THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Knowledge that will change your world

The Power of Biospecimens in Understanding Disease Progression in ALS

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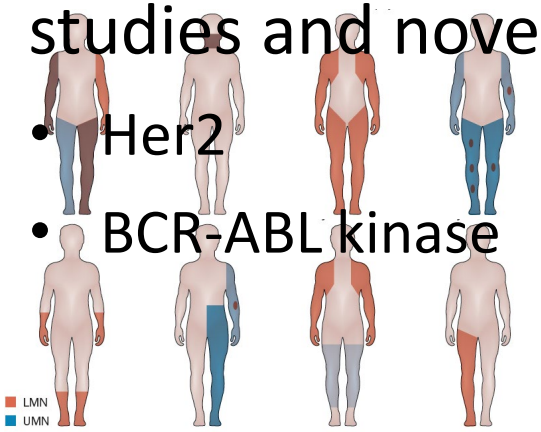
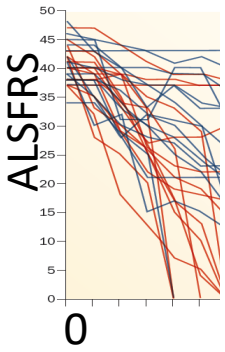
Objectives

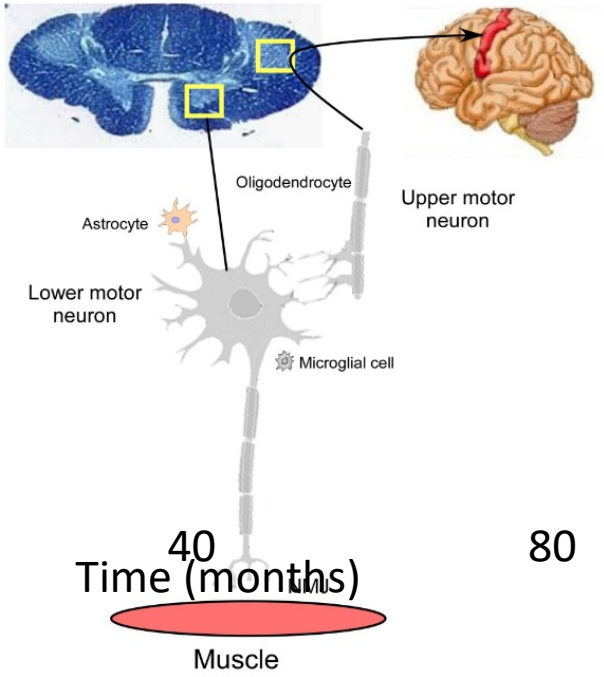
- General concepts of disease marker discovery
- Sharing the UAB/BVAMC experience in using ALS tissue
  - Example 1: Discovery of muscle biomarkers and new directions for understanding disease mechanisms
  - Example 2: Validation of HuR as a new regulator of central neuroinflammation in ALS
  - Example 3: Validation of peripheral neuroinflammation in ALS and support for a novel treatment direction

Importance of Biomarker discovery by “omics”

- Markers for clinical assessment
- Reveals novel molecular pathways
- Provides direction for mechanistic studies and novel therapies
  - Her2
  - BCR-ABL kinase





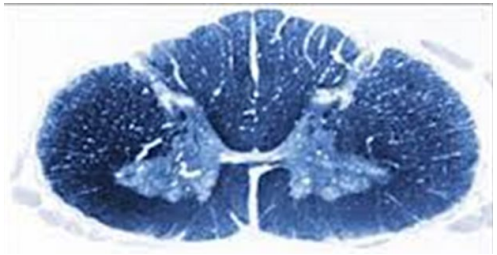


Swinnen, Nature Rev, 2014

Post Mortem Biospecimens in ALS

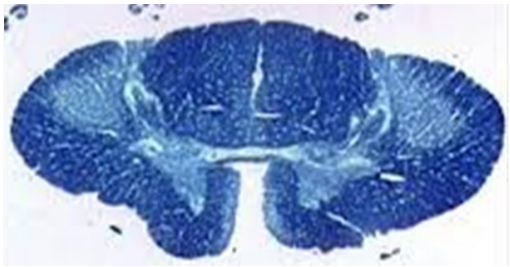
Normal

Before



ALS

After



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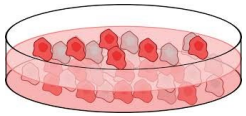
F5 tornado

Aerial bombing

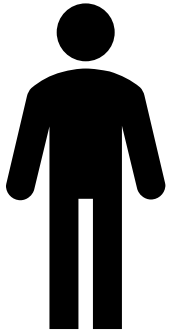
King and Mitsumoto, 1996

ALS Tissue: Discovery and Validation

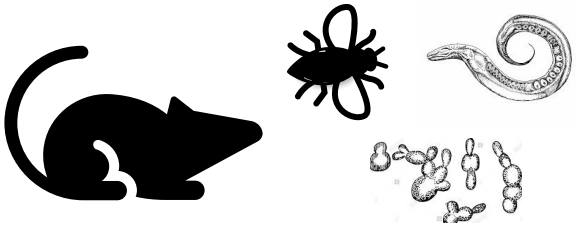


IPSC

- ALS patient derived
- Sporadic or familial
- Rapid testing
- Mechanistic
- Not an organism
- No aging effect



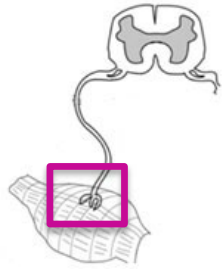
- "Gold" standard
- Sporadic form
- Microenvironment
- End-stage
- QC RNA, protein



- Temporal and spatial evolution
- Microenvironment
- Mechanistic
- Genetic manipulation
- Genetic based
- Incomplete recapitulation of pathology

Example 1: Biomarker Discovery

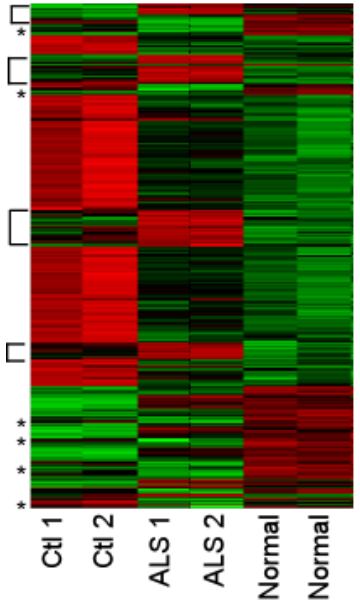
Discovery



Muscle samples[a].

	Normal	ALS	Myopathy	Neuropathy
Number	22	39	12	15
Age Range	32–74	27–82	38–74	33–88
Mean Age (y)	54 ± 11	59 ± 12	56 ± 14	60 ± 11
Gender (M:F)	2.6:1	1.2:1	1.1:1	3.3:1
Diagnosis	–	Spinal (77%)	Inflammatory	Axonal neuropathy
		Bulbar (23%)	Mitochondrial	Plexopathy
			Necrotizing	CIDP
				GBS (1)
			Non-specific	GBS
				Non-specific

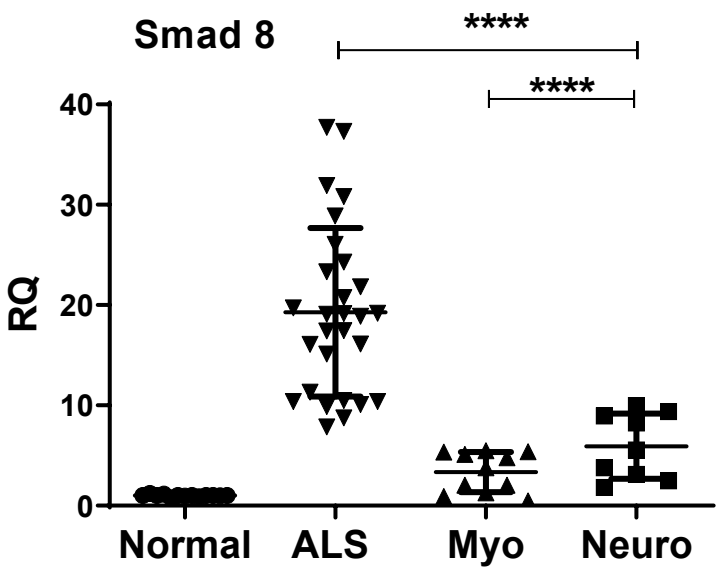
[a] Patient samples were used for either qPCR or western blot analysis. CIDP, Chronic inflammatory demyelinating polyradiculoneuropathy; GBS, Guillain Barre syndrome.

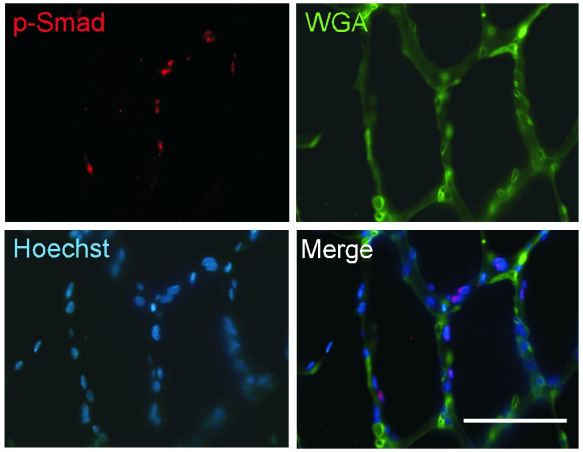


Si et al., Ann Clin Transl Neurol. 2014

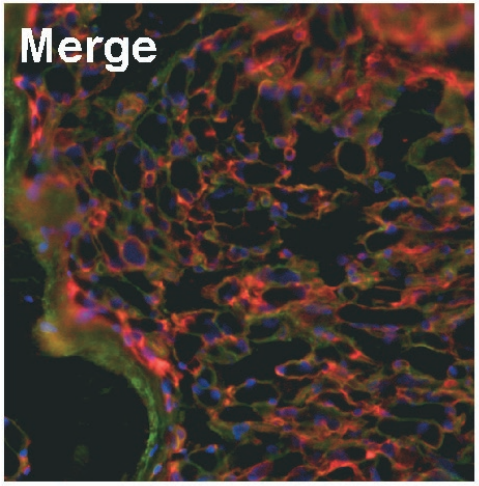
Smad 8 Validation

qPCR





Biopsy

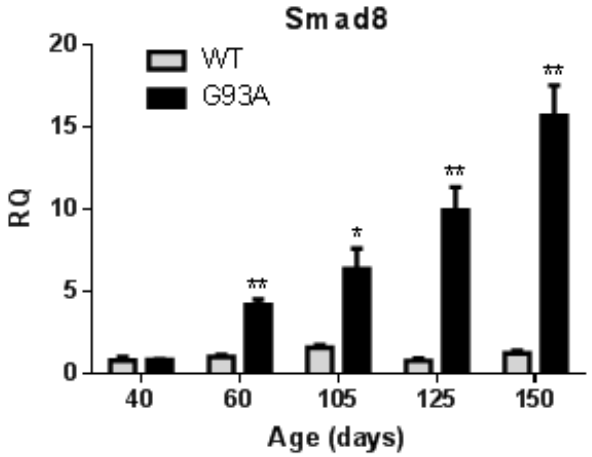


Autopsy

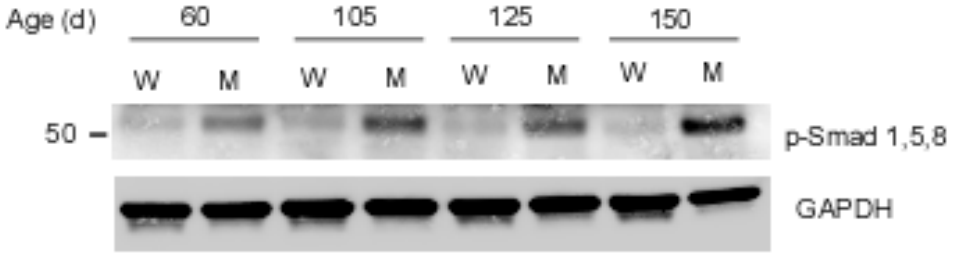
Si et al., Ann Clin Transl Neurol. 2014

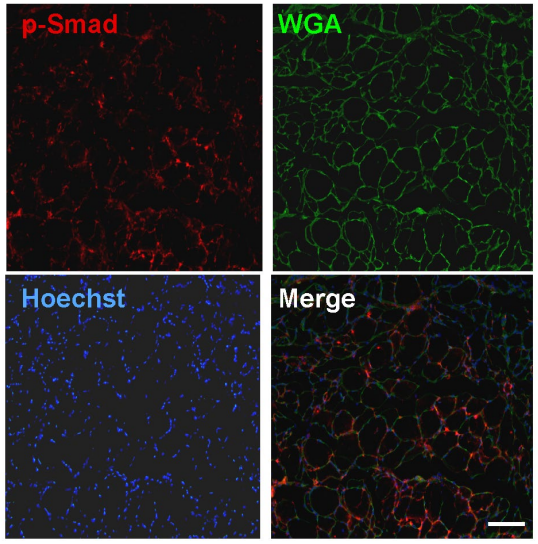
Smad8 in the G93A SOD1 mouse

ALS mouse



qPCR

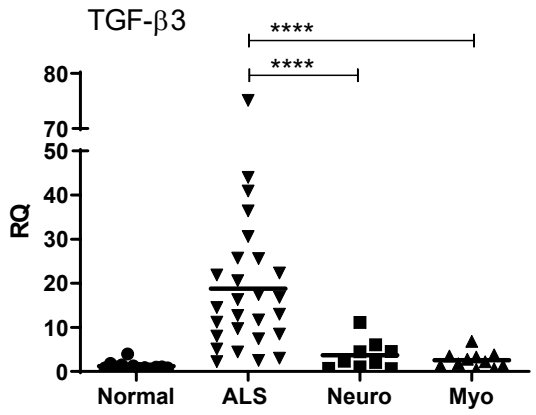


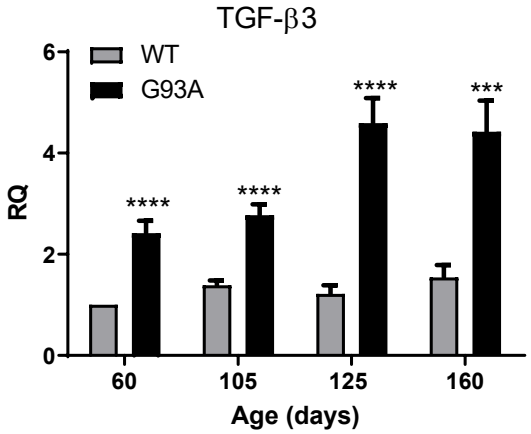


IHC

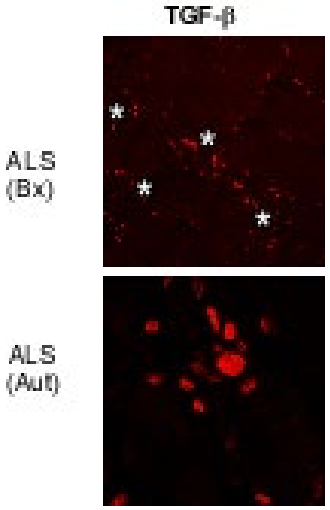
Si et al., Ann Clin Transl Neurol. 2014

TGF-β

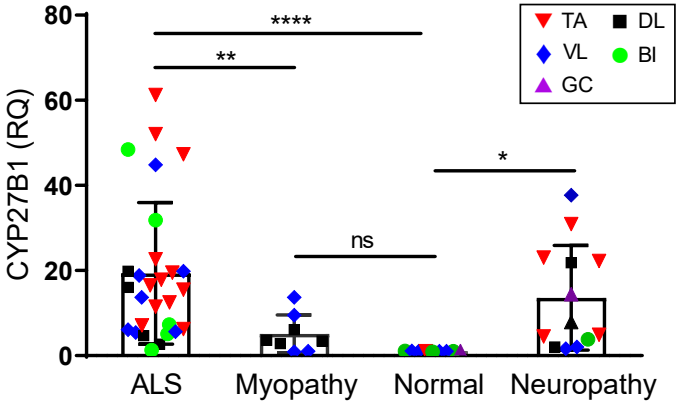


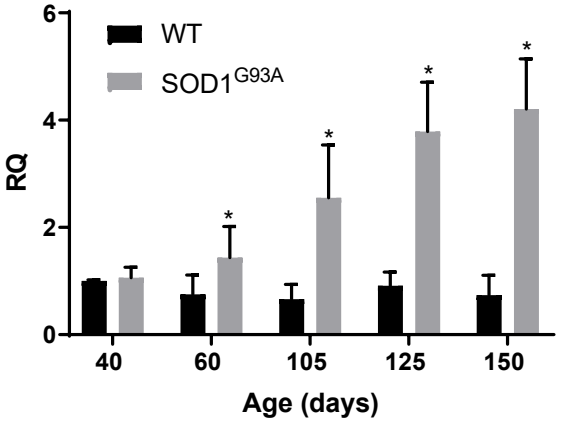


Human ALS (VL)

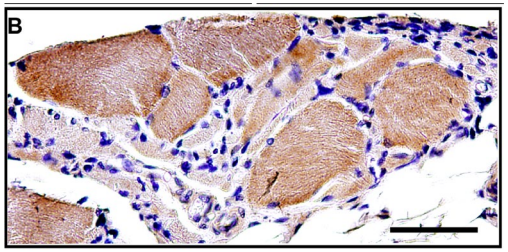


CYP27B1





Human ALS (VL)



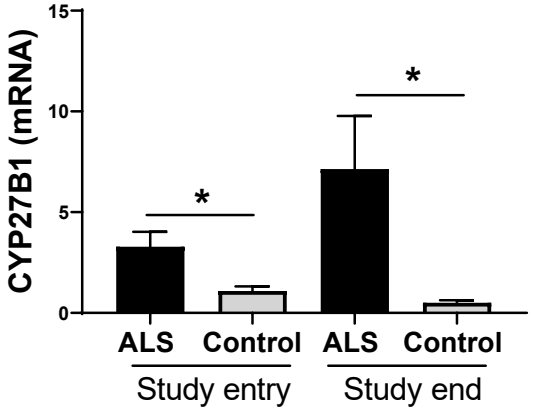
Si et al., Plos One, 2015

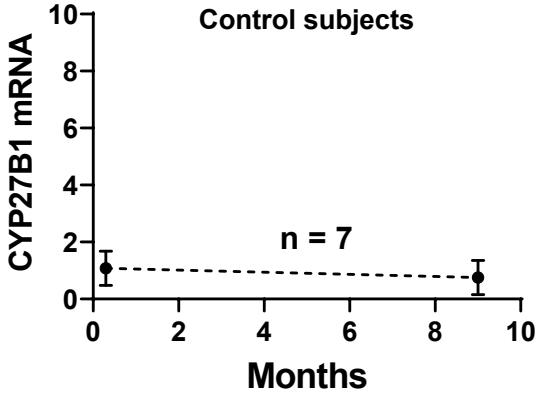
Si et al.,J Steroid Bioch, 2020

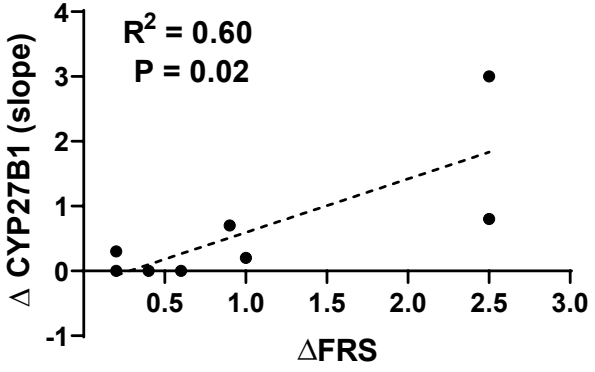
CYP27B1 and ALS Disease progression: a prospective study

Study patients

Sex	Age	Onset	[a]Duration (m)	Study duration (m)	[c]ALSFRS-R		[d]ΔFRS	[e]Muscle
					Entry	End		
M	59	Bulbar	7	6[g]	28	13	2.5	DL
M	64	Bulbar	8	6[g]	36	21	2.5	DL
M	41	Spinal	15	12	24	12	1.0	TA
M	44	Spinal	17	12	30	19	0.9	DL
M	54	Spinal	47	12	34	27	0.6	BI
M	66	Spinal	17	12	44	42	0.2	DL
F	53	Spinal	26	12	43	40	0.3	DL
M	62	Spinal	16	12	29	27	0.2	DL







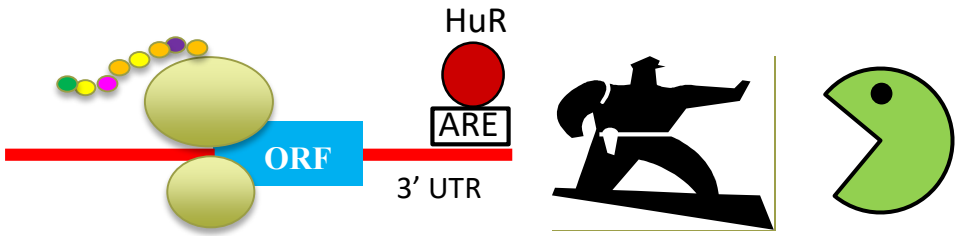
Si et al.,J Steroid Bioch, 2020

Lessons Learned

- Coordinated molecular program initiated in skeletal muscle in ALS at early pre-symptomatic stages
- Diversity of novel disease-associated pathways
  - Smads in muscle denervation/reinnervation and miRNA regulation
  - Role of local Vitamin D in denervated skeletal muscle
  - TGF-β: role in muscle fibrosis and inflammation; link to Smads
- Potential markers for tracking disease progression

Example 2 Central neuroinflammation: validation

- HuR is an RNA binding protein in the ELAV family
- Binds to AUUUA sequences and stabilizes mRNA and increases translation
- Translocates to cytoplasm when activated
- Expressed in microglia/macrophages

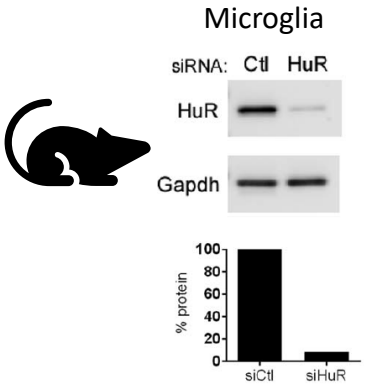


TNF-α

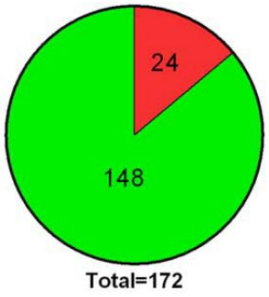
GAUUAUUUAUUAUUUAUU
UAUUAUUUAUUUAUUUAC
AGAUGAAUGUAUUUAUUU

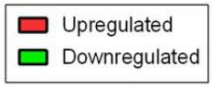
Gene	*Name*	*HuR Binding Sites*[4]
IL-1β	Interleukin 1β	4
LIF	Leukemia inhibitory factor	20
IL-6	Interleukin 6	4
TNF-α	Tumor necrosis factor alpha	12
CXCL1	C-X-C motif chemokine ligand 1	9
CCL2	C-C motif ligand 2	4
MMP12	Matrix metalloproteinase-12	9

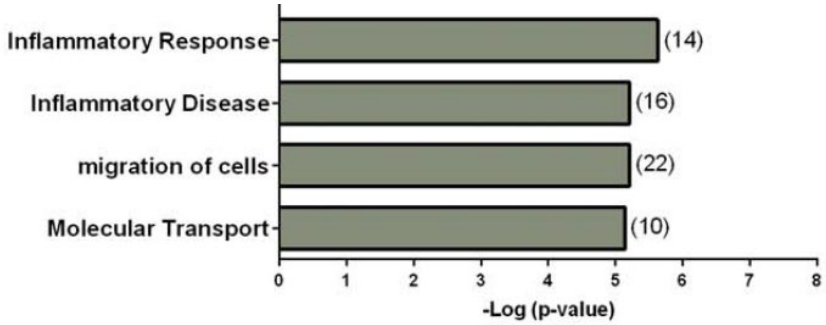
HuR and Neuroinflammation



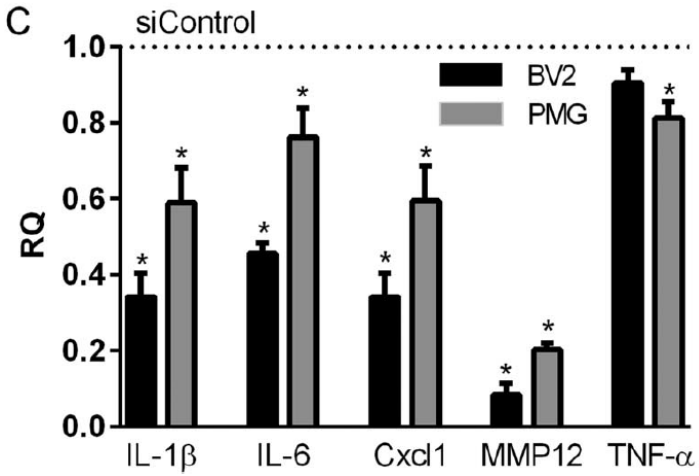
RNA Sequencing of microglia





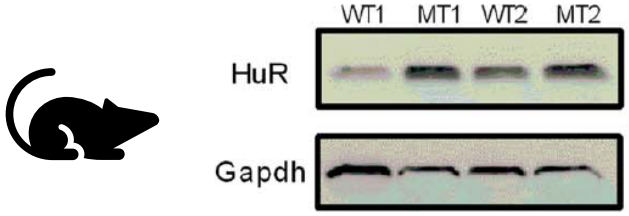


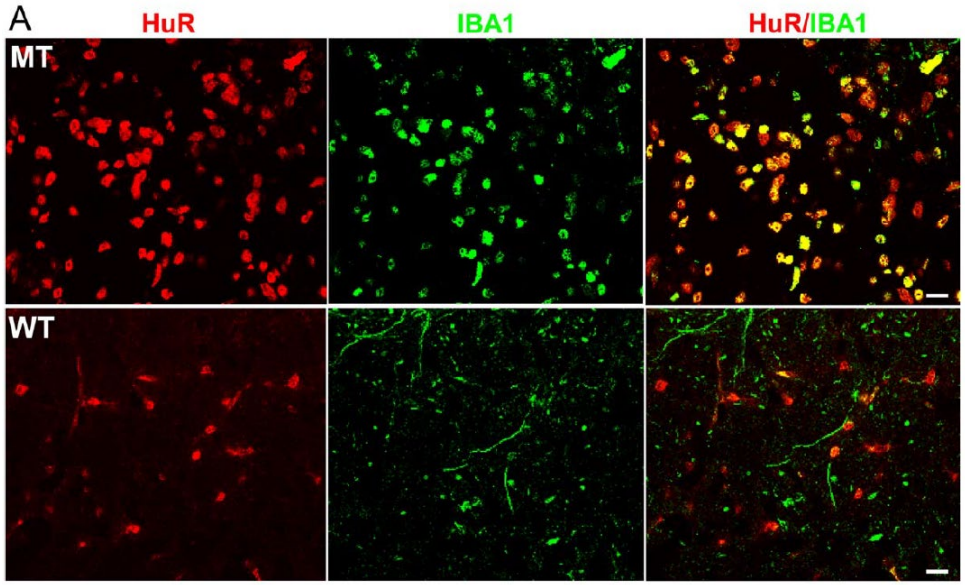
Validation of targets

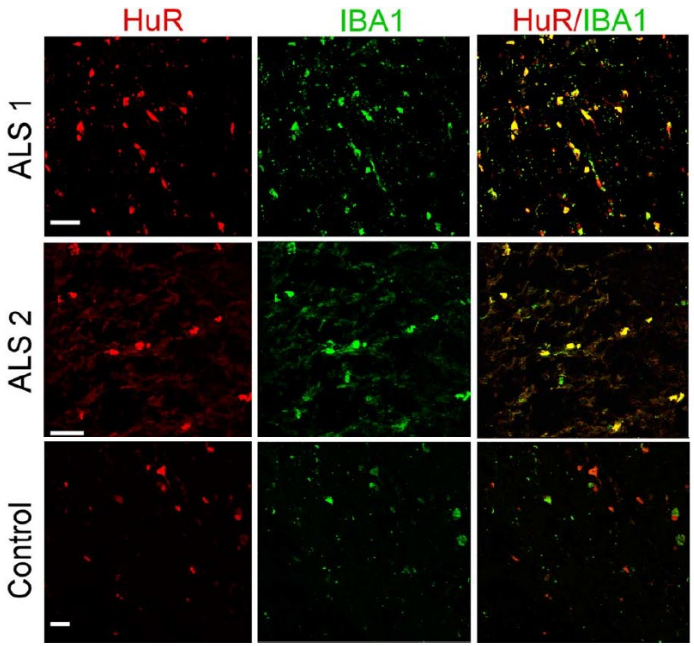


Matsye et al., Glia, 2017

HuR and ALS


WT1
MT1
WT2
MT2
HuR
Gapdh

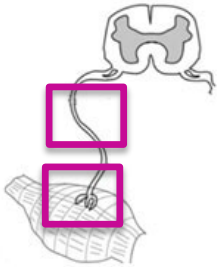

A
HuR
IBA1
HuR/IBA1
MT
WT


HuR
IBA1
HuR/IBA1
ALS 1
ALS 2
Control

Lessons Learned

- HuR is a major regulator of inflammatory cytokine production through posttranscriptional pathways
- Human tissue validation of ALS mouse findings: HuR is activated and upregulated in microglia.
- HuR may be a therapeutic target for slowing disease progression in ALS

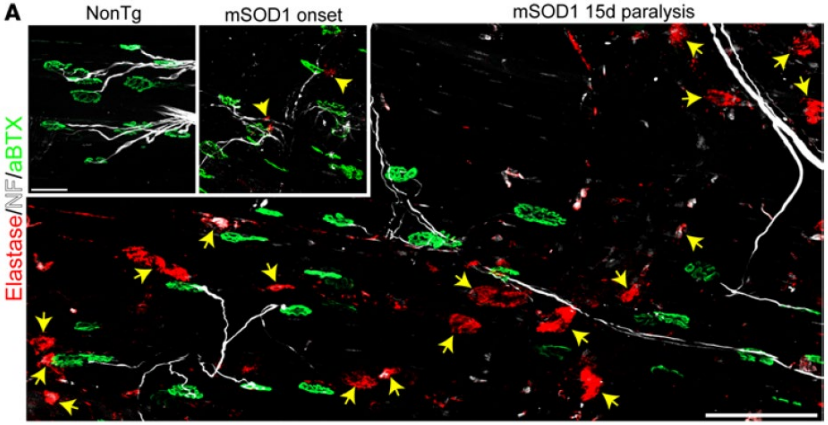
Example 3 Peripheral inflammation in ALS: validation

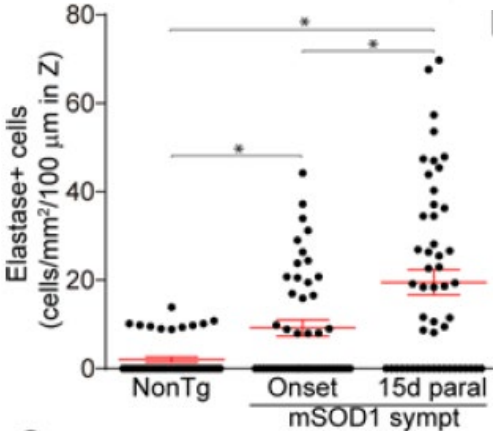


Neutrophils

G93A Rat ALS muscle

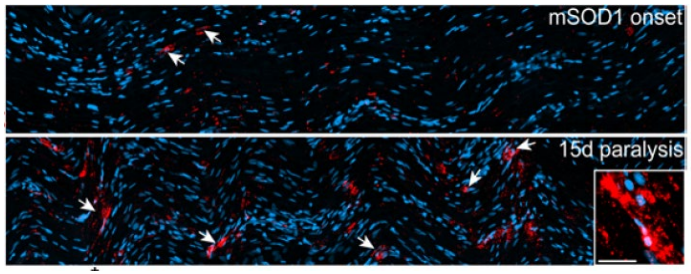
αBT
Elastase
Myelin

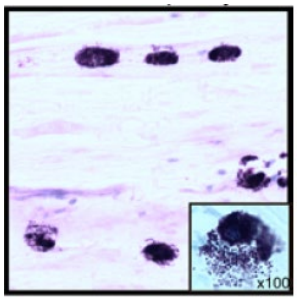




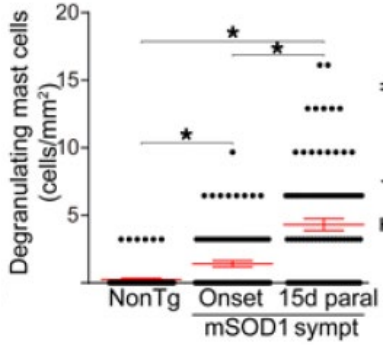
Mast cells

G93A Rat ALS Sciatic nerve





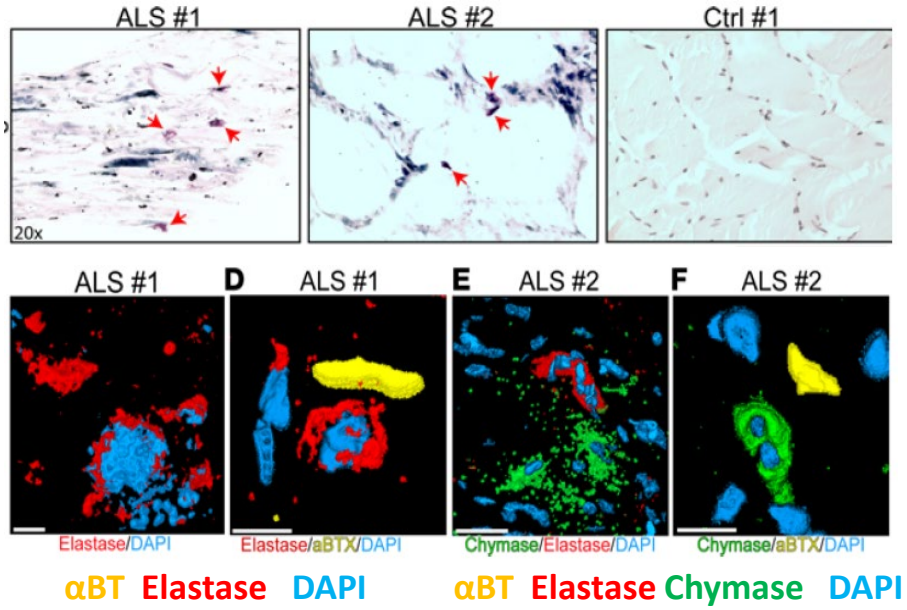
Tryptase DAPI

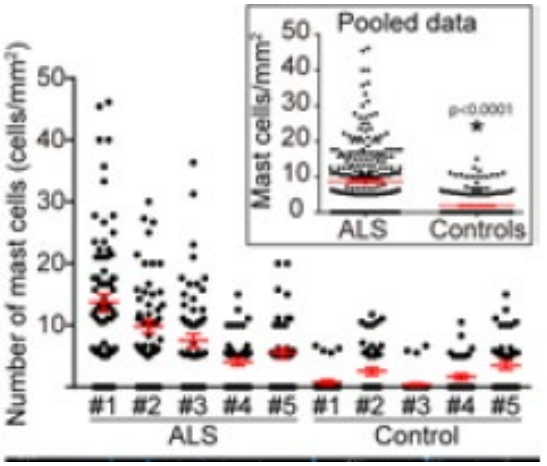


Trias et al. JCI Insight, 2018

Validation in Human ALS

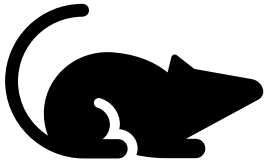
NMJ/muscle

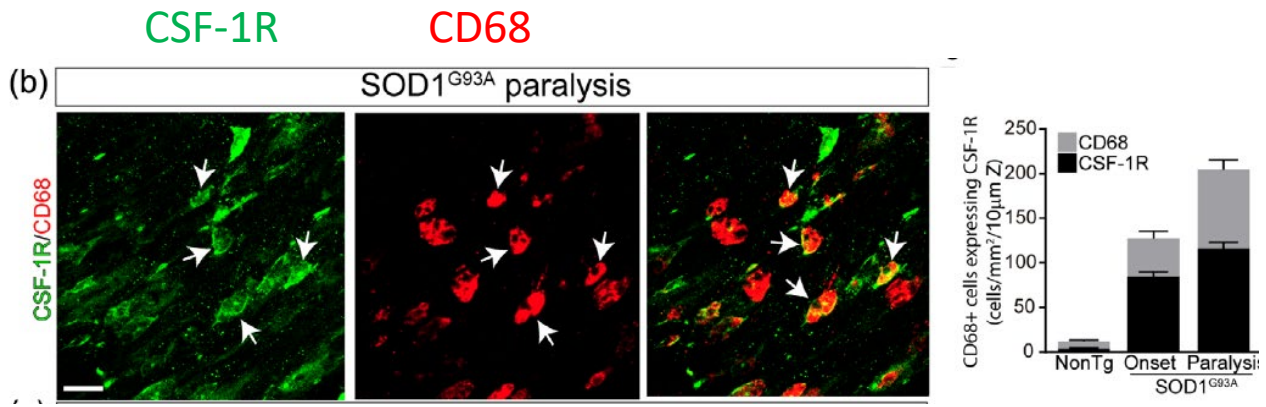


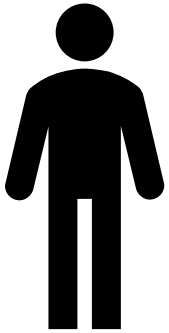


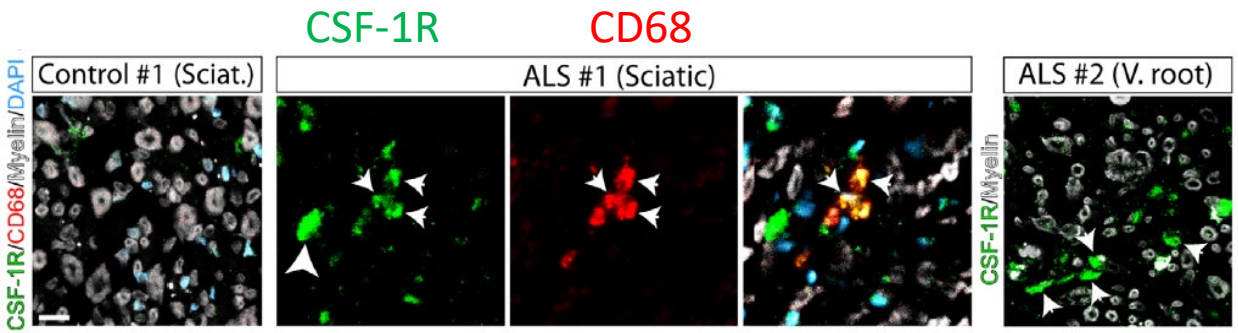
JCI Insight, 2018

CD68+, CSF-1R+ Macrophages in ALS Nerve Roots





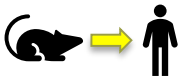




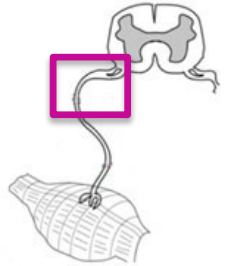
Trias et al., 2019

RESEARCH ARTICLE GLIA

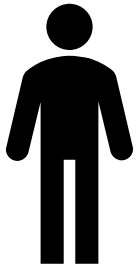
Schwann cells orchestrate peripheral nerve inflammation through the expression of CSF1, IL-34, and SCF in amyotrophic lateral sclerosis



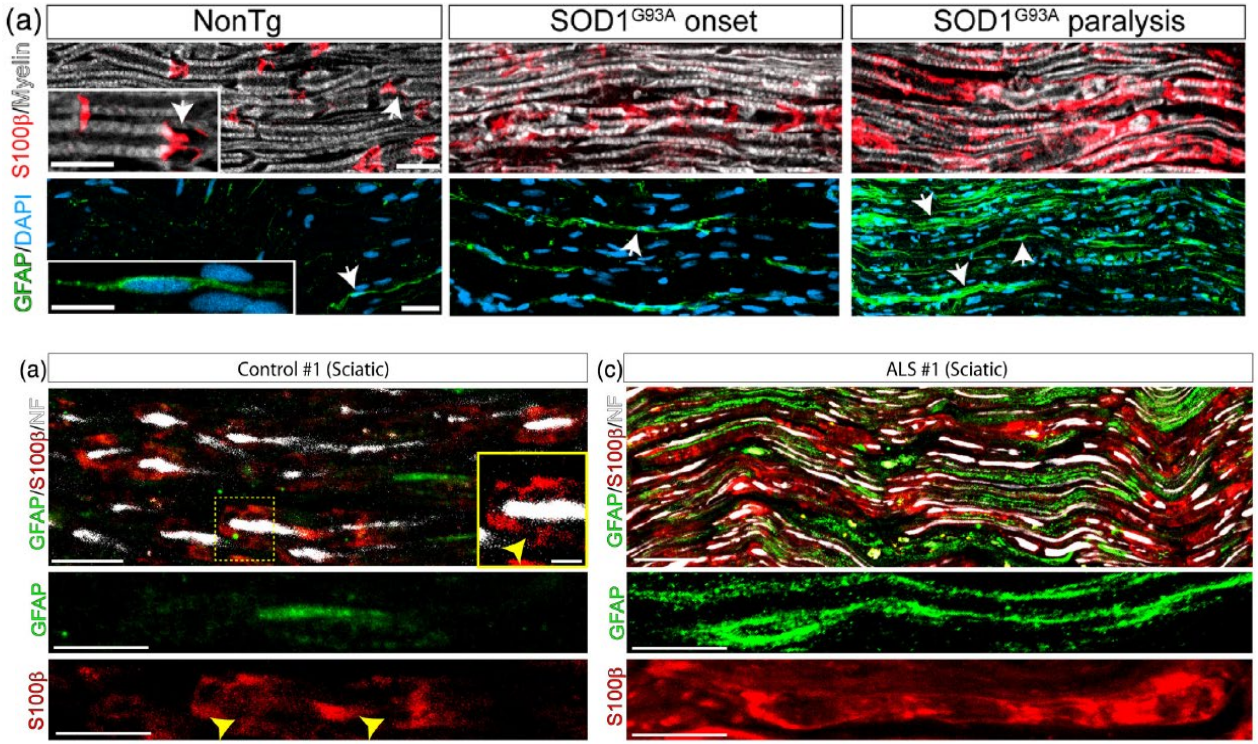
Validation



Sciatic nerve



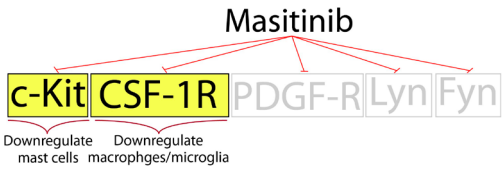
S100β GFAP DAPI Myelin

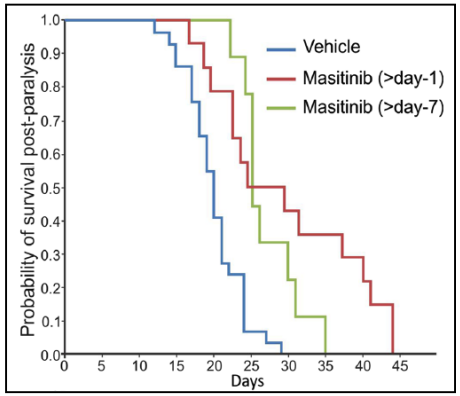


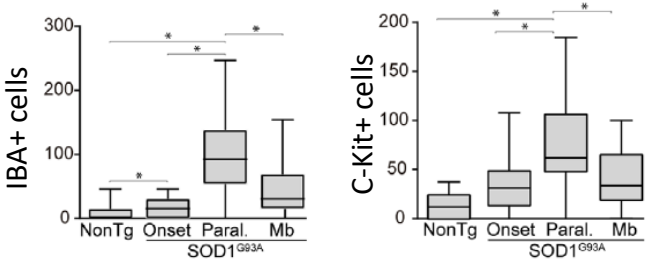
Trias et al., Glia, 2019

Lessons learned

- Peripheral neuromuscular inflammation in Rat ALS model is validated in human ALS tissue
- Rat model indicates an <u>evolution</u> of peripheral inflammation with disease progression
- Provide rationale for masitinib







Conclusions

- Post mortem ALS tissue is essential for discovery of new pathways and validation of pathways discovered in non-human ALS models
- The importance of animal models for assessing temporal evolution of biomarkers
- The importance of normal controls and disease controls
- Discovery in ALS will not move forward without the cooperation and courage of our patients

Patient Acknowledgement

associated factors. Microglia play multiple roles in normal brain and neuroinflammatory/degenerative diseases, some beneficial and some detrimental (Cherry, Olschowka, & O'Banion, 2014; Pena-Altamira et al., 2015; Streit, 2002; Wake et al., 2013). The roles may change depending on the disease type and stage. In the G93A mouse model of ALS, for example, microglia are neuroprotective in the early stages of the disease and later become deleterious (Henkel et al., 2009; Zhao et al., 2013). In models of AD and MS, migration of microglia to diseased brain and phagocytic removal of debris (Aβ in the former and myelin breakdown products in the latter) may be beneficial (El Khoury et al., 2007; Neumann, Kotter, & Franklin, 2009). On the other hand, in spinal cord injury, early upregulation of IL-1β and TNF-α (microglia being a major source) contributes to neuronal toxicity (David and Kroner, 2011). Taken together, these findings suggest that HuR may be a therapeutic target depending on the disease and stage of disease. HuR expression is not limited to microglia, and thus it remains to be seen whether other cells could be adversely affected by its inhibition.

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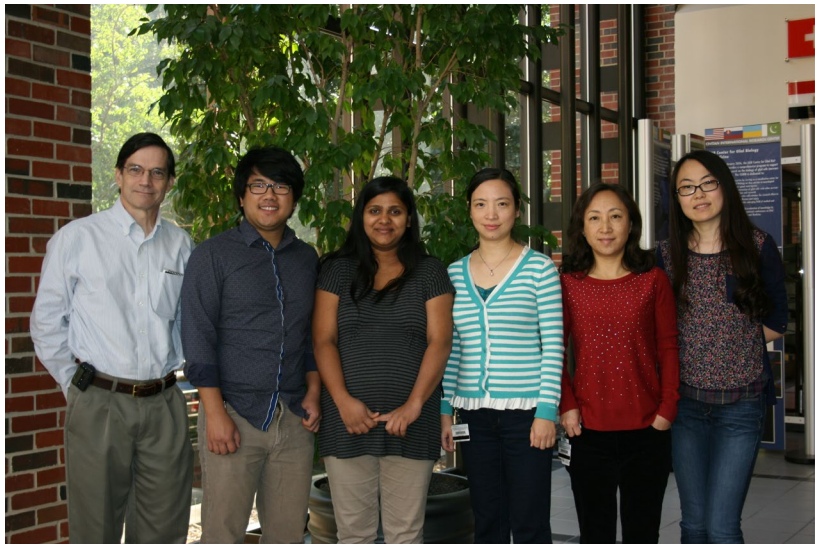
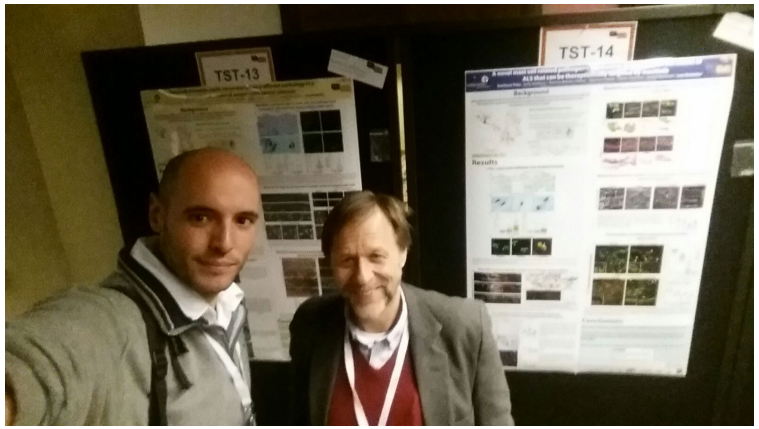
"…We are also grateful to our patients who donated their spinal cord tissues postmortem for ALS research"

Collaborative Teams

Laboratory



BVAMC ALS Clinic



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