

# Autoantibodies in Myositis

Neil McHugh

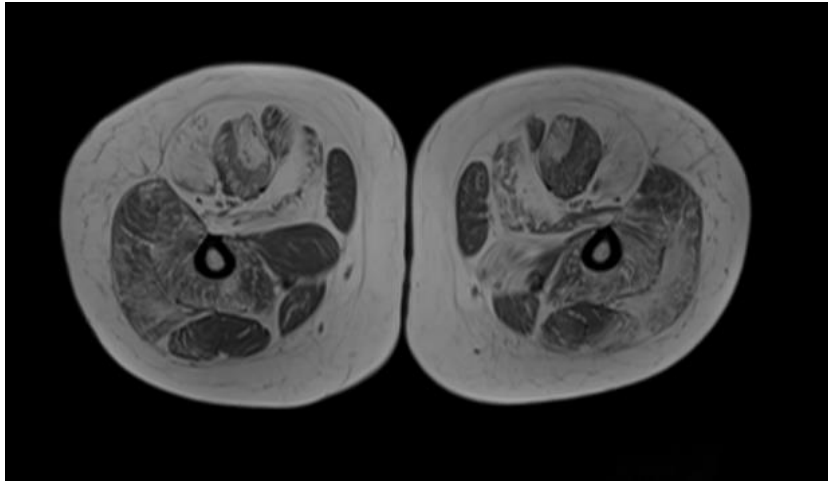
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# Idiopathic Inflammatory Myositis Spectrum Disease



Muscle inflammation



Skin disorder



Interstitial lung disease

Myositis spectrum disease autoantibodies (MSDA)!

# Myositis Spectrum Disease

- **Polymyositis**
  - Anti-synthetase syndrome
  - Immune-mediated necrotising myopathy
- **Dermatomyositis**
  - Clinically amyopathic dermatomyositis (CADM)
  - Cancer associated myositis (CAM)
- **Inclusion Body Myositis**
- **Juvenile Dermatomyositis**
- **Myositis associated with connective tissue disease**
- **Otherj**
  - Granulomatous, eosinophilic, focal, orbital, macrophagic, myofasciitis

# Autoantibodies in Myositis Spectrum Disease

- MSDA (myositis spectrum disorder autoantibodies)
  - Anti-tRNA synthetases (e.g. anti-Jo-1)
  - Anti-Mi-2
  - Anti-signal recognition particle
  - Anti-SAE
  - Anti-TIF1- $\gamma$
  - Anti-NXP2
  - Anti-MDA5
  - Anti-HMGCR
  - Anti-cN-1A
- MAA (myositis associated autoantibodies)
  - Anti-PM-Scl
  - Anti-U1RNP
  - Anti-Ku
  - Anti-U3RNP
  - Anti-Ro (SSA)

# MSDAs and target autoantigens I

Autoantibodies	Target autoantigen	Autoantigen function	Clinical phenotype
<b>Anti-ARS</b> Anti-Jo-1 Anti-PL-7 Anti-PL-12 Anti-EJ Anti-OJ Anti-KS Anti-Zo Anti-YRS	<b>tRNA synthetase</b> Histidyl Threonyl Alanyl Glycyl Isoleucyl Asparaginylyl Phenylalanyl Tyrosyl	<b>Intracytoplasmic protein synthesis</b> <b>Binding between an amino acid and its cognate tRNA</b>	<b>ASS</b> <b>Myositis</b> <b>Interstitial pneumonia</b> <b>Mechanics hands</b> <b>Arthritis</b> <b>Fever</b> <b>Raynauds</b>
<b>Anti-Mi-2</b>	<b>Helicase protein part of the NuRD complex</b>	<b>Nuclear transcription</b>	<b>Adult and juvenile DM</b> <b>Hallmark cutaneous disease</b>
<b>Anti-SRP</b>	<b>SRP</b> <b>6 polypeptides and ribonucleoprotein 7SLRNA</b>	<b>Intracytoplasmic protein translocation (endoplasmic reticulum)</b>	<b>Severe necrotizing myopathy</b>
<b>Anti-HMGCR</b>	<b>3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase</b>	<b>Biosynthesis of cholesterol</b>	<b>Necrotising myopathy associated with statin use</b>

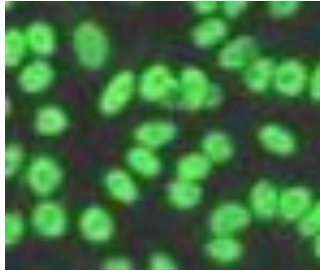
## MDSAs and target autoantigens II

<b>Autoantibodies</b>	<b>Target autoantigen</b>	<b>Autoantigen function</b>	<b>Clinical phenotype</b>
<b>Anti-p155/140</b>	<b>TIF1-<math>\gamma</math></b>	<b>Nuclear transcription Cellular differentiation</b>	<b>Severe cutaneous disease in juvenile DM and cancer in adults</b>
<b>Anti-p140 (MJ)</b>	<b>NXP-2</b>	<b>Nuclear transcription (tumour suppressor gene p53)</b>	<b>Juvenile DM</b>
<b>Anti-SAE</b>	<b>SAE</b>	<b>Post-translational modification – targets include nuclear transcription factors</b>	<b>Adult DM May present with CADM first</b>
<b>Anti-CADM-140</b>	<b>MDA5</b>	<b>Viral RNA recognition</b>	<b>CADM Interstitial pneumonia</b>
<b>Anti-Mup44</b>	<b>Cytosolic 5'nucleotidase 1A (cN-1A)</b>	<b>Hydrolysis of AMP</b>	<b>Inclusion body myositis (Sjogren's)</b>

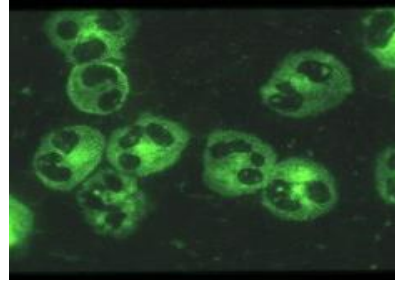
# Methods for detecting autoantibodies

## Autoantibody Screening by Indirect Immunofluorescence

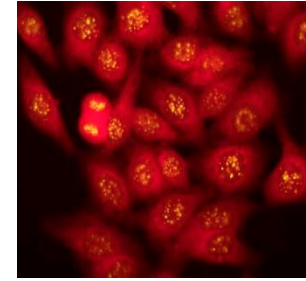
Hep-2



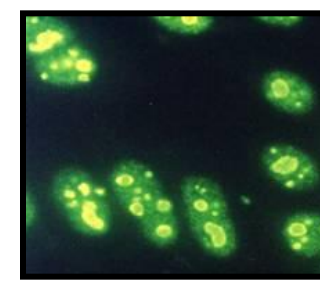
Human neutrophil



Hep-2

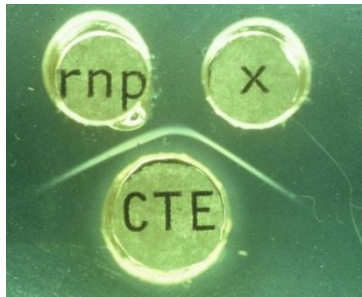


Hep-2



## Autoantibody identification by second technique

Immunodiffusion



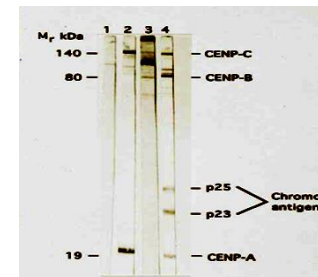
ENA  
anti-RNP

ELISA



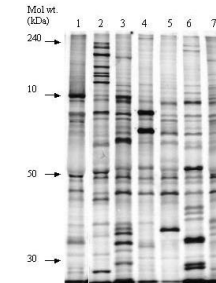
anti-PR3

Immunoblot



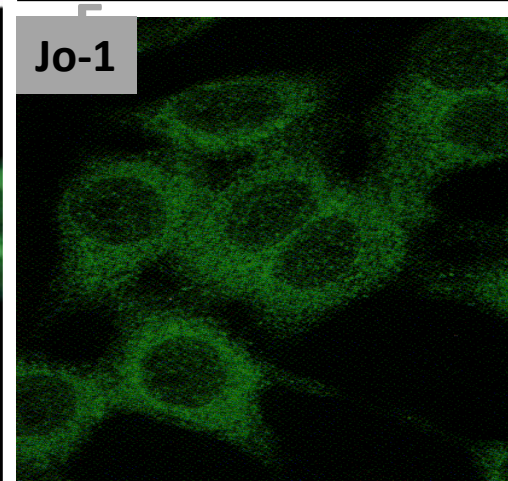
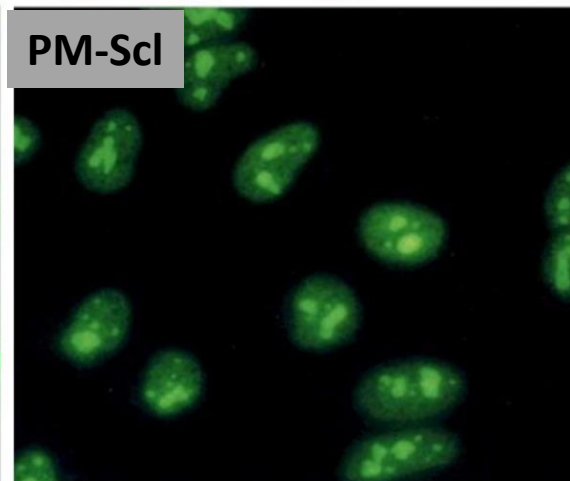
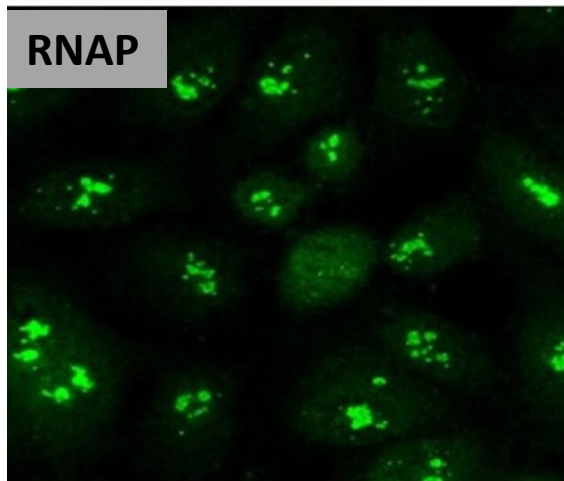
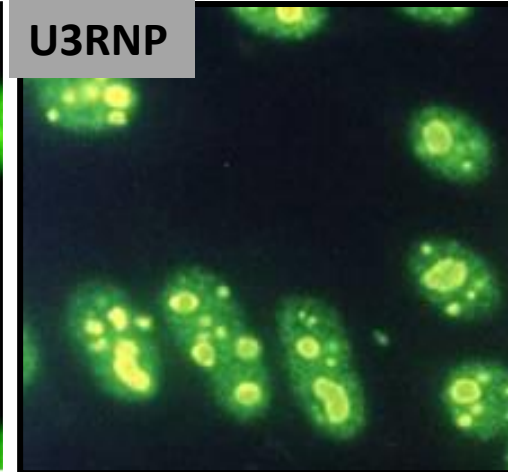
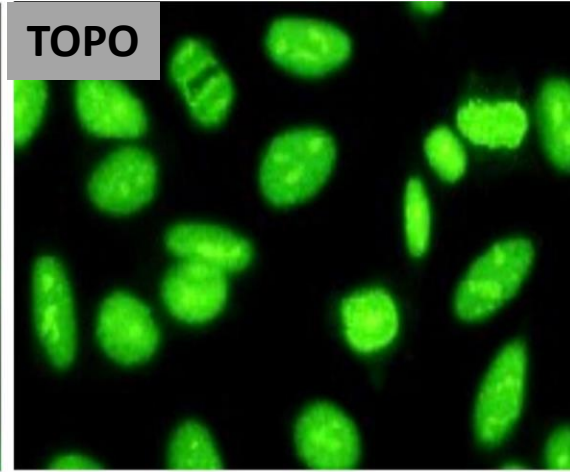
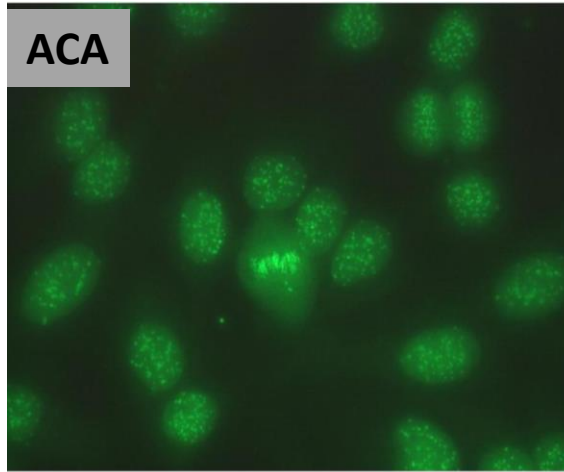
anti-centromere

Immunoprecipitation



Anti-fibrillarin  
U3RNP

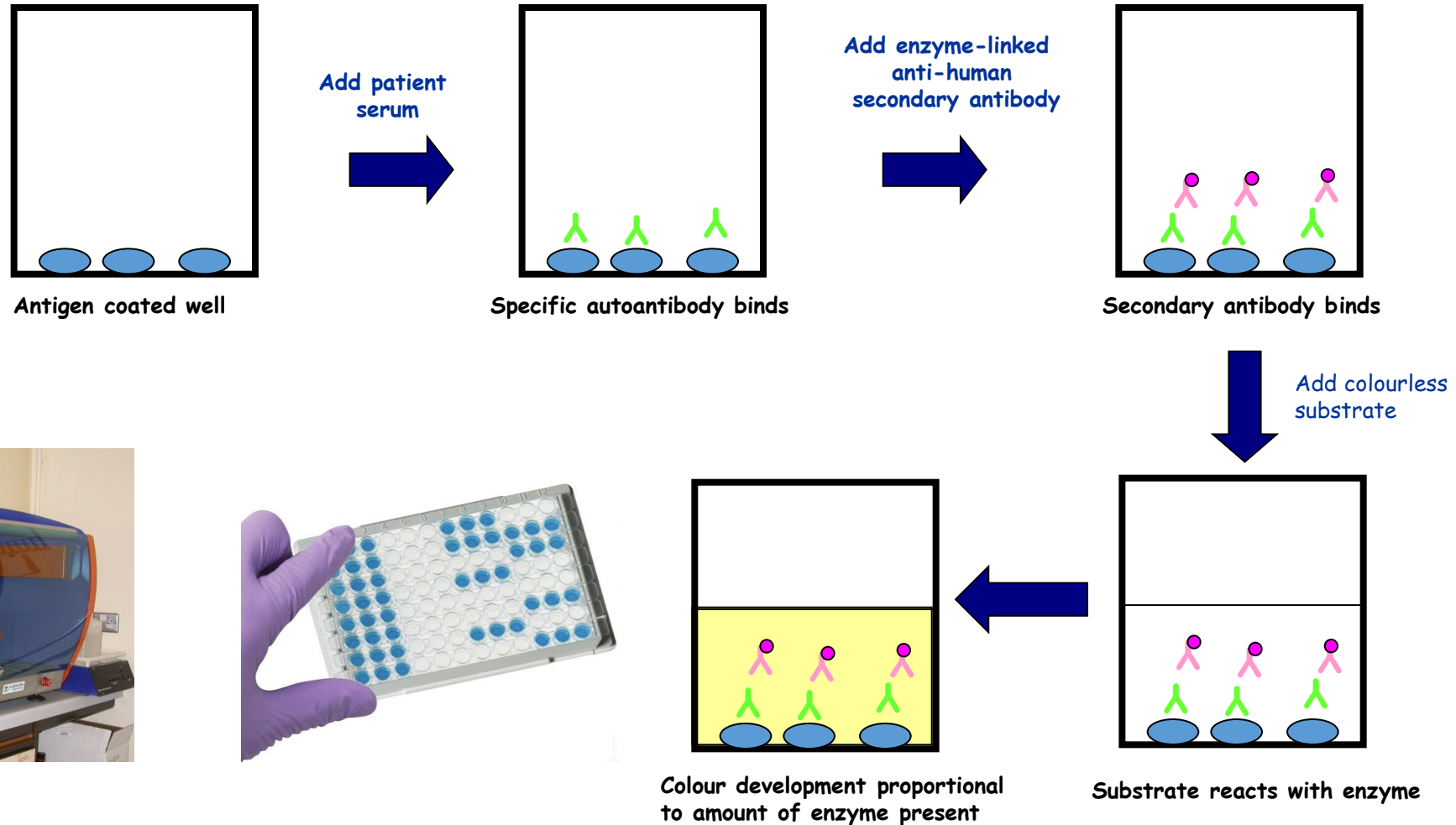
# Indirect immunofluorescence



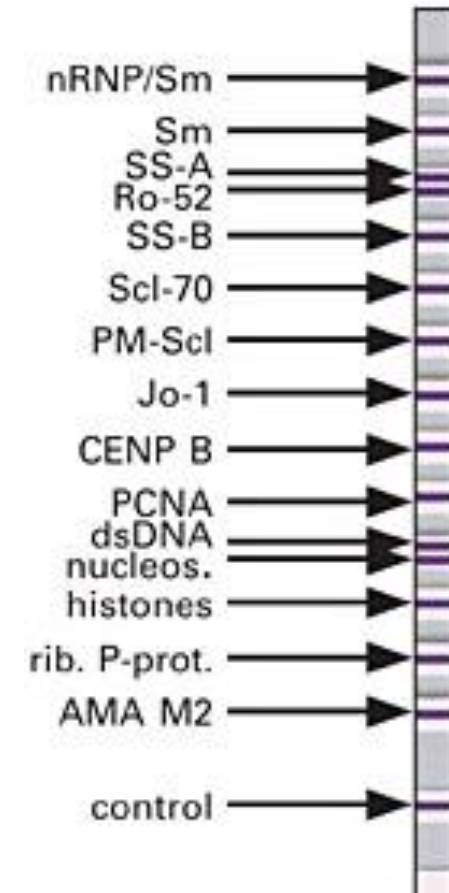
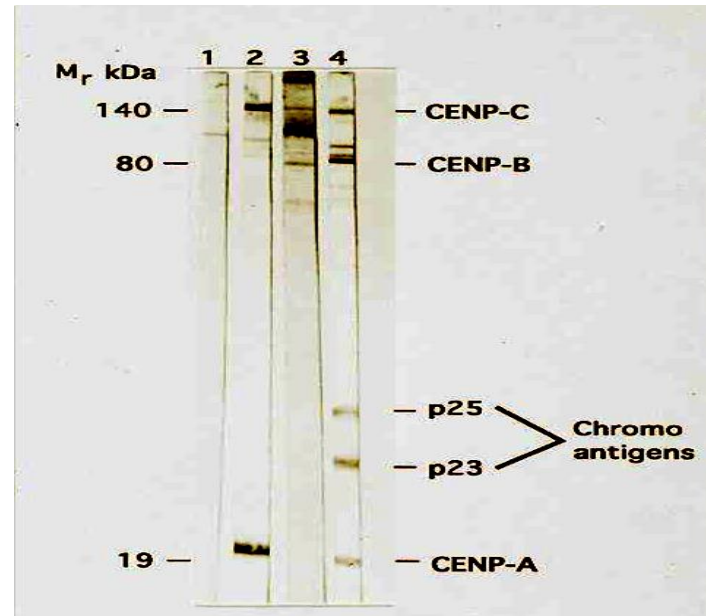
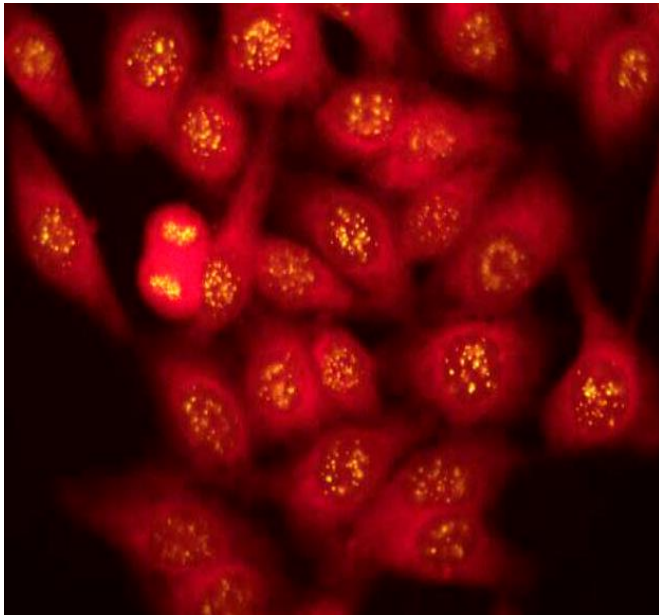
- If test positive the patient will be reported as having an antinuclear antibody (ANA)
- Sometimes the pattern will reveal the type of ANA (specificity) but usually another method will be necessary for exact identity



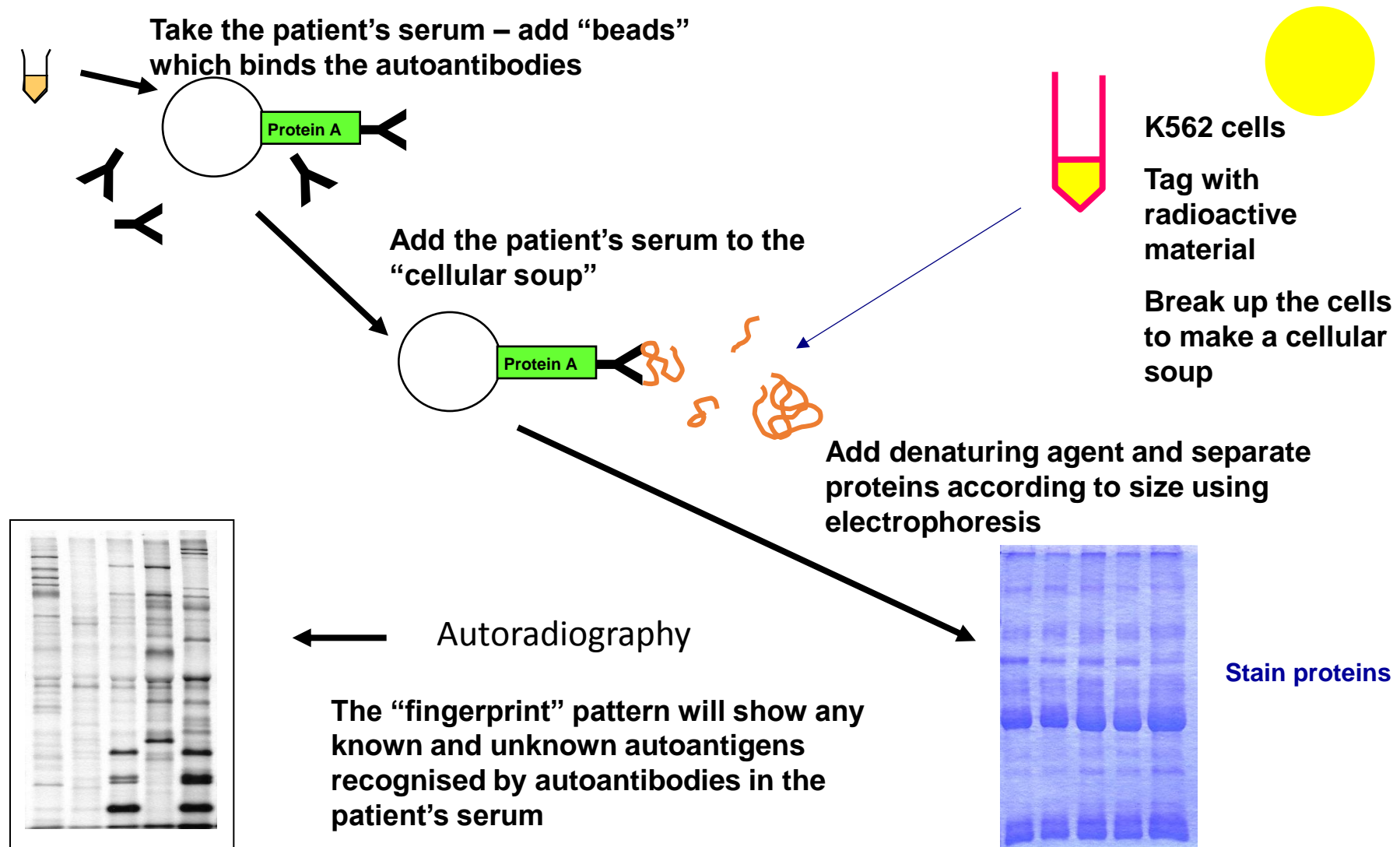
# Enzyme-linked immunosorbent assay (ELISA)



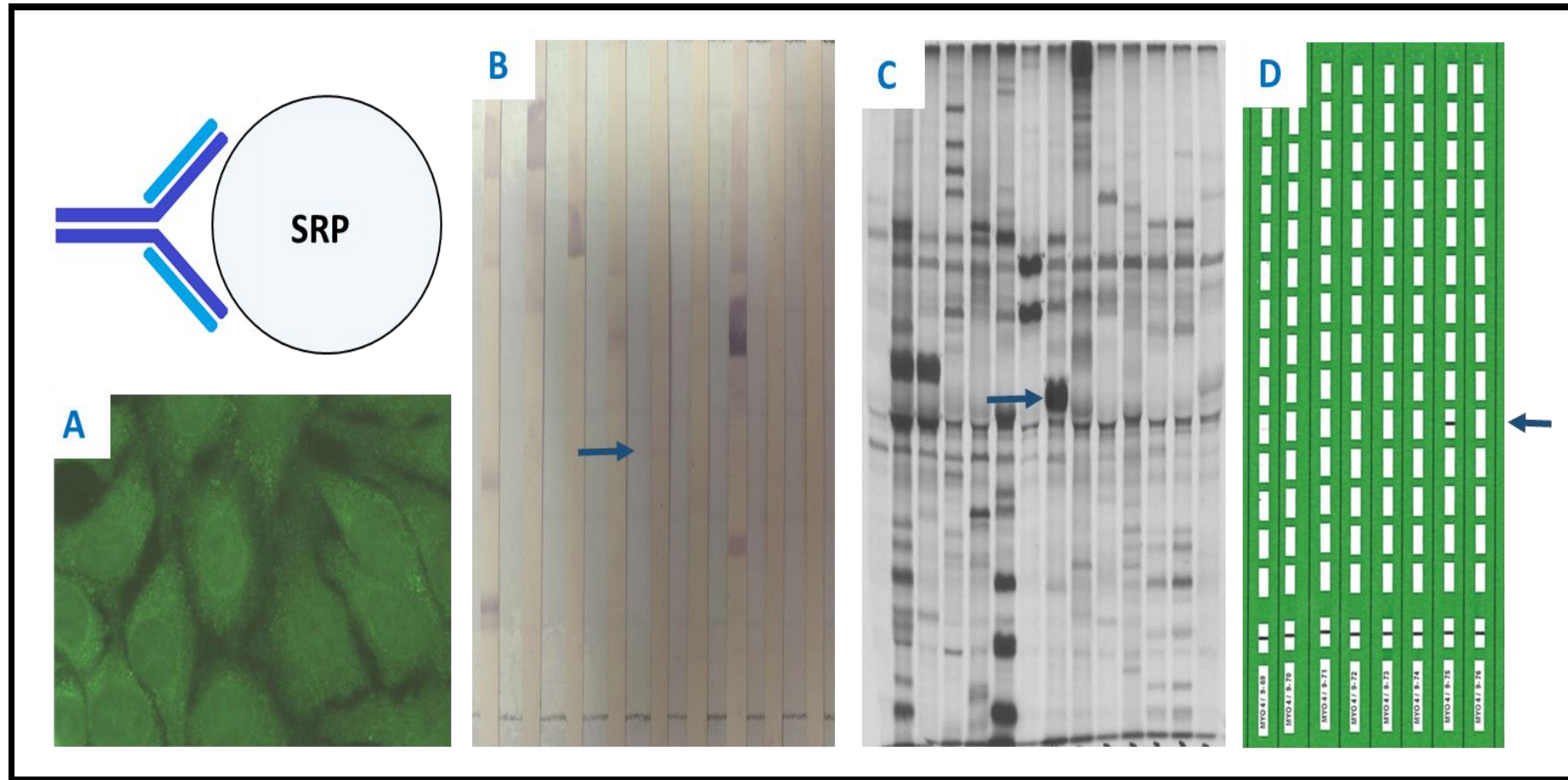
# Immunoblot and Lineblot



# Autoantibody detection by protein immunoprecipitation



# Anti-signal recognition particle (SRP) by different assays

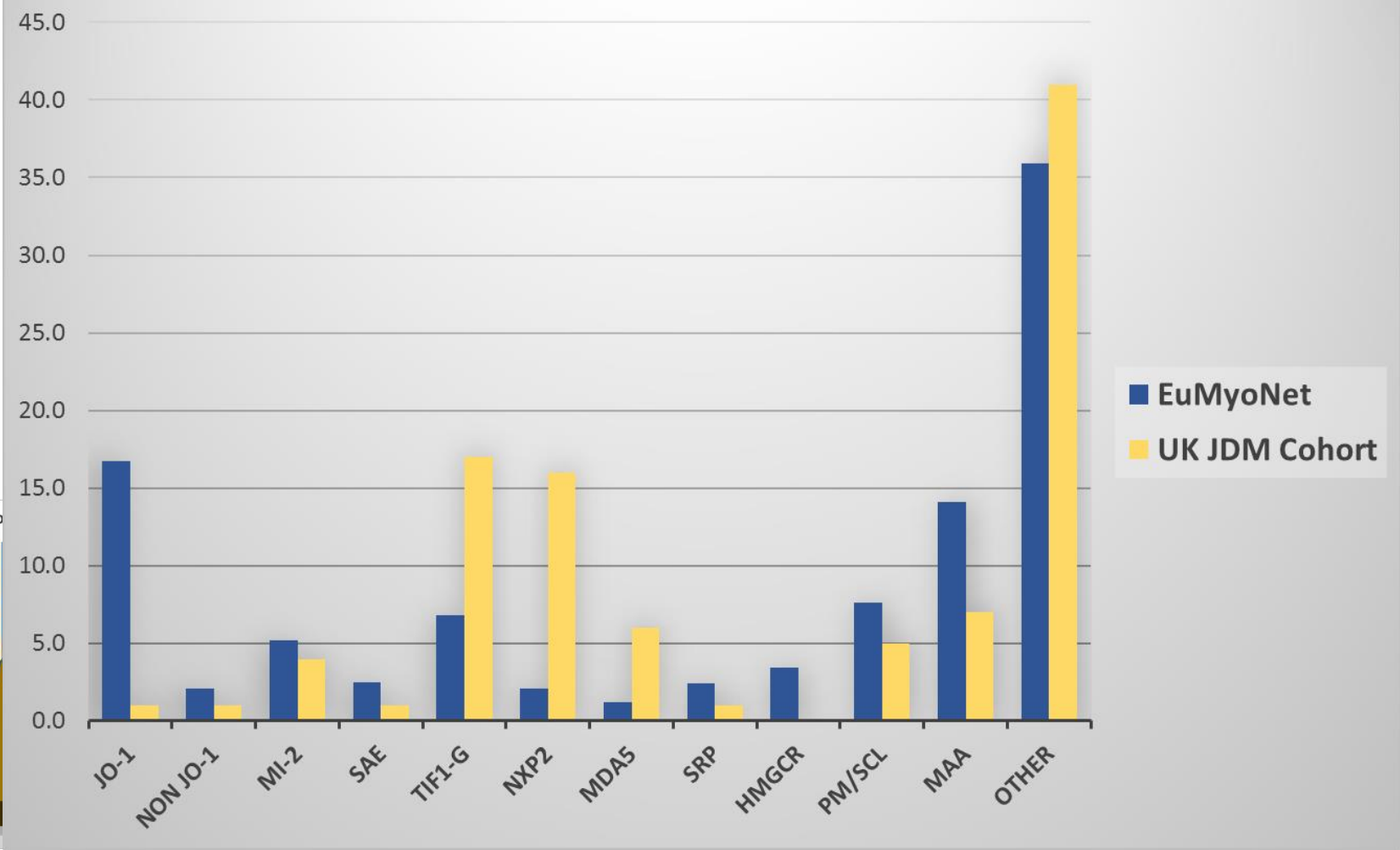


# Patterns of Juvenile versus Adult MSD

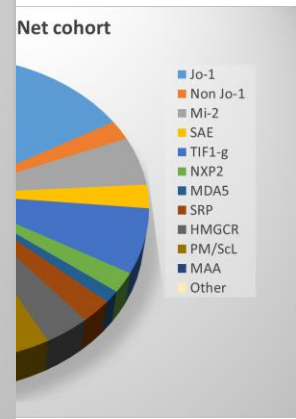
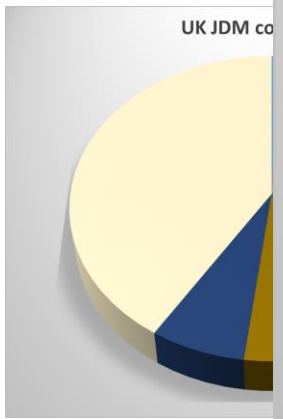
- Juvenile

- 45.0
- 40.0
- 35.0
- 30.0
- 25.0
- 20.0
- 15.0
- 10.0
- 5.0
- 0.0

MSDA in Adult versus Juvenile myositis



■ EuMyoNet  
■ UK JDM Cohort



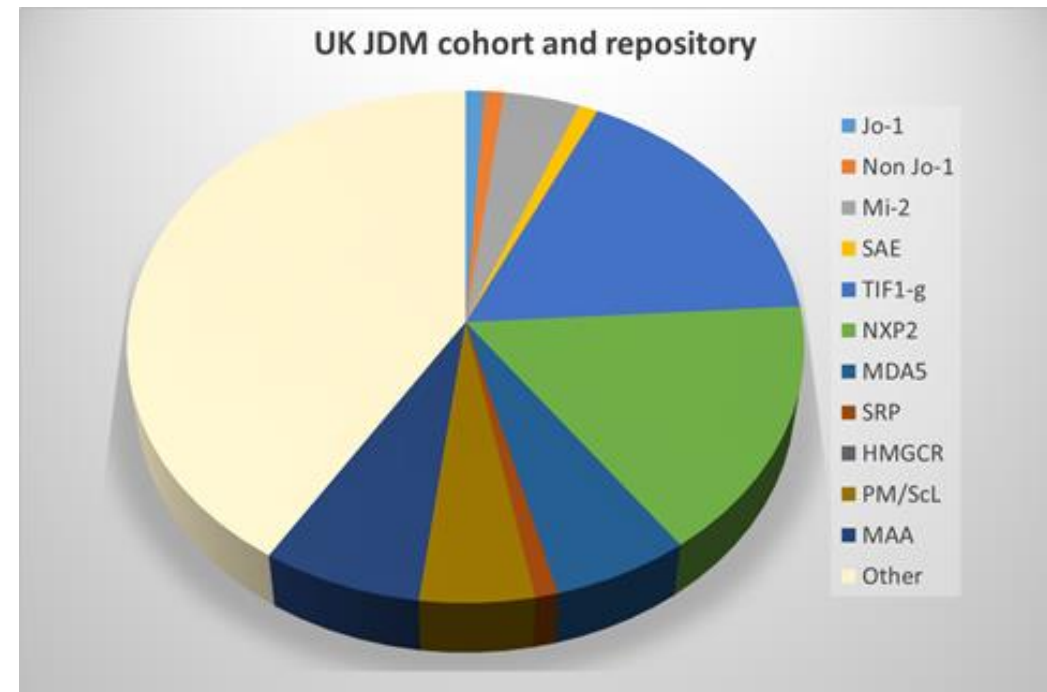
UK JDM Cohort and Biomarker Study n= 347

EUMYONET n = 1616

# Autoantibodies in Juvenile MSD



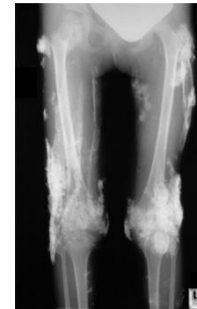
- MSDAs
  - Anti-TIF1g
  - Anti-NXP2
  - Anti-MDA5
  - Anti-Mi-2
  - Low frequency of anti-synthetase and anti-SRP
- MAAs
  - Overlap syndromes with scleroderma/lupus
    - Anti-PmScl
    - Anti-U1RNP



UK JDM Cohort and Biomarker study n = 347

# MDSAs in Juvenile MSD

- TIF1- $\gamma$ 
  - 17-33% of cases
    - More severe skin disease, ulceration, generalised lipodystrophy
- NXP2
  - 18-36% of cases
    - Calcinosis, contractures, muscle atrophy
- MDA5
  - 7-38% of cases
    - Skin and Oral Ulcers, Arthritis, Milder Muscle Disease, ILD
- Mi-2
  - 2-5% of cases
    - Milder disease course

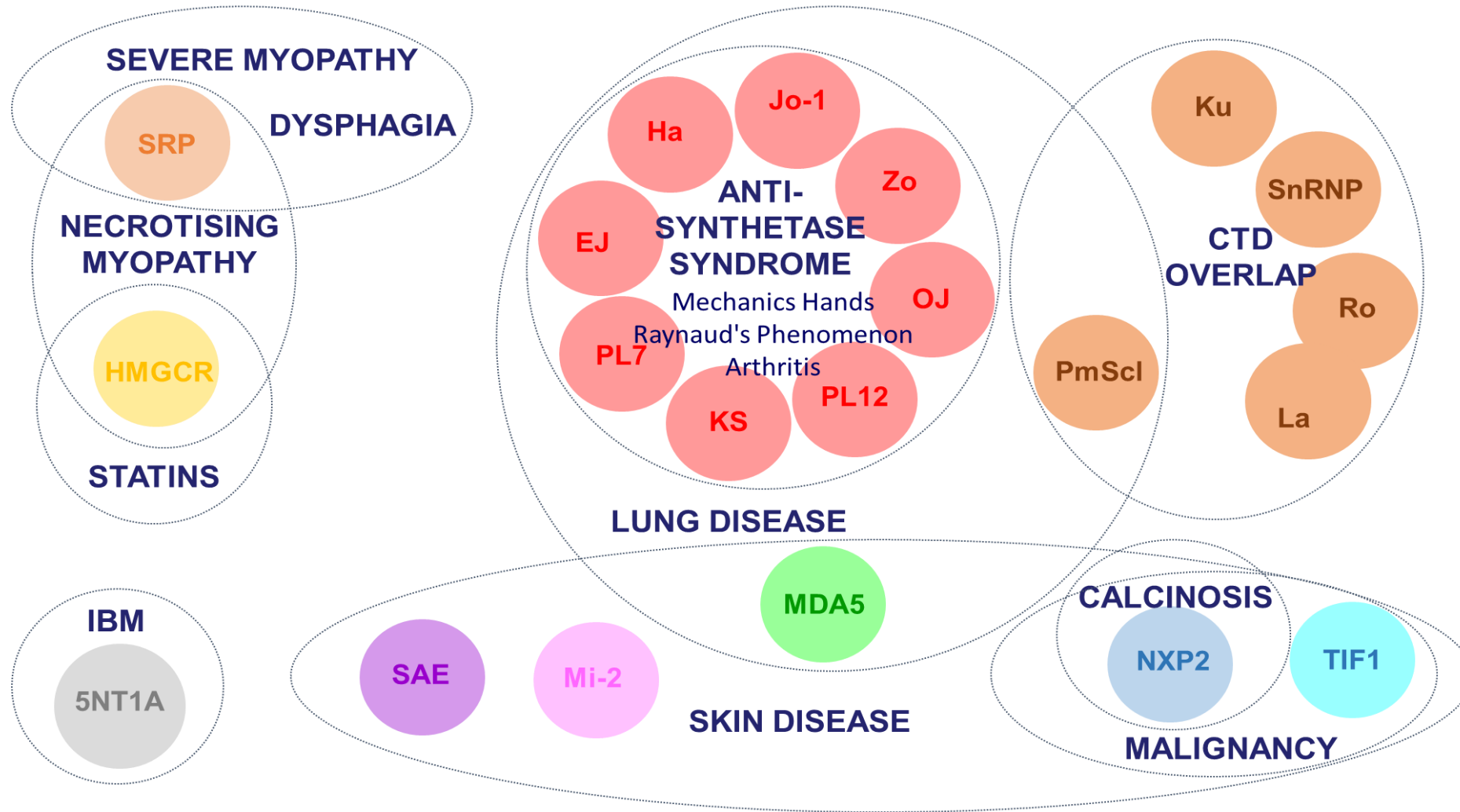


**Bingham Medicine Baltimore 2008;87(2):70-86**  
**Gunawardena Rheumatology 2008;47(3):324-8**  
**Espada J Rheumatol 2009;36:2547-51**  
**Rider Medicine Baltimore 2013 92(4) 223-43**  
**Kobayashi et al J Pediatr 2011;158:675-7**  
**Tansley Rheumatology 2014;53(12):2204-8**  
**Tansley Arthritis Res Ther 2014;16:R138**

# MSDAs and Adult Myositis



# MSDA/MAAs and clinical associations in adult myositis

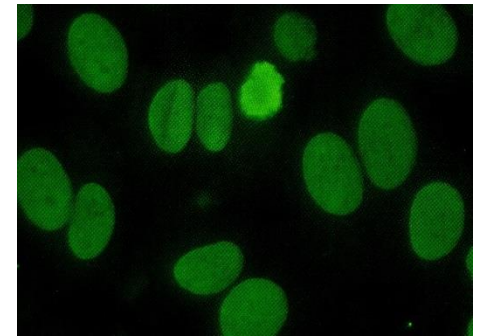
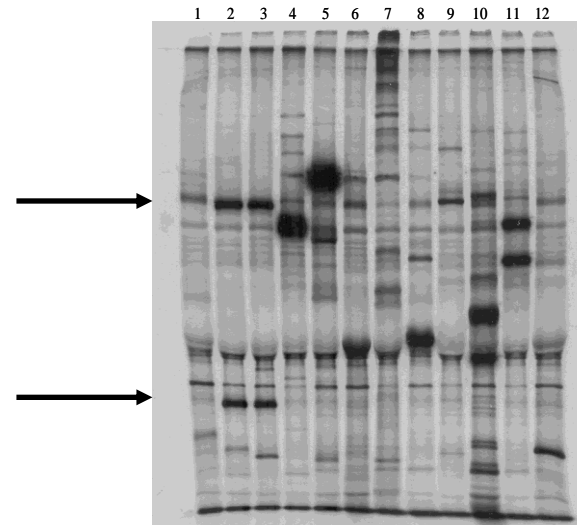




# Anti-SAE

Q9UBT2	ULE1B _Human	Ubiquitin like 1 activating enzyme E1B SUMO1 (activating enzyme subunit 2)	71.179
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Q9UBE0	ULE1A _Human	Ubiquitin like 1 activating enzyme E1A SUMO1 (activating enzyme subunit 1)	38.425
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Betteridge *et al* Arthritis Rheum. 2007. 56:3132-3137

Ann Rheum Dis 2009;68:1621-5

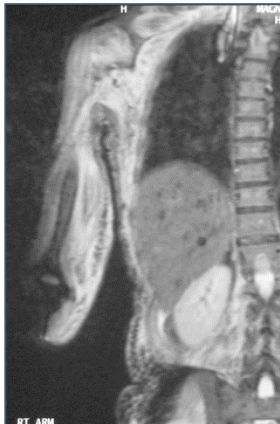
# Anti-SAE Autoantibodies

**Clinically amyopathic DM (CADM)**

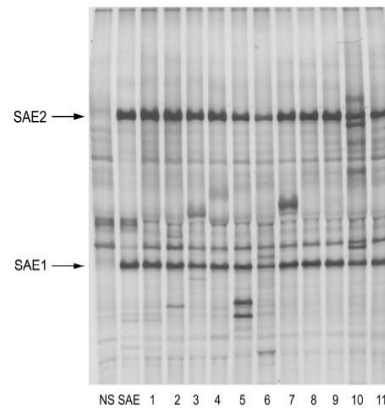
**? Higher frequency of systemic involvement - dysphagia, GI disease (UK cohort)**

**Not associated with cancer**

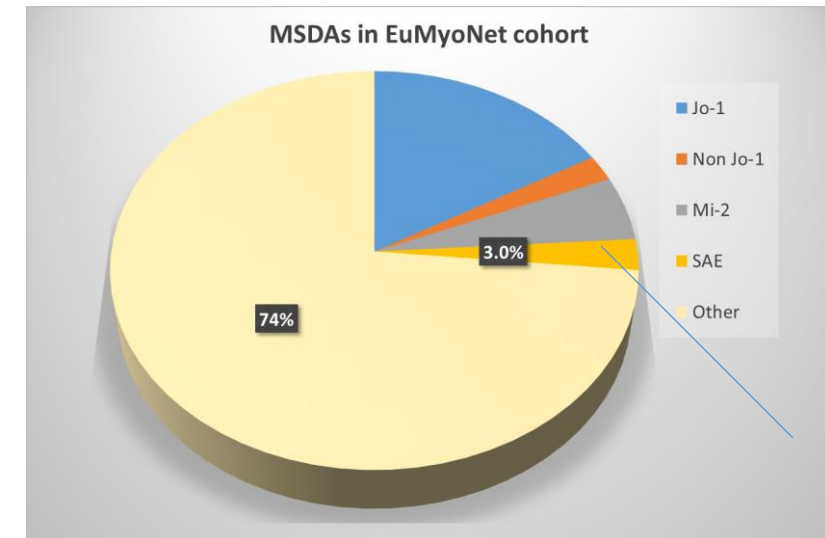
**Low frequency of ILD in Caucasians (0-18%), but higher in Asian cohorts (50-71%)**



				EUMYONET		
	Target Autoantigen	Adult Myositis (%)	Juvenile Myositis (%)	IIM (%)	PM (%)	DM (%)
SAE	Small Ubiquitin Like Modifier Activating Enzyme	6-8 DM 2 Asian	<0.1	2.5	0.1	5.4



Betteridge ZE *et al.* Ann Rheum Dis 2009;68:1621-5.  
 Tarricone E *et al.* J Immunol Methods 2012; 384:128-34.  
 Muro Y *et al.* Autoimmunity 2013; 46:279-84  
 Fujimoto *et al.* Ann Rheum Dis 2103;72:151-3

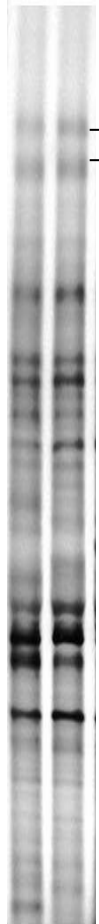


Anti-Transcriptional intermediary  
factor 1 gamma (TIF1- $\gamma$ )

# Anti-TIF1 Autoantibodies

Originally described by Targoff *et al* and Kaji *et al* in two separate studies

Targets Transcription Intermediary Factor 1



155 kDa gamma subunit

140 kDa alpha subunit

Beta subunit (~100kDa) targeted in some patients

Malignancy (Adults)

older age especially

CADM and DM

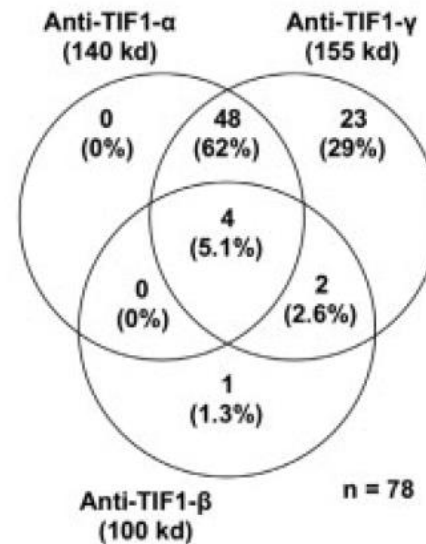
Diffuse photoerythema

Scalp Rash

Facial Rash

V Sign

Shawl Sign



Malignancy was more frequent in patients with autoantibodies to both TIF1-alpha and TIF1-gamma compared with TIF1-gamma alone (73% vs 50%,  $p < 0.05$ )

Manabu Fujimoto *et al* Arthritis Rheum 2011

Targoff IN *et al*. Arthritis Rheum 2006;54:3682-3689.

Kaji K *et al*. Rheumatology 2007;46:25-28

Trallero-Aragua's E *et al*. Medicine (Baltimore) 2010;89:47-52

Fiorentino et al J Am Acad Dermatol 2015;72:449-55

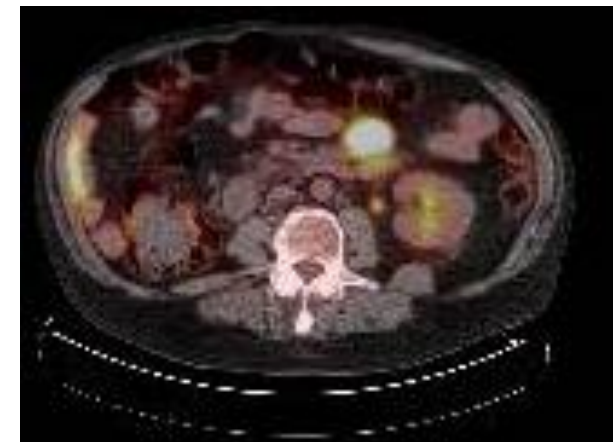
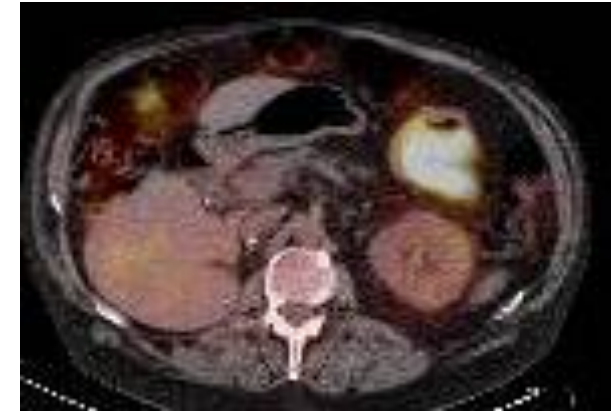
Rider *et al* 2013, Medicine Baltimore 92(4) 223-43

Gunawardena et al Rheumatology 2008;47(3):324-8.

Bingham et al Medicine Baltimore 2008;87(2):70-86

# Case D male born 1953

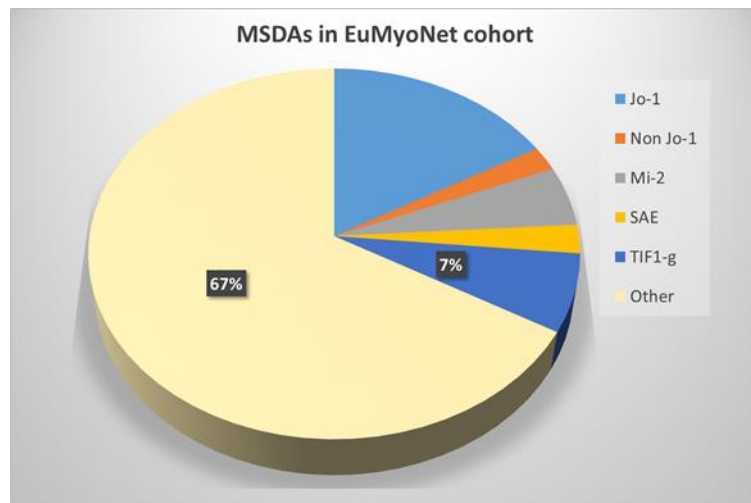
- **Acute admission March 2014**
  - PUO
  - 4/12 fatigue, muscle aching and weakness, weight loss
  - Worsening anaemia Hb 85
  - CRP 90, PV 2.71, normal myeloma screen, CK, CEA, CA19.9
  - Normal CT scans, colonoscopy and temporal artery biopsy
  - MR thighs – muscle atrophy
  - Positive anti-TIF-1 $\gamma$  autoantibody
- **PMHx**
  - Type 2 diabetes
  - Renal cell carcinoma in 2011
    - Nephrectomy (SOURCE RCT Sorafenib vs placebo)
    - Three monthly follow-up in remission
- **Laparoscopic biopsy**
  - Recurrent renal cell carcinoma
  - Removal of lesion has led to a sustained recovery



# Clinical Associations of TIF1 in EuMyoNet (first 1616 cases – unpublished)

		EUMYONET				
	Target Autoantigen	Published Frequency	IIM (%)	PM (%)	DM (%)	Juvenile Myositis (%)
TIF1	Transcription Intermediary Factor 1 gamma / alpha	13-31% DM	7.1	0.5	14.8	22-29%

Clinical Feature	TIF1 Negative	TIF1 Positive	p value
Gottrons	29.7%	79.3%	<0.0001
Heliotrope Rash	29.3%	77.3%	<0.0001
ILD	31.2%	16.0%	=0.0038
Cancer (ever)	8.0%	32.2%	<0.0001
CAM	2.3%	20.5%	<0.0001
CADM	0.8%	5.2%	=0.0028





Anti-MDA5

# Anti-MDA5 Autoantibodies

## Clinical Associations (Children)

Skin and Oral Ulcers

Arthritis

Milder Muscle Disease

ILD (RP-ILD)?

## Clinical Associations (Adults)

ILD (67-100%)

Rapidly Progressing ILD (RP-ILD) (22-100%)

Skin manifestations

DM

Gottron's Papules

Periungal

Ulceration (skin and mouth)

Hand Swelling

Arthritis

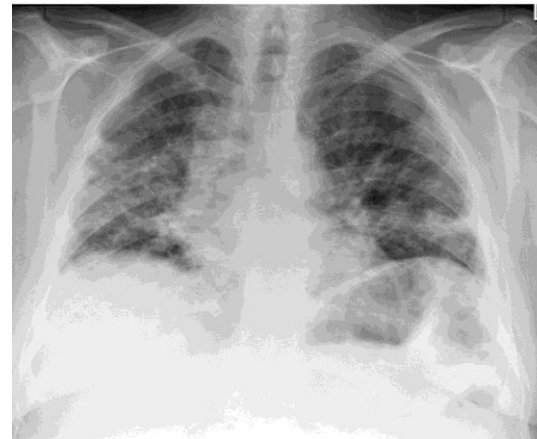
Palmar Papules

Mechanic's Hands

Panniculitis

Alopecia

				EUMYONET		
	Target Autoantigen	Adult Myositis (%)	Juvenile Myositis (%)	IIM (%)	PM (%)	DM (%)
MDA5	Melanoma Differentiation Associated Gene 5	10-48 DM (Asian) 0-13 DM (Caucasian)	7-38	1.2%	0.0%	2.7%



Fiorentino *et al* J Am Acad Dermatol 2011;65:25-34

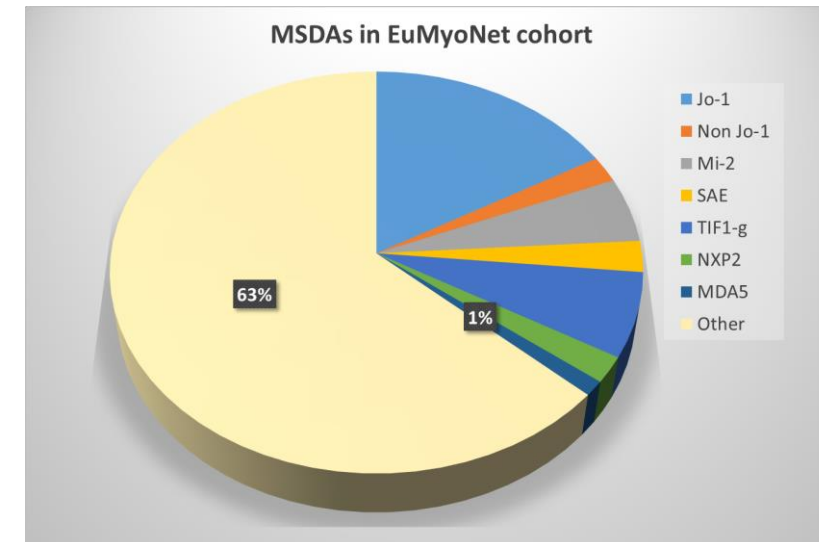
Sato *et al* Arthritis Rheum 2005;52:1571-6

Nakashima *et al* Rheumatol 2010;49:433-40

Kobayashi *et al* J Pediatr 2011;158:675-7

Tansley *et al* Arthritis Res Ther 2014;16:R138

Moghadam *et al* Arthritis Care Res 2015

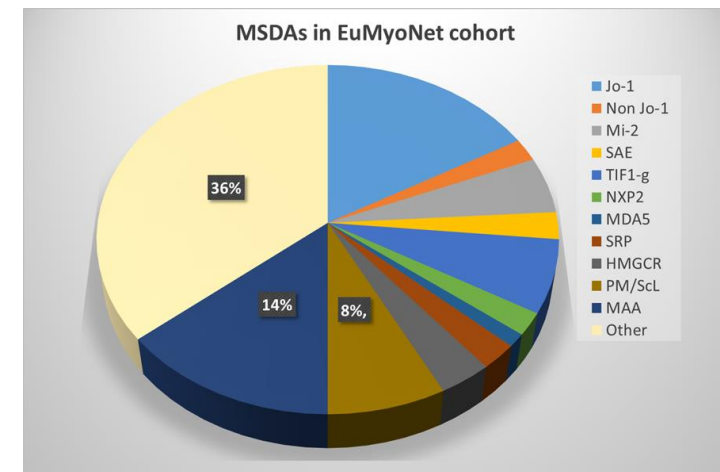
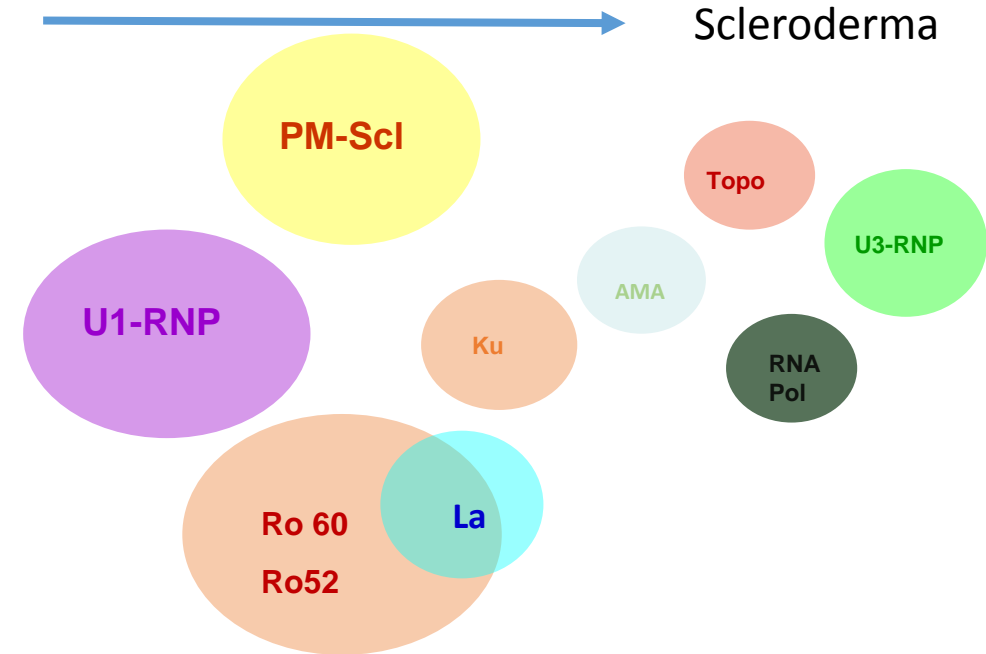


# Myositis Associated Autoantibodies

# Myositis Associated Autoantibodies

**Table 1: Myositis-associated autoantibodies, autoantigen targets and clinical features**

Autoantibodies	Autoantigen and function	Clinical Phenotype
Anti-U1-RNP	snRNP ribonucleoprotein complex Splicing mRNA	Mixed connective tissue disease – overlap features including myositis, arthritis, sclerodacty, Raynaud's, diffuse parenchymal lung disease, pulmonary hypertension
Anti-Ro52/60	Ro ribonucleoprotein complex (proteins hYRNA)  Binding to DNA and transcription factors	SLE, Sjogren's, overlap myositis, reported in association with anti-ARS autoantibodies
Anti-PM-Scl	Nucleolar multi-protein Biogenesis of ribosomes	Myositis-scleroderma overlap
Anti-U3-RNP	Nucleolar fibrillarin ribonucleoprotein Pre-ribosomal RNA processing	Myositis-scleroderma overlap
Anti-Ku	Heterodimer Ku-p350 complex DNA repair and phosphorylation of transcription factors	Myositis-scleroderma overlap



## EUMYONET: Distribution of autoantibodies in all myositis n = 1639

	Exclusive	Jo-1	Non Jo-1 ASA	SRP	Mi-2	SAE	NXP2	MDA5	TIF1	PMScl	MAA
Jo-1	245		1	0	0	0	0	0	0	0	75
Non Jo-1 ASA	45	1	0	0	0	1	0	0	1	0	10
SRP	38	0	0		0	0	0	0	0	0	1
Mi-2	84	0	0	0		0	0	0	0	0	5
SAE	41	0	1	0	0		0	0	0	0	1
NXP2	32	0	0	0	0	0		0	0	0	8
MDA5	21	0	0	0	0	0	0		0	0	0
TIF1	105	0	1	0	0	0	0	0		0	10
PMScl	119	0	0	0	0	0	0	0	0		11
MAA	126	75	10	1	5	1	8	0	10	11	80

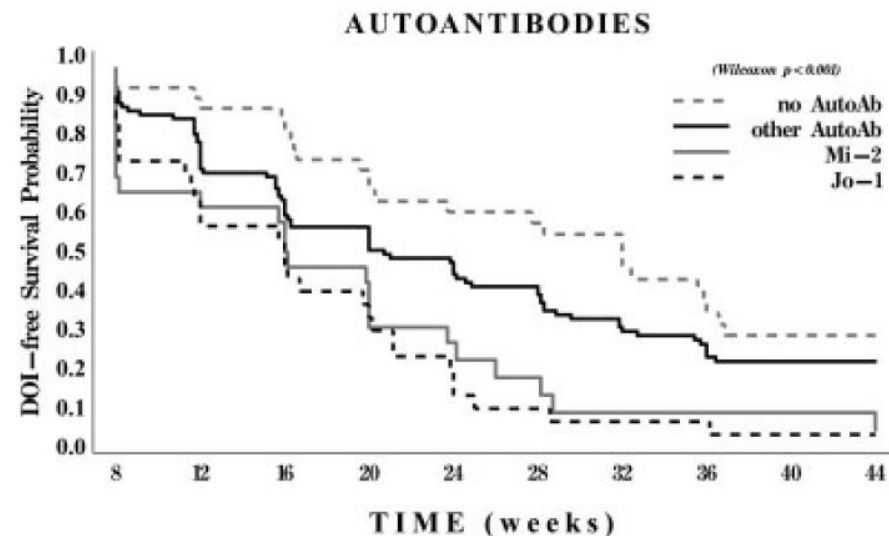
MAA plus MAA = 80    MDSA plus MAA = 120    MDSA plus MDSA = 3

Does the presence of a particular  
MSDA predict response to  
treatment?

# Predictors of Clinical Improvement in Rituximab-Treated Refractory Adult and Juvenile Dermatomyositis and Adult Polymyositis

Rohit Aggarwal,<sup>1</sup> Andriy Bandos,<sup>1</sup> Ann M. Reed,<sup>2</sup> Dana P. Ascherman,<sup>3</sup> Richard J. Barohn,<sup>4</sup>  
Brian M. Feldman,<sup>5</sup> Frederick W. Miller,<sup>6</sup> Lisa G. Rider,<sup>6</sup> Michael O. Harris-Love,<sup>7</sup>  
Marc C. Levesque,<sup>1</sup> the RIM Study Group, and Chester V. Oddis<sup>1</sup>

- Anti-Jo-1 and anti-Mi2 predicted a shorter time to improvement with Rituximab treatment
- Inception cohort studies needed



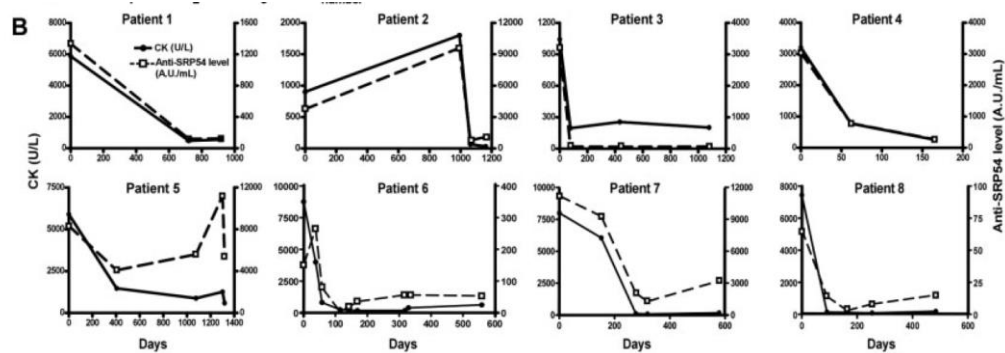
Do autoantibody levels reflect  
disease activity?



# MSDA levels and disease activity

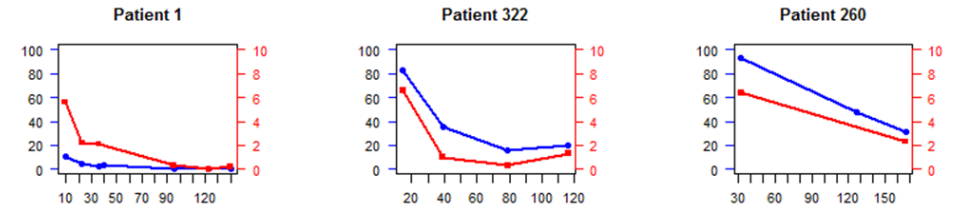
0.001). In multiple regression analyses of 11 patients with serial samples, anti-Jo-1 antibody levels correlated significantly with CK levels ( $R^2 = 0.65$ ,  $P = 0.0002$ ), myositis VAS ( $R^2 = 0.53$ ,  $P = 0.0008$ ), arthritis VAS ( $R^2 = 0.53$ ,  $P = 0.006$ ), pulmonary VAS ( $R^2 = 0.69$ ,  $P = 0.005$ ), global VAS ( $R^2 = 0.63$ ,  $P = 0.002$ ), and global MITAX ( $R^2 = 0.64$ ,  $P = 0.0003$ ).

Stone et al Arthritis Rheum 2007

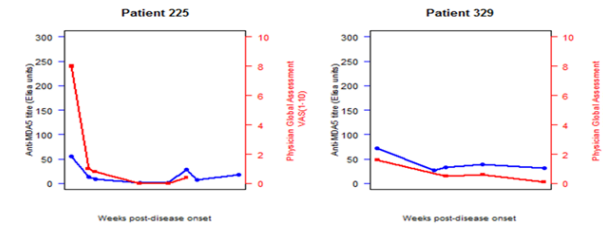


Anti-SRP correlates with CK levels  
Benveniste et al Arthritis Rheum 2011

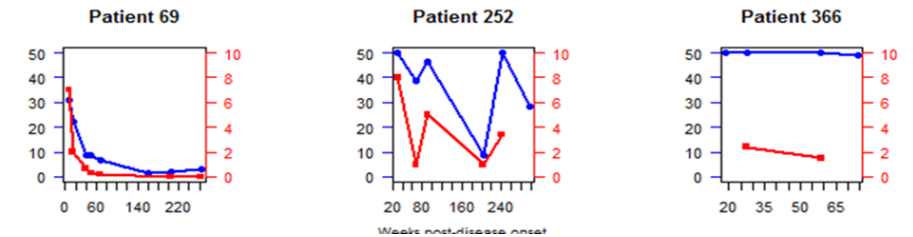
NXP2



MDA5



TIF1- $\gamma$



Tansley et al unpublished

# Summary

- Myositis autoantibodies (MSDA and MAA)
  - Present in 60% of cases of myositis spectrum disorders
  - May be valuable in diagnosis
  - Newer specificities (TIF1, NXP2, MDA5) account for 40% of cases in JDM
  - Have different prevalence and associations across the myositis spectrum dependent on age of onset of disease
  - Profiles may predict response to therapy
  - Levels may reflect disease activity
  - Provide insights into genetic and environmental mechanisms of disease



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