New autoantibodies

and the role of **IP-MS** in routine diagnostics ?

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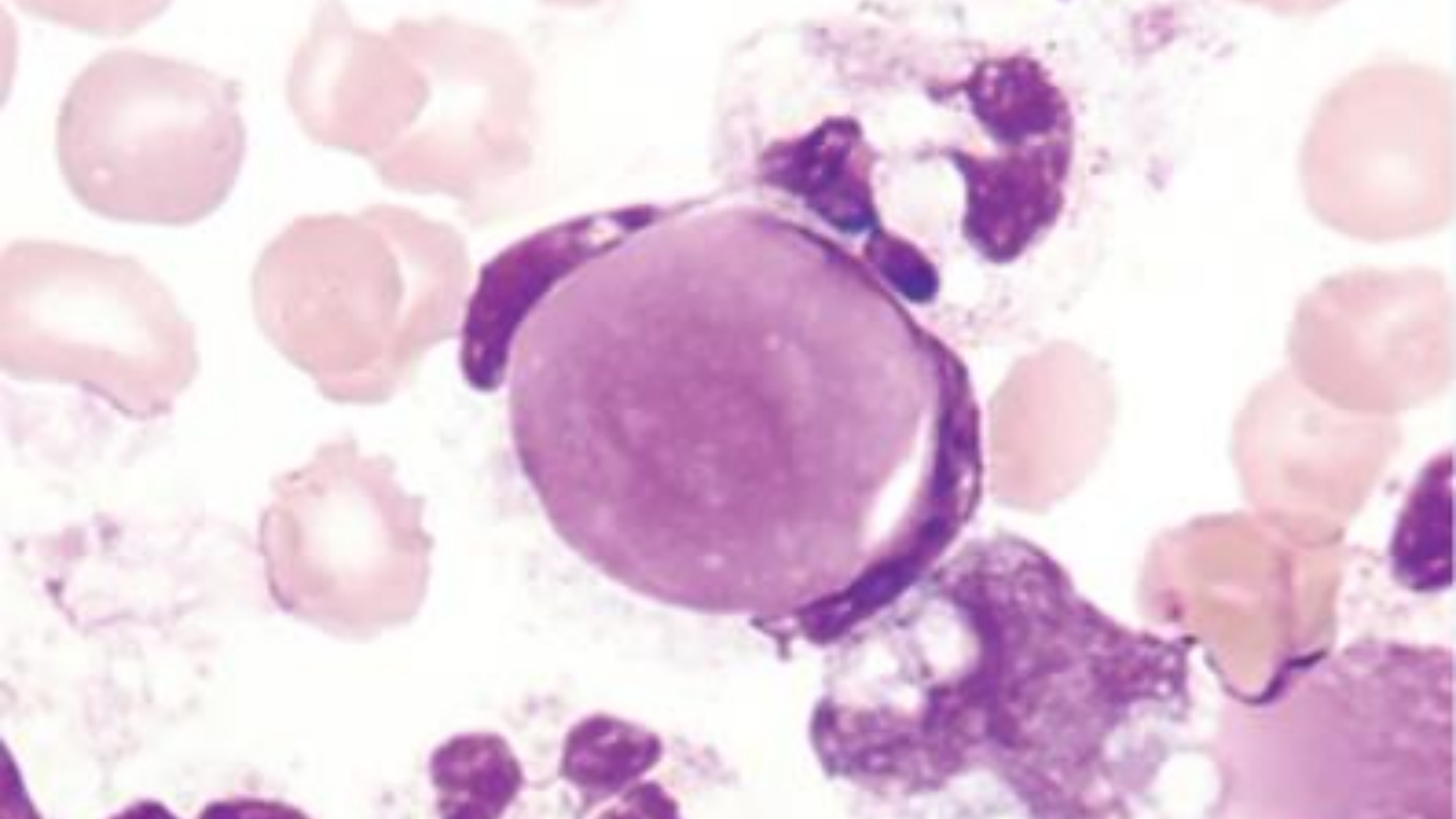
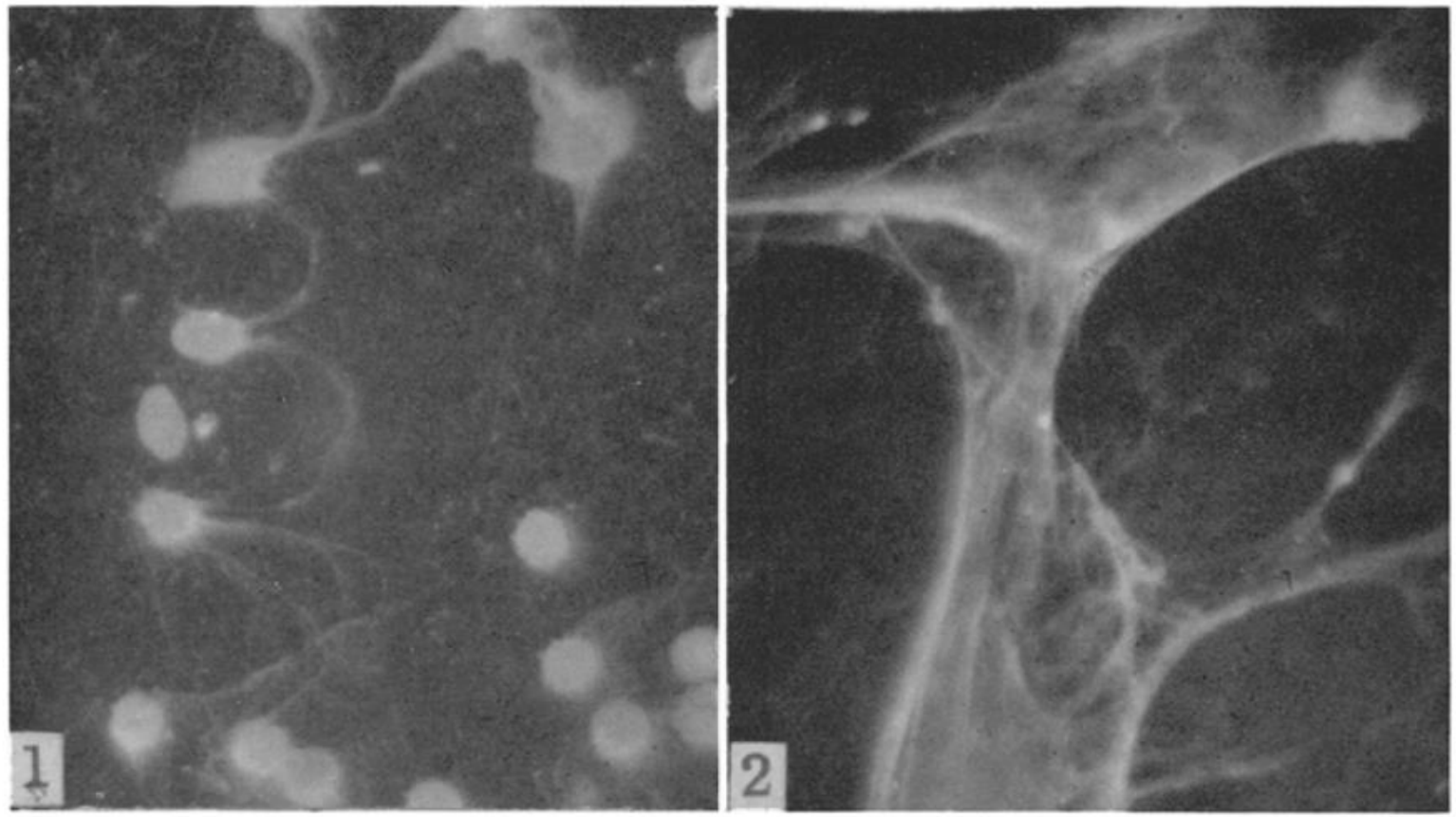
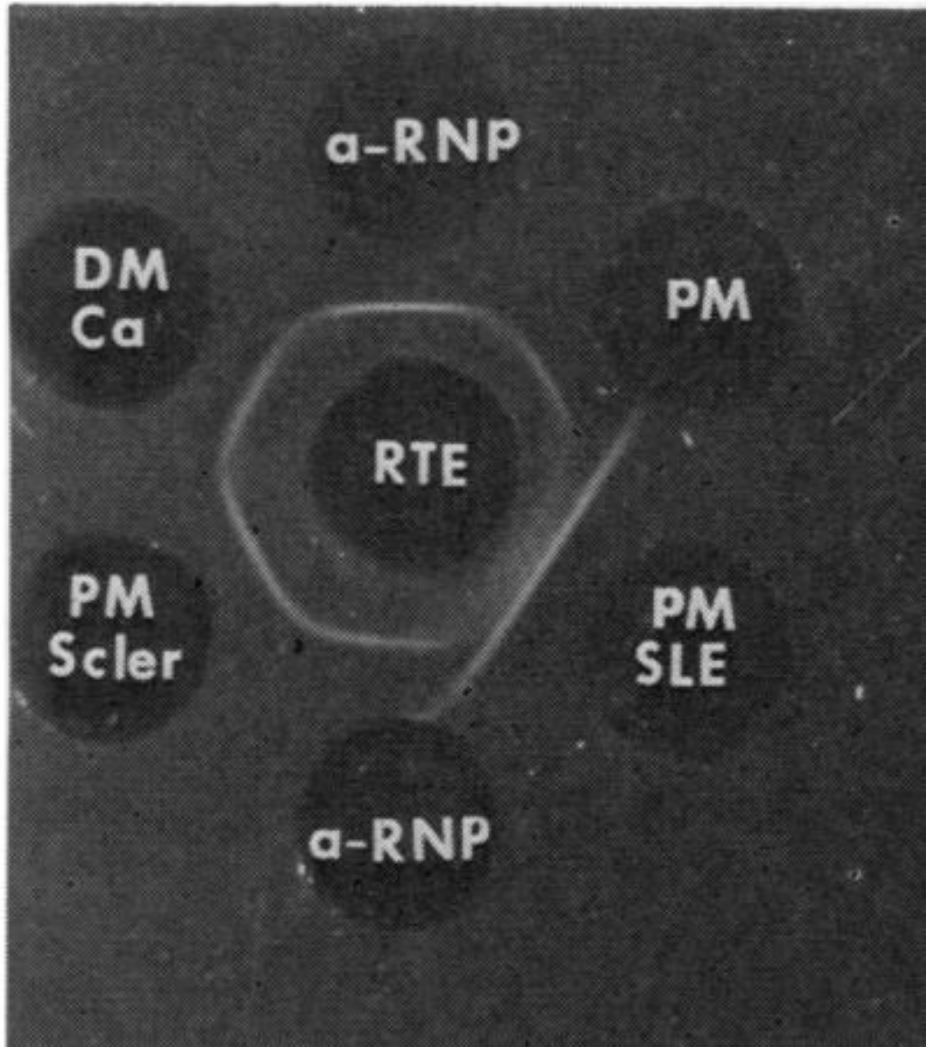
AZ Delta Roeselare

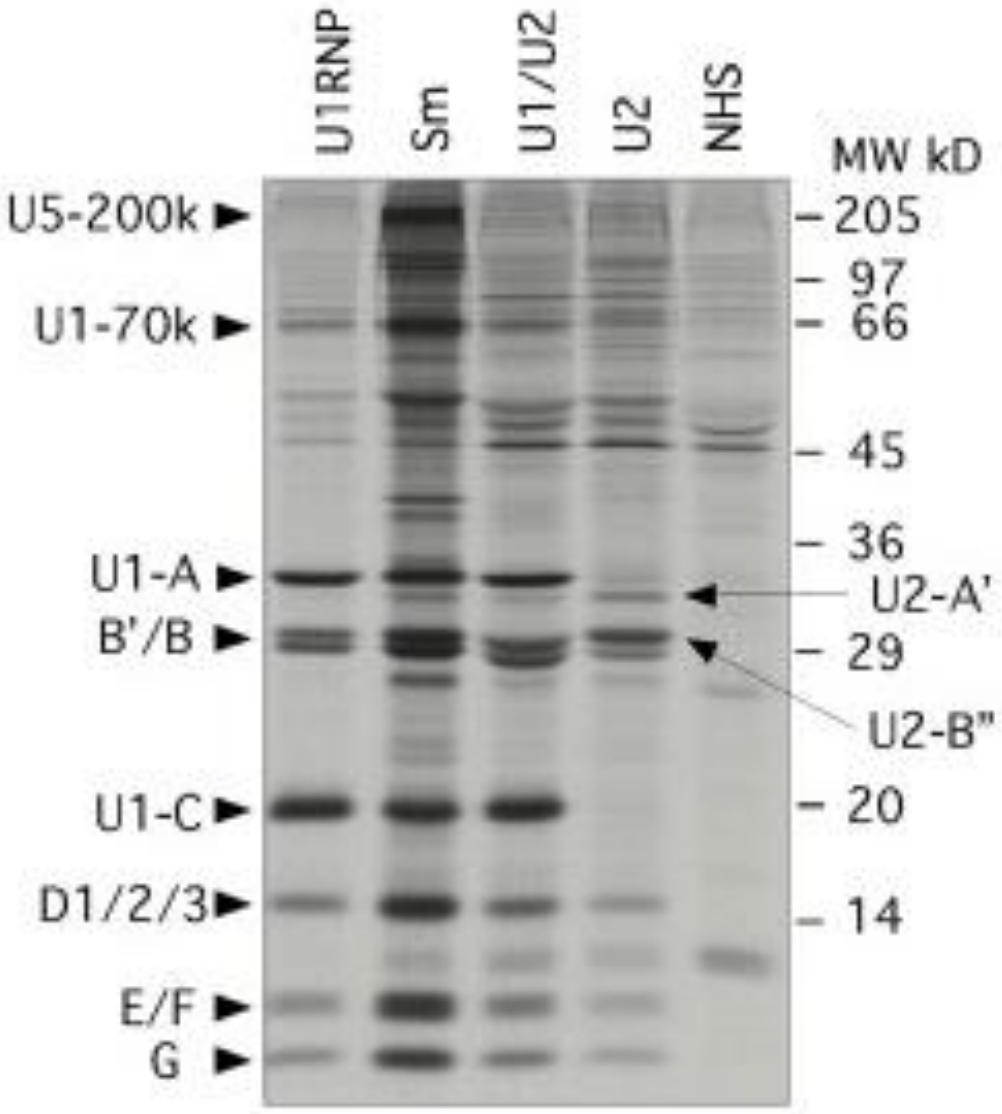




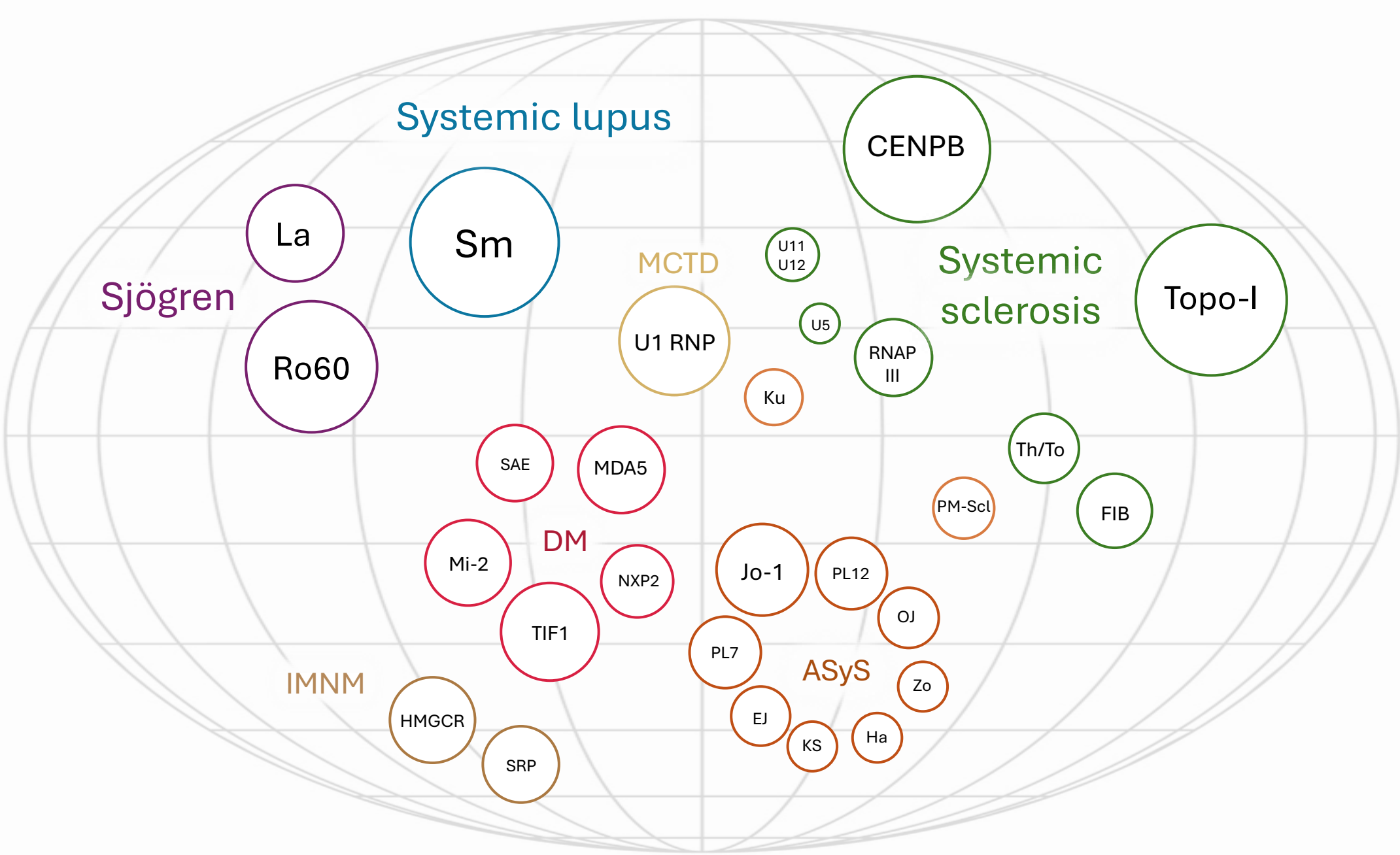
Friou et al., J Immunol 1958



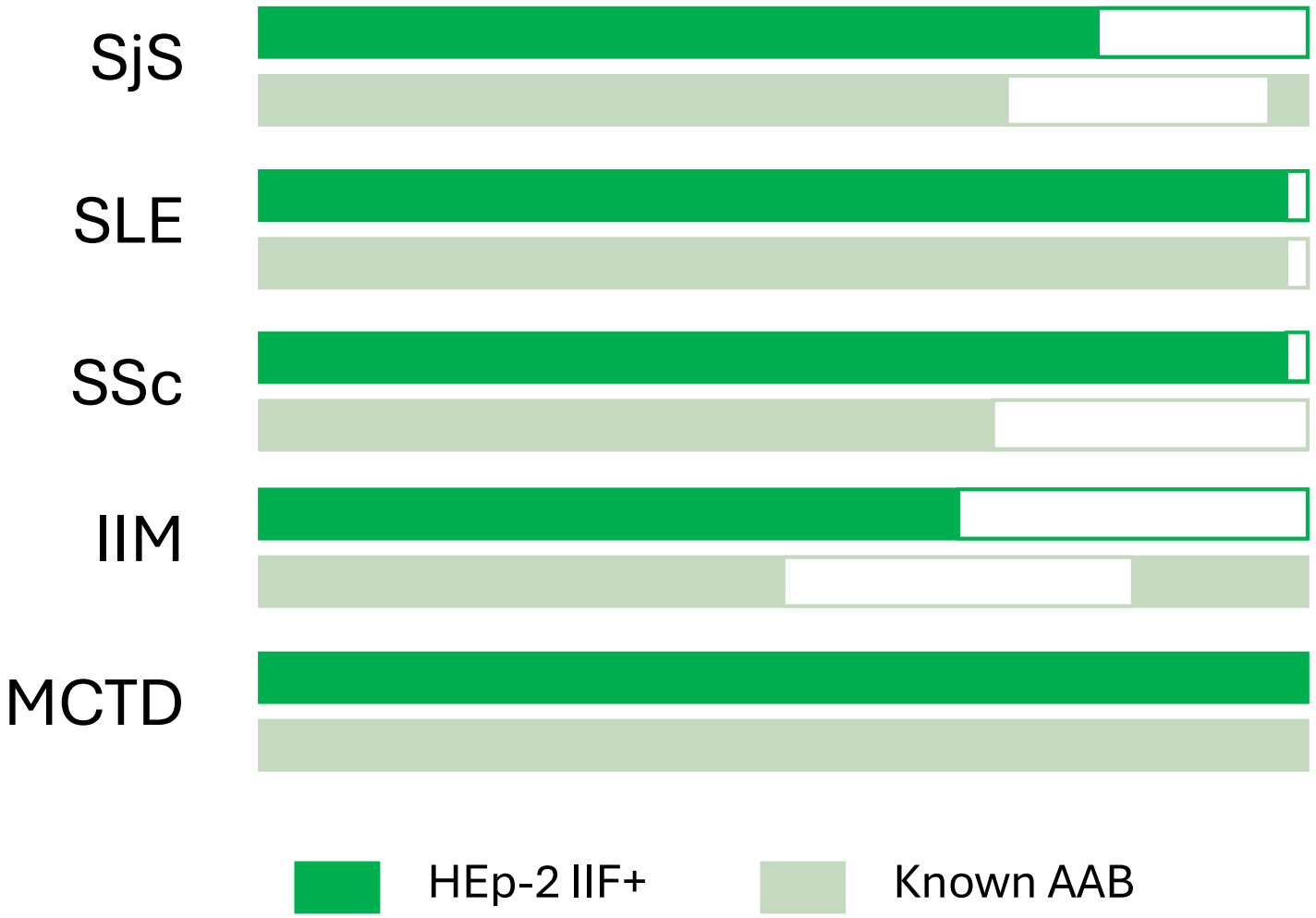
Venables et al., ARD 1981

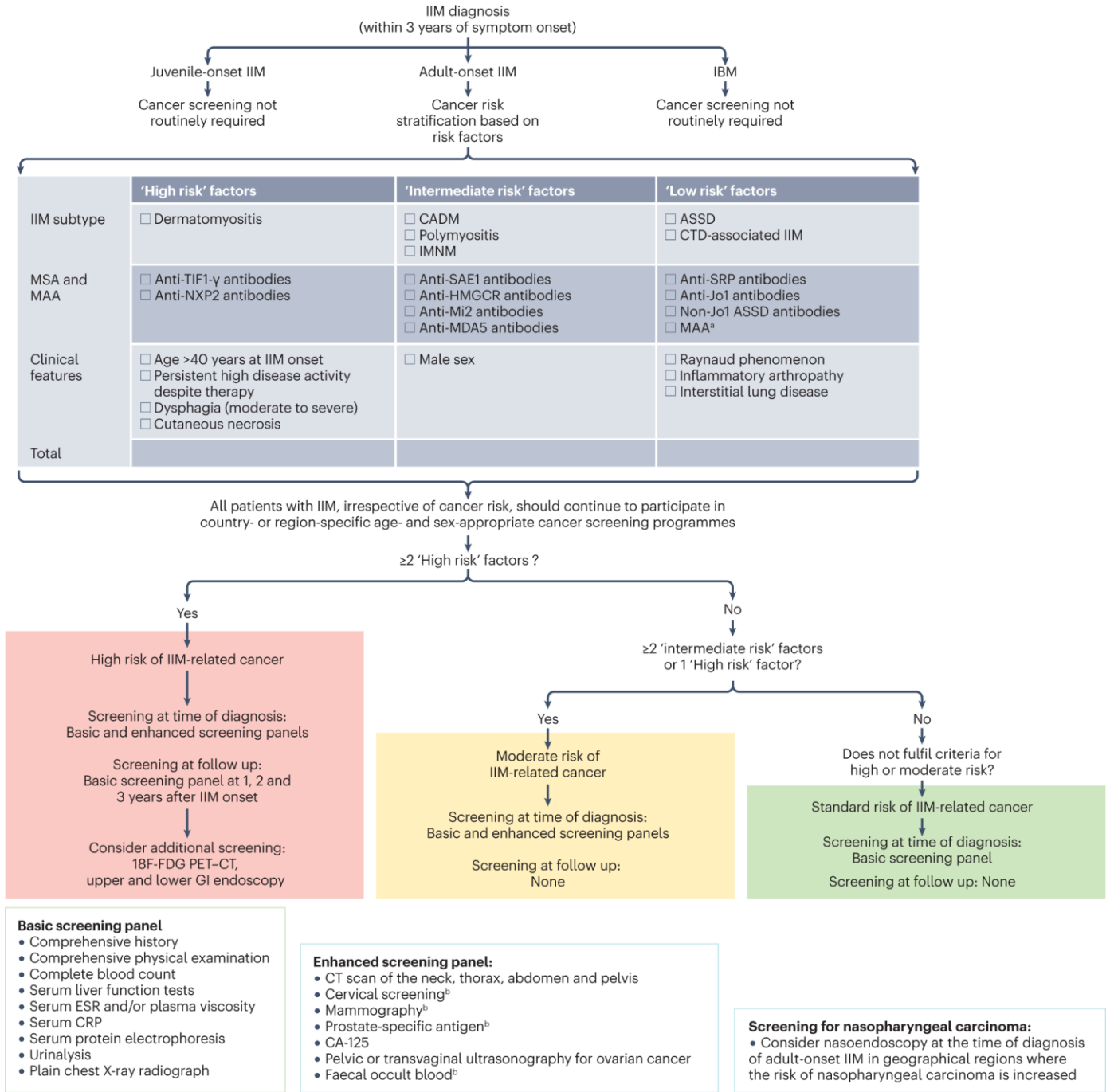


Satoh et al., Autoantibodies 2014

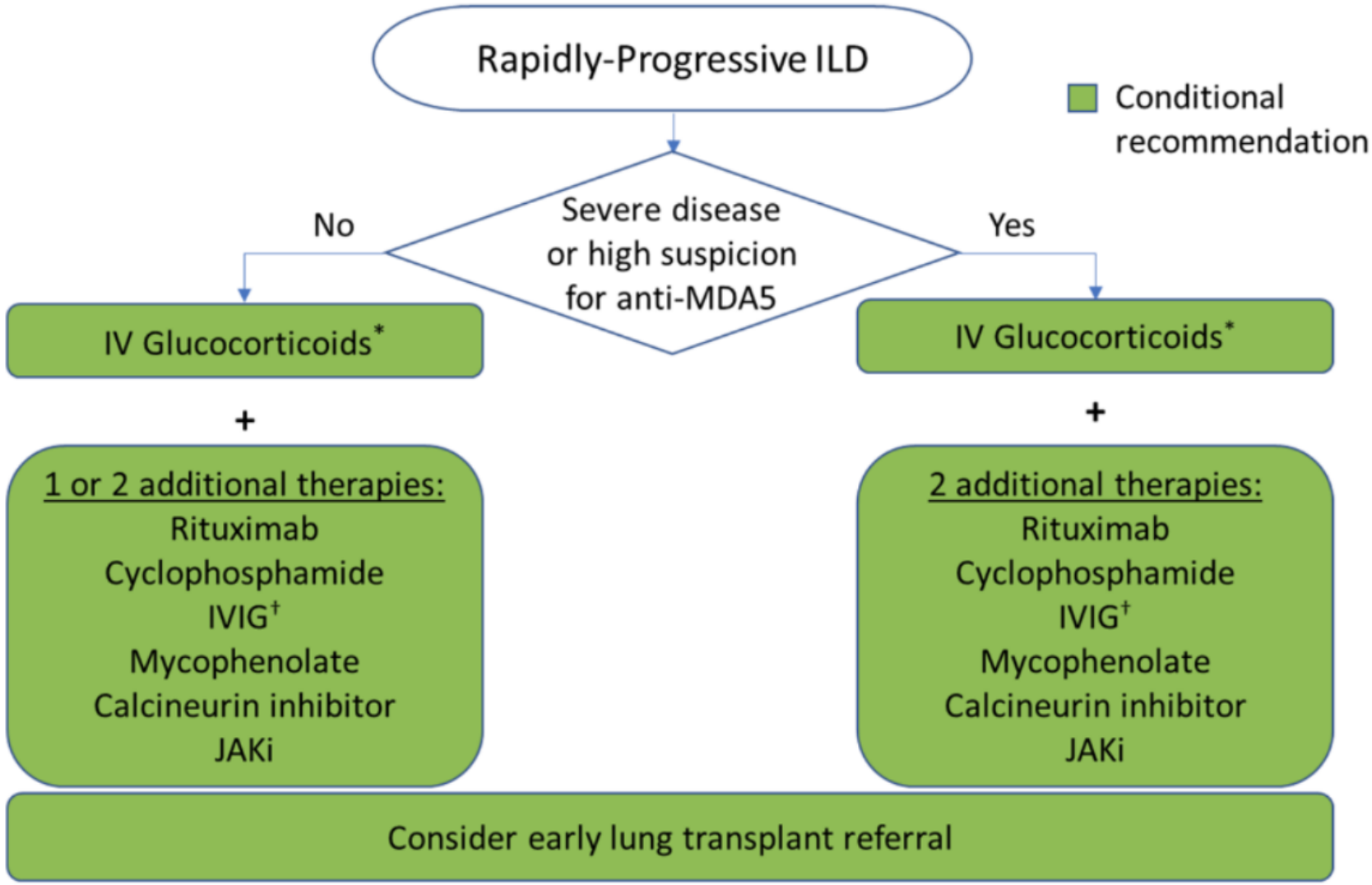

Systemic lupus
CENPB
La
Sm
Sjögren
MCTD
U11
U12
Systemic
sclerosis
Topo-I
U5
U1 RNP
RNAP
III
Ro60
Ku
Th/To
SAE
MDA5
PM-Scl
FIB
DM
Mi-2
Jo-1
PL12
NXP2
OJ
TIF1
PL7
ASyS
IMNM
Zo
HMGCR
EJ
KS
Ha
SRP

Anno 2024: a **seronegative** gap remains





Oldroyd et al.,
Nat Rev Rheumatol
2023



ACR Guidelines 2023

Anno 2024: a brewing solid-phase storm

- Discordance between solid-phase assays Vulsteke et al., Ann Rheum Dis 2018
- Discordance solid-phase assays and immunoprecipitation
- Lower pretest probabilities

Table 1 Agreement between LIA and IP derived from three studies

	Espinosa-Ortega *et al*	Cavazzana *et al*	Mahler *et al*	Combined	
MSA	DP/IP/LP/DN *Kappa* (95% CI)				Interpretation (*kappa*)
Jo-1	11/1/7/91 0.69 (0.50 to 0.89)	3/5/7/42 0.21 (–0.11 to 0.52)	NA	14/6/14/133 0.52 (0.33 to 0.70)	Minimal–Moderate
TIF1y	1/2/0/107 0.56 (0.29 to 0.83)	4/0/2/51 0.78 (0.49 to 1.00)	11/4/4/138 0.71 (0.51 to 0.90)	16/6/6/296 0.71 (0.55 to 0.86)	Weak–Strong
SRP	3/1/0/106 0.85 (0.57 to 1.00)	0/3/7/47 –0.08 (–0.15 to –0.01)	14/1/8/134 0.73 (0.56 to 0.89)	17/5/15/287 0.60 (0.44 to 0.76)	Weak–Moderate
MDA5	1/2/0/107 0.49 (–0.12 to 1.00)	3/1/2/51 0.64 (0.26 to 1.00)	14/1/5/137 0.80 (0.65 to 0.96)	18/4/7/295 0.75 (0.61 to 0.89)	Moderate–Strong
PM/Scl	2/3/1/104 0.48 (0.05 to 0.92)	NA NA	NA NA	2/3/1/104 0.48 (0.05 to 0.92)	NA
Ku	3/0/1/106 0.86 (0.57 to 1.00)	NA NA	NA NA	3/0/1/106 0.86 (0.57 to 1.00)	NA
SAE	3/0/1/106 0.86 (0.57 to 1.00)	0/2/1/54 –0.02 (–0.06 to 0.01)	15/0/3/139 0.90 (0.79 to 1.00)	18/2/5/299 0.83 (0.70 to 0.95)	Moderate–Almost perfect
NXP2	0/0/2/108 0.00	5/3/2/47 –0.62 (0.31 to 0.92)	13/2/0/142 0.92 (0.81 to 1.00)	18/5/4/297 0.79 (0.65 to 0.92)	Moderate–Almost perfect
Mi-2	2/0/6/102 0.38 (0.00 to 0.76)	3/0/5/49 0.51 (0.45 to 0.87)	12/3/9/133 0.62 (0.43 to 0.82)	17/3/20/284 0.56 (0.41 to 0.72)	Weak–Moderate
EJ	0/0/0/110 NA	1/2/0/54 0.49 (–0.11 to 1.00)	7/3/1/146 0.76 (0.54 to 0.99)	8/5/1/310 0.72 (0.50 to 0.93)	Weak–Almost perfect
OJ	0/1/0/109 0.00	0/2/0/55 0.00	NA	0/3/0/164 NA	NA
PL-7	0/0/0/110 NA	0/0/2/55 0.00	12/3/0/142 0.88 (0.74 to 1.00)	12/3/2/307 0.82 (0.67 to 0.97)	Moderate–Almost perfect
PL-12	0/0/1/109 0.00	0/0/3/54 0.00	14/1/5/137 0.80 (0.65 to 0.96)	14/1/9/300 0.72 (0.56 to 0.89)	Weak–Strong

95% CIs were reconstructed based on the given prevalence.

Mahler et al., ARD 2021

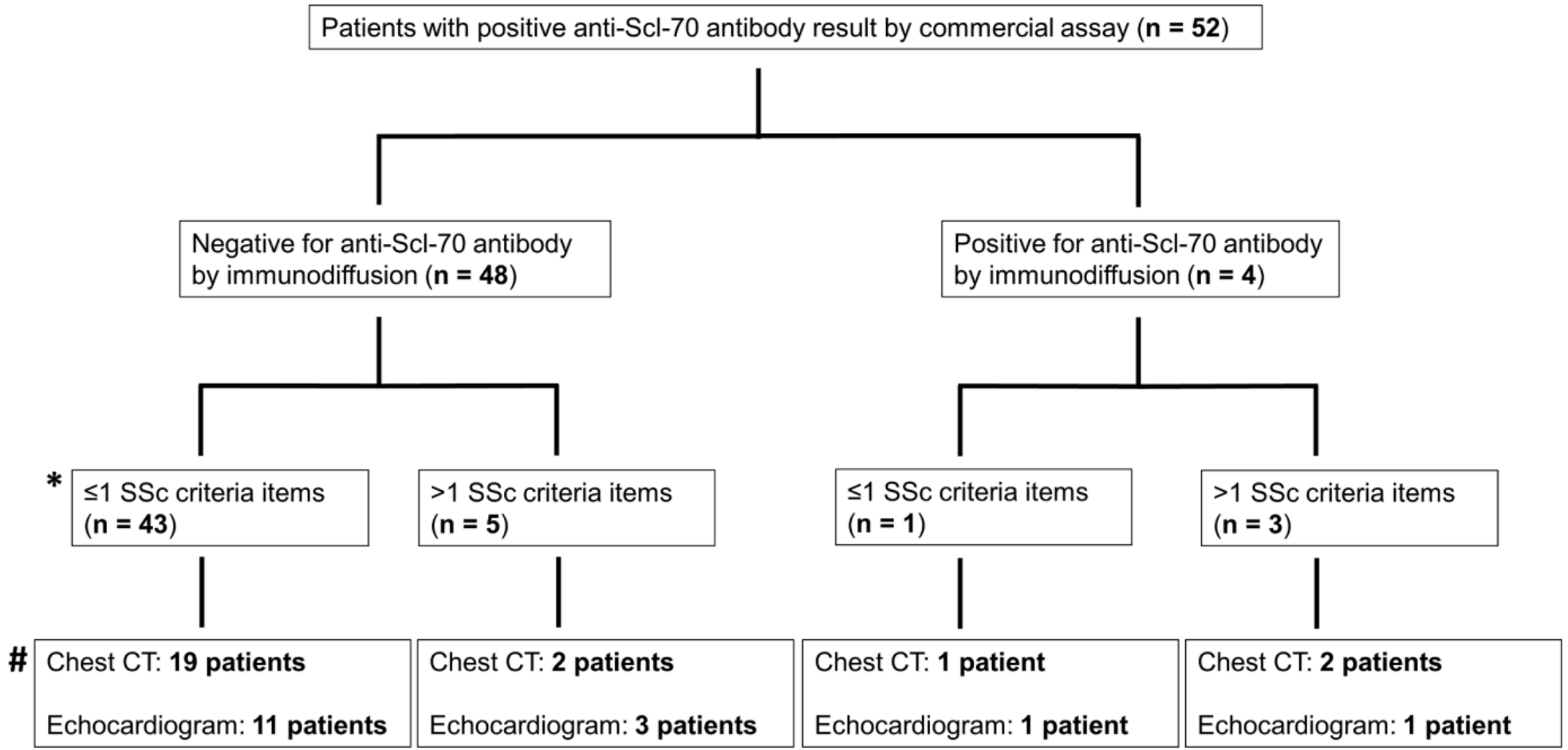


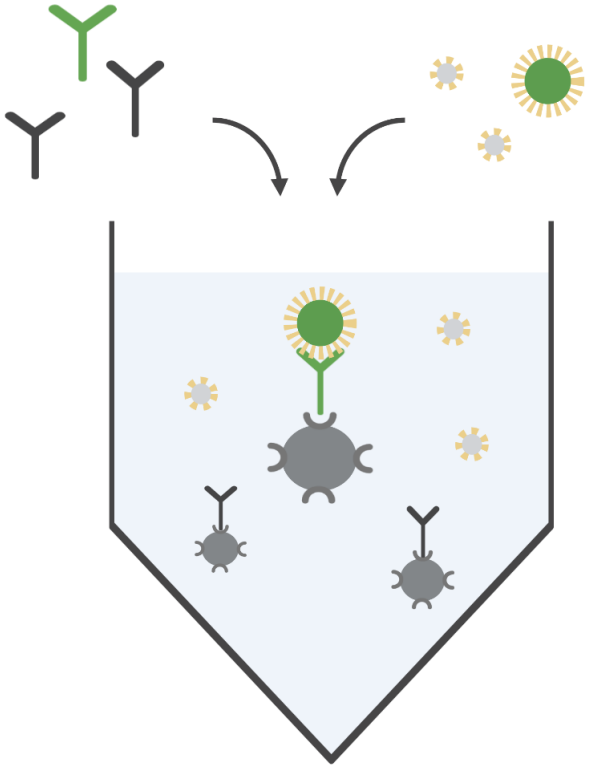
Fig. 2. Flow diagram showing numbers of patients who underwent chest CT and/or echocardiogram within patient subgroups, by Scl-70 immunodiffusion results and number of ACR/EULAR systemic sclerosis classification criteria items. *Commercial Scl-70 antibody results were not counted towards the criteria items, as noted in the Methods section. #Refers to the numbers of patients who underwent chest CT and/or echocardiogram prior to evaluation in our clinic.

Immunoprecipitation-mass spectrometry or IP-MS

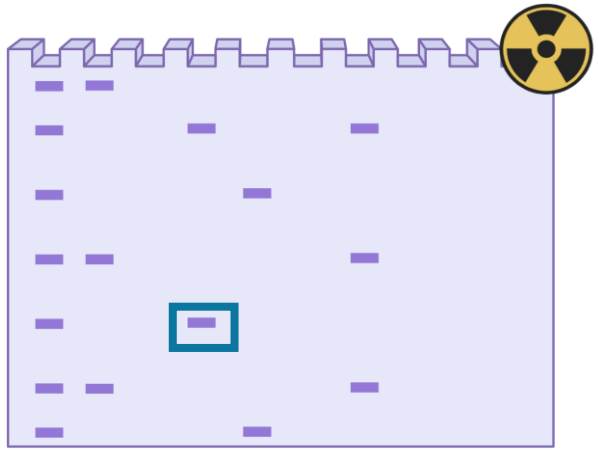
Immunoprecipitation

Serum

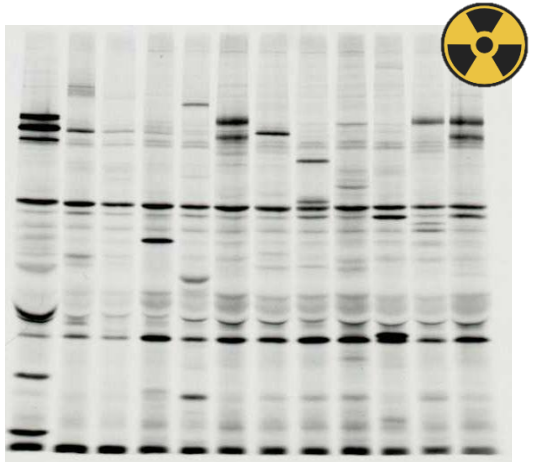
Radioactive cell extract



Gel electrophoresis



Autoradiography



Mass spectrometry

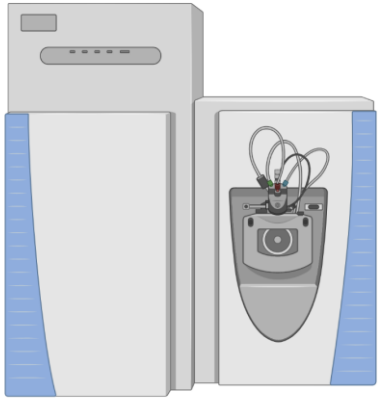
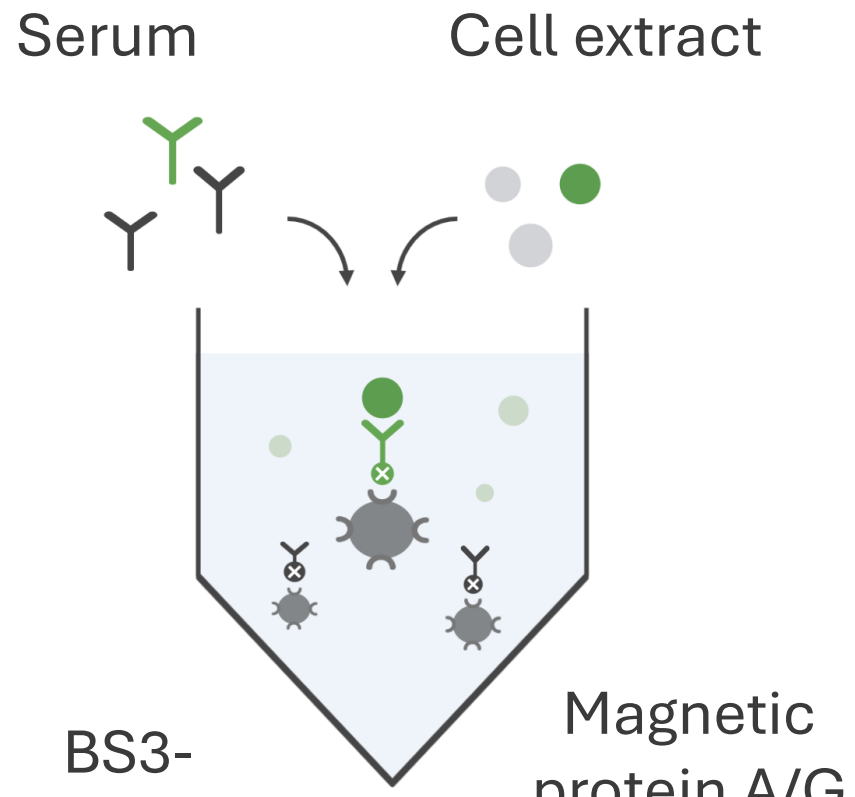
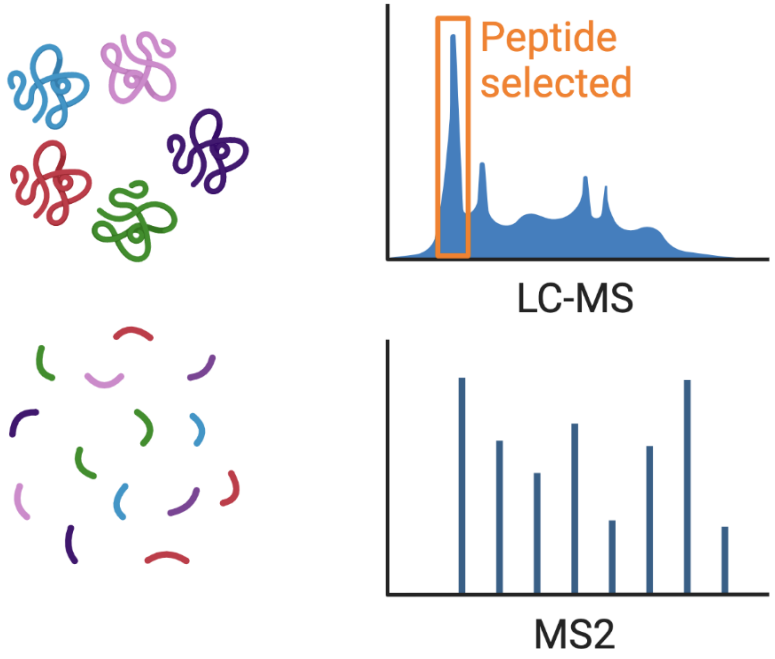


Image made with BioRender

Immunoprecipitation



LC-MS/MS



Data-dependent acquisition

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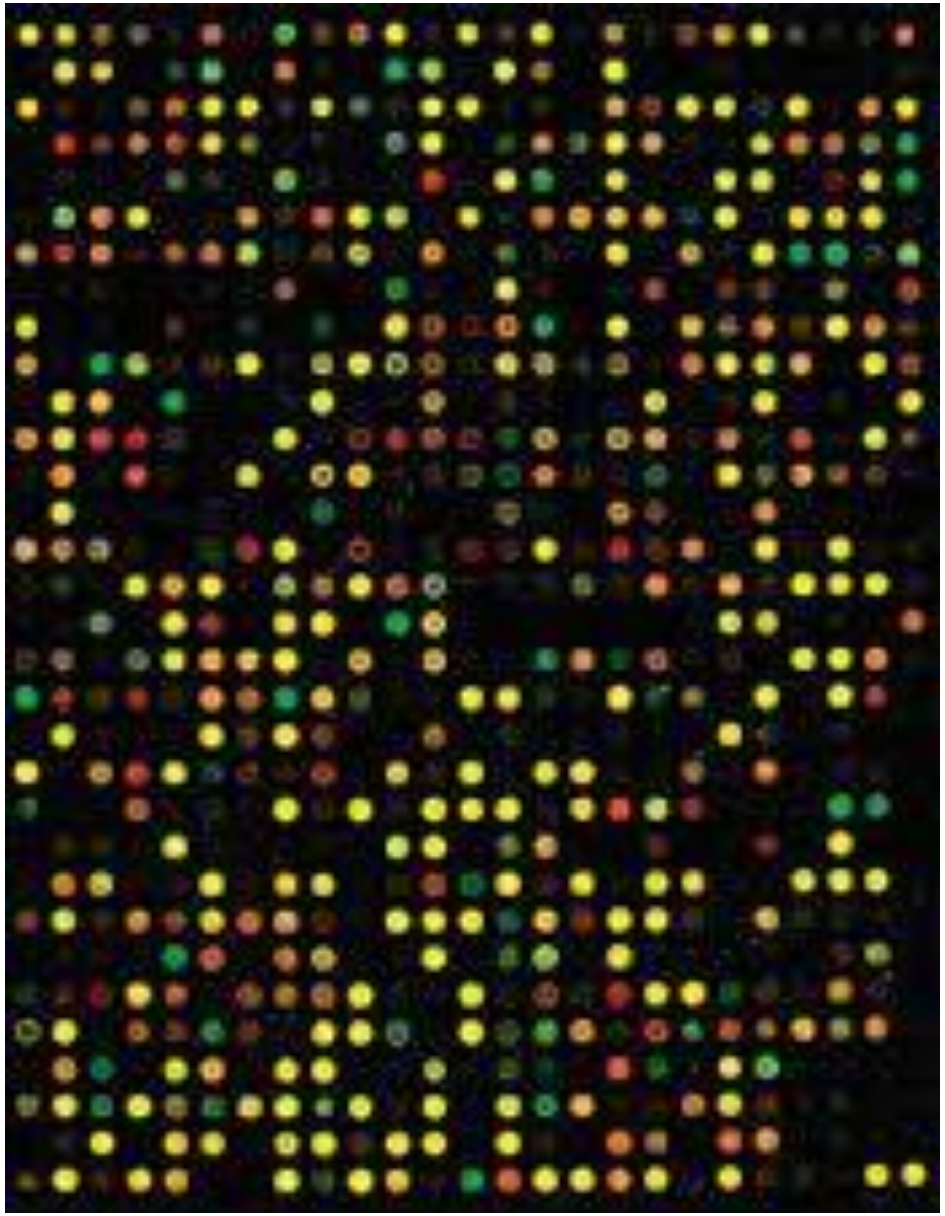
Why not protein arrays?

- None of the established AABs have been identified with protein arrays
- Conformational dependence?



Quaternary

autoepitopes?

Jo-1

Ro60

PM-Scl

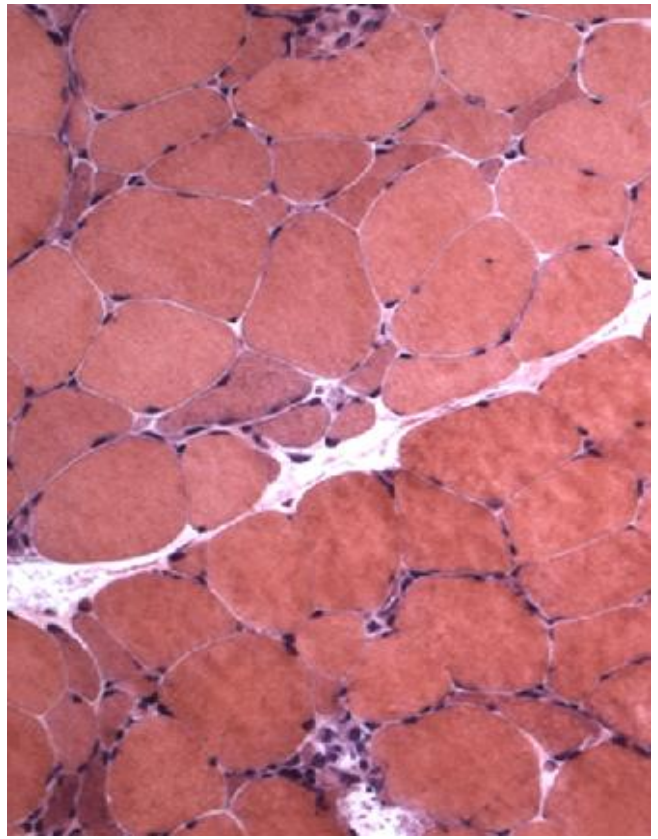
RuvBL

OJ

Heterogeneity

Mr. N

72 y

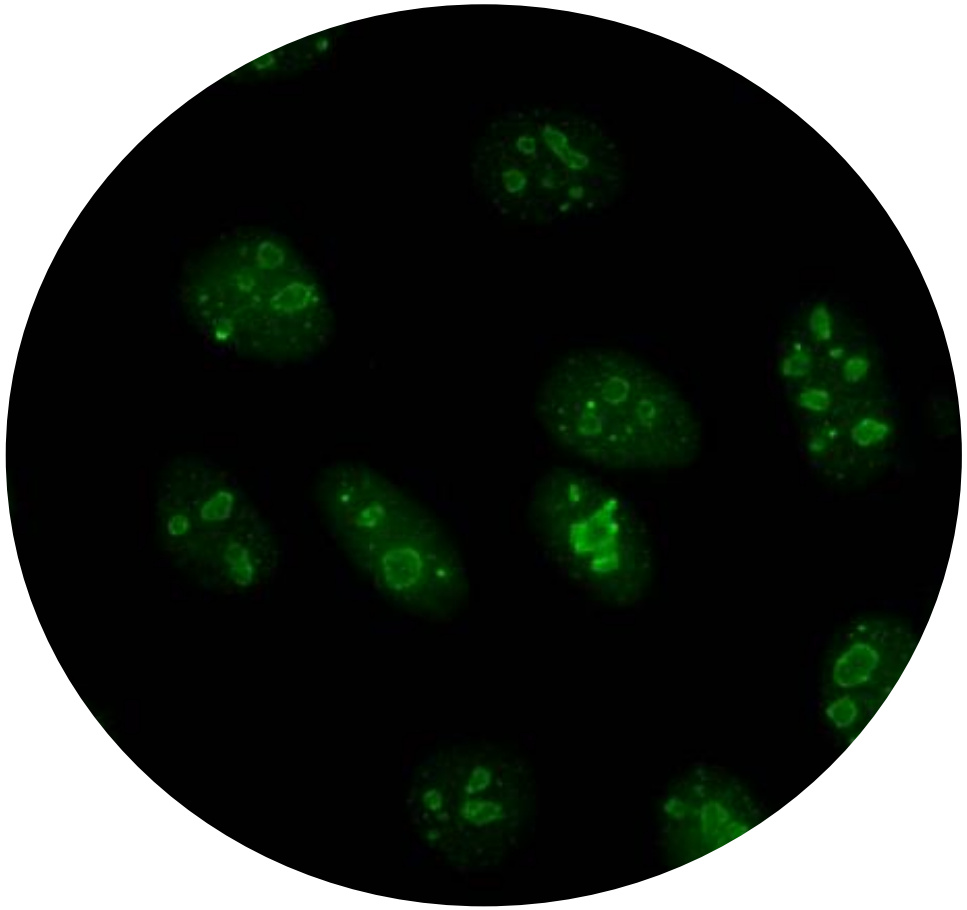


Mr. N

Cluster of Vimentin	VIM	369	314	314	167	303	70	127	88	84	92	105	85	71	91	145	90	127
RNA transcription, translation and transport factor protein	RTRAF	105	103	94	97	84	66	71	55	13	63	78	60	32	83	76	28	70
Signal recognition particle 14 kDa protein	SRP14	66	57	66	46	56	49	41	44	20	39	46	25	17	38	48	51	37
Cluster of Actin, cytoplasmic 1	ACTB	65	57	64	51	43	20	38	24	27	28	44	22	13	28	37	25	39
Pre-mRNA-processing factor 19	PRPF19	50	32	61	44	39	38	16	20	5	14	27	16	15	40	25	12	58
Non-POU domain-containing octamer-binding protein	NONO	59	71	61	52	70	29	28	16	15	42	35	12	22	19	29	52	48
Cluster of Prohibitin	PHB	66	48	60	30	52	42	33	30	17	31	47	27	17	40	50	25	39
40S ribosomal protein S18	RPS18	69	56	54	47	56	25	36	26	25	41	49	21	24	25	39	35	33
Cleavage and polyadenylation specificity factor subunit 5	NUDT21	50	54	49	21	49	33	31	30	18	27	35	18	7	32	32	13	32
Cluster of Heat shock cognate 71 kDa protein	HSPA8	42	40	46	28	36	21	21	18	23	14	21	20	24	29	11	16	23
Splicing factor, proline- and glutamine-rich	SFPQ	44	46	44	44	42	32	25	22	31	32	24	31	34	40	39	30	35
Cluster of Uncharacterized protein DKFZp686N02209		13	12	44	59	140	63	43	48	61	67	67	103	56	52	90	13	136
DNA damage-binding protein 1	DDB1	56	48	43	36	34	31	18	33	16	25	19	19	26	29	20	8	27
Cluster of Polyadenylate-binding protein 1	PABPC1	43	36	42	18	33	15	15	16	1	15	20	14	8	23	21	5	10
Prohibitin-2	PHB2	41	31	37	19	33	29	23	25	24	24	28	23	21	21	23	13	21
Cluster of Heat shock 70 kDa protein 1A	HSPA1A	35	41	34	25	35	28	24	28	28	22	27	28	31	33	17	12	22
40S ribosomal protein S3	RPS3	30	31	32	25	30	33	23	32	16	29	29	16	11	24	22	36	18
Pyruvate kinase PKM	PKM	34	29	31	30	29	42	35	33	13	32	31	27	21	28	31	13	15
40S ribosomal protein S5	RPS5	41	37	29	19	21	22	14	7	2	11	22	5	0	6	28	24	12
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	DDOST	29	34	29	22	23	14	12	4	4	8	21	0	0	12	16	5	9
Nucleolar and coiled-body phosphoprotein 1	NOLC1	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cluster of Myosin light polypeptide 6	MYL6	29	21	25	11	17	8	10	7	8	6	9	10	9	12	8	5	7
40S ribosomal protein SA	RPSA	26	26	24	25	24	24	18	22	16	22	24	24	16	24	16	13	34
60S ribosomal protein L13	RPL13	27	30	24	23	27	12	18	7	15	13	13	18	20	18	16	22	4
NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	NDUFS3	6	14	22	9	18	12	9	13	2	7	13	6	2	10	8	1	5
Ribosomal RNA small subunit methyltransferase NEP1	EMG1	21	17	19	12	15	9	8	9	0	6	10	6	2	15	9	3	9
40S ribosomal protein S7	RPS7	23	10	18	7	9	0	0	1	0	0	2	0	0	1	9	1	2
60S acidic ribosomal protein P2	RPLP2	30	20	18	8	13	7	5	7	6	6	16	15	4	23	13	9	16
Cluster of General transcription and DNA repair factor IIH helicase subunit XPB	ERCC3	1	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RuvB-like 1	RUVBL1	13	18	17	6	16	4	7	4	1	0	10	1	2	2	2	0	2
60S ribosomal protein L23	RPL23	14	19	17	23	12	11	16	11	13	11	12	12	9	14	9	8	10
Replication factor C subunit 4	RFC4	13	8	17	3	10	5	2	0	2	5	2	6	4	5	4	0	3
Cytochrome c oxidase subunit 7A2, mitochondrial	COX7A2	15	15	17	15	15	7	9	7	0	8	11	10	0	4	10	5	7

Mr. N

Cluster of Vimentin	VIM	369	314	314	167	303	70	127	88	84	92	105	85	71	91	145	90	127
RNA transcription, translation and transport factor protein	RTRAF	105	103	94	97	84	66	71	55	13	63	78	60	32	83	76	28	70
Signal recognition particle 14 kDa protein	SRP14	66	57	66	46	56	49	41	44	20	39	46	25	17	38	48	51	37
Cluster of Actin, cytoplasmic 1	ACTB	65	57	64	51	43	20	38	24	27	28	44	22	13	28	37	25	39
Pre-mRNA-processing factor 19	PRPF19	50	32	61	44	39	38	16	20	5	14	27	16	15	40	25	12	58
Non-POU domain-containing octamer-binding protein	NONO	59	71	61	52	70	29	28	16	15	42	35	12	22	19	29	52	48
Cluster of Prohibitin	PHB	66	48	60	30	52	42	33	30	17	31	47	27	17	40	50	25	39
40S ribosomal protein S18	RPS18	69	56	54	47	56	25	36	26	25	41	49	21	24	25	39	35	33
Cleavage and polyadenylation specificity factor subunit 5	NUDT21	50	54	49	21	49	33	31	30	18	27	35	18	7	32	32	13	32
Cluster of Heat shock cognate 71 kDa protein	HSPA8	42	40	46	28	36	21	21	18	23	14	21	20	24	29	11	16	23
Splicing factor, proline- and glutamine-rich	SFPQ	44	46	44	44	42	32	25	22	31	32	24	31	34	40	39	30	35
Cluster of Uncharacterized protein DKFZp686N02209		13	12	44	59	140	63	43	48	61	67	67	103	56	52	90	13	136
DNA damage-binding protein 1	DDB1	56	48	43	36	34	31	18	33	16	25	19	19	26	29	20	8	27
Cluster of Polyadenylate-binding protein 1	PABPC1	43	36	42	18	33	15	15	16	1	15	20	14	8	23	21	5	10
Prohibitin-2	PHB2	41	31	37	19	33	29	23	25	24	24	28	23	21	21	23	13	21
Cluster of Heat shock 70 kDa protein 1A	HSPA1A	35	41	34	25	35	28	24	28	28	22	27	28	31	33	17	12	22
40S ribosomal protein S3	RPS3	30	31	32	25	30	33	23	32	16	29	29	16	11	24	22	36	18
Pyruvate kinase PKM	PKM	34	29	31	30	29	42	35	33	13	32	31	27	21	28	31	13	15
40S ribosomal protein S5	RPS5	41	37	29	19	21	22	14	7	2	11	22	5	0	6	28	24	12
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	DDOST	29	34	29	22	23	14	12	4	4	8	21	0	0	12	16	5	9
Nucleolar and coiled-body phosphoprotein 1	NOLC1	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cluster of Myosin light polypeptide 6	MYL6	29	21	25	11	17	8	10	7	8	6	9	10	9	12	8	5	7
40S ribosomal protein SA	RPSA	26	26	24	25	24	24	18	22	16	22	24	24	16	24	16	13	34
60S ribosomal protein L13	RPL13	27	30	24	23	27	12	18	7	15	13	13	18	20	18	16	22	4
NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	NDUFS3	6	14	22	9	18	12	9	13	2	7	13	6	2	10	8	1	5
Ribosomal RNA small subunit methyltransferase NEP1	EMG1	21	17	19	12	15	9	8	9	0	6	10	6	2	15	9	3	9
40S ribosomal protein S7	RPS7	23	10	18	7	9	0	0	1	0	0	2	0	0	1	9	1	2
60S acidic ribosomal protein P2	RPLP2	30	20	18	8	13	7	5	7	6	6	16	15	4	23	13	9	16
Cluster of General transcription and DNA repair factor IIH helicase subunit XPB	ERCC3	1	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RuvB-like 1	RUVBL1	13	18	17	6	16	4	7	4	1	0	10	1	2	2	2	0	2
60S ribosomal protein L23	RPL23	14	19	17	23	12	11	16	11	13	11	12	12	9	14	9	8	10
Replication factor C subunit 4	RFC4	13	8	17	3	10	5	2	0	2	5	2	6	4	5	4	0	3
Cytochrome c oxidase subunit 7A2, mitochondrial	COX7A2	15	15	17	15	15	7	9	7	0	8	11	10	0	4	10	5	7

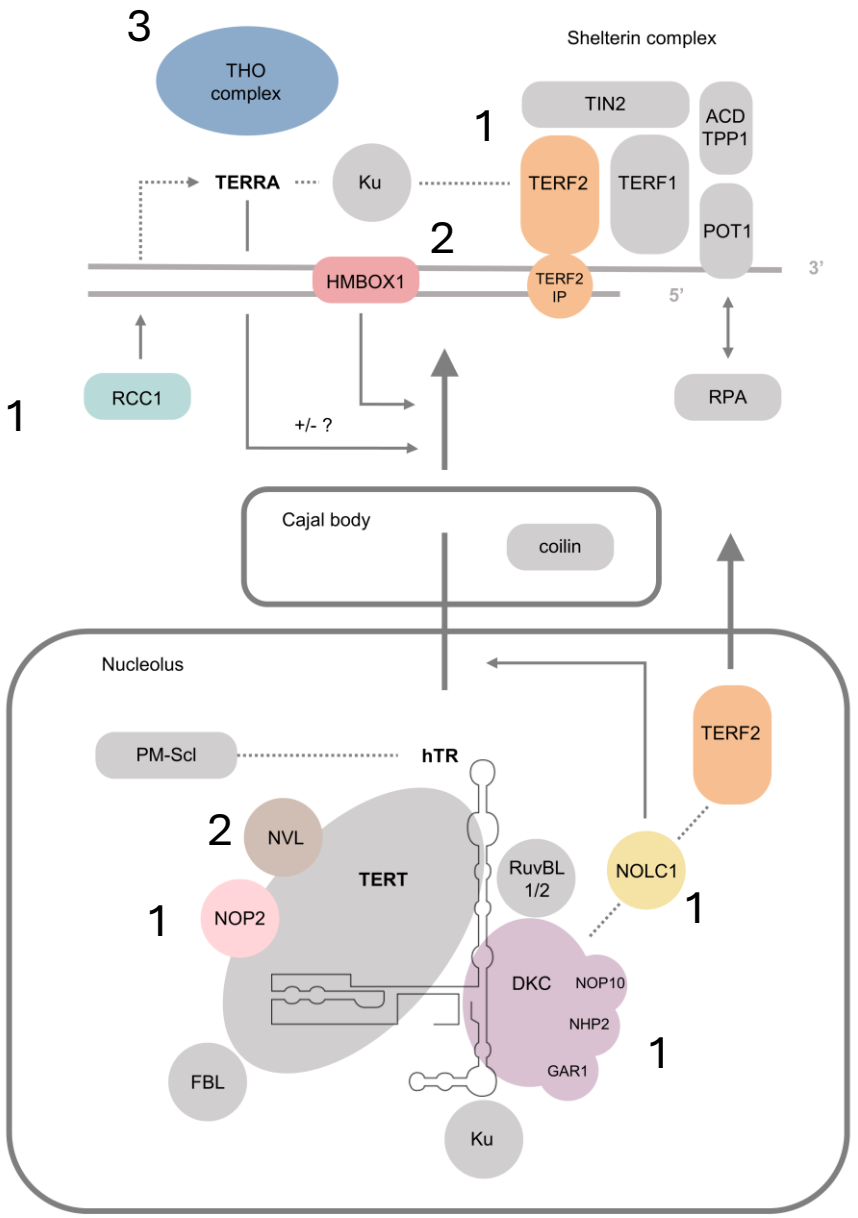


Nucleolar and coiled-body phosphoprotein 1

Mr. N

VIM	369	314	314	167	303	70	127	88	84	92	105	85	71	91	145	90	127
RTRAF	105	103	94	97	84	66	71	55	13	63	78	60	32	83	76	28	70
SRP14	66	57	66	46	56	49	41	44	20	39	46	25	17	38	48	51	37
ACTB	65	57	64	51	43	20	38	24	27	28	44	22	13	28	37	25	39
PRPF19	50	32	61	44	39	38	16	20	5	14	27	16	15	40	25	12	58
NONO	59	71	61	52	70	29	28	16	15	42	35	12	22	19	29	52	48
PHB	66	48	60	30	52	42	33	30	17	31	47	27	17	40	50	25	39
RPS18	69	56	54	47	56	25	36	26	25	41	49	21	24	25	39	35	33
NUDT21	50	54	49	21	49	33	31	30	18	27	35	18	7	32	32	13	32
HSPA8	42	40	46	28	36	21	21	18	23	14	21	20	24	29	11	16	23
SFPQ	44	46	44	44	42	32	25	22	31	32	24	31	34	40	39	30	35
	13	12	44	59	140	63	43	48	61	67	67	103	56	52	90	13	136
DDB1	56	48	43	36	34	31	18	33	16	25	19	19	26	29	20	8	27
PABPC1	43	36	42	18	33	15	15	16	1	15	20	14	8	23	21	5	10
PHB2	41	31	37	19	33	29	23	25	24	24	28	23	21	21	23	13	21
HSPA1A	35	41	34	25	35	28	24	28	28	22	27	28	31	33	17	12	22
RPS3	30	31	32	25	30	33	23	32	16	29	29	16	11	24	22	36	18
PKM	34	29	31	30	29	42	35	33	13	32	31	27	21	28	31	13	15
RPS5	41	37	29	19	21	22	14	7	2	11	22	5	0	6	28	24	12
DDOST	29	34	29	22	23	14	12	4	4	8	21	0	0	12	16	5	9
NOLC1	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MYL6	29	21	25	11	17	8	10	7	8	6	9	10	9	12	8	5	7
RPSA	26	26	24	25	24	24	18	22	16	22	24	24	16	24	16	13	34
RPL13	27	30	24	23	27	12	18	7	15	13	13	18	20	18	16	22	4
NDUFS3	6	14	22	9	18	12	9	13	2	7	13	6	2	10	8	1	5
EMG1	21	17	19	12	15	9	8	9	0	6	10	6	2	15	9	3	9
RPS7	23	10	18	7	9	0	0	1	0	0	2	0	0	1	9	1	2
RPLP2	30	20	18	8	13	7	5	7	6	6	16	15	4	23	13	9	16
ERCC3	1	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RUVBL1	13	18	17	6	16	4	7	4	1	0	10	1	2	2	2	0	2
RPL23	14	19	17	23	12	11	16	11	13	11	12	12	9	14	9	8	10
RFC4	13	8	17	3	10	5	2	0	2	5	2	6	4	5	4	0	3
COX7A2	15	15	17	15	15	7	9	7	0	8	11	10	0	4	10	5	7

Identified proteins	Gene name	Proteomic dataset				Total spectral counts per individual serum												
		A	B1	B2	C	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	H1	H2
THO complex subunit 1	THOC1	•				79	86	51	0	0	0	0	0	0	0	0	0	0
THO complex subunit 2	THOC2	•	•			71	45	36	0	0	0	0	0	0	0	0	0	0
THO complex subunit 3	THOC3	•	•			31	40	12	0	0	0	0	0	0	0	0	0	0
THO complex subunit 5	THOC5	•				162	175	113	0	0	0	0	0	0	0	0	0	0
THO complex subunit 6	THOC6	•	•			138	158	130	0	0	0	0	0	0	0	0	0	0
THO complex subunit 7	THOC7	•				32	33	17	0	0	0	0	0	0	0	0	0	0
Telomeric repeat-binding factor 2	TERF2	•*	•*	•*		0	0	0	111	0	0	0	0	0	0	0	0	0
Telomeric repeat-binding factor 2-interacting protein 1	TERF2IP	•	•	•		0	0	0	137	0	0	0	0	0	0	0	0	0
Homeobox-containing protein 1	HMBOX1		•			0	0	0	0	30	11	0	0	0	0	0	0	0
Importin subunit beta-1	KPNB1		•			0	0	1	5	9	1	21	0	0	2	0	0	0
GTP-binding nuclear protein Ran	RAN		•			46	41	48	41	13	55	276	34	42	38	35	36	40
E3 SUMO-protein ligase RanBP2	RANBP2					0	0	0	0	0	0	6	0	0	0	0	0	0
Ran GTPase-activating protein 1	RANGAP1					9	5	15	19	13	25	294	12	13	11	3	5	2
Regulator of chromosome condensation	RCC1		•	•		0	0	0	0	4	0	674	0	0	0	0	0	0
DNA-directed RNA polymerase I subunit RPA34	CD3EAP		•	•		0	0	0	0	0	0	0	18	0	0	0	0	0
DNA excision repair protein ERCC-3	ERCC3					0	0	0	0	0	0	0	5	0	0	0	0	0
General transcription factor IIH subunit 4	GTF2H4					11	5	3	13	0	10	0	37	5	10	4	9	10
Nucleolar and coiled-body phosphoprotein 1	NOLC1			•		0	3	2	0	0	0	0	308	7	0	0	1	0
U3 small nucleolar RNA-associated protein 4 homolog	UTP4			•		0	0	0	0	0	0	0	29	0	0	0	0	0
H/ACA ribonucleoprotein complex subunit DKC1	DKC1			•	•	0	0	0	0	0	0	0	11	203	0	0	0	0
H/ACA ribonucleoprotein complex subunit 1	GAR1		•	•	•	0	0	0	0	0	0	0	0	29	0	0	0	0
H/ACA ribonucleoprotein complex subunit 2	NHP2		•	•	•	0	0	0	0	0	0	0	20	112	0	0	0	0
H/ACA ribonucleoprotein complex subunit 3	NOP10				•	0	0	0	0	0	0	0	0	100	0	0	0	0
60S ribosome subunit biogenesis protein NIP7 homolog	NIP7			•		0	0	0	0	0	0	0	0	0	54	0	0	0
Probable 28S rRNA (cytosine(4447)-C(5))-methyltransferase	NOP2		•	•		0	0	0	0	0	0	0	0	0	197	0	0	0
Nuclear valosin-containing protein-like	NVL		•	•	•	0	0	0	0	0	0†	0	0	0	0	0†	0	0


3
THO complex
Shelterin complex
1
TIN2
ACD TPP1
TERRA
Ku
TERF2
TERF1
POT1
2
HMBOX1
TERF2 IP
3'
5'
RCC1
1
+/- ?
RPA
Cajal body
coilin
Nucleolus
TERF2
PM-Scl
hTR
2
NVL
TERT
RuvBL 1/2
NOLC1
1
1
NOP2
DKC
NOP10
NHP2
GAR1
1
FBL
Ku

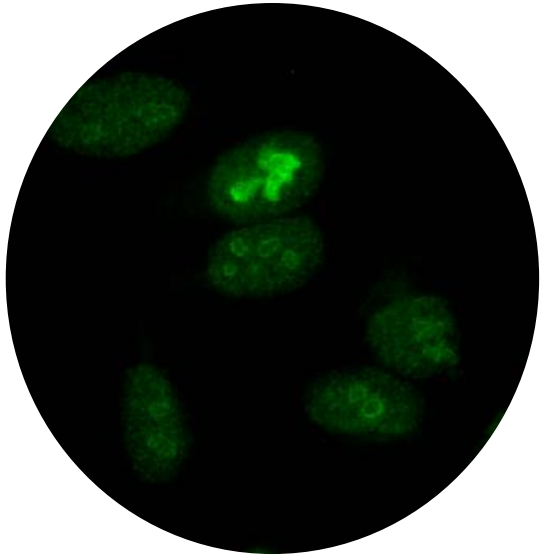
Anti-NVL autoantibodies in systemic sclerosis

	Vulsteke et al. *Jan 2023*	Preruna-Prieto et al. *Sep 2023*	Matsuda et al. *July 2023 (preprint)*	
Number of patients	**2**	**6**	**5**	➔ **13 cases**
Nucleolar HEp-2 IIFA pattern	2/2	6/6	5/5	➔ Nucleolar pattern
Limited cutaneous	2/2	6/6	5/5	➔ Limited cutaneous
Interstitial lung disease	0/2	2/6	0/5	➔ Few ILD
Calcinosis	1/2	6/6	0/5	➔ Unclear
Cancer or premalignancy	2/2	4/6	NA	➔ **Potential association**

Nuclear valosin-containing protein-like

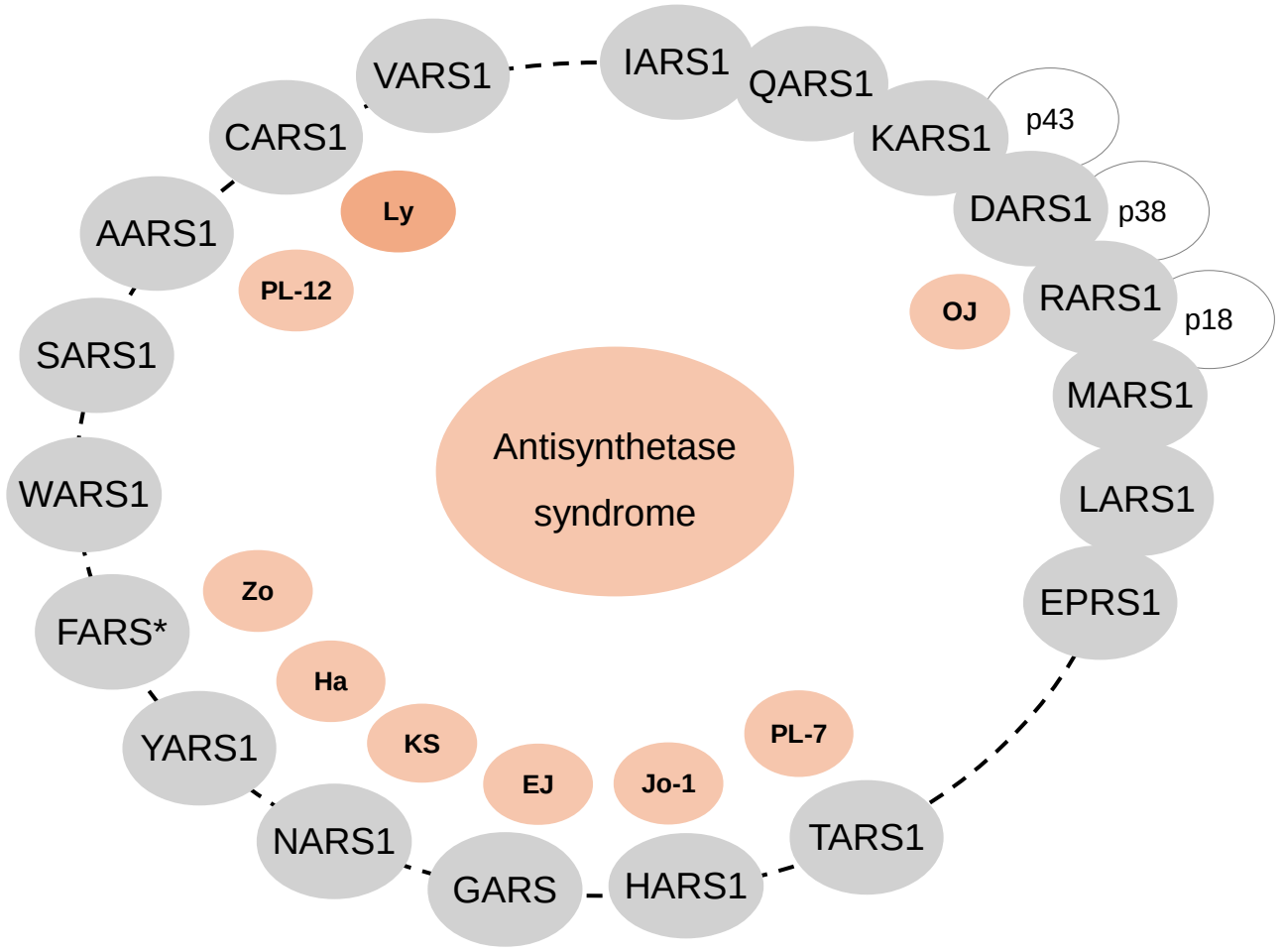
Telomerase assembly and function

Ribosome biogenesis



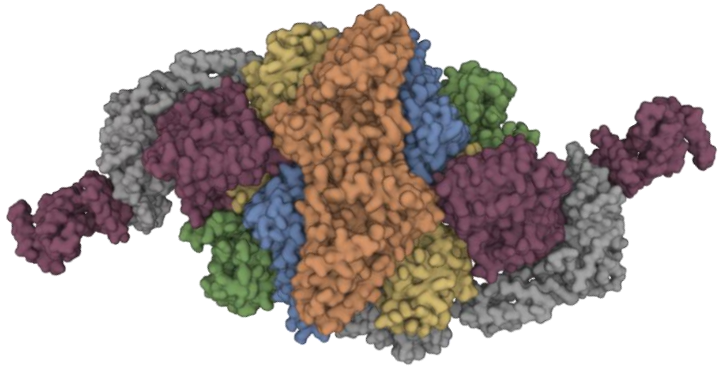
@JBVulsteke

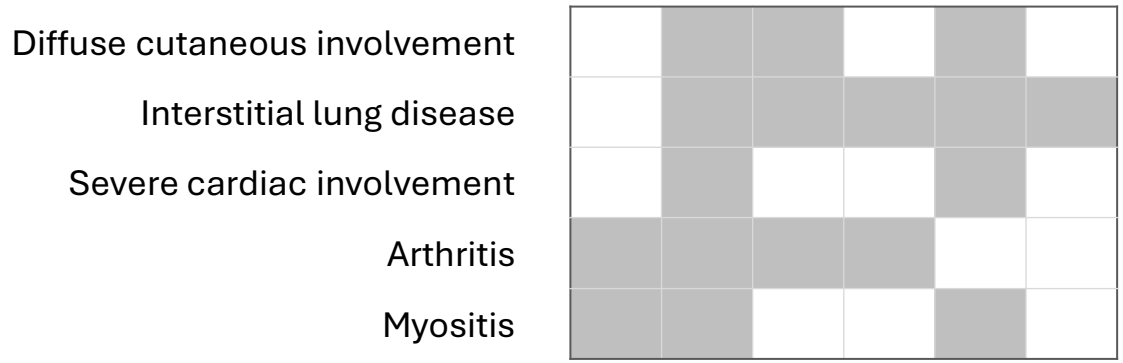
			Total spectral count per individual serum																		
			Possible ASyS, ASA-					Jo-1 (DIA)			PL-7 (DIA)		PL-12 (DIA)			EJ (DIA)			Healthy controls			
Identified protein	Abbr.	MW	U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	U12	U13	U14	U15	U16	U17	U18	U19	U20	U21
Cluster of Cysteinyl-tRNA synthetase	CARS1	85 kDa	51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Valine--tRNA ligase	VARS1	140 kDa	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bifunctional glutamate/proline--tRNA ligase	EPRS1	171 kDa	2	26	47	1	2	2	5	3	9	1	2	1	4	2	3	3	1	2	2	3
Glutamine--tRNA ligase	QARS1	88 kDa	0	18	28	0	2	1	1	1	1	1	0	1	2	0	0	1	1	0	1	0
Aspartate--tRNA ligase	DARS1	57 kDa	5	16	21	6	4	4	4	5	9	8	5	5	8	5	7	6	2	4	2	4
Lysine--tRNA ligase	KARS1	68 kDa	0	14	6	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Arginine--tRNA ligase	RARS1	75 kDa	3	13	43	3	2	2	3	1	2	4	3	2	5	2	1	2	1	2	0	0
Isoleucine--tRNA ligase	IARS1	145 kDa	1	8	19	1	1	1	1	1	3	0	1	0	5	1	0	0	1	2	0	0
Methionine--tRNA ligase	MARS1	101 kDa	0	2	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leucine--tRNA ligase	LARS1	134 kDa	0	0	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aminoacyl tRNA synthase complex-IMP1	AIMP1	34 kDa	2	10	11	1	2	1	0	2	4	0	1	1	1	0	1	1	0	0	2	0
Eukaryotic translation elongation factor 1 epsilon-1	EEF1E1	20 kDa	3	5	7	2	2	2	2	2	3	3	2	2	3	2	2	3	2	2	2	2
Aminoacyl tRNA synthase complex-IMP2	AIMP2	35 kDa	1	3	5	0	2	1	1	1	2	2	2	2	2	1	1	1	0	0	1	0
Phenylalanine--tRNA ligase alpha subunit	FARSA	58 kDa	2	2	3	28	2	2	2	2	2	2	1	1	1	2	2	2	2	2	2	2
Phenylalanine--tRNA ligase beta subunit	FARSB	66 kDa	0	0	0	26	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Asparagine--tRNA ligase	NARS1	63 kDa	0	0	0	0	22	0	0	4	1	0	0	0	1	0	0	0	0	0	0	0
Histidine--tRNA ligase	HARS1	57 kDa	0	2	0	0	0	32	21	21	0	0	0	0	0	0	0	0	0	0	0	0
Cluster of Threonine--tRNA ligase 1	TARS1	83 kDa	1	1	2	1	3	0	2	2	100	58	3	1	2	1	1	1	3	2	3	2
Alanine--tRNA ligase	AARS1	107 kDa	0	0	0	0	0	0	0	0	0	0	48	57	60	1	0	0	0	0	0	0
Glycine--tRNA ligase	GARS1	83 kDa	0	0	1	1	1	1	1	0	1	1	0	0	0	15	1	23	0	0	0	0
Acetyl-CoA carboxylase 1	ACACA	266 kDa	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cluster of ATPase family protein 2 homolog	SPATA5	98 kDa	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spermatogenesis-associated protein 5-like protein 1	SPATA5L1	81 kDa	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Uncharacterized protein C1orf109	C1ORF109	23 kDa	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RuvB-like 1	RUVBL1	50 kDa	1	5	3	1	3	5	1	2	3	3	2	1	4	4	72	3	0	2	1	4
RuvB-like 2	RUVBL2	51 kDa	1	2	2	3	2	3	3	2	3	1	2	3	3	2	71	3	2	3	1	3

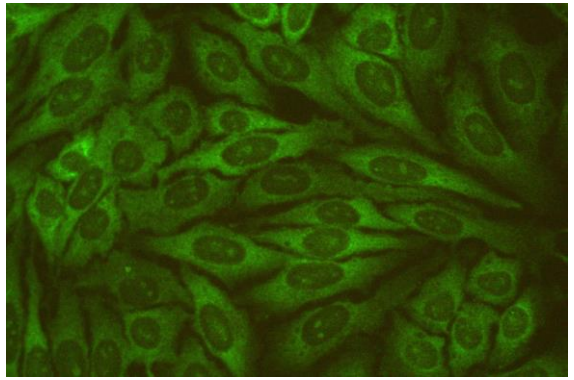

Antisynthetase syndrome
VARS1
IARS1
QARS1
KARS1
p43
DARS1
p38
RARS1
p18
MARS1
LARS1
EPRS1
TARS1
HARS1
GARS
NARS1
YARS1
FARS*
WARS1
SARS1
AARS1
CARS1
Ly
PL-12
OJ
Zo
Ha
KS
EJ
Jo-1
PL-7

eIF2B

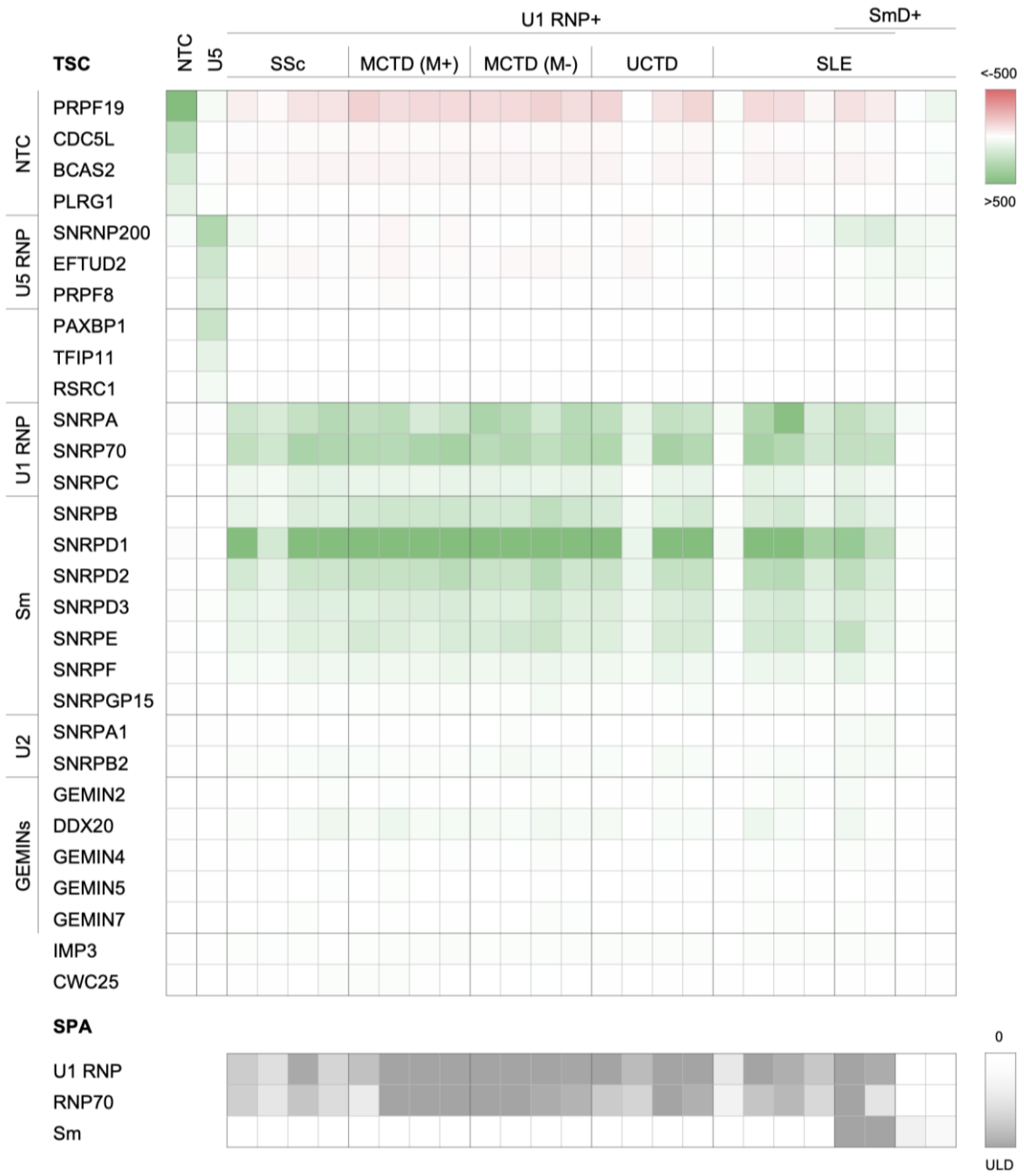
		MW	#1	#2	#3	#4	#5	#6	H1	H2
eIF2B alpha	EIF2B1	34 kDa	5	4	7	225	42	103	0	0
eIF2B beta	EIF2B2	39 kDa	3	28	19	17	21	3	0	0
eIF2B gamma	EIF2B3	50 kDa	9	25	33	11	13	4	1	1
eIF2B delta	EIF2B4	60 kDa	18	41	38	16	9	11	0	0
eIF2B epsilon	EIF2B5	43 kDa	13	23	27	23	5	8	0	1

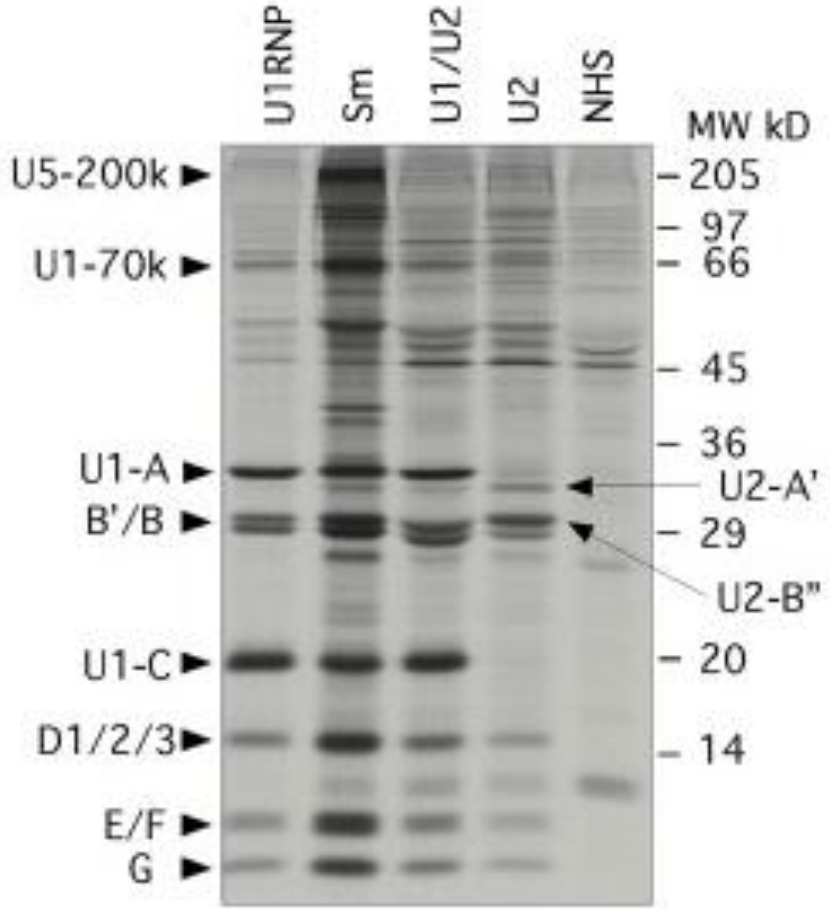






Kenner et al., Science 2019
Vulsteke et al., Rheumatology 2023

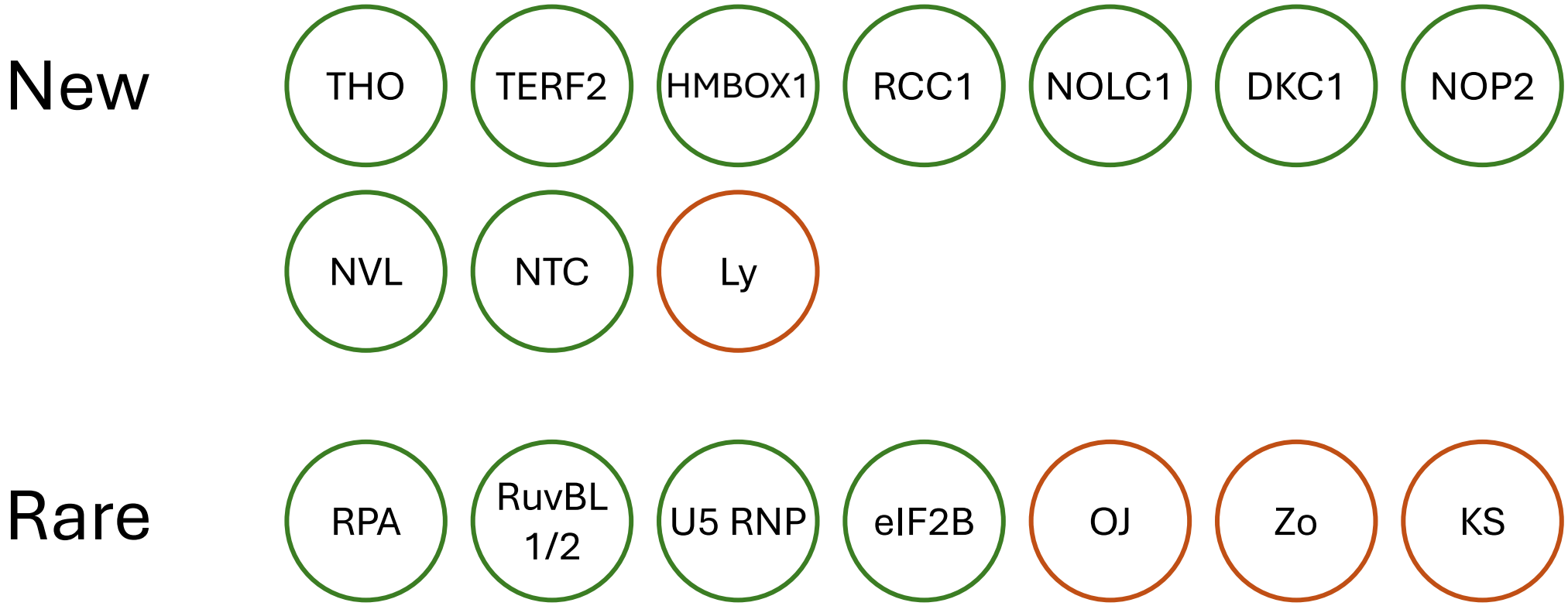




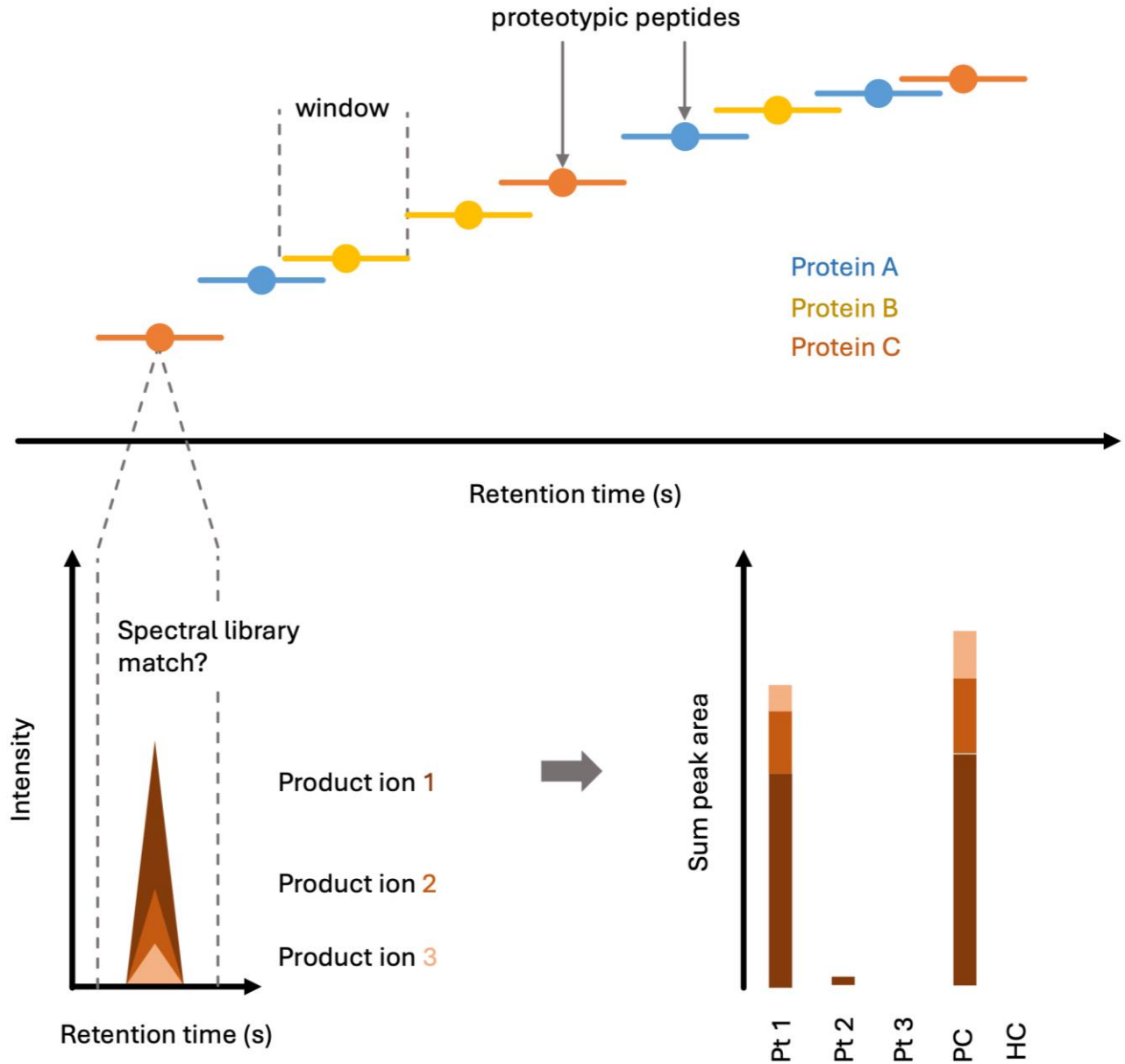
Vulsteke et al., J Autoimmun 2023

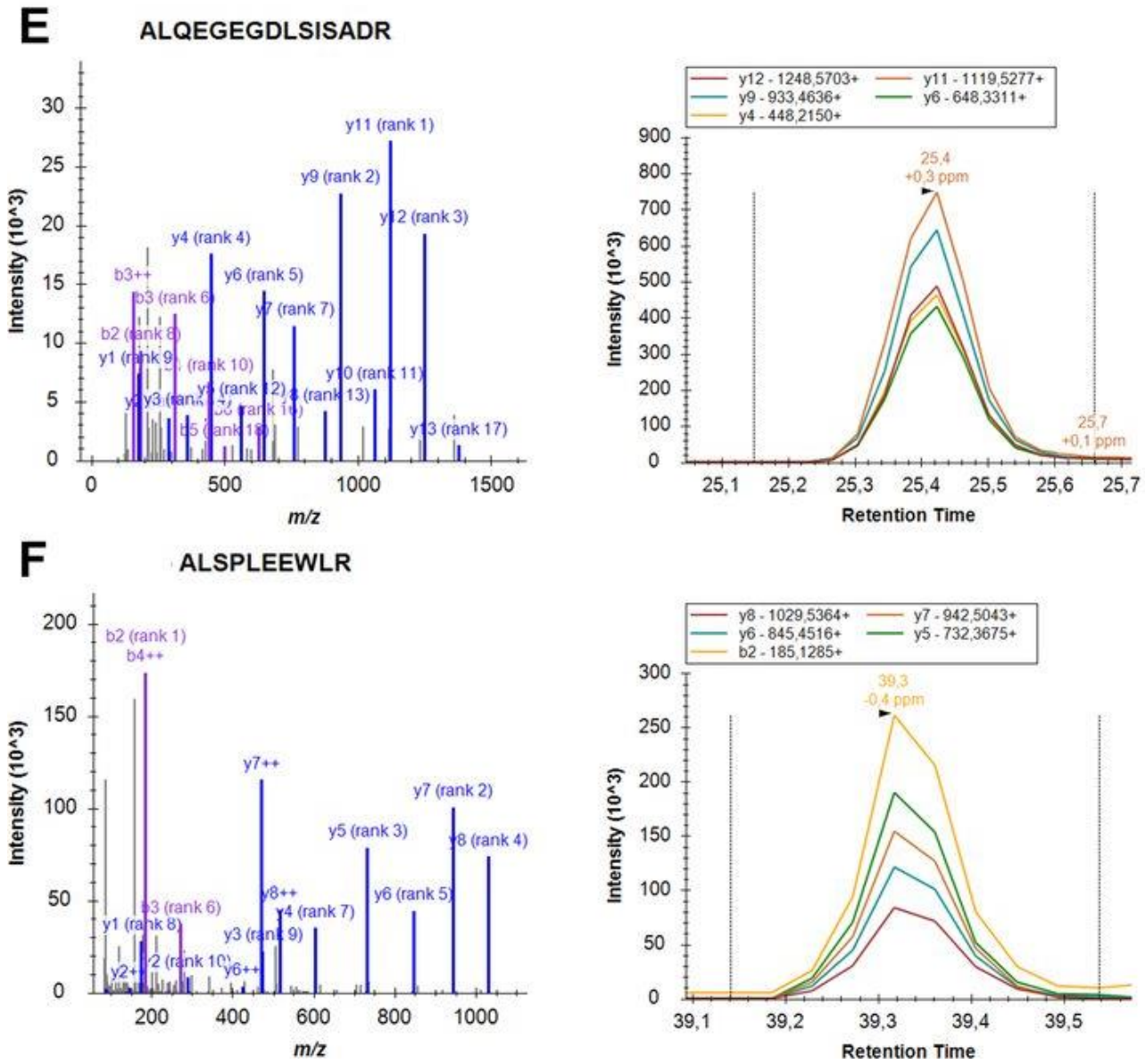
Satoh et al., Autoantibodies 2014

The story of IP-MS thus far

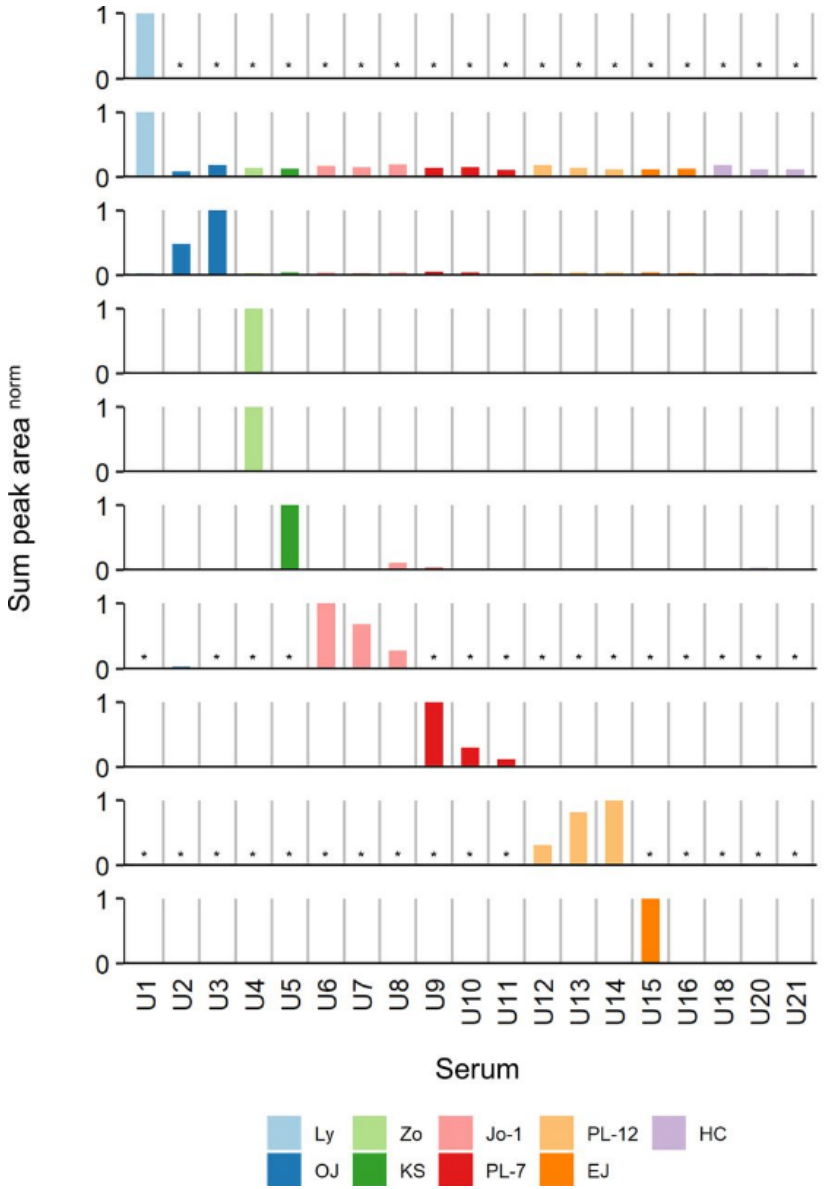


Targeted IP-MS: one step further


proteotypic peptides
window
Protein A
Protein B
Protein C
Retention time (s)
Spectral library match?
Intensity
Product ion 1
Product ion 2
Product ion 3
Retention time (s)
Sum peak area
Pt 1
Pt 2
Pt 3
PC
HC

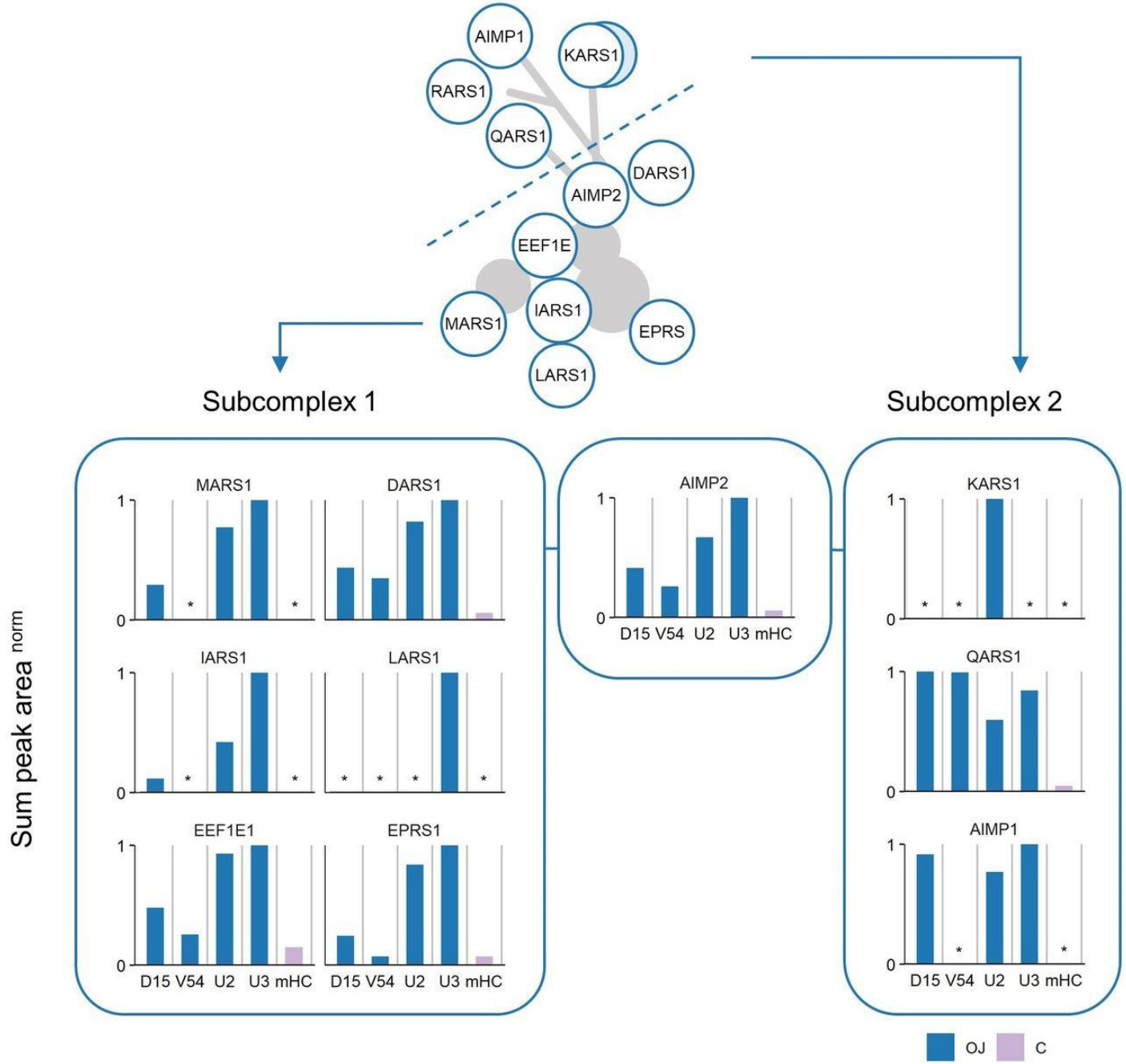


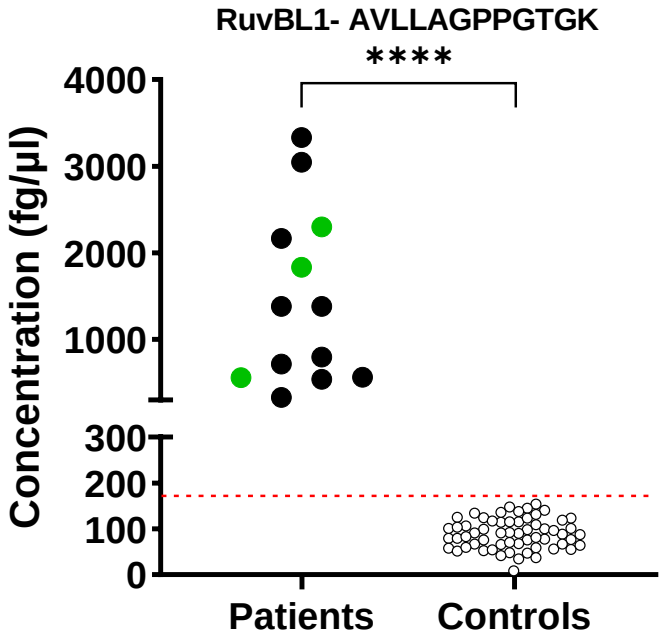
Vulsteke JB, et al. Ann Rheum Dis. 2022:annrheumdis-2022-222686.

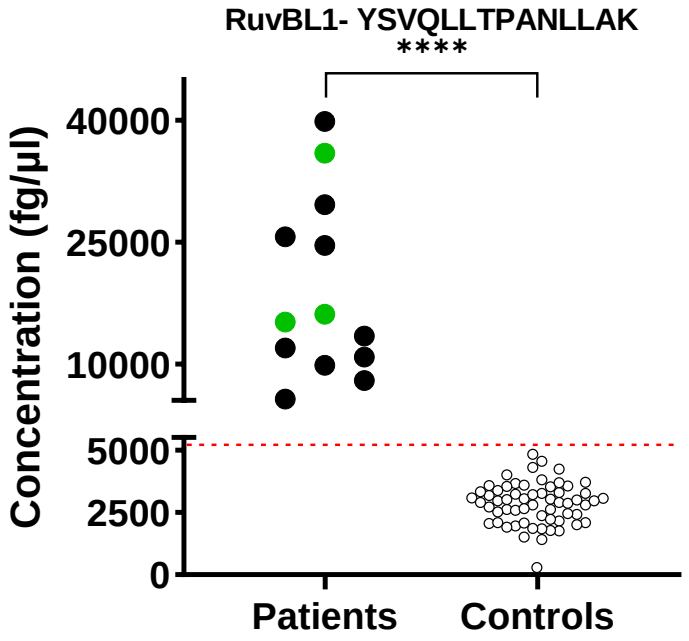


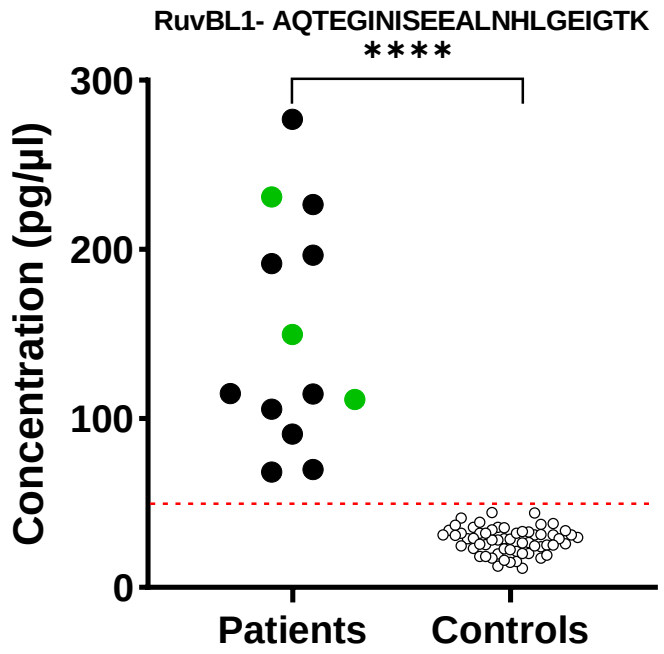
CARS1 ALQEGEGDLSISADR	Cysteine-tRNA synthetase	anti-Ly
VARS1 ALSPLEEWLR		
IARS1 LFLNETQTQEITEDIPVK	Isoleucine-tRNA synthetase	anti-OJ
FARSA SLQALGEVIEAELR		
FARSB ASEGPAFFPGR	Phenylalanine-tRNA synthetase	anti-Zo
NARS1 EGIDPTPYYWYTDQR	Asparagine-tRNA synthetase	anti-Ks
HARS1 LLFEYLTLFGIDDK	Histidine-tRNA synthetase	anti-Jo1
TARS1 FLGDIEVWDQAEK	Threonine-tRNA synthetase	anti-PL-7
AARS1 NVGcLQEALQLATSFAQLR	Alanine-tRNA synthetase	anti-PL-12
GARS1 TLYVEEVVPNVIEPSFGLGR	Glycine-tRNA synthetase	anti-EJ

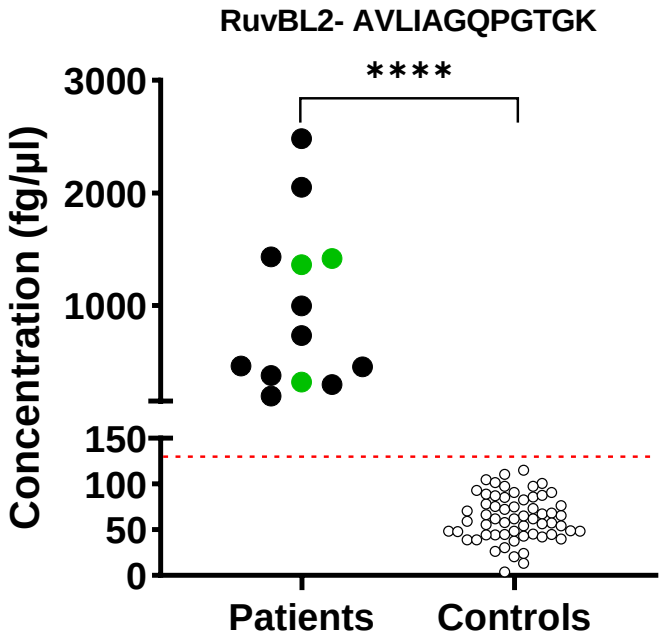
Vulsteke JB, et al. Ann Rheum Dis. 2022:annrheumdis-2022-222686.

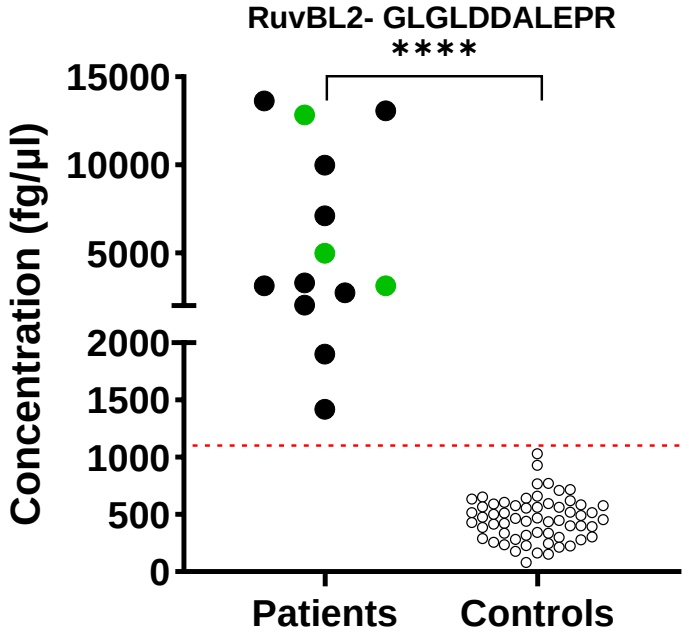

AIMP1
KARS1
RARS1
QARS1
DARS1
AIMP2
EEF1E
MARS1
IARS1
EPRS
LARS1
Subcomplex 1
Subcomplex 2
Sum peak area norm
MARS1
DARS1
IARS1
LARS1
EEF1E1
EPRS1
AIMP2
KARS1
QARS1
AIMP1
D15 V54 U2 U3 mHC
OJ
C

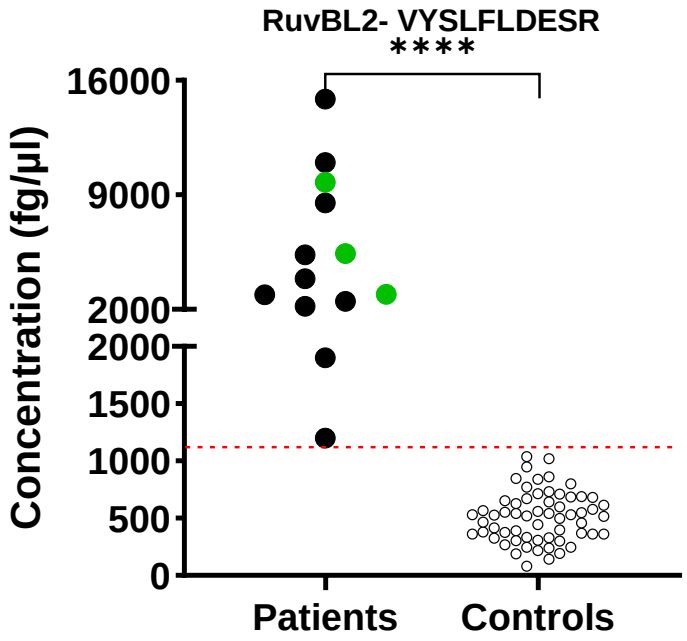












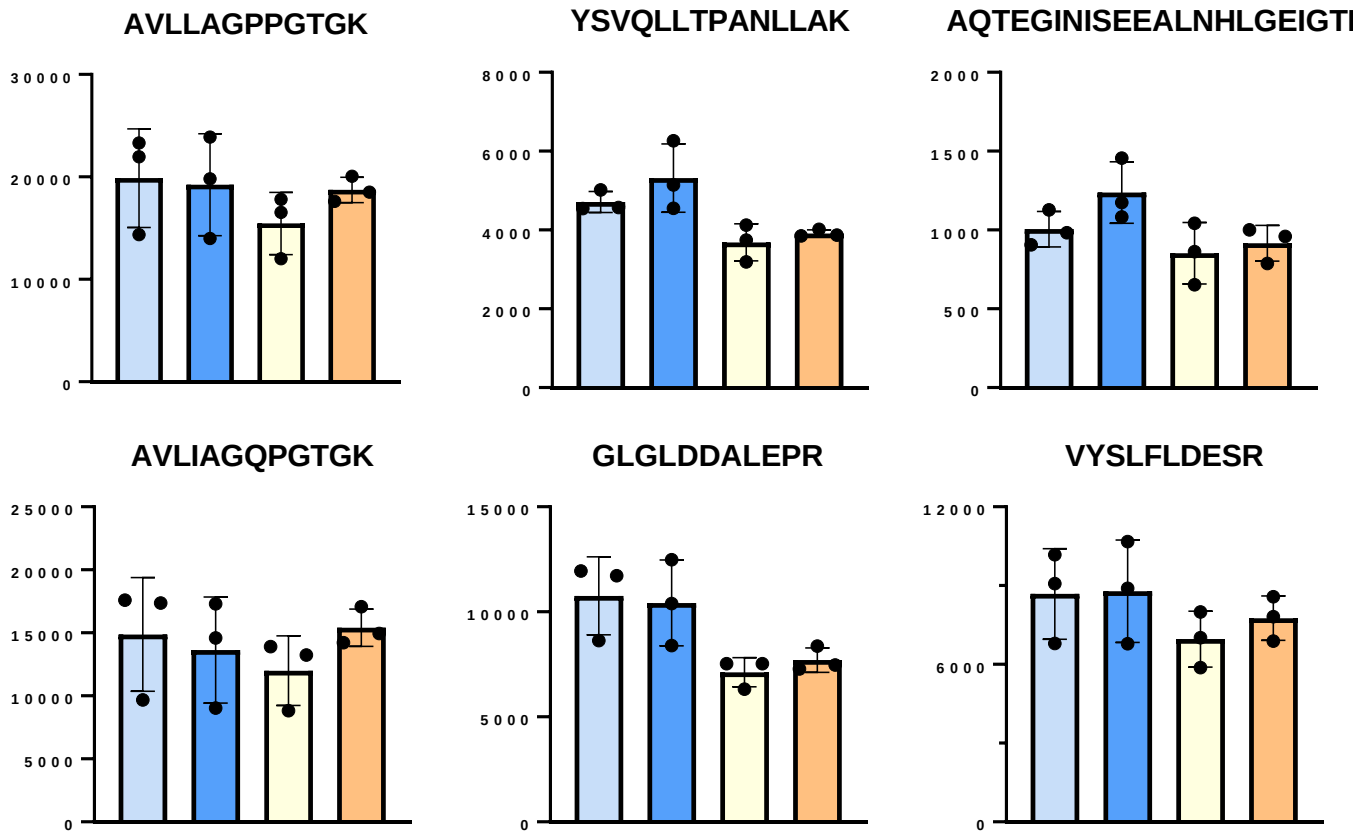
(n = 13 anti-RuvBL1/2 patients, n = 60 Disease Controls)

Nuclear HeLa extract 1

Nuclear HeLa extract 2

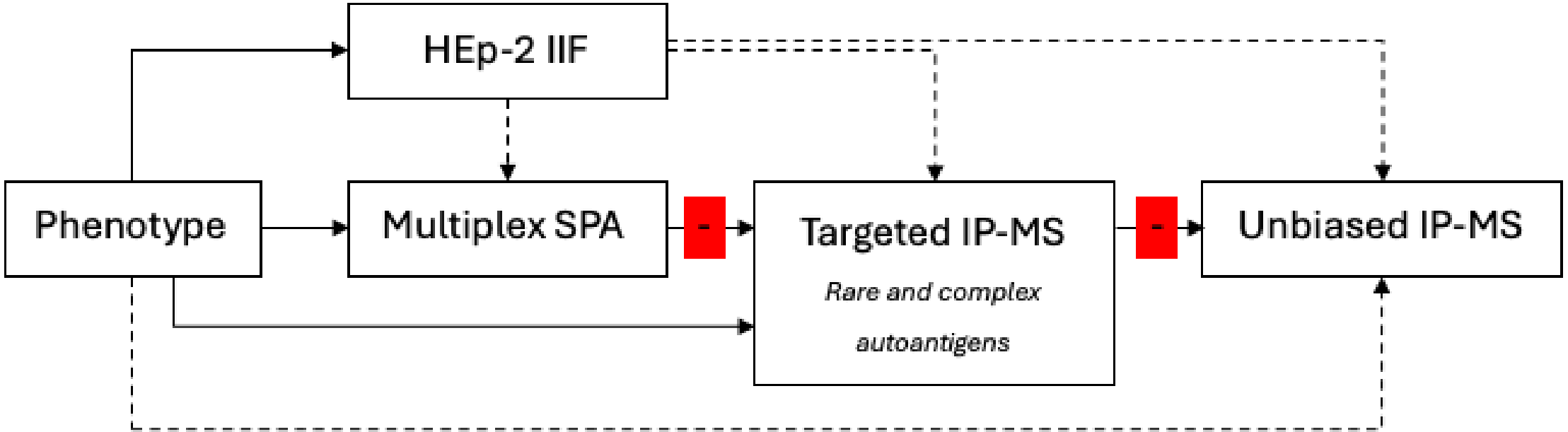
Cytoplasmatic HeLa extract 1

Cytoplasmatic HeLa extract 2



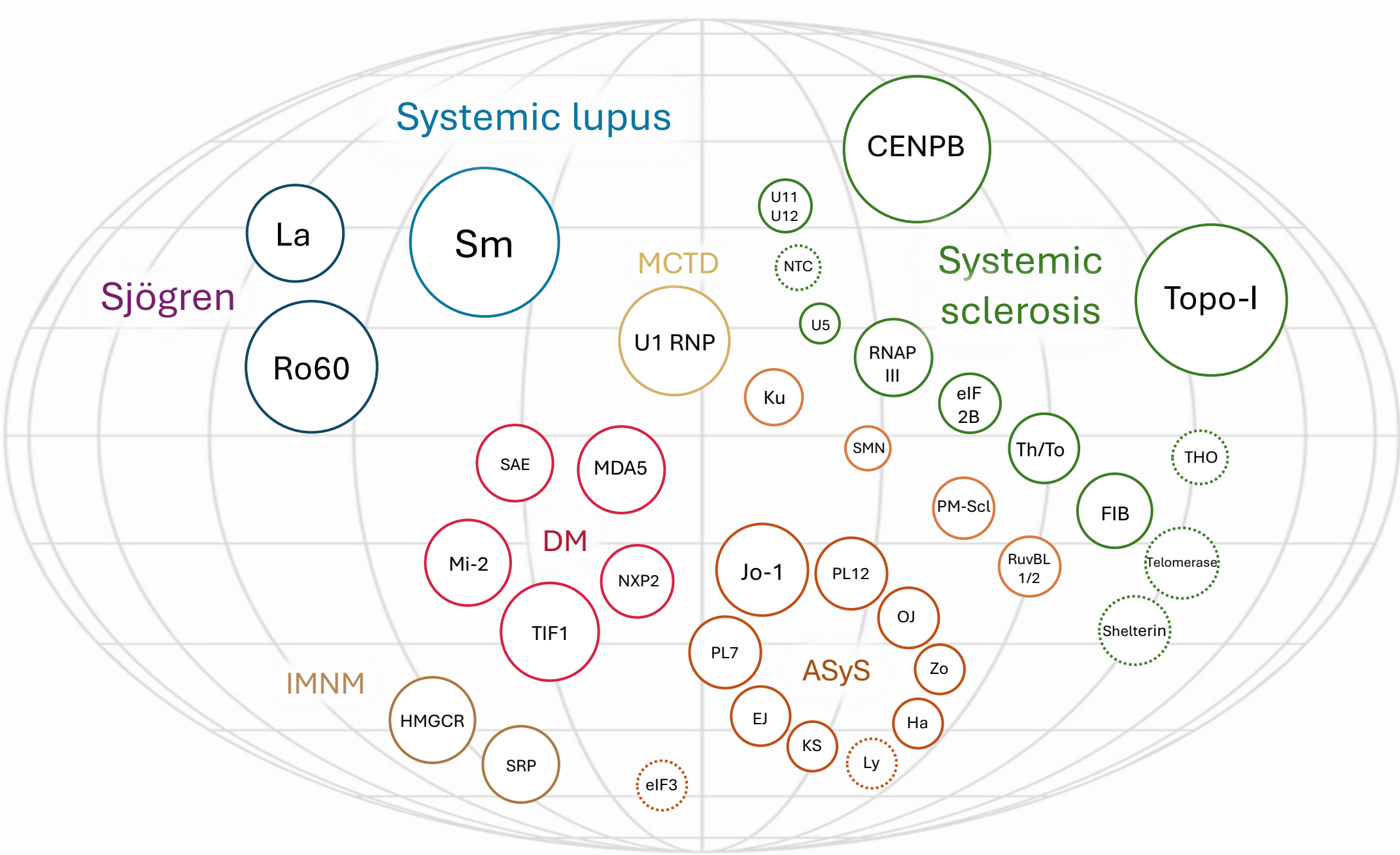
Imprecision assessment of Immunoprecipitation protocol

Is IP-MS ready for routine diagnostics?


HEp-2 IIF
Phenotype
Multiplex SPA
-
Targeted IP-MS
Rare and complex
autoantigens
-
Unbiased IP-MS

BEL AIR

The **Belgian** Autoantigen Identification Registry


Systemic lupus
Sjögren
La
Ro60
Sm
MCTD
U1 RNP
U11
U12
NTC
U5
CENPB
Systemic
sclerosis
Topo-I
RNAP
III
Ku
eIF
2B
SMN
Th/To
THO
FIB
PM-Scl
RuvBL
1/2
Telomerase
Shelterin
SAE
MDA5
DM
Mi-2
NXP2
TIF1
Jo-1
PL12
OJ
Zo
Ha
PL7
ASyS
EJ
KS
Ly
IMNM
HMGCR
SRP
eIF3

Fonds Joël Hurlet

Clinical and Diagnostic Immunology

Xavier Bossuyt

Greet Wuyts

Doreen Dillaerts

Maaike Cockx

Tom Dehaemer

Birthe Michiels

KU Leuven SyBioMa

Rita Derua

Kusay Arat

Sebastien Carpentier

Skeletal Biology Engineering and Research Center

Ellen De Langhe

Rik Lories

Patrick Verschueren

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Petra De Haes

Kristl Claeys

Jan Lenaerts

Steven Vanderschueren

Wim Wuyts

Ghent University Hospital

Vanessa Smith

Carolien Bonroy

Yves Piette

Leen Moens

Lina Ancheva

Minoru Satoh

Nicole Fabien

Frédéric Coutant

Sylvain Ducubquoi

David Goncalves

Benoit Nespola

Questions?

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