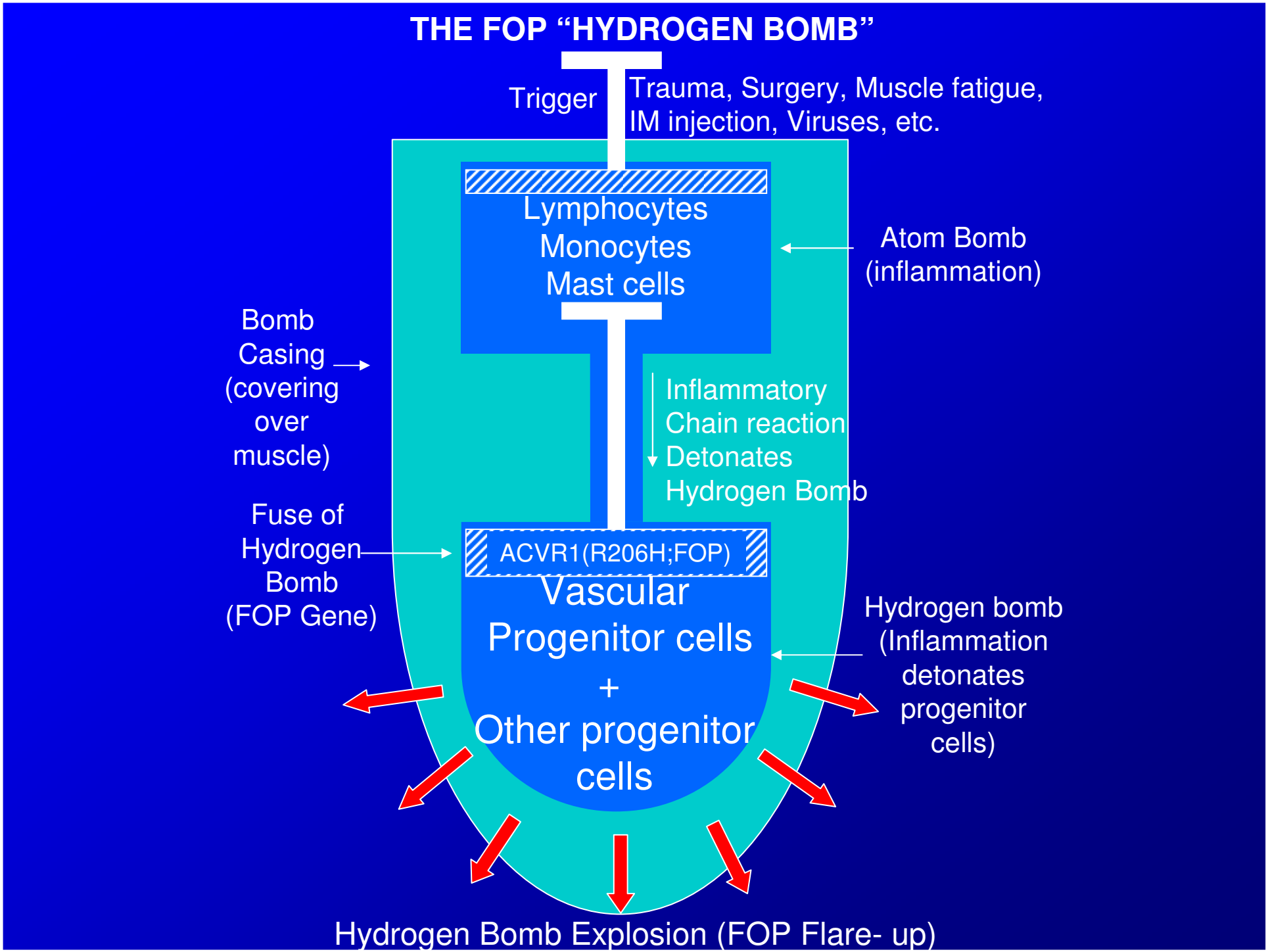
- Inflammatory cells of hematopoietic origin trigger soft tissue metamorphosis to heterotopic bone.

Principles of Skeletal Metamorphosis in FOP

- Cells of the innate immune system, specifically of the monocyte/macrophage lineage, induce metamorphic changes in a BMP conducive environment.
- Cells of the adaptive immune system, specifically of the lymphocyte lineage, propagate the growth and expansion of metamorphic changes in a BMP conducive environment.
- Immunosuppression ameliorates heterotopic ossification in a genetically susceptible host.

Principles of Skeletal Metamorphosis in FOP

- Bone marrow transplantation does not cure FOP and is ineffective in abrogating its progression.
- A normal immune system is sufficient to trigger heterotopic ossification in a genetically susceptible host.
- Connective tissue progenitor cells of vascular origin transduce inflammatory signals in a BMP conducive environment and contribute to every stage in the evolution of a heterotopic anlagen.
- Therapeutic regulation of progenitor cell populations involved in FOP lesions holds promise for treatment of FOP and possibly other disorders of heterotopic ossification.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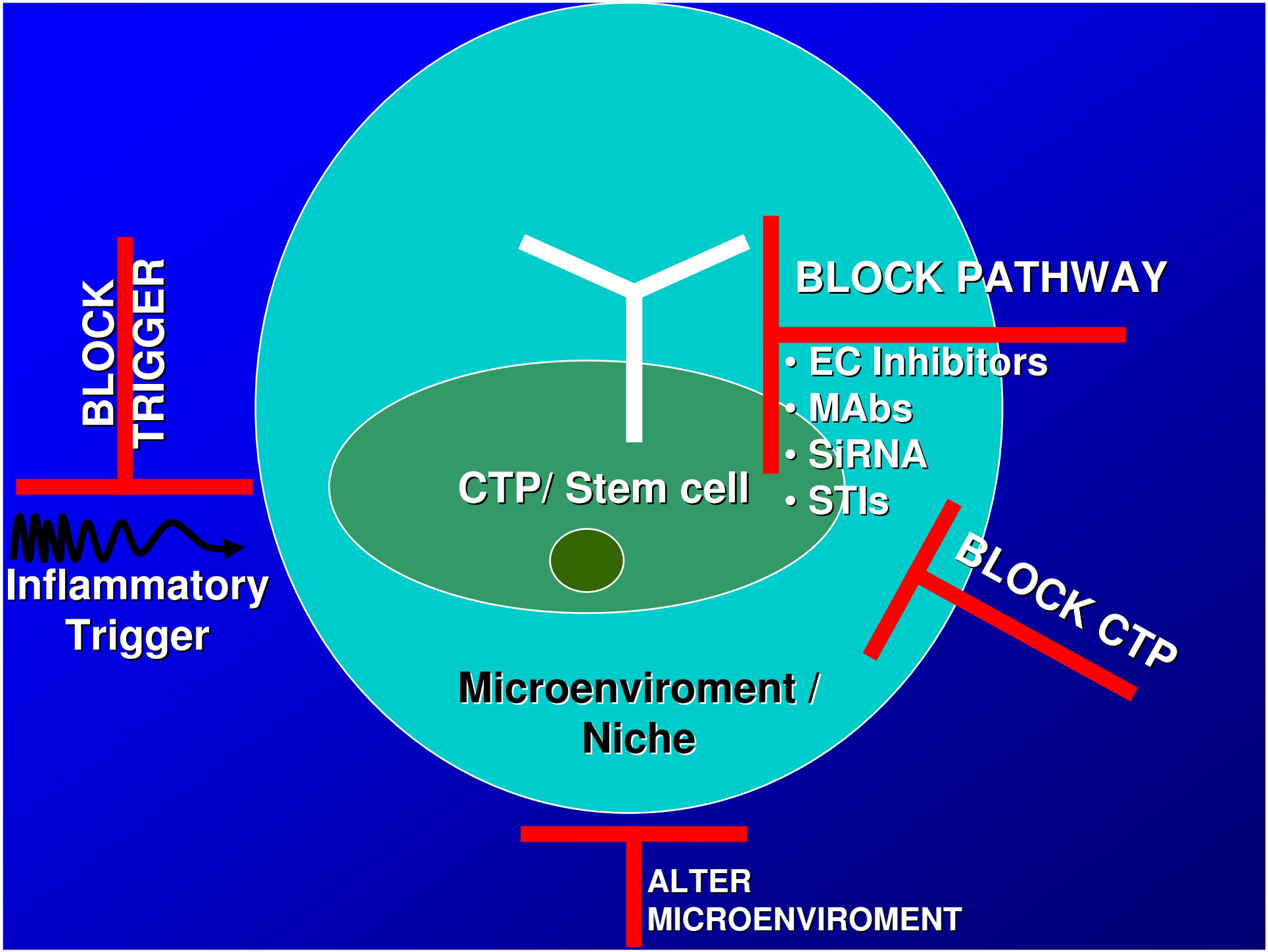
Hydrogen Bomb Explosion (FOP Flare- up)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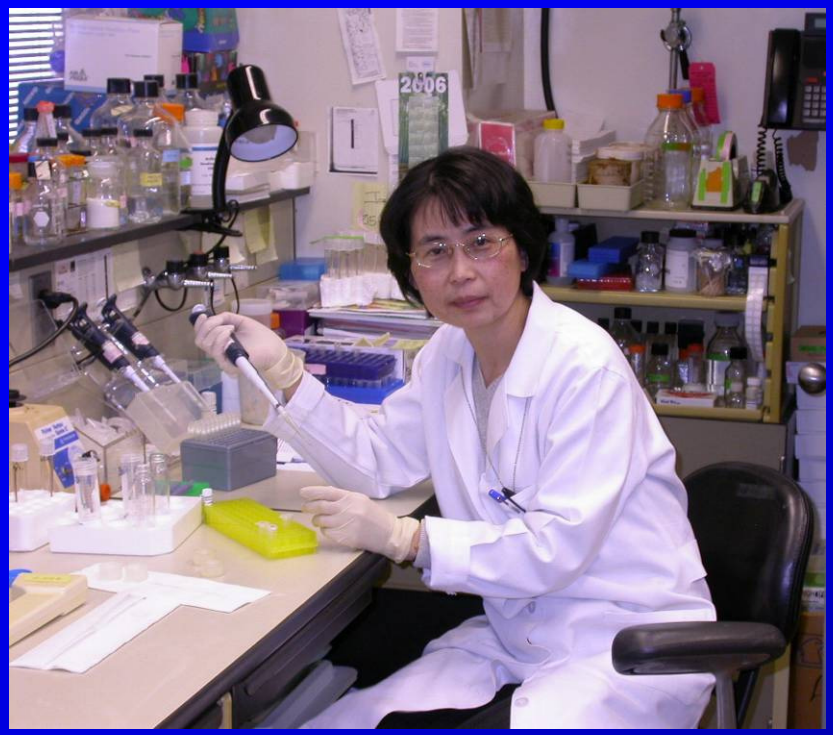
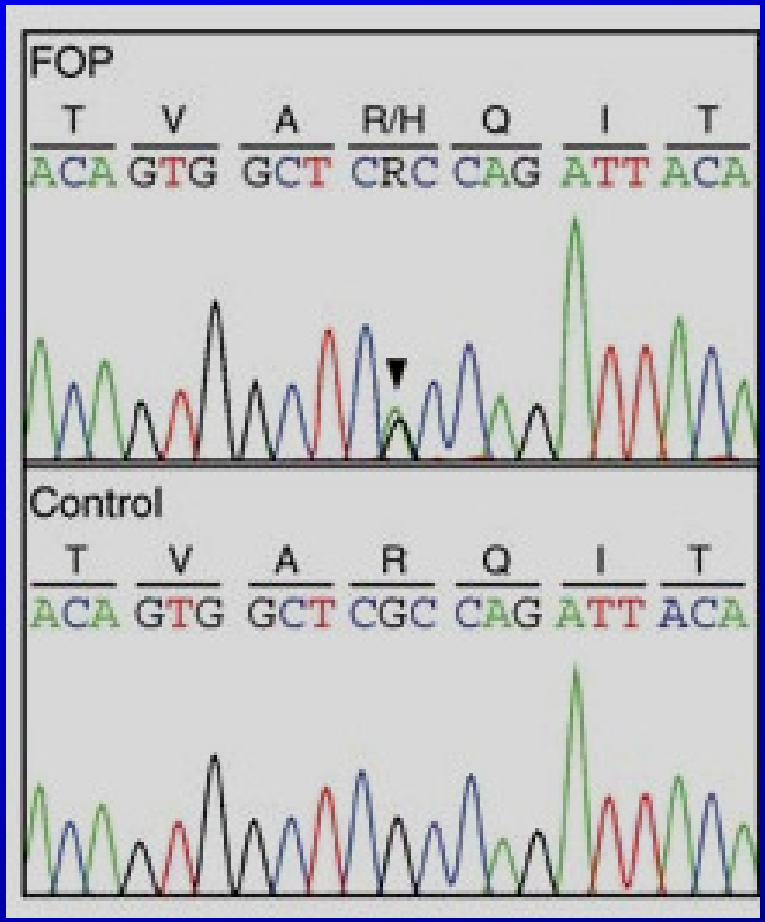
ACVR1 (c.617 G>A; R206H)





One misspelled letter in 6 billion

One of the most highly specific disease causing mutations in the human genome

"All the News That's Fit to Print"

The New York Times

Late Edition
New York: **Today**, partly cloudy and breezy, high 66. **Tonight**, spotty showers to the east, low 52. **Tomorrow**, seasonable, light winds, high 67. **Yesterday**, high 63, low 53. Details, Page D8.

VOL. CLV . . . . No. 53,574

NEW YORK, TUESDAY, MAY 9, 2006

ONE DOLLAR

Finally, With Genetic Discovery, Hope for Escape From a Prison of Bone

By MICHAEL MASON

Peering into the hollow stump of a redwood tree, Hayden Pheif, 5, finds a cache of treasured river rocks exactly where he left them.

It's a luminous afternoon in Mill Valley, Calif., perfect for tossing a few of them back into the creek that runs through this small park. But Hayden's mother, Megan Pheif, knows better than to let her son scramble down the steep embankment to the stream.

Hayden can barely bend forward, and he cannot raise his arms much above his shoulders. Once down that slope, he may not be able to get back up. So she lifts him, over loud protests, back onto the walking trail, lingering for a moment over the hunch that has begun to form on his back. In Hayden's body, too, there are pockets of stone.

"It's upsetting, obviously," said Ms. Pheif, 41, a sales representative for a textiles company. "The childhood you thought your kid would have isn't possible. The doctors don't have a cure, and they can't tell you what's

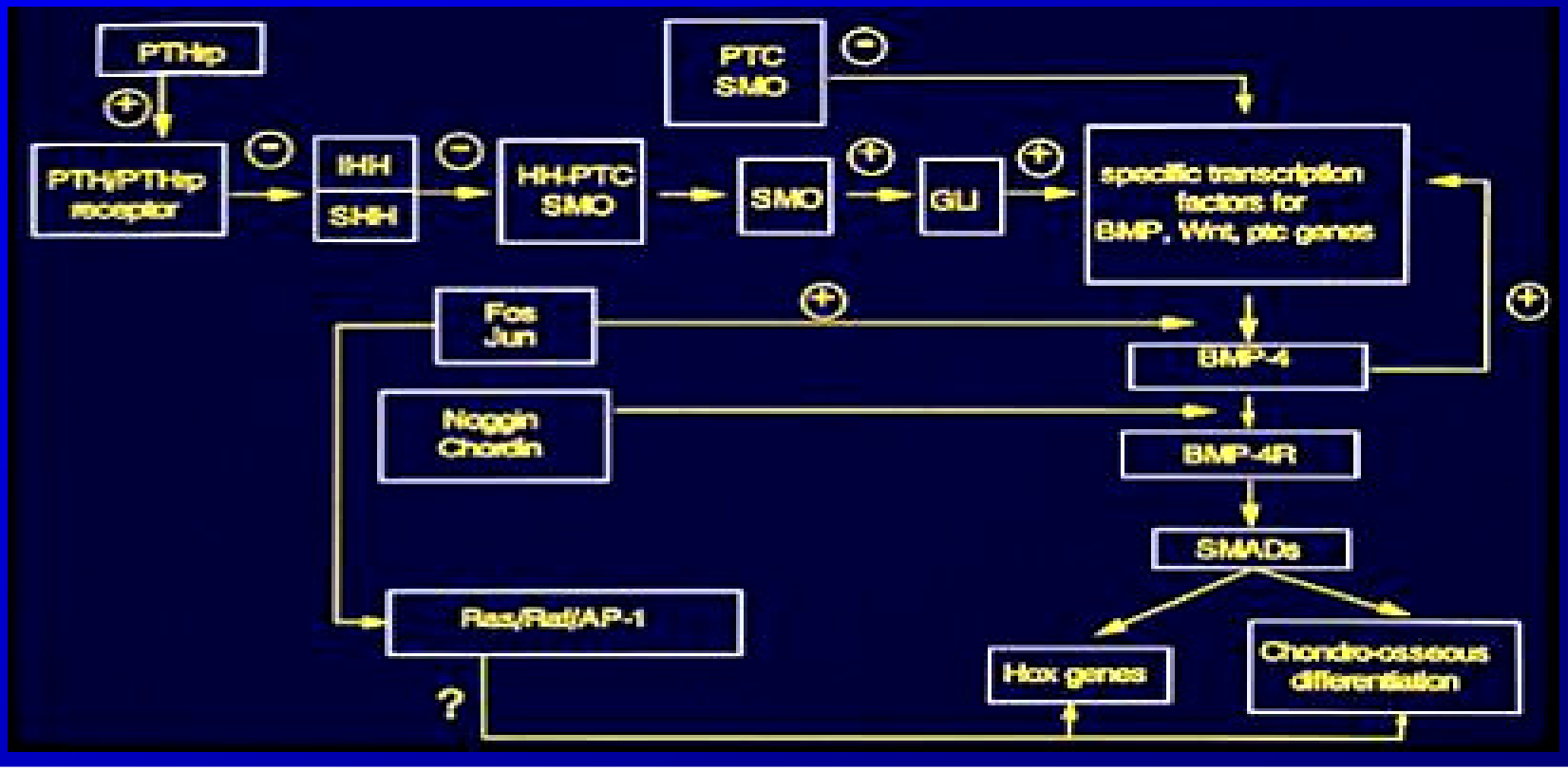
The skeleton explodes in bodies that eventually become living statues.

Peter DaSilva for The New York Times; The New England Journal of Medicine (below)

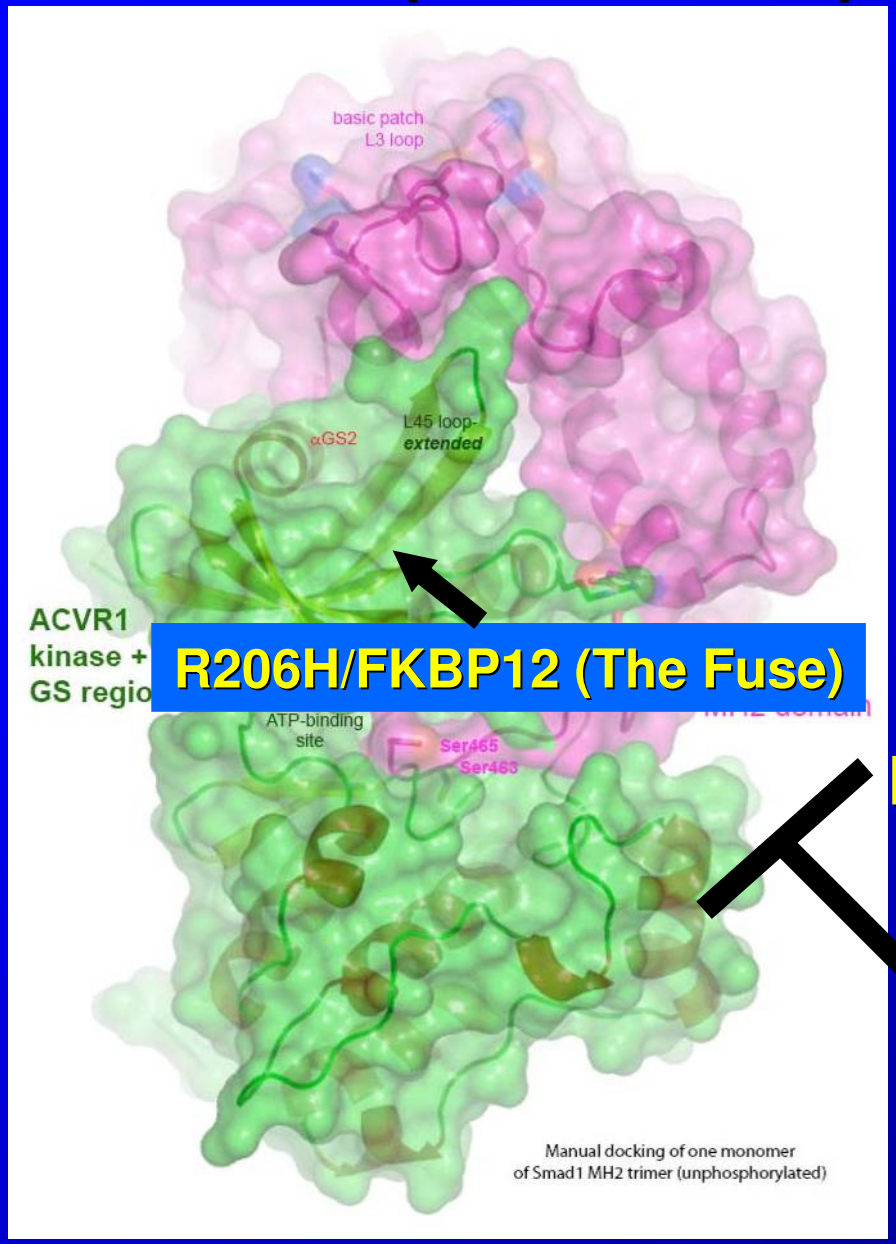
MUSCLES TO BONE Hayden Pheif, 5, at his home in Mill Valley, Calif., has F.O.P., a rare disease that transforms his soft tissue into bone, as illustrated below in the skeleton.

With so much being discovered about how The BMPs act, it might be possible to Develop drugs that would block some part Of the BMP-4 pathway, and therefore Prevent the progression of what is a Horrible nightmare disease.

- Brigid Hogan
***Science* 273:1170,1996**



ACVR1 (The Bomb)



R206H/FKBP12 (The Fuse)

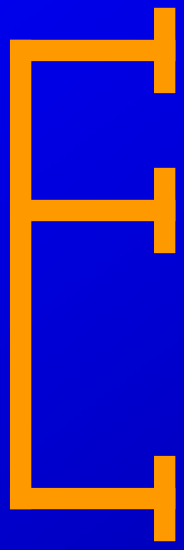
INFLAM
(Trigger)

pH/ Hypoxia
(Fuel)

STI

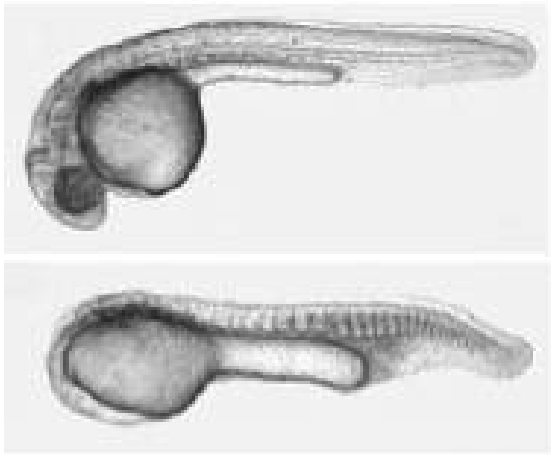
ACTIVIN- LIKE KINASES

ALK1	TSR1	Smads 1,5,8
2	*ACVR1*	*1,5,8*
3	BMPRIA	1,5,8
4	ACVR1B	2,3
5	TGF-βRI	2,3
6	BMPRIB	1,5,8
7	ACVR1C	2,3



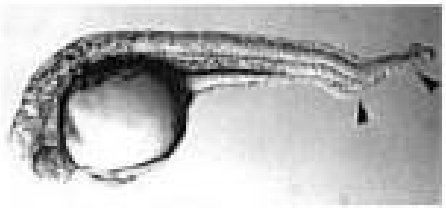

JOURNAL OF BONE & JOINT SURGERY
For reference

Zebrafish Embryogenesis Can Be Used As An In Vivo Screen for BMP Activity



Nguyen et al., 1998

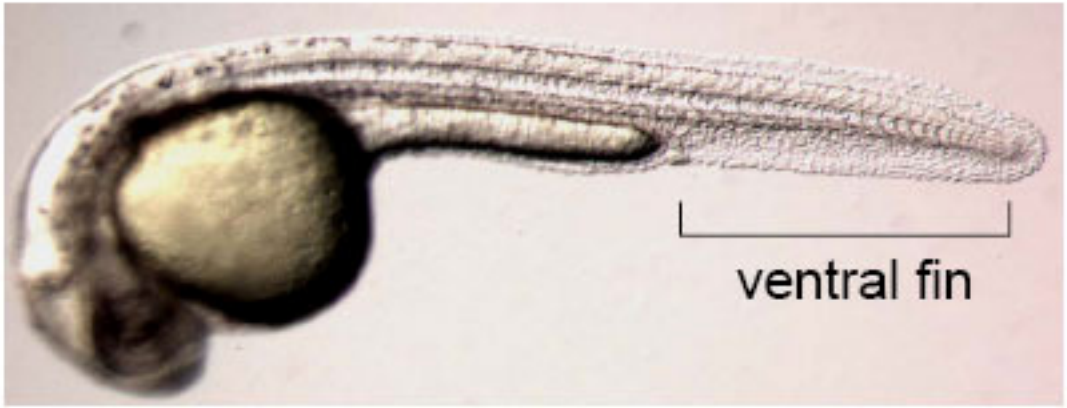
BMP agonism ventralizes



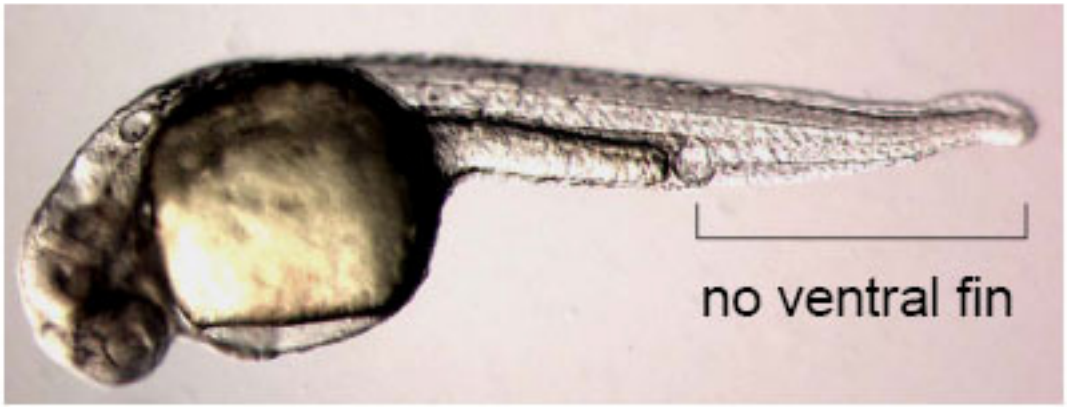
Furthaueret al., 1999

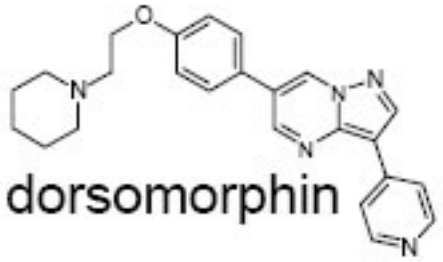
BMP antagonism dorsalizes

Identification of a dorsalizing compound



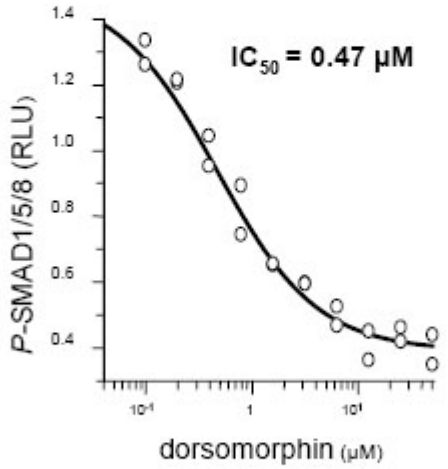
untreated

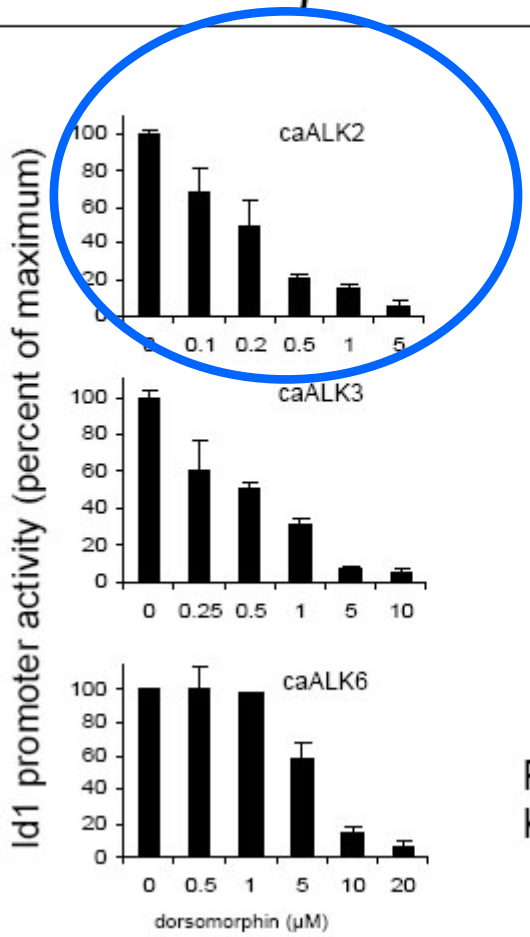




dorsomorphin

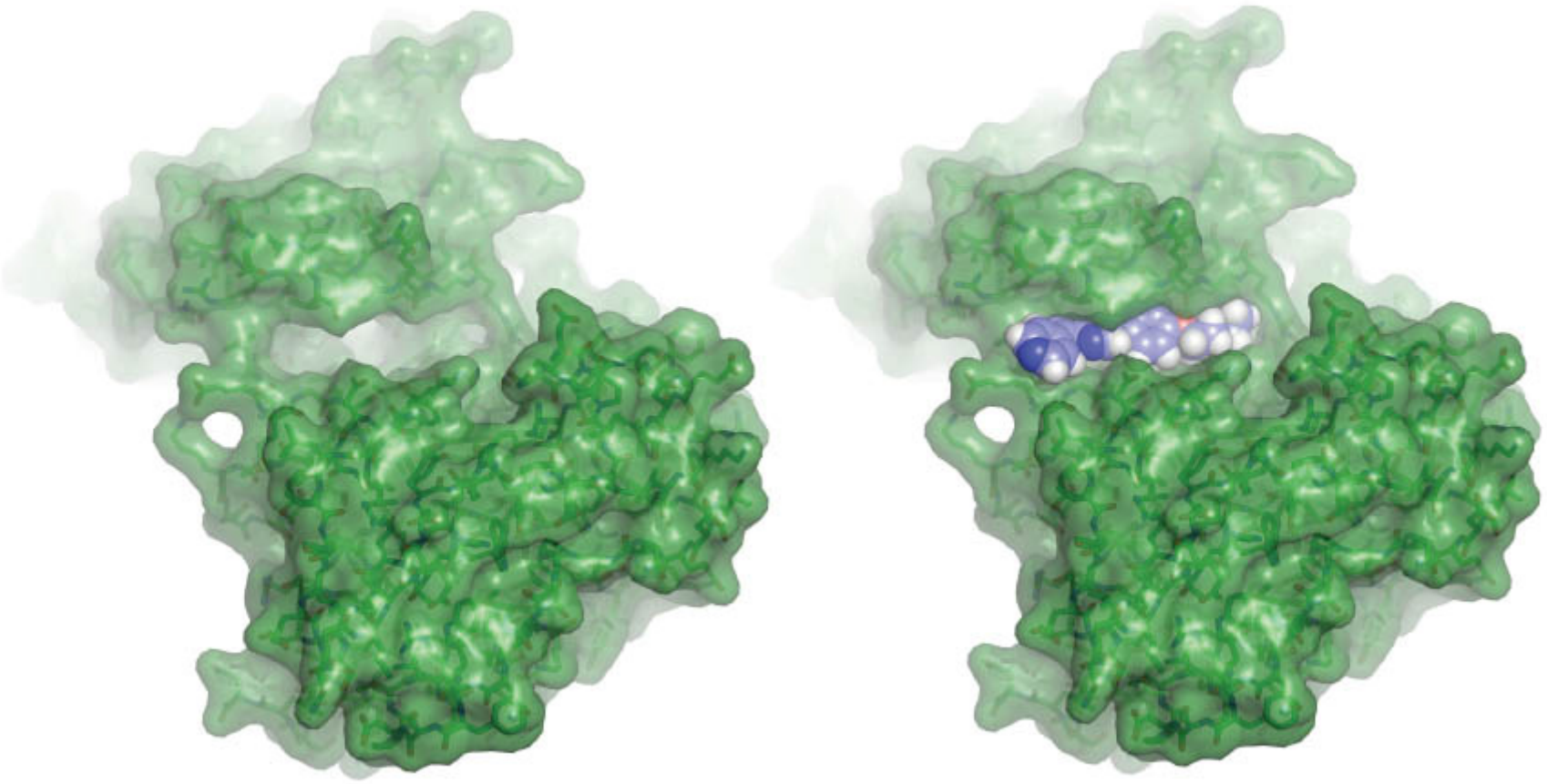
Dorsomorphin inhibits constitutively active type I BMP receptors





Paul Yu
Ken Bloch

Computational Docking of Dorsomorphin to ACVR1 Kinase

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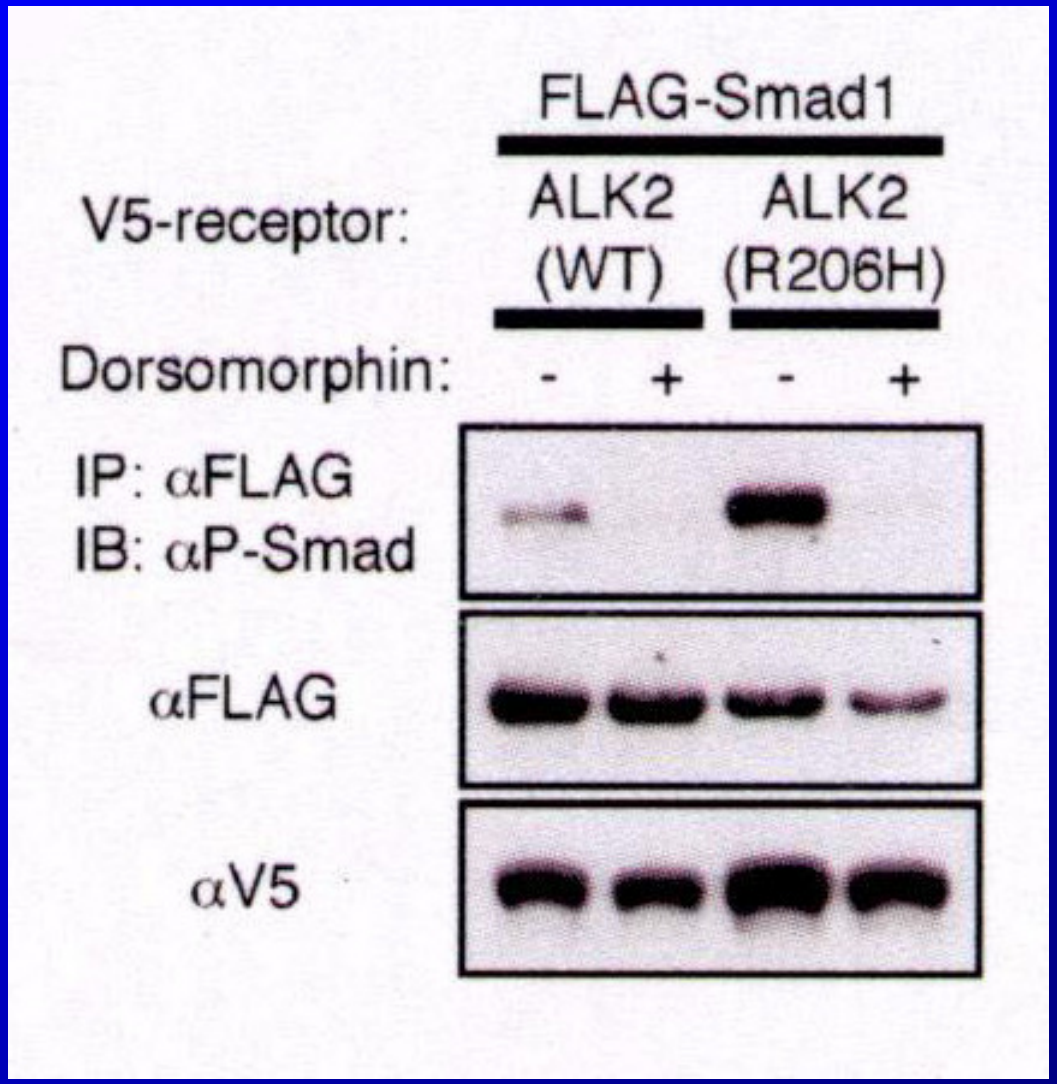
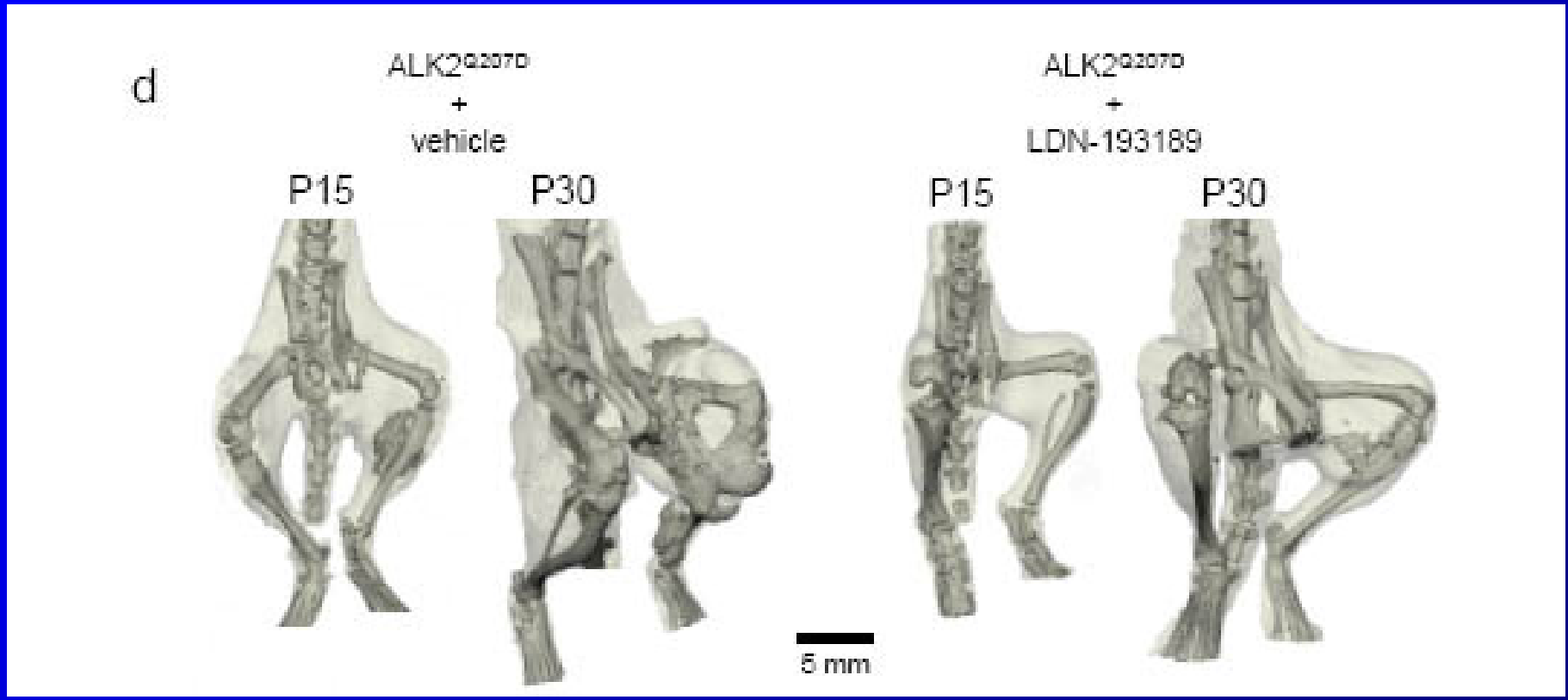
Dorsomorphin Inhibits BMP-Smad Signaling in FOP Cells

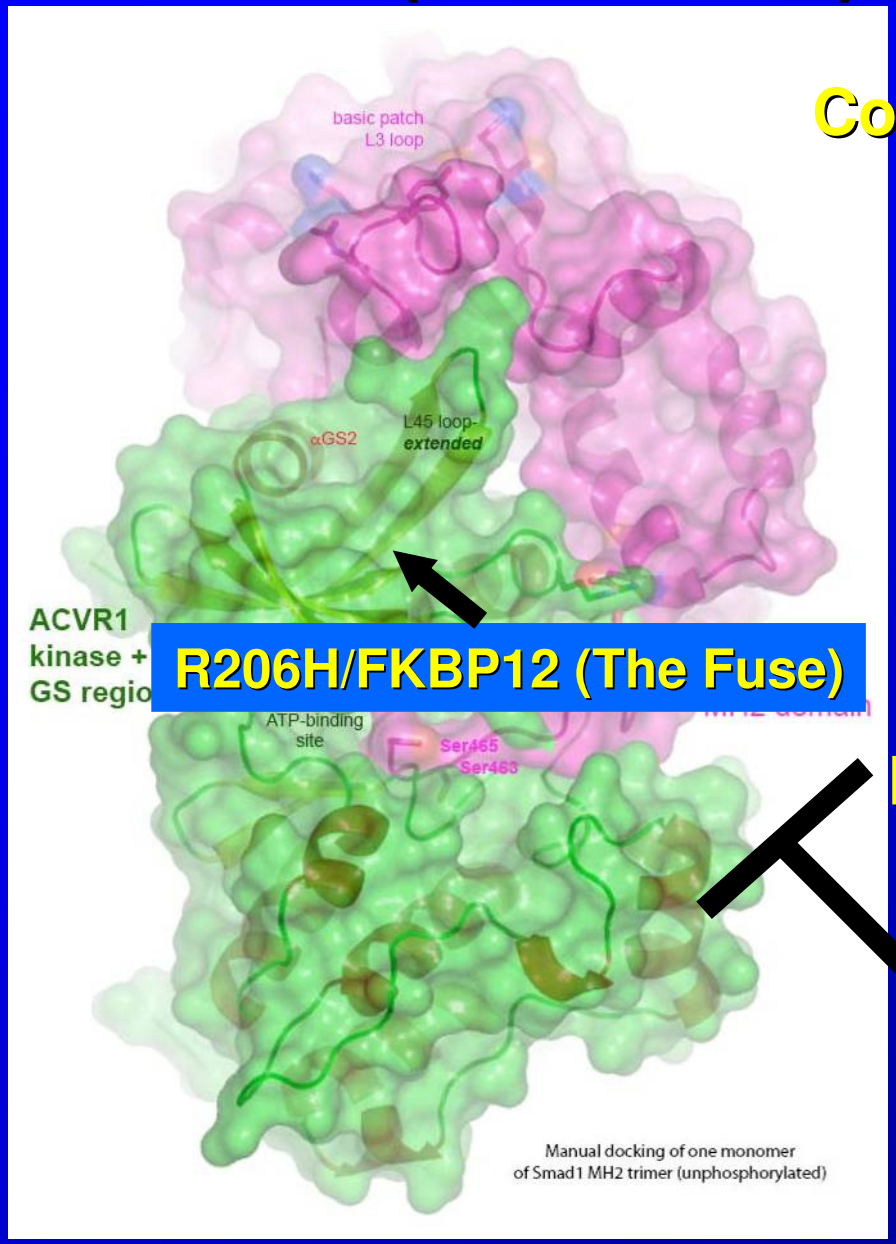


DM-3189 Partly Inhibits H.O. in Conditional caAlk2 mice



& So Do Corticosteroids

ACVR1 (The Bomb)



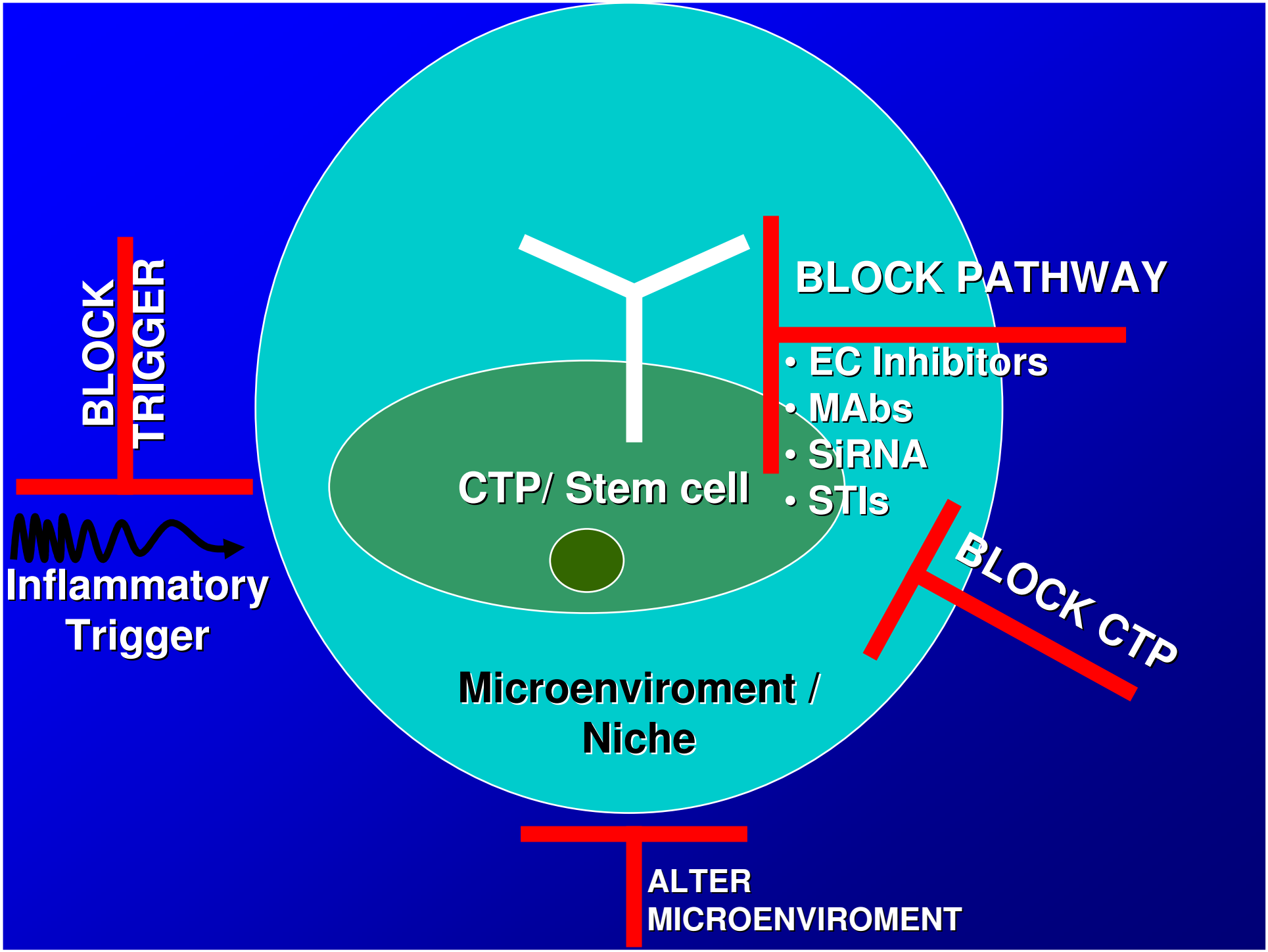
R206H/FKBP12 (The Fuse)

Corticosteroids

INFLAM
(Trigger)

pH/ Hypoxia
(Fuel)

STI


BLOCK
TRIGGER
Inflammatory
Trigger
BLOCK PATHWAY
• EC Inhibitors
• MAbs
• SiRNA
• STIs
CTP/ Stem cell
BLOCK CTP
Microenviroment /
Niche
ALTER
MICROENVIROMENT

ACKNOWLEDGEMENTS

- The The International FOP Association
- Friends and Families of FOP Patients Worldwide
- The Isaac & Rose Nassau Professorship of Orthopaedic Molecular Medicine
- The Ian Cali Endowment
- The Weldon Family Endowment
- Association Pierre-Yves
- FOPe.v.
- University of Oxford FOP Research Fund
- The Sarah Cameron Fund
- The Stephen Roach- Whitney Weldon Fellowship
- The Allison Weiss Fellowship
- Born- Lotke- Zasloff Fellowship
- The Roemex Fellowship
- The Grampian Fellowship
- The People of Santa Maria
- The National Institutes of Health

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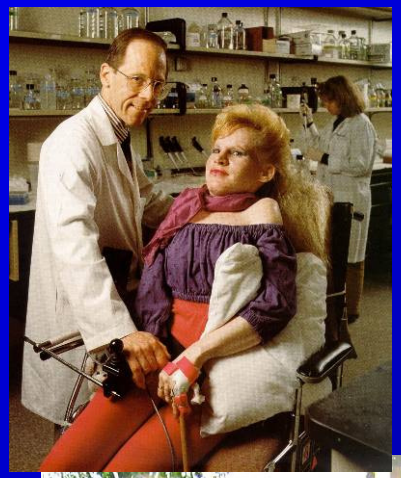
Nature



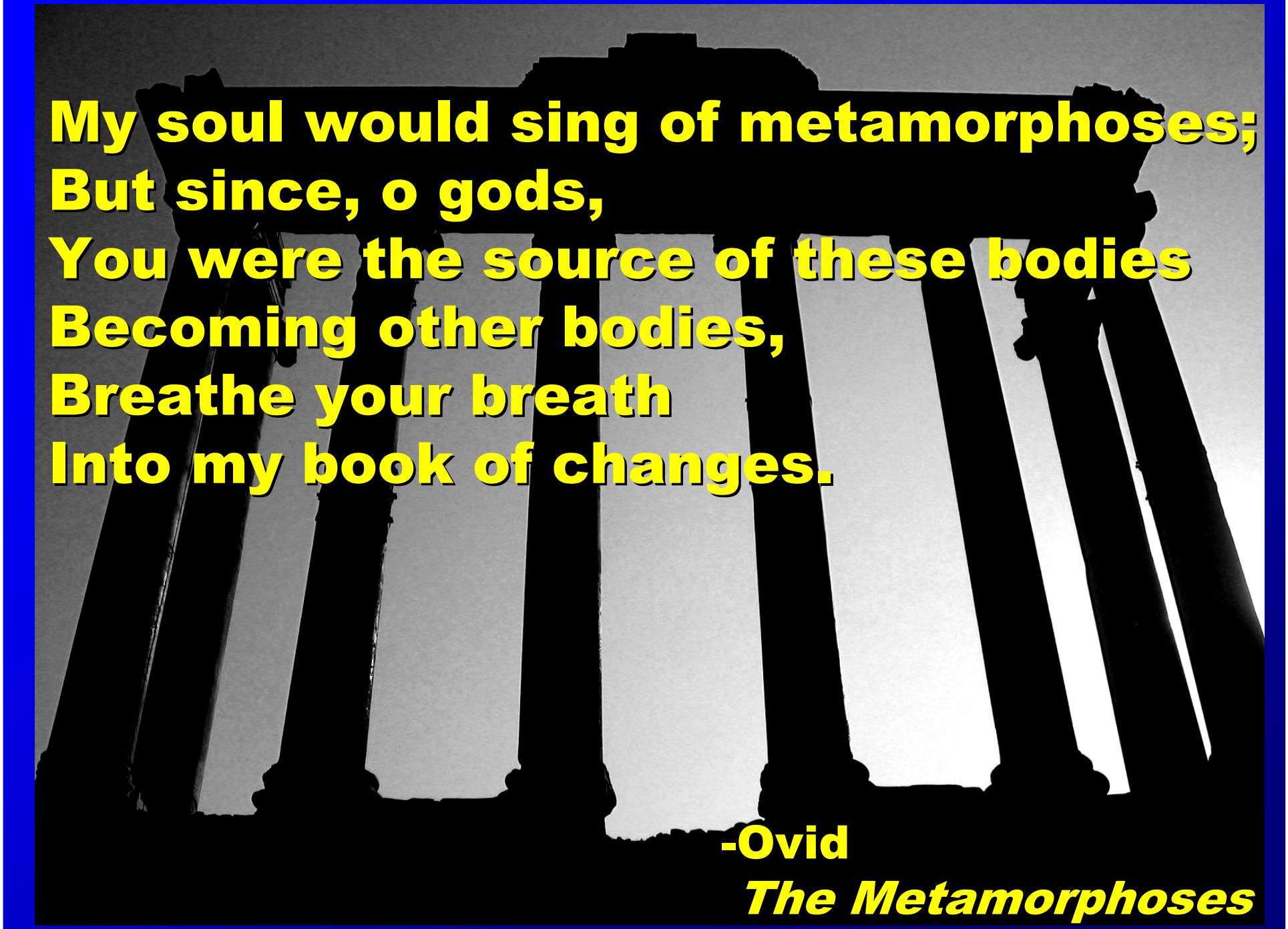




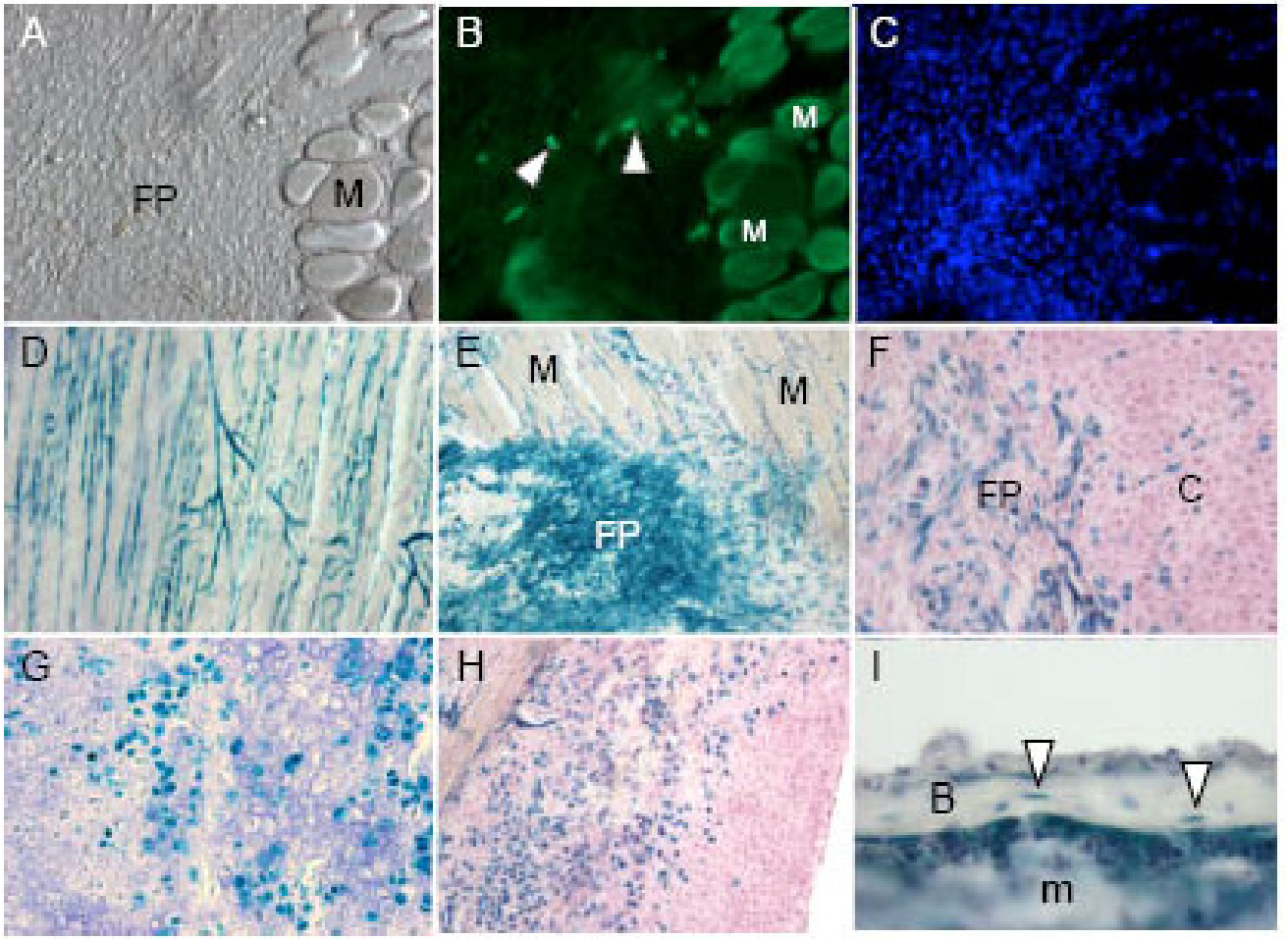




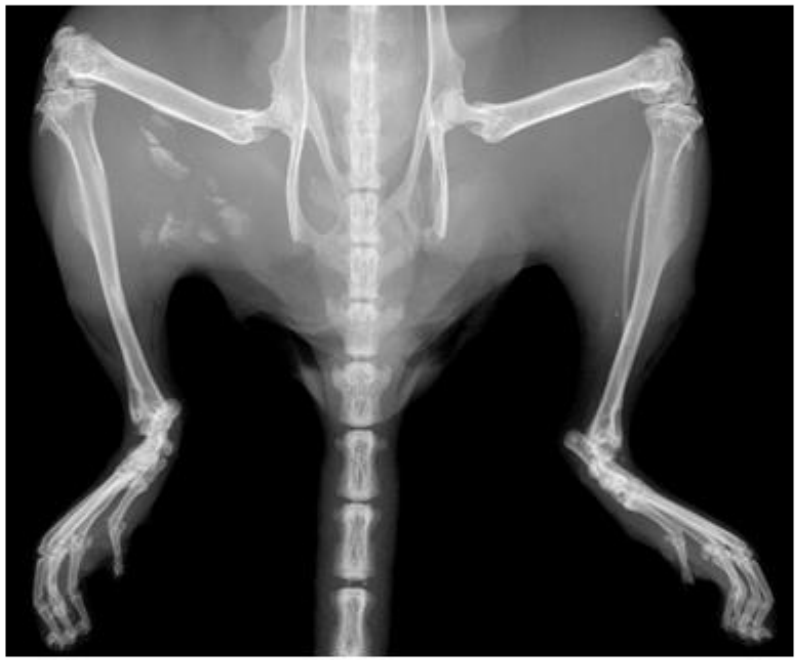

Extras


My soul would sing of metamorphoses;
But since, o gods,
You were the source of these bodies
Becoming other bodies,
Breathe your breath
Into my book of changes.
-Ovid
The Metamorphoses

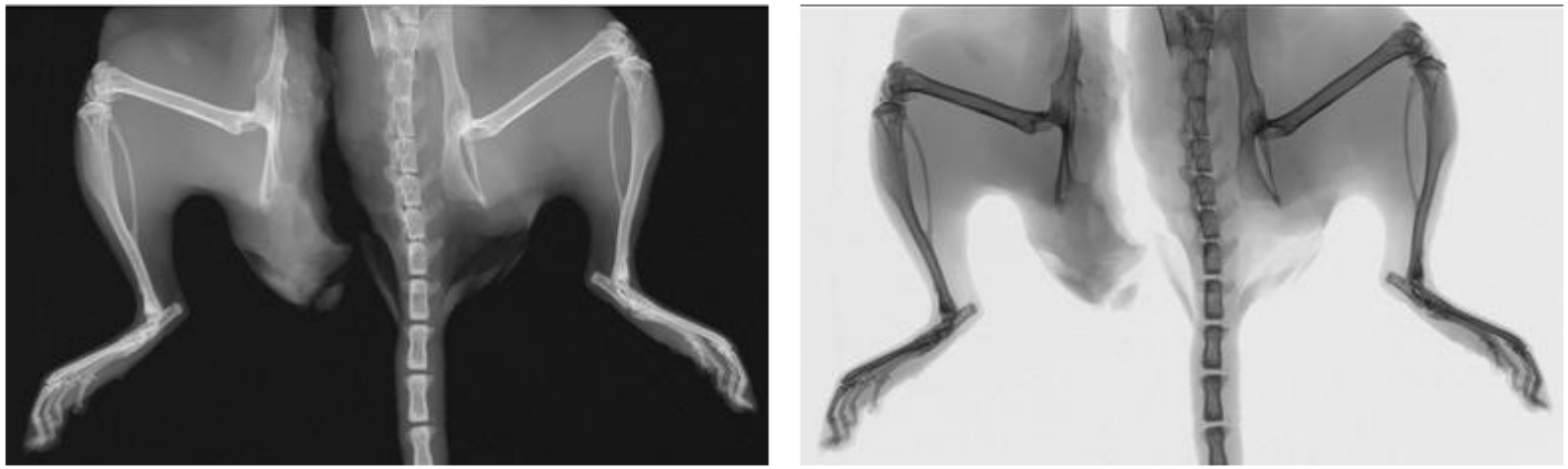
Contribution of MyoD+ and Tie2+ Cells to Heterotopic Ossification Following Intra muscular Injection of BMP2



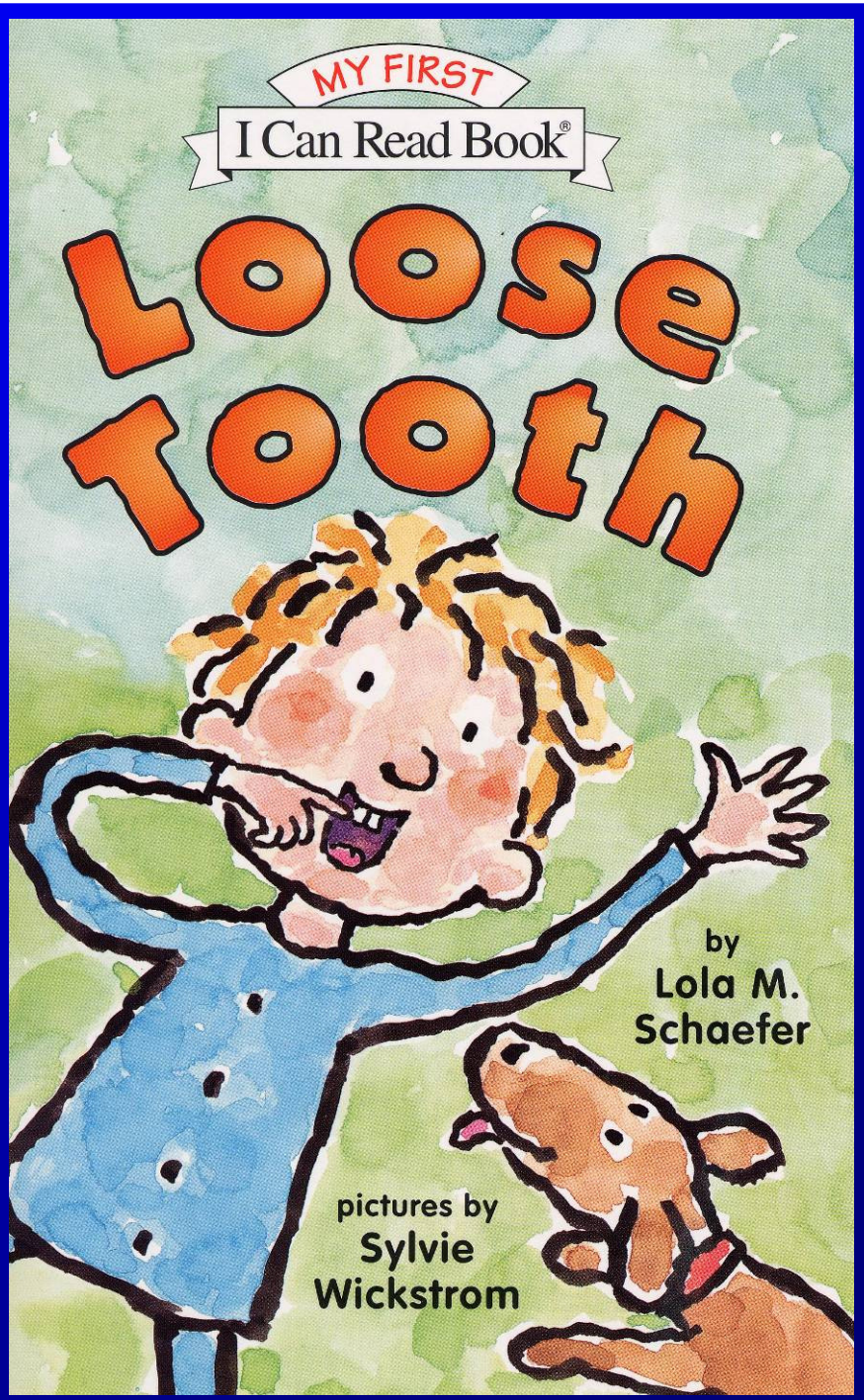
Radiograph of caALK2 mouse made three weeks after AV-Cre injection



Radiograph of caALK2 mouse made three weeks after AV-Cre injection



Mouse was treated with dorsomorphin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Schaefer
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Sylvie
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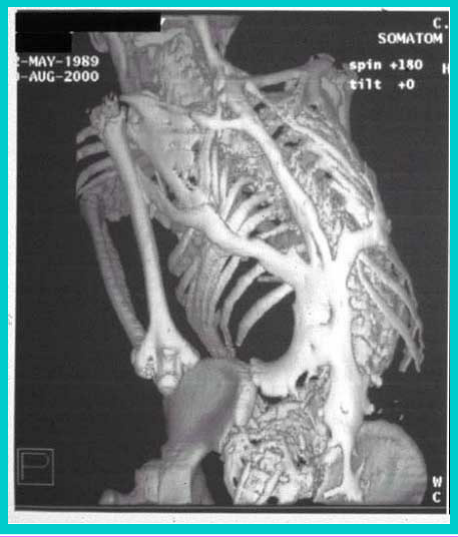
FOP Cells

- Increased expression of BMP4 (NEJM)
- Failure to upregulate BMP4 antagonists (JBJS)
- Failure to regulate concentration of BMP4 in extracellular space (JBJR)
- Increased concentration of BMP type I receptors at cell surface (JBMR)
- Failure to internalize & degrade BMP type I receptors in presence of ligand (JBMR)
- Basal leakiness of BMP signaling through Smad pathway in absence of ligand (JBMR)
- Hyper-responsiveness of BMP signaling through p38 MAPK pathway in presence on ligand (JBMR)

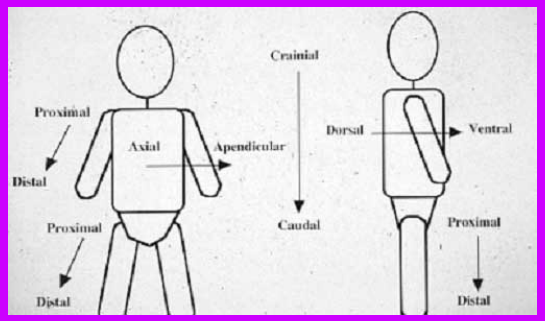
ACVR1 (R206H)

Ectopic Skeletogenesis

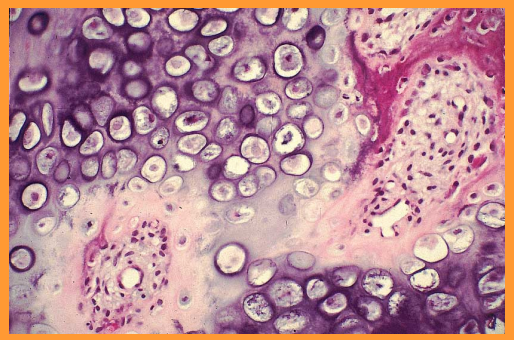


Great Toe Malformation

Pattern Formation



Endochondral Ossification



FOP MUTATION MUST EXPLAIN

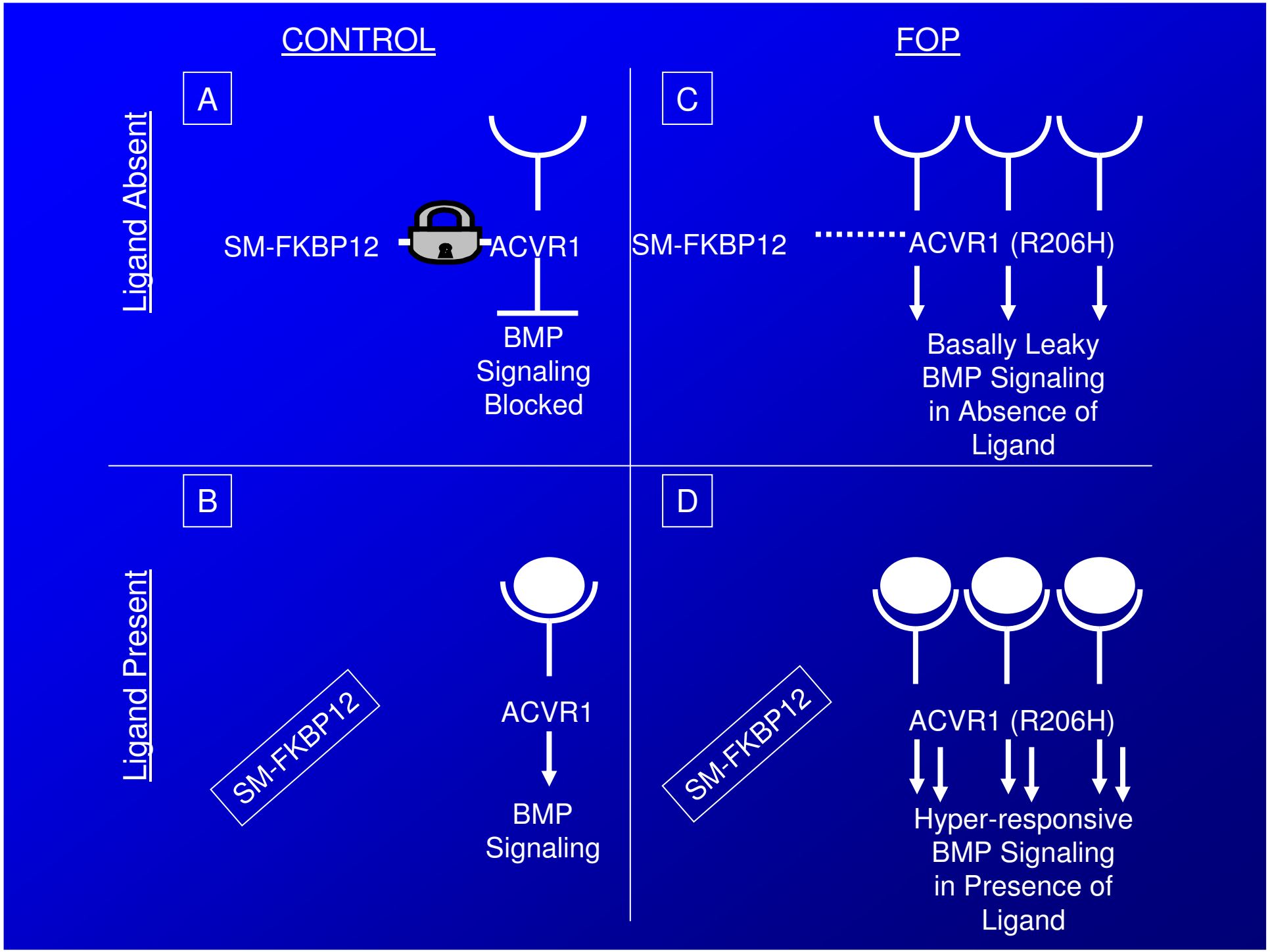
- Basal Leakiness of BMP Signaling in absence of ligand
- Increased Responsiveness of BMP Signaling in presence of ligand
- Decreased BMP Receptor Internalization/ Degradation

How Does the Switch Work?

FKBP12 may be part of the key !

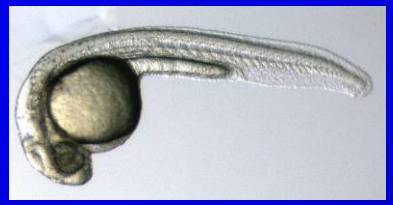
GS Domain of ACVR1 Binds FKBP12, Which

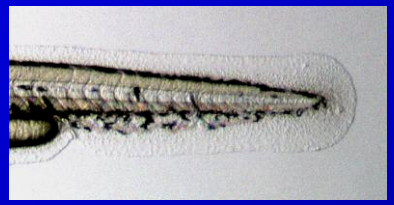
- Prevents basal leakiness in absence of ligand
- Induces receptor degradation by acting as an adaptor molecule for Smad7 – Smurf2 which promotes receptor ubiquitination to terminate signaling


CONTROL
FOP
A
C
Ligand Absent
SM-FKBP12
ACVR1
BMP
Signaling
Blocked
SM-FKBP12
ACVR1 (R206H)
Basally Leaky
BMP Signaling
in Absence of
Ligand
B
D
Ligand Present
SM-FKBP12
ACVR1
BMP
Signaling
SM-FKBP12
ACVR1 (R206H)
Hyper-responsive
BMP Signaling
in Presence of
Ligand

Functional Effects of ACVR1 c.617G>A (R206H) Mutation
- in vivo analyses: zebrafish

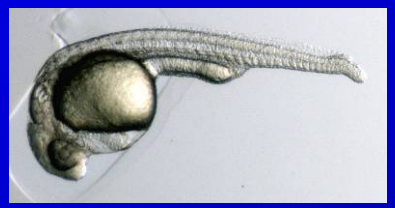
WT Alk8





normal

Alk8-/-



mildly dorsalized

Alk8-/-
+
wt ACVR1 (c.617G)

~75% rescue (shift to more ventral)

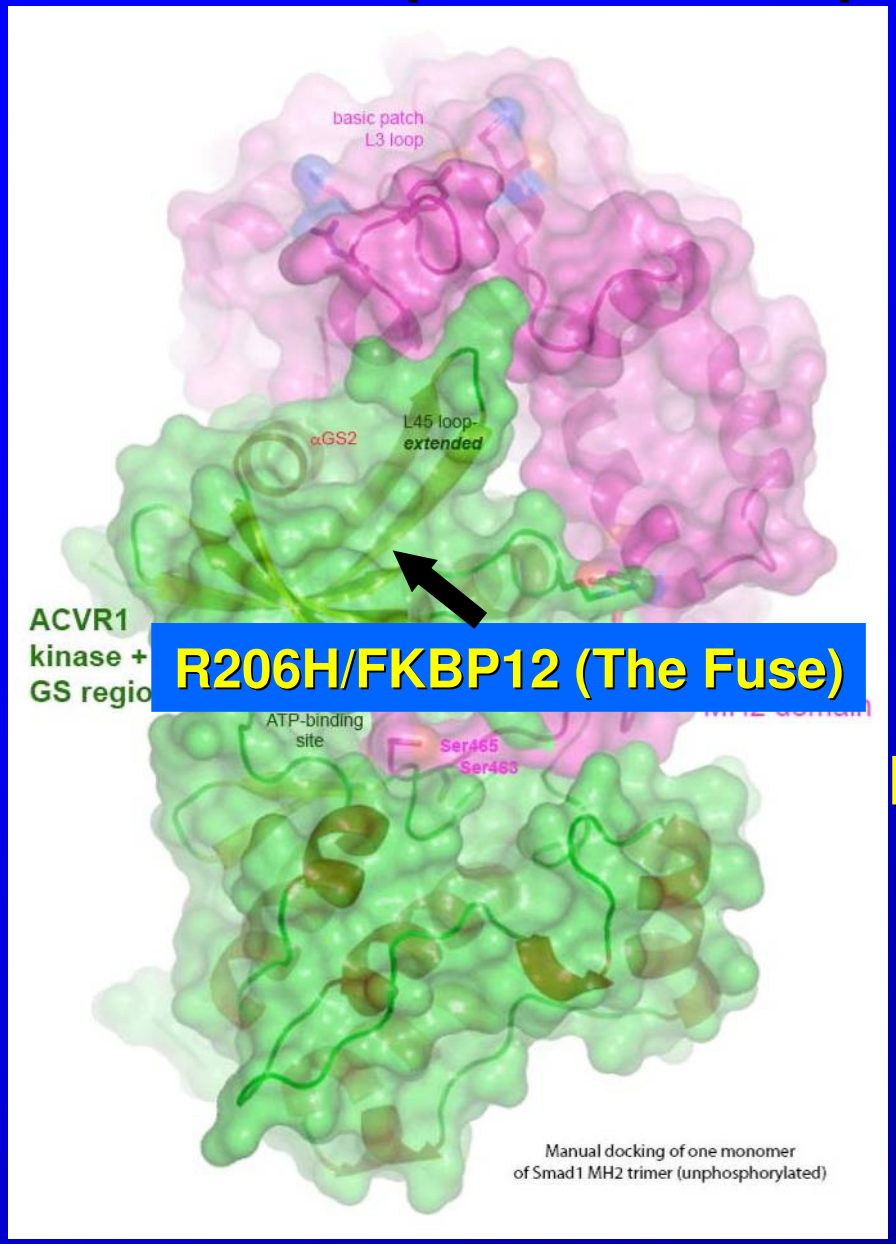
Alk8-/-
+
mt ACVR1 (c.617A)

hyper-activity of BMP signaling

ventralized

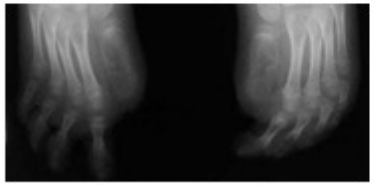
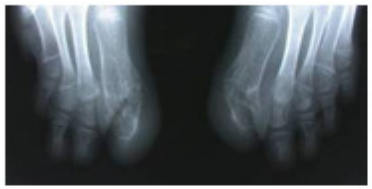
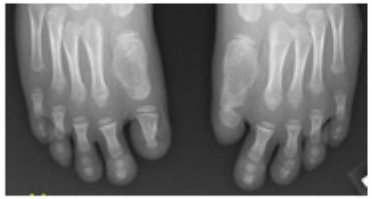
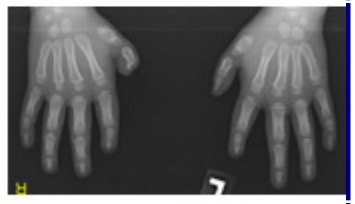
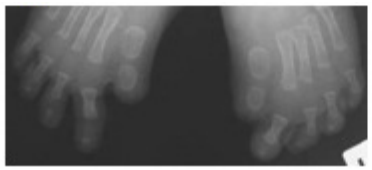
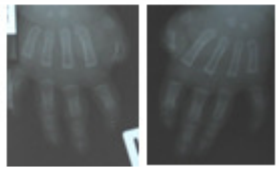
Shen, Little et al. unpubl.

ACVR1 (The Bomb)



INFLAM (Trigger)

pH/ Hypoxia (Fuel)

	FOP VARIANTS	FEET		HANDS	
A	G328R (Pt #8)				
B	G328R (Pt #10)				
C	G328W (Pt #11)				
D	G328E (Pt #13)				
E	G328E (Pt #14)				
F	G356D (Pt #16)				
G	G356D (Pt #17)				

nature genetics

A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva

Eileen M Shore[1–3], Meiqi Xu[1,2], George J Feldman[1,2], David A Fenstermacher[4–6], The FOP International Research Consortium, Matthew A Brown[7] & Frederick S Kaplan[1,2,8],

RESEARCH ARTICLE

Human Mutation

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www.hgvs.org

Classic and Atypical Fibrodysplasia Ossificans Progressiva (FOP) Phenotypes Are Caused by Mutations in the Bone Morphogenetic Protein (BMP) Type I Receptor ACVR1

Frederick S. Kaplan,[1,2,19] Meiqi Xu,[1,19] Petra Seemann,[4] J. Michael Connor,[5] David L. Glaser,[1,19] Liam Carroll,[6] Patricia Delai,[7] Elisabeth Fastnacht-Urban,[8] Stephen J. Forman,[9] Gabriele Gillessen-Kaesbach,[10] Julie Hoover-Fong,[11] Bernhard Köster,[12] Richard M. Pauli,[13,20] William Reardon,[14] Syed-Adeel Zaidi,[15] Michael Zasloff,[1,19] Rolf Morhart,[16] Stefan Mundlos,[4,17] Jay Groppe,[18] and Eileen M. Shore[1,3,19]*

From April, 2006 to December 2008, members of the FOP Research Consortium have:

- **Discovered the FOP gene**
- **Identified major clinical and molecular variants of FOP**

- **Modeled the structure of the mutant protein encoded by the FOP gene and identified a previously unrecognized and unstable switch enabled by the FOP mutation.**

- **Demonstrated that the mutant FOP receptor has leakey BMP signaling at rest and hyperresponsive BMP signaling when triggered by inflammatory signals in the cellular microenvironment.**

- **Unveiled a key co-conspiratory protein, FKBP12, that binds less efficiently to the FOP fuse and permits leaky signaling in the absence of BMPs**

- **Recognized that circulating monocytes and tissue macrophages are critical inflammatory triggers of FOP flare- ups**

- **Revealed progenitor cells of vascular origin that contribute to every stage of the FOP lesion**

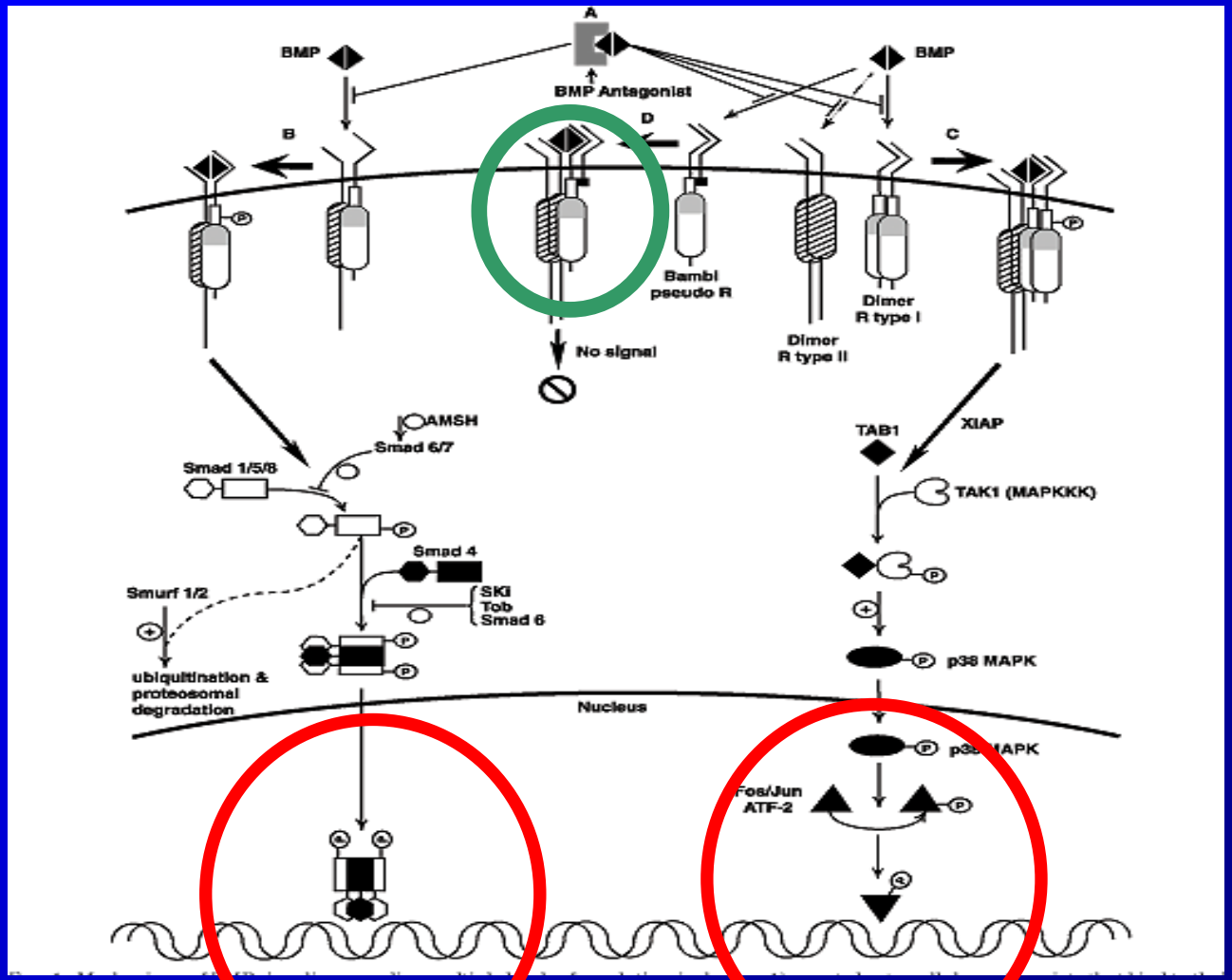
- **Showed that hypoxia dramatically enhances BMP signaling in FOP cells**

- **Rescued a lethal ACVR1 knockout in zebrafish with the mutant FOP gene and thereby demonstrated functional overactivity of the FOP gene in an animal model**

- **Developed a chimeric mouse model of FOP**

- **Identified a class of compounds that inhibit FOP-like lesions in animal models and that may serve as a basis for future drug development**

BMP Signaling Pathways



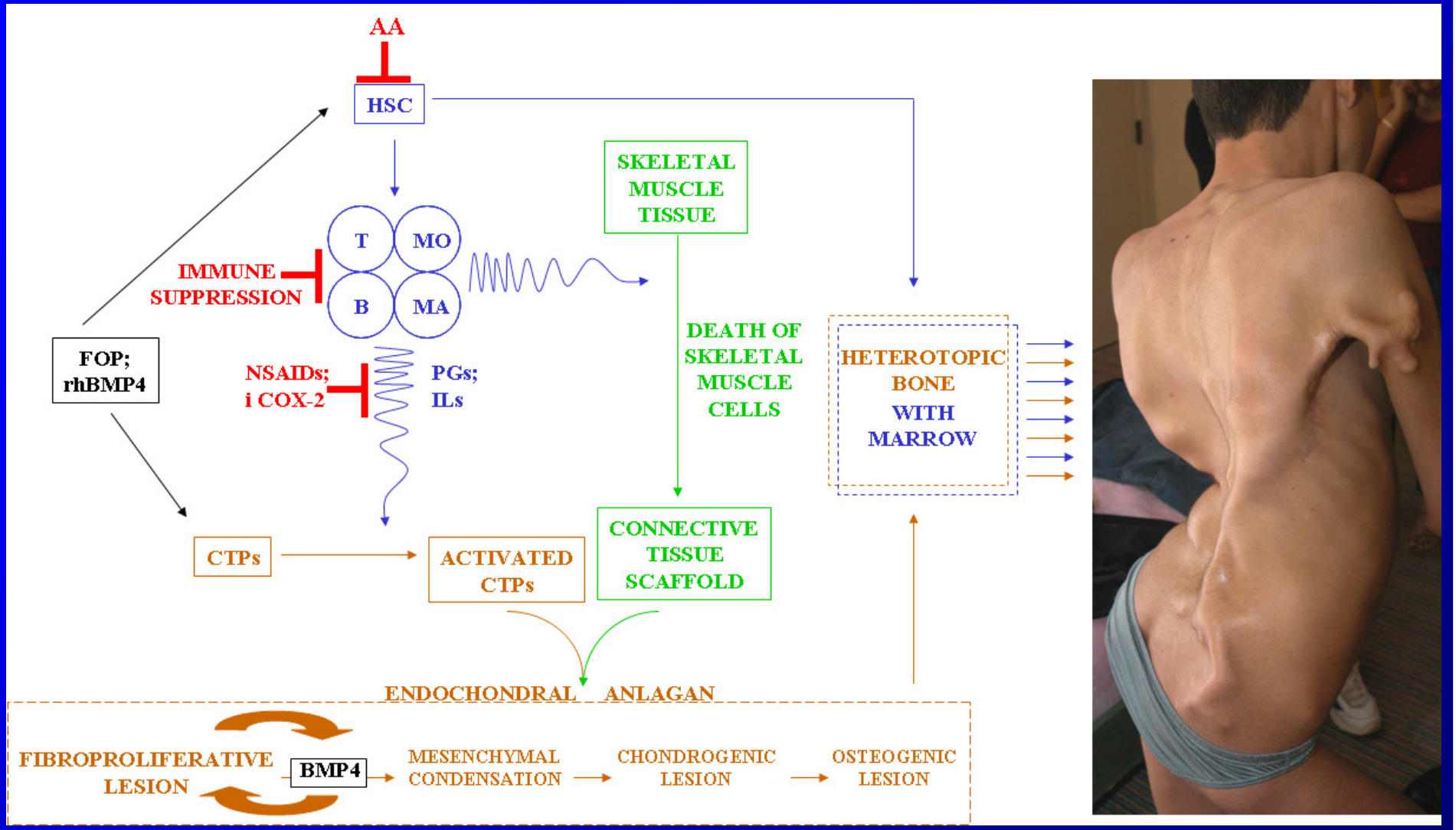
Leaky

Hyper-responsive

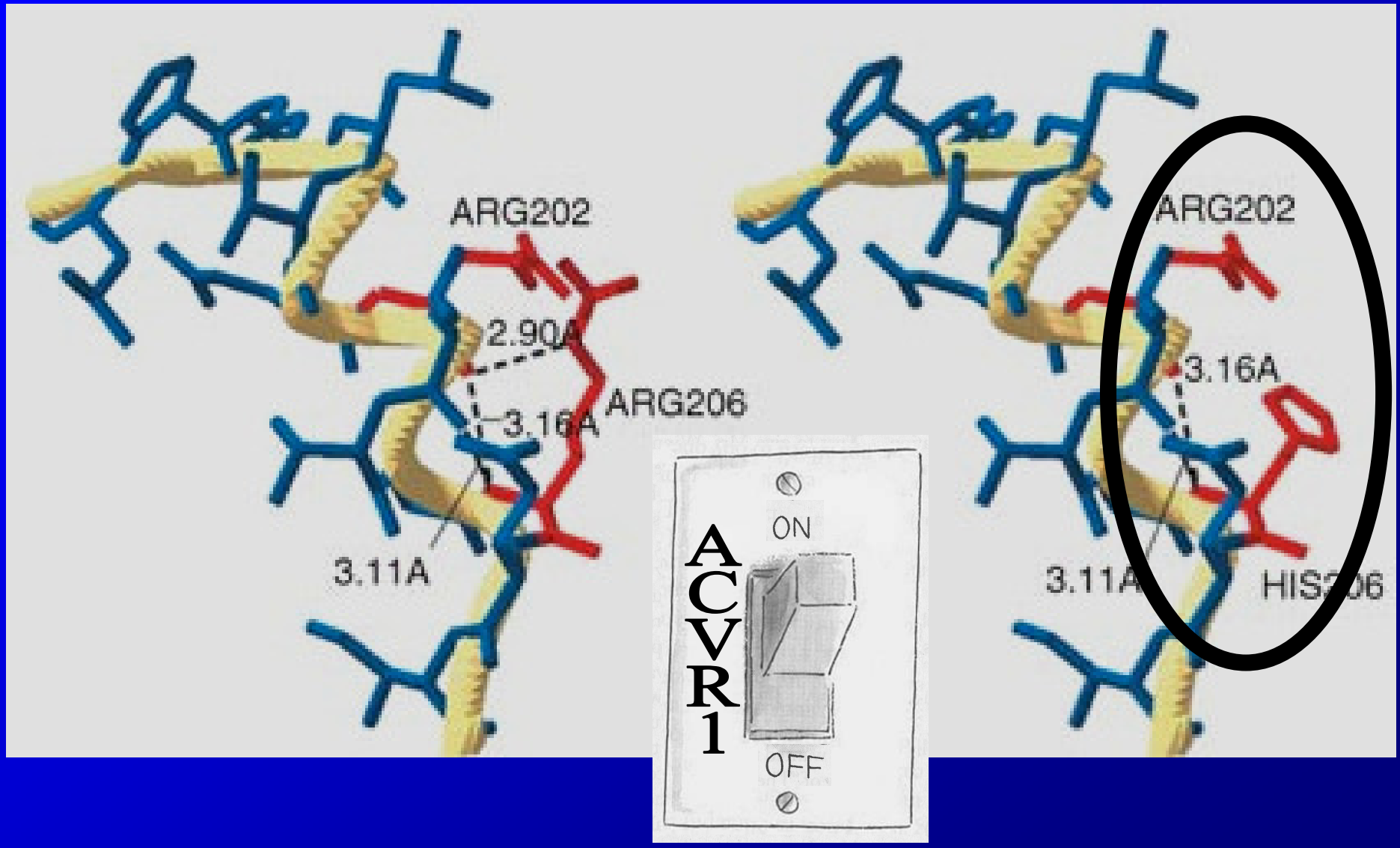
METAMORPHOSIS

The transformation
of one normal tissue
into another

FOP Is A Stem Cell Disease

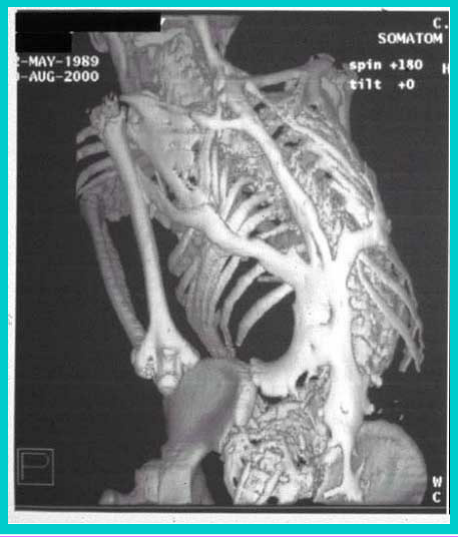


The Broken Switch



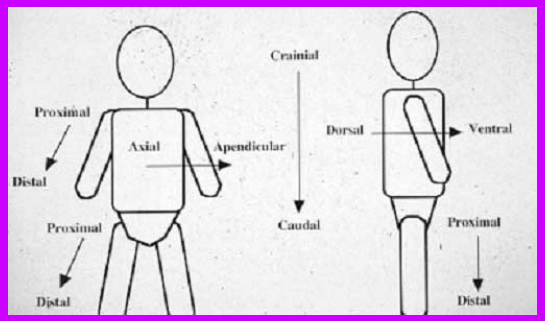
ACVR1 (R206H)

Ectopic Skeletogenesis

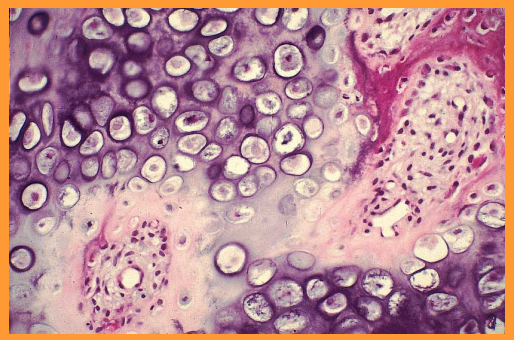


Great Toe Malformation

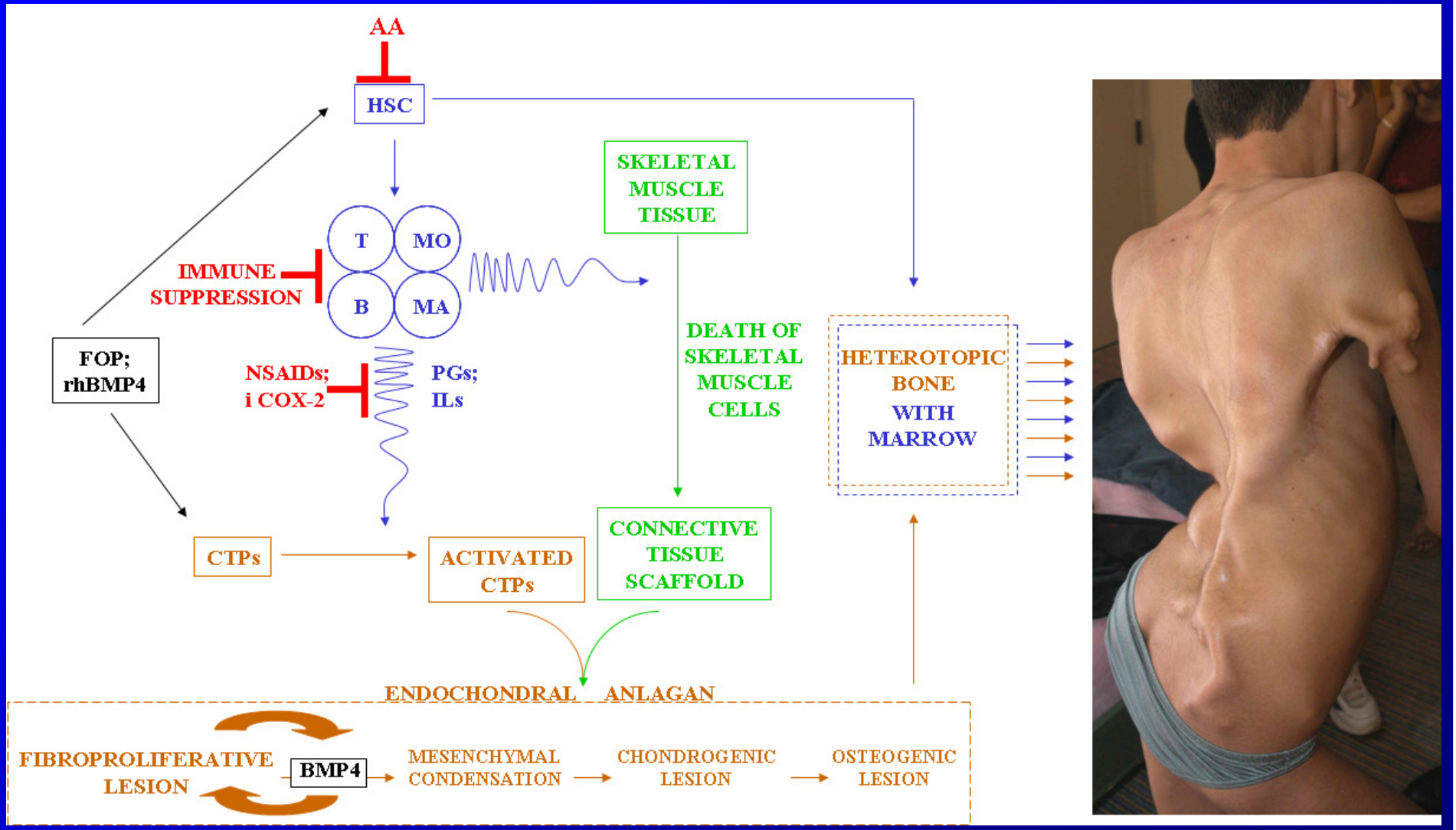
Pattern Formation



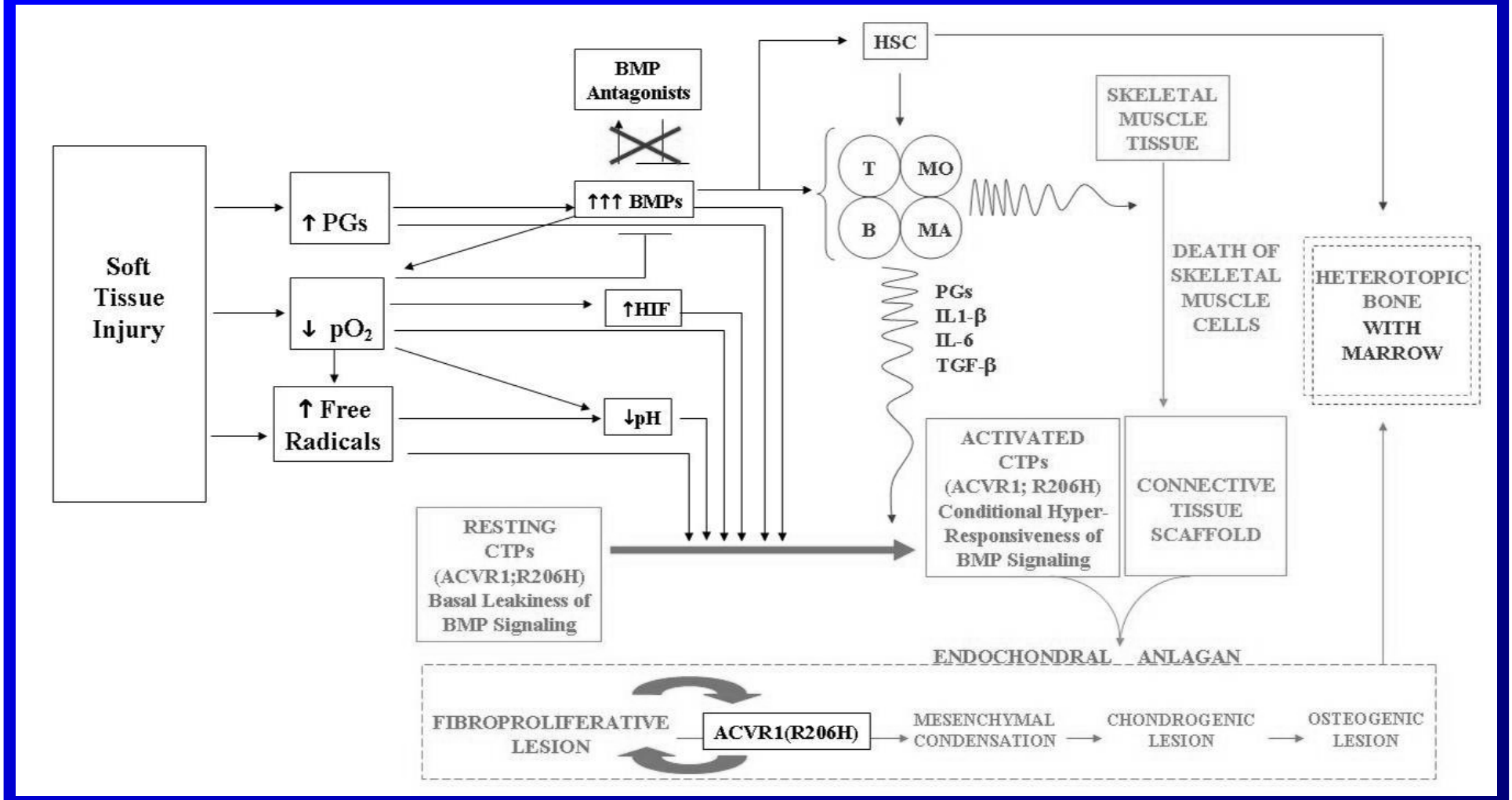
Endochondral Ossification



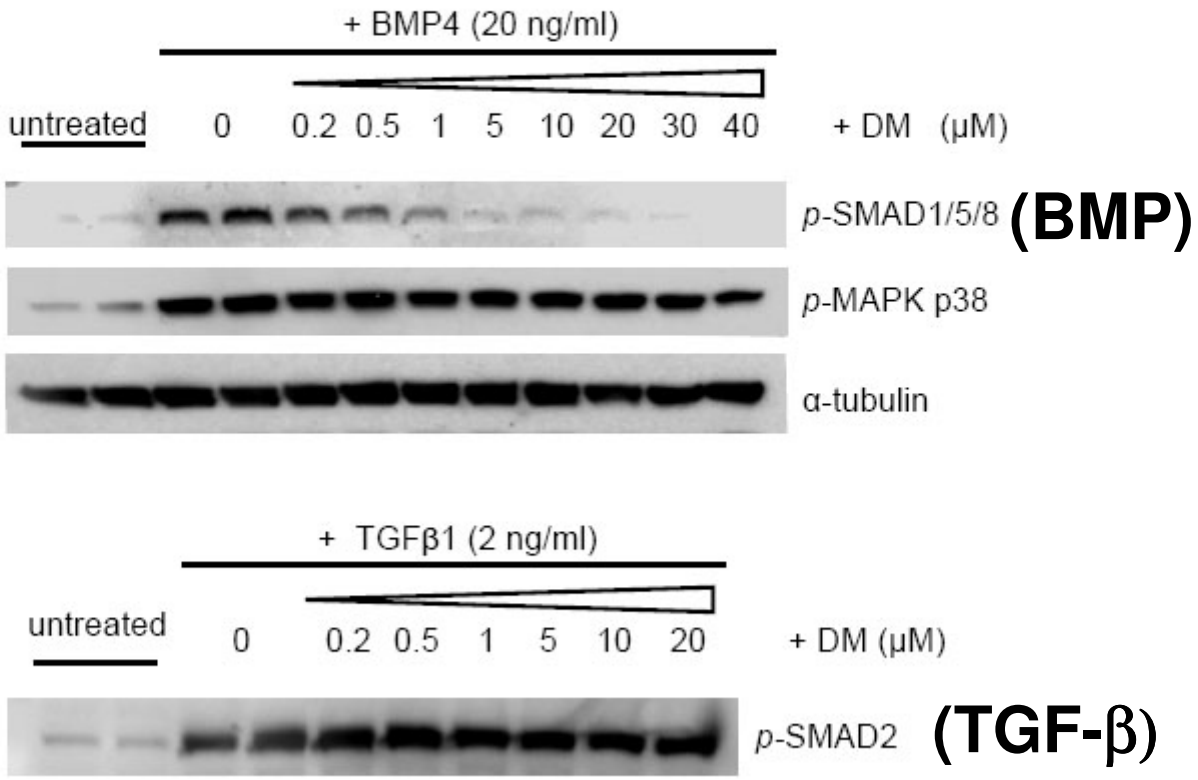
FOP Is A Stem Cell Disease



Inflammatory Triggers/ Signals Activate Stem/ Progenitor Cells



Dorsomorphin exhibits selectivity for BMP inhibition over TGFβ inhibition



Paul Yu, Ken Bloch

Dm-3189 Is A Selective & Potent Inhibitor of BMP type I Receptor Activity