



BOMARIN®

Human primary chondrocytes, a relevant model of MPS IVA, internalize GALNS (N-Acetylgalactosamine-6-Sulfatase) into lysosomes, resulting in clearance of keratan sulfate

Melita Dvorak-Ewell, Chuck Hague, Danielle Crippen & Michael Vellard

MPS IVA (Morquio A):

A lysosomal storage disease caused by the inability to degrade keratan sulfate

Biochemistry

- ∅ deficiency of lysosomal GALNS (N-Acetylgalactosamine-6-Sulfatase)
- ∅ inability to degrade keratan sulfate (KS)

Pathophysiology

- ∅ lysosomal accumulation of KS primarily in cartilage and cornea
- ∅ impaired cellular function of chondrocytes leads to growth plate abnormalities



Major clinical features

- ∅ bone dysplasia
 - ∅ dwarfism, genu valgum, pectus carinatum, odontoid hypoplasia, hearing loss
- ∅ ligamentous laxity
- ∅ cardiac valve defects
- ∅ corneal opacity

Autosomal recessive

1:200 000 – 1:300 000

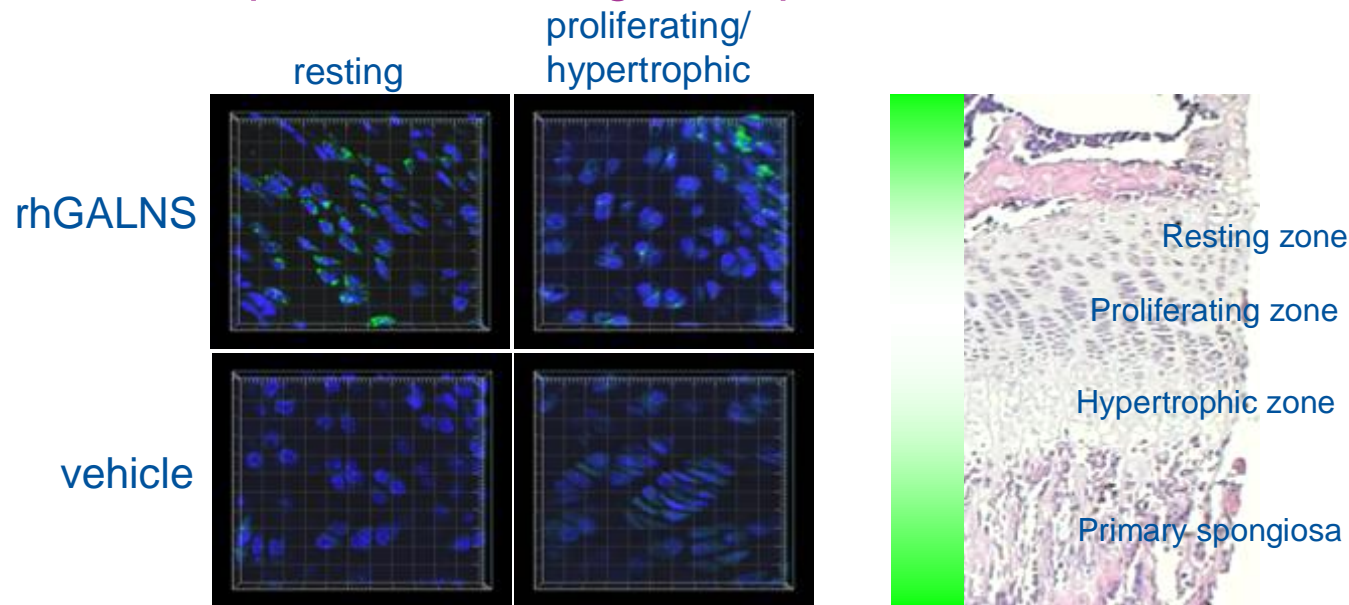


BIOMARIN GALNS is an investigational candidate that has not been approved by the FDA for the treatment of MPS IVA

rhGALNS for MPS IVA enzyme replacement therapy

- ∅ functional
- ∅ long in vitro serum $t_{1/2}$ at 37°C (~200 hours)
- ∅ hydroxyapatite affinity
- ∅ uptake by synoviocytes ($K_{\text{uptake}} \sim 6 \text{ nM}$)

rhGALNS penetrates the growth plate and heart valve cartilage



Dvorak-Ewell, Wendt, et al POSTER



Can we show rhGALNS efficacy?

- ∅ KS clearance, improved cellular function
- ∅ Model of MPS IVA



Development of the MPS IVA model

GALNS-null mice (*Tomatsu et al 2003, 2007, 2008*)

- ∅ No significant accumulation of KS in cartilage
- ∅ No skeletal phenotype
- ∅ Not a relevant model of human MPSIVA

In vitro model of human MPS IVA

∅ Primary human MPS IVA fibroblasts

- » Do not accumulate KS
- » Irrelevant cell type

∅ Immortalized human chondrosarcoma cell lines

- » Endogenous GALNS expression
- » ~~Sarcoma phenotype~~

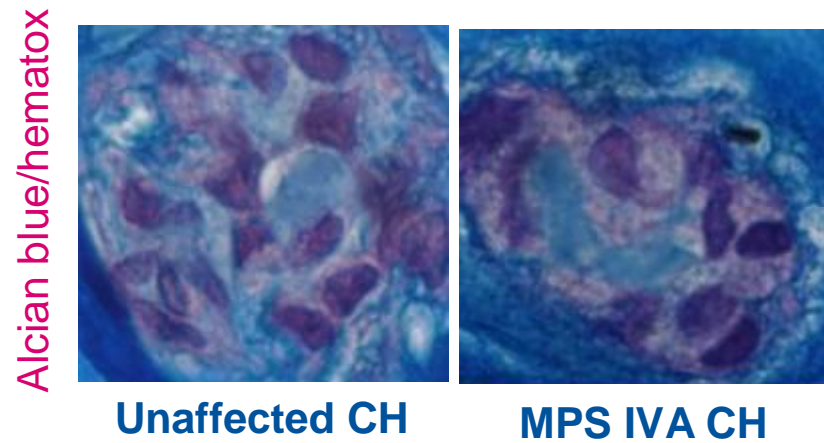
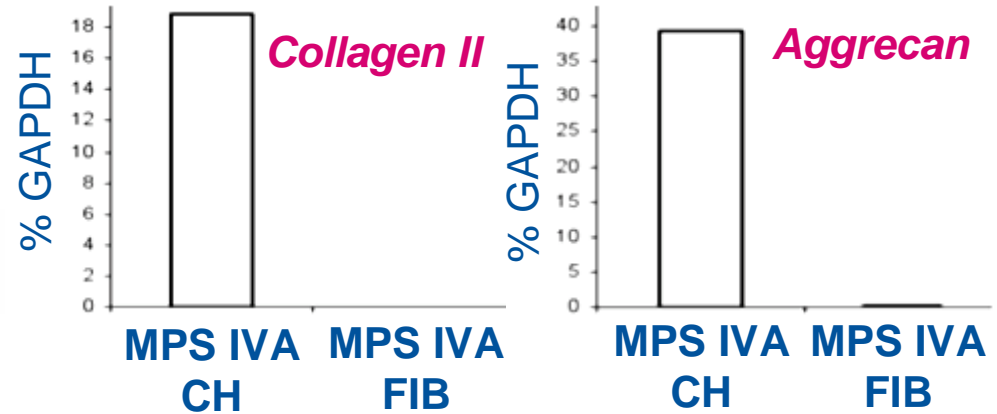
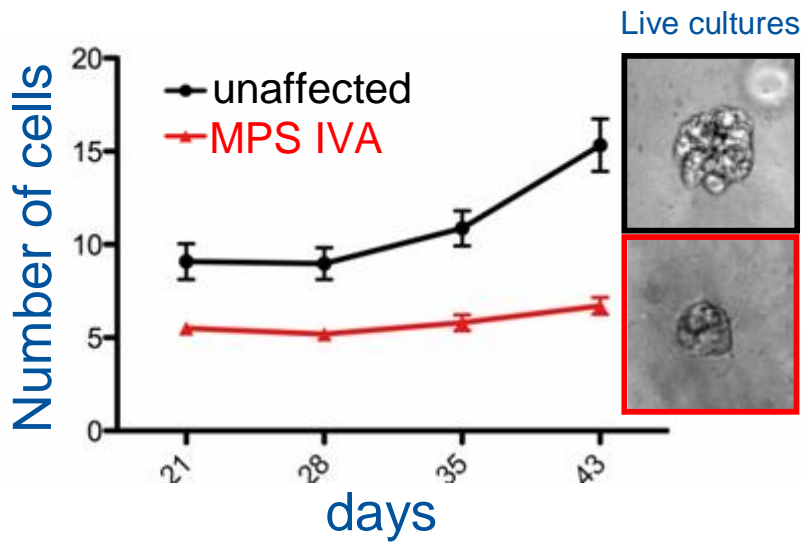
∅ MPS IVA chondrocytes

- ∅ Primary human MPS IVA chondrocytes from iliac crest biopsy (Dr. Wilcox, Cedars-Sinai, LA)
- » Difficult to maintain chondrogenic phenotype
- » Long term cultures
- » Limited number of cell

Unaffected control cells

- ∅ Primary human knee chondrocytes from healthy donors

Human Primary MPS IVA chondrocytes in alginate cultures



Decreased proliferation

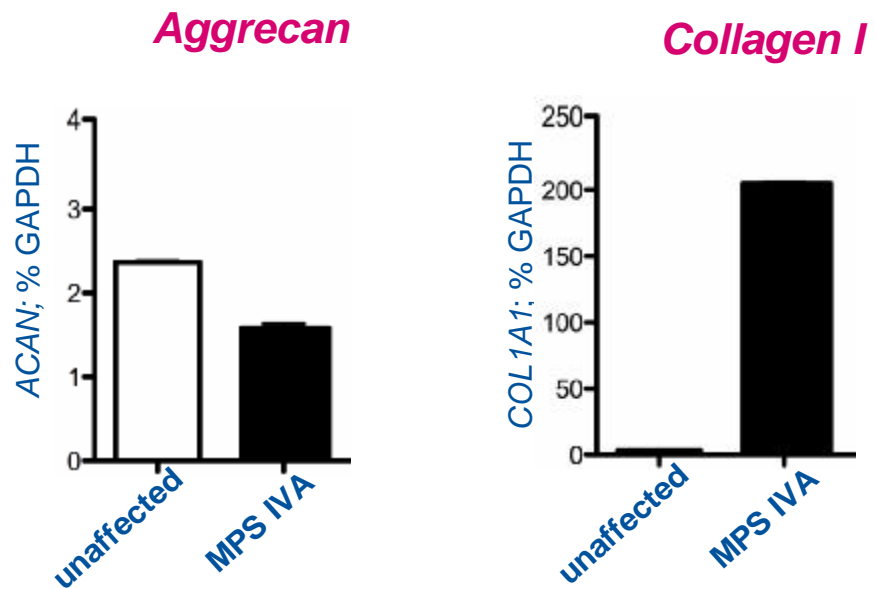
» Limited number of cells for experiments

Chondrogenic differentiation



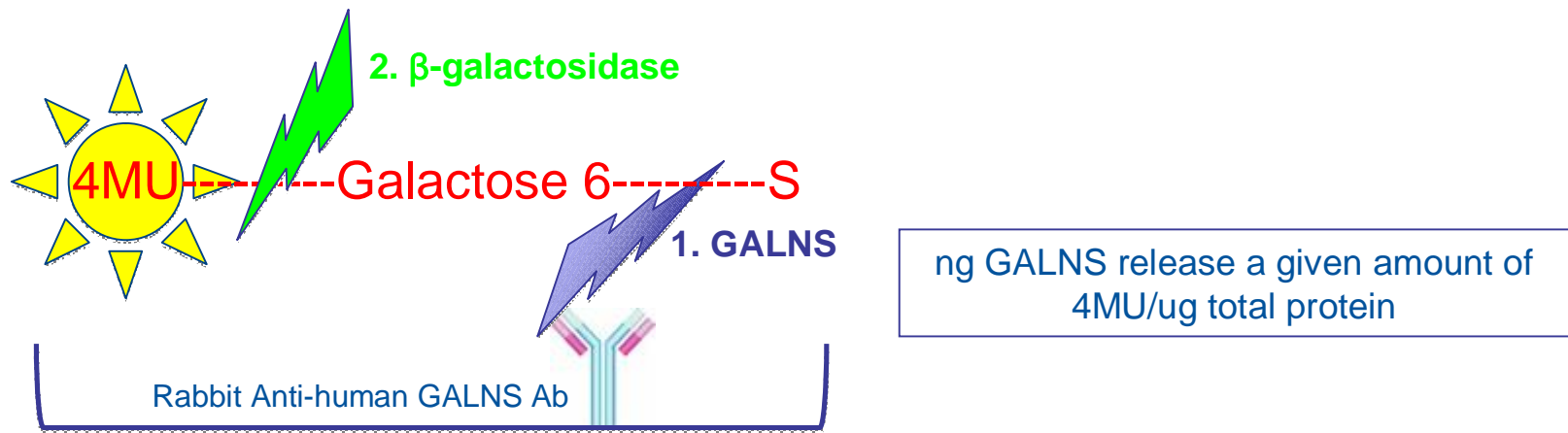
MPS IVA chondrocytes exhibit altered expression of chondrogenic markers

- 11 weeks cultures, qRT-PCR



MPS IVA chondrocytes lack GALNS

- GALNS capture activity ELISA



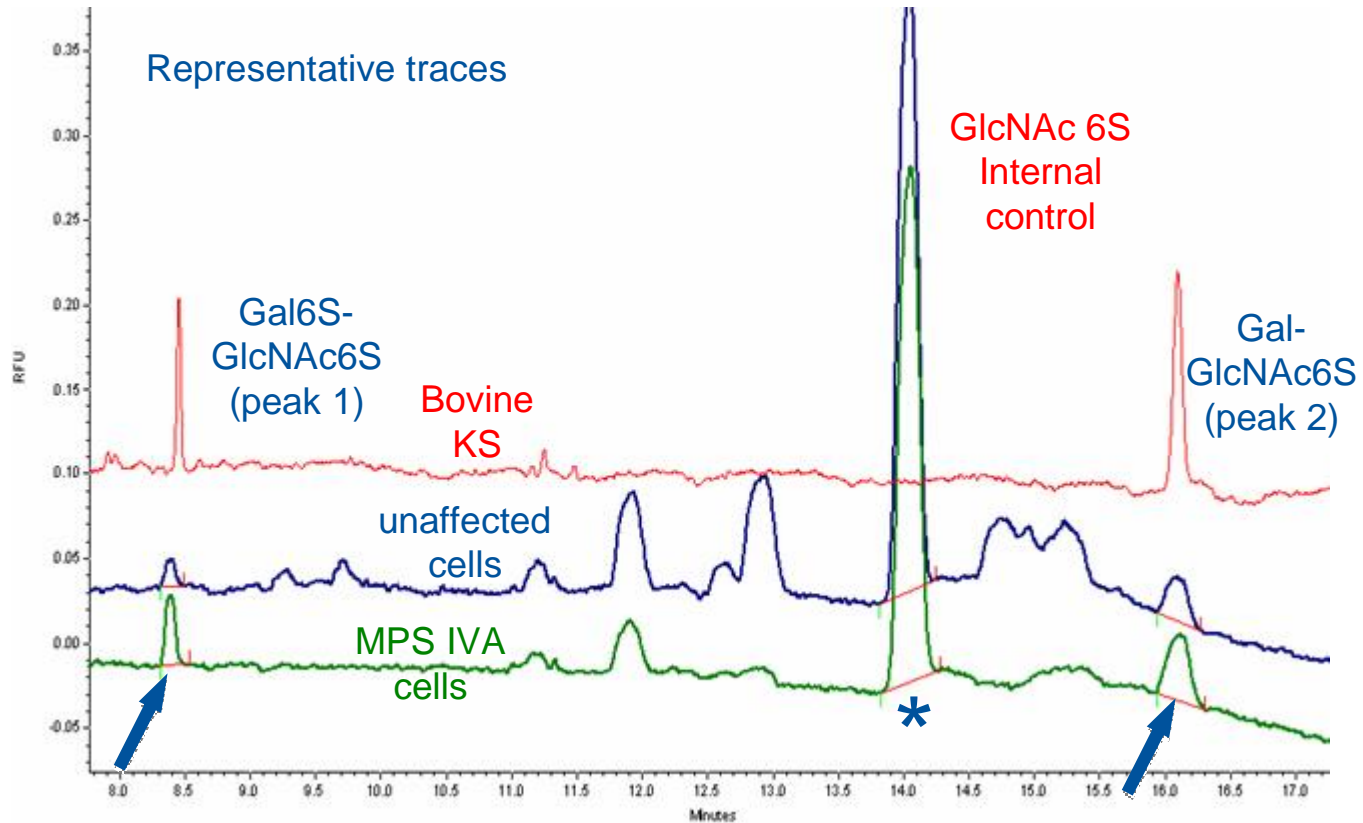
conditions	Active GALNS (ng)/total protein (ug)	
	MPS IVA	Unaffected cells
monolayers ^a	none detected	0.1±0.003
alginate suspension ^b	none detected	0.32

^a Cell lysates obtained from subconfluent monolayers (n=3)

^b Cell lysates obtained from 15-week alginate suspension cultures (n=1)

MPS IVA chondrocytes accumulate KS

- 6 week alginate cultures, capillary electrophoresis of keratanase II digests



	Peak 1	Peak 2
MPS IVA	1950 ±310	2170 ±360
unaffected	170 ±40	400 ±80

*normalized for internal control/ug protein

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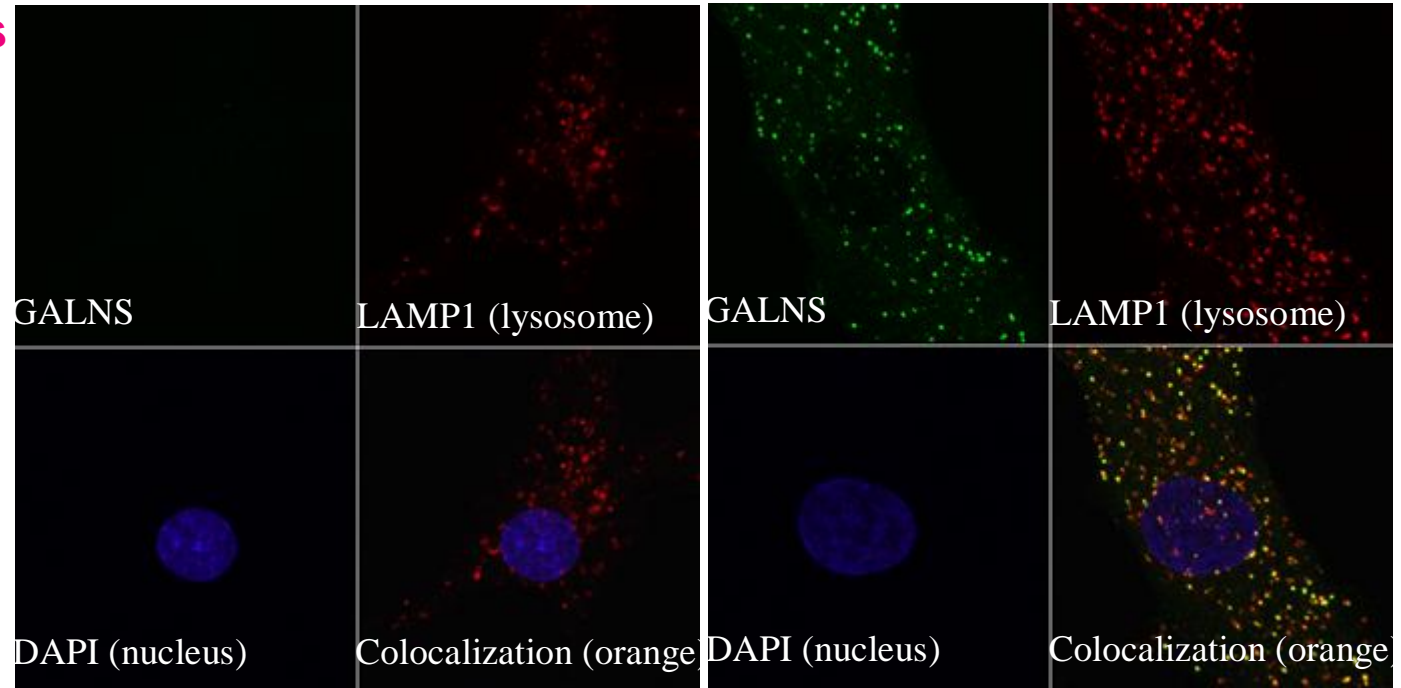
MPS IVA chondrocytes uptake rhGALNS into lysosomes

- Short term monolayer cultures (3 days)

A: Confocal Microscopy;
MPS IVA cells

untreated

10 nM rhGALNS



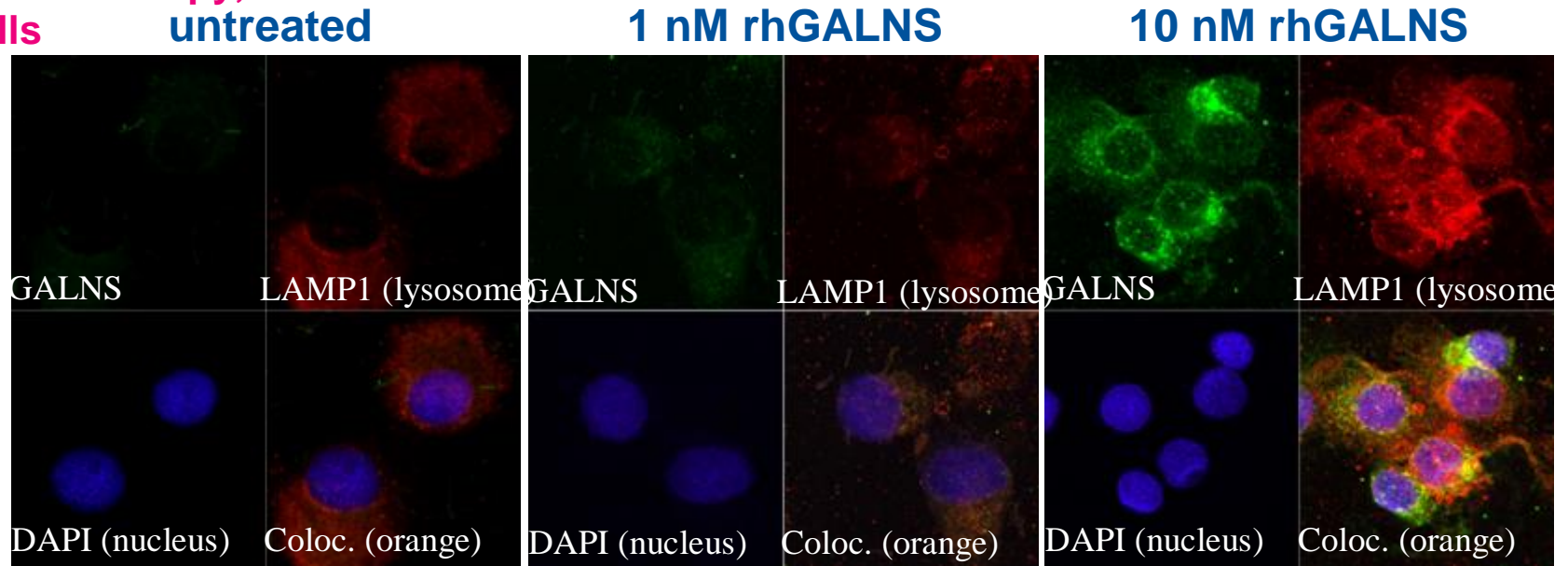
B: Capture ELISA;
n=3

Active GALNS/total protein	MPS IVA	unaffected
No treatment control	none detected	0.1±0.003 ng activity/ug protein
10nM rhGALNS	2.15±0.18 ng activity/ug protein	2.16±0.03 ng activity/ug protein

MPS IVA chondrocytes uptake rhGALNS into lysosomes

- Long term alginate cultures (6-15 weeks)

A: Confocal Microscopy;
MPS IVA cells



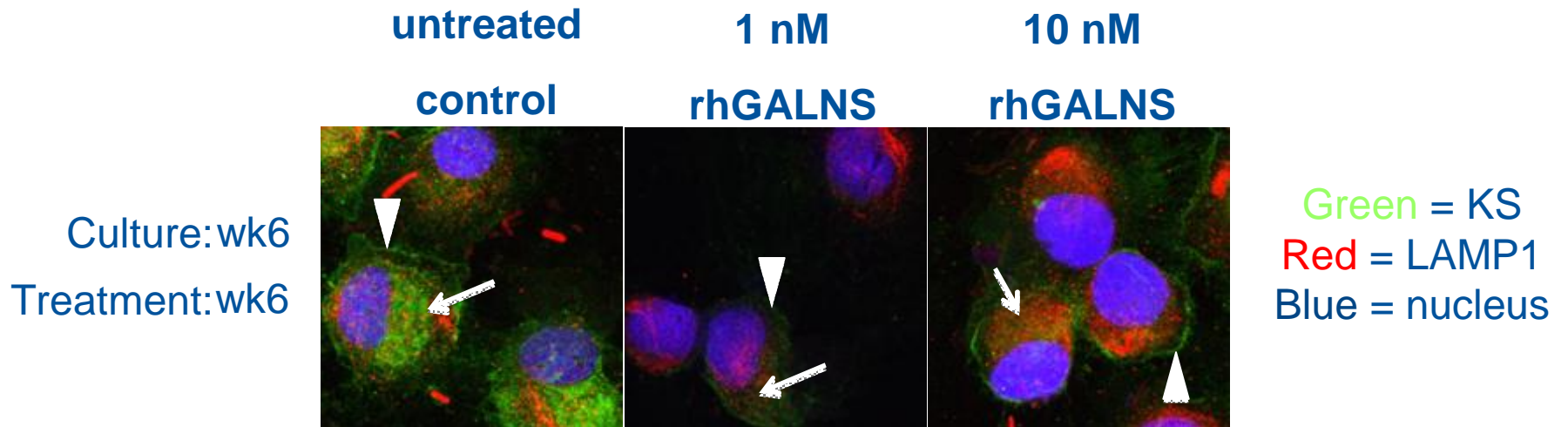
- Treatment: 6 weeks, twice/wk throughout culture
Similar findings throughout culture (weekly samples)

B: Capture ELISA;
n=1

	Active GALNS/total protein	MPS IVA	unaffected
No treatment control		none detected	0.32 ng activity/ug protein
10nM rhGALNS		1.48 ng activity/ug protein	1.57 ng activity/ug protein

rhGALNS reduces KS accumulation in MPS IVA chondrocytes

- Long term alginate cultures (6 weeks), confocal microscopy; MPS IVA cells

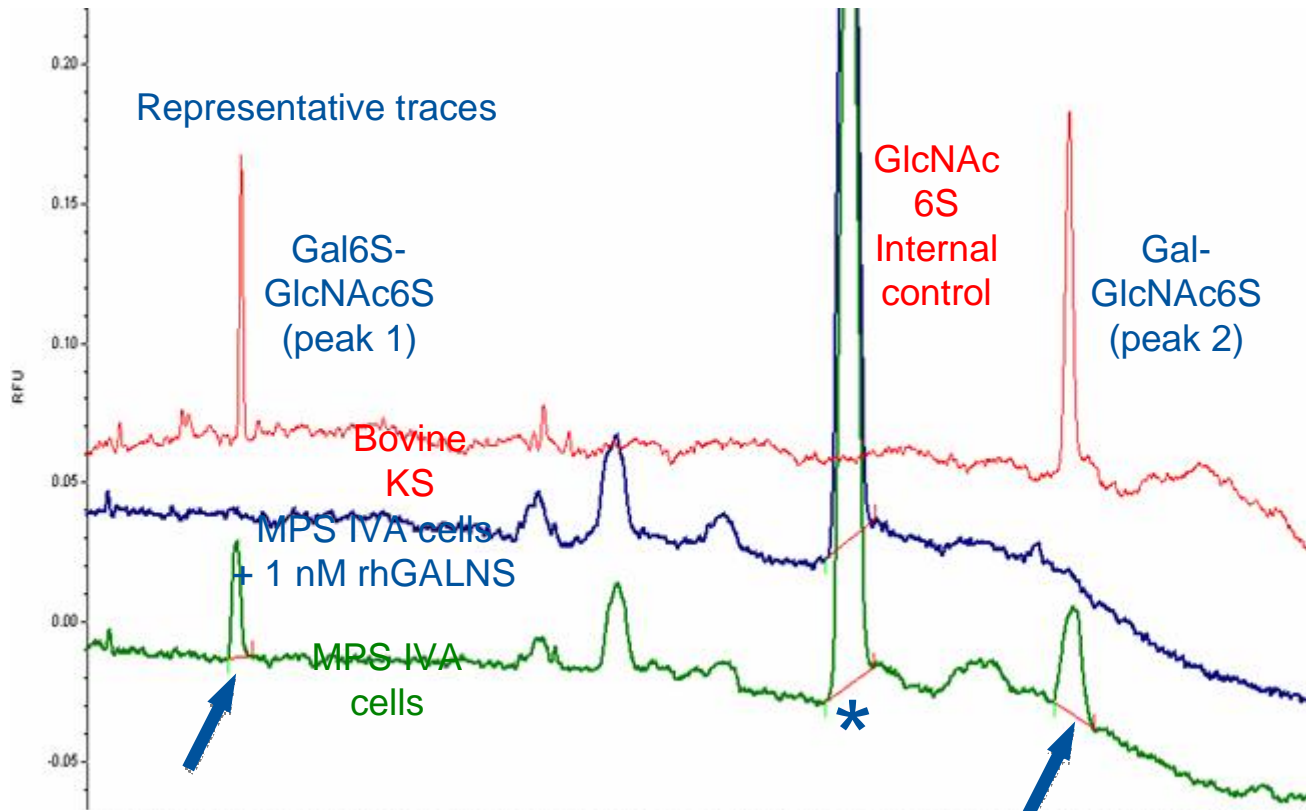


- Treatment: 6 weeks, twice/wk throughout culture

Similar findings throughout culture (weekly samples)
No change in KS in unaffected cells

rhGALNS reduces KS accumulation in MPS IVA chondrocytes

- Long term alginate cultures (6 weeks), capillary electrophoresis n=2/*1



rhGALNS/nM	MPS IVA		Unaffected cells	
	Peak 1	Peak 2	Peak 1	Peak 2
0	1950 ±310	2170 ±360	170 ±40	400 ±80
1	270*	460*	370 ±90	850 ±80
10	210 ±30	320 ±40	280 ±80	440 ±150

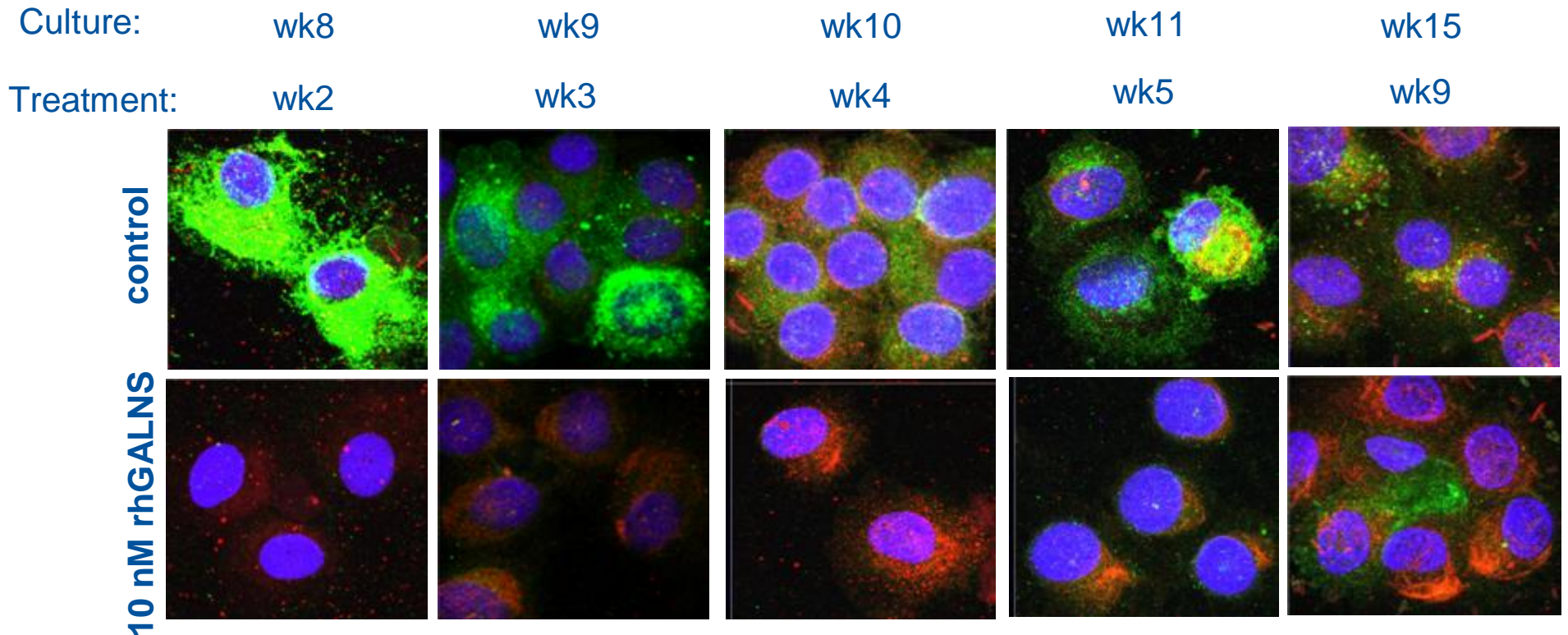
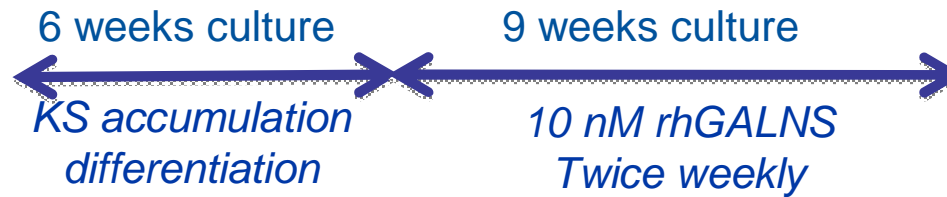
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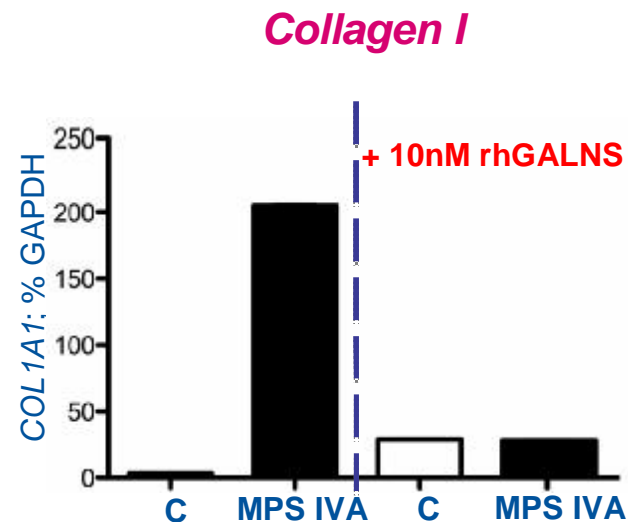
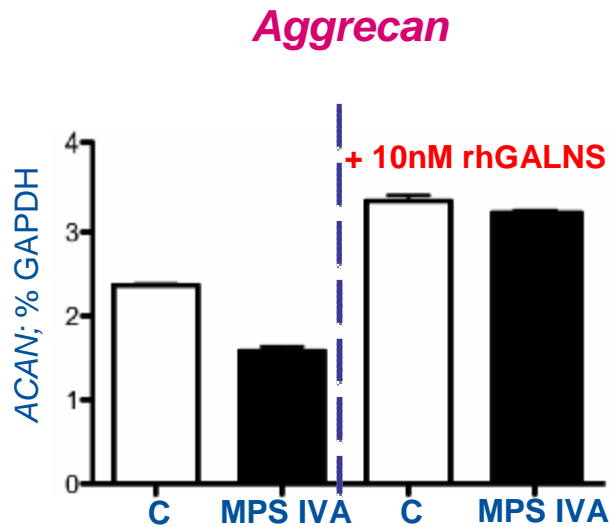
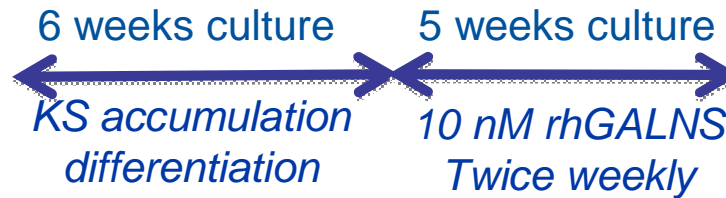
rhGALNS clears accumulated KS in MPS IVA chondrocytes

- Long term alginate cultures, confocal microscopy



rhGALNS restores aberrant gene expression of chondrogenic markers by MPS IVA cells

- qRT-PCR





SUMMARY

MPS IVA cells grown in alginate suspension cultures are a valid MPS IVA model *in vitro*

- Exhibit chondrocyte phenotype
- No detectable GALNS
- Accumulate KS
- Aberrant cellular function

rhGALNS is internalized by MPS IVA lysosomes

- Dose-dependent

rhGALNS clears KS

- Reduces accumulation
- Clears accumulated KS

Preliminary evidence shows rhGALNS restores function of MPS IVA cells

- Gene expression



BioMarin Pharmaceutical Inc.

Michael Vellard
Terri Christianson
Melita Dvorak-Ewell
Eskil Eskilsson
Shinong Long
Terrence Satterfield
Ben Schooler
Melanie Williams
Yan Yan

Emil Kakkis
Dan Wendt
Chuck Hague
Vish Koppaka
Florence Lorget

BioMarin Morquio Core Team

The Buck Institute

Danielle Crippen
Cathy Vitel

Cedar-Sinai Medical Center, LA

Bill Wilcox