Validation of 3 Novel Biomarker Candidates for Systemic Sclerosis

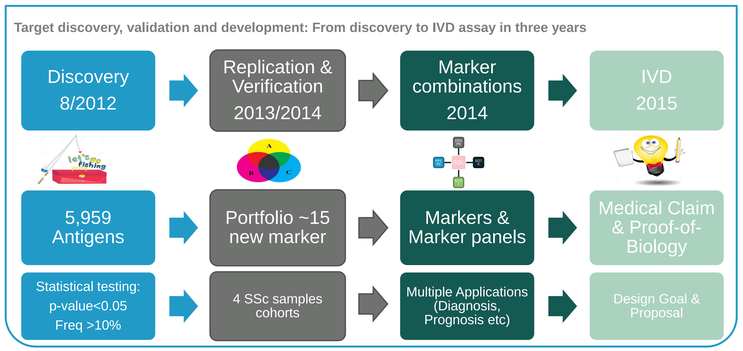
J. Schulte-Pelkum[1], P. Budde*[1], H.-D. Zucht[1], D. Wirtz[1], P. Schulz-Knappe[1], O. Distler[2], S. Jordan[2], N. Hunzelmann[3], M.Schneider[4], B. Maurer[2]

[1]Protagen AG, Dortmund, Germany, [2]Division of Rheumatology, University Hospital Zürich, Zürich, Switzerland, [3]Department of Dermatology, University of Cologne, Cologne, [4]Rheumatology, Heinrich-Heine-University, Düsseldorf, Germany

EULAR2016 SAT0549

Introduction

Systemic sclerosis (SSc) is a systemic autoimmune disease that manifests as progressive fibrosis of the skin and internal organs. SSc is associated with the presence of several autoantibodies (aab) to intracellular targets, with the three most important SSc-specific being anti-centromere, anti-Scl70 and anti-RNA polymerase III antibodies, which occur in over 50% of SSc patients. Autoantibody specificities are strongly associated with pattern of organ involvement and disease outcome, making autoantibodies an essential tool in the clinical management of SSc. This highlights the need for additional specific and sensitive diagnostic and prognostic biomarkers in SSc. We have recently conducted high-content autoantibody profiling studies of SSc, systemic autoimmune diseases (AID), and healthy controls and found novel SSc associated autoantibodies.



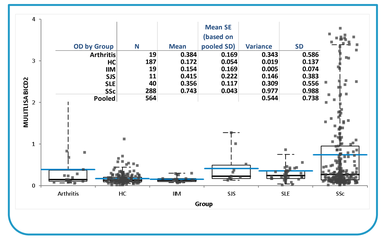
The Protagen Biomarker Discovery workflow for the development of novel biomarker targets. SeroTag® autoantibody discovery process is based on a bead-based array screening of recombinant human autoantigens. Assay development is based on ELISA technology

Methods

Three novel identified biomarker candidates, namely Lysine (K)-specific demethylase 6B (KDM6B), Protein Phosphatase Inhibitor 2 (PPP1R2) and Bicaudal Drosophila Homolog 2 (BICD2) were developed into ELISA Kits using highly purified recombinant antigens and evaluated. Autoantibody specificities were analyzed using an independent and well characterized cohort containing sera of patients suffering from SSc (n=288), IIM (n=19), SjS (n=11), RA (n=19), SLE (n=40) and healthy blood donors (n=187). Assay threshold levels were calculated using a receiver operating characteristic analysis and set for specificities of around 95%.

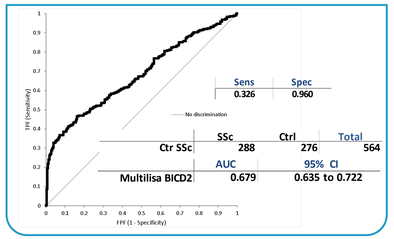
Results

Using the respective cut-off for the ELISA tests we were able to find autoantibody reactivity against BICD2 in 32.6% of SSc patients and 4% of control samples. Anti-BICD2 and anti-PPP1R2 reactivities co-migrated with anti-centromere aab, but were also found in anti-centromere and anti-Scl-70 negative samples. Interestingly, both anti-BICD2 and anti-PPP1R2 aab were found in SSc samples tested negative for anti-RNA Polymerase III auto-reactivity (data not shown).



OD by Group	N	Mean	Mean SE (based on pooled SD)	Variance	SD
Arthritis	19	0.384	0.169	0.343	0.586
HC	187	0.172	0.054	0.019	0.137
IIM	19	0.154	0.169	0.005	0.074
SjS	11	0.415	0.222	0.146	0.383
SLE	40	0.356	0.117	0.309	0.556
SSc	288	0.743	0.043	0.977	0.988
Pooled	564			0.544	0.738

Scatter plot of anti-BICD2 autoantibody reactivity in SSc, IIM, SjS, Arthritis, SLE and healthy control serum samples. Specifically elevated reactivity against BICD2 was observed in the group of the SSc patients



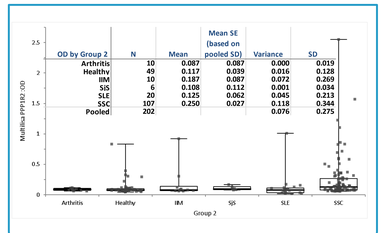
	Sens	Spec
	0.326	0.960

	SSc	Ctrl	Total
Ctr SSc	288	276	564

	AUC	95% CI
Multilisa BICD2	0.679	0.635 to 0.722

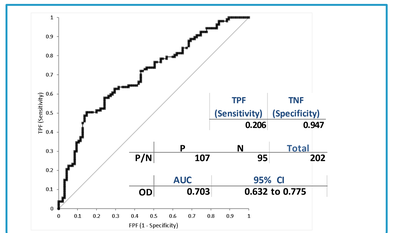
ROC analysis of Multilisa BICD2 tested in SSc (n=288) and compared against healthy donor sera (n=187) and disease control sera (n=89) revealed an AUC of 0.679 corresponding to a sensitivity of 32.6% and a specificity of 96%

Optimized solid and liquid phase of the Multilisa PPP1R2 showed high diagnostic performance. In a reduced study cohort (n=196) Multilisa PPP1R2 reached a sensitivity and specificity of 20.6% and 94.4%, respectively.



OD by Group 2	N	Mean	Mean SE (based on pooled SD)	Variance	SD
Arthritis	10	0.087	0.087	0.000	0.019
Healthy	49	0.117	0.039	0.016	0.128
IIM	10	0.187	0.087	0.072	0.269
SjS	6	0.108	0.112	0.001	0.034
SLE	20	0.125	0.062	0.045	0.213
SSc	107	0.250	0.027	0.118	0.344
Pooled	202			0.076	0.275

Scatter plot of anti-PPP1R2 autoantibody reactivity in the context of different Systemic autoimmune diseases and healthy control serum samples. High reactivity against PPP1R2 was observed in the group of the SSc patients



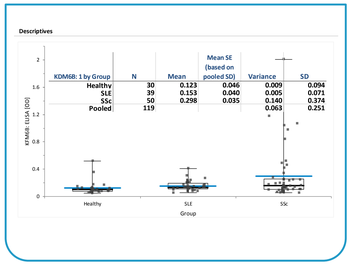
	TPF (Sensitivity)	TNF (Specificity)
	0.206	0.947

P/N	P	N	Total
	107	95	202

	AUC	95% CI
OD	0.703	0.632 to 0.775

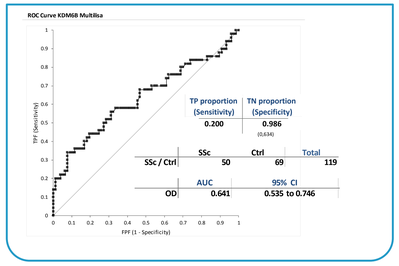
PPP1R2 prototype assay yields 20.6% sensitivity at 94.7% specificity for SSc patients vs. Healthy controls & AID sera.

In a reduced study cohort size tested with SSc sera against healthy controls and sera from SLE patients the Multilisa KDM6B reached a sensitivity of 20% and a specificity of 98.6%, respectively.



KDM6B: 1 by Group	N	Mean	Mean SE (based on pooled SD)	Variance	SD
Healthy	30	0.123	0.046	0.009	0.094
SLE	39	0.153	0.040	0.005	0.071
SSc	50	0.298	0.035	0.140	0.374
Pooled	119			0.063	0.251

Anti-KDM6B reactivity is specifically elevated in SSc patient samples



TP proportion (Sensitivity)	TN proportion (Specificity)
0.200	0.986
(0.034)	

SSc / Ctrl	SSc	Ctrl	Total
	50	69	119

OD	AUC	95% CI
	0.641	0.535 to 0.746

ROC Analysis of Multilisa KDM6B data for 50 SSc patients versus healthy (n=30) and SLE (n=39) controls revealed an AUC value of 0.634

Anti-BICD2 aab showed significant inverse correlation with pulmonary fibrosis (high resolution CT scan) and may be associated with a decreased incidence of severe lung disease.

BICD2: Index [AU] by MRSS stage	N	Mean	Mean SE (based on pooled SD)	Variance
0	13	1.61	0.52	2.97
1	72	1.63	0.22	4.34
2	15	0.28	0.49	0.09
3	1	0.30	1.89	.
Pooled	101			3.56

Scatter plot of anti-BICD2 reactivity compared to modified Rodnan skin score stages in SSc patients (data available for 101 patients). Autoantibodies against BICD2 are elevated in groups of patients with MRSS of 0 and 1

Kruskal-Wallis test

Evo.3.BICD2.OD by Pulmonary fibrosis, HRCT, lung	n	Rank sum	Mean rank
negative	65	14193.2	218.36
positive	71	12993.8	183.01

H statistic	17.51
X^2 approximation	17.51
DF	1
p-value	<0,0001

Scatter plot of reactivity of the novel antigen BICD2 compared to High resolution CT results for lung fibrosis in SSc patients. Anti-BICD2 autoantibodies significantly correlate with lowered risk of pulmonary fibrosis

When analyzed for Skin thickening measured by modified Rodnan Skin score (MRSS), anti-BICD2 reactivity was found elevated in patients with MRSS stages of 0 and 1 respectively, anti-BICD2 positive SSc patients seem to show a more moderate skin involvement.

Conclusion

In this study we were able to validate the diagnostic value and high specificity of the three newly discovered autoantigens using ELISA technology. Anti-BICD2 aab showed correlations with a more moderate disease course of SSc. Search for clinical associations of the other newly discovered SSc-associated autoreactivities is ongoing.