Development of a Connective Tissue Disease Array for Differential Diagnosis of ANA-Positive Patients

A. Lueking[1], P. Budde[1], S. Vordenbäumen[2], H.-D. Zucht[1], M. von Darl[1], H. Göhler[1], P. Rengers[1], M. Gamer[1], K. Marquart[1], A. Telaar[1], D. Chamrad[1], C. Theek[1], M. Schneider[2], P. Schulz-Knappe [1]

[1]Protagen AG, Dortmund, Germany, [2] Heinrich-Heine-Universität, Poliklinik für Rheumatologie, Düsseldorf, Germany

Introduction

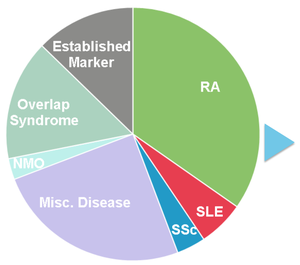
A current challenge is to identify individuals at risk to develop a Connective Tissue Disease (CTD), to predict future organ involvement and response to treatment. When a CTD is suspected, a limited number of disease associated autoantibodies (AAB) is tested. Since CTDs are associated with a large number of autoantibodies, multiplexing technologies now offer the opportunity to comprehensively measure clinically relevant AAB and to simultaneously assess the utility of novel biomarkers. Here, we describe the development of a single-step bead-based CTD array enabling the multiplex analysis of up to 400 AABs (Fig.1)

SeroTag® CTD Array

Comprehensive, but flexible design
50-400 novel & established antigens
Low serum volume (5 µl)

Deep characterization of autoantibody reactivity in patient subgroups

RA: P0111 Fri. – March 28
SLE: P0039 Sat. - March 29



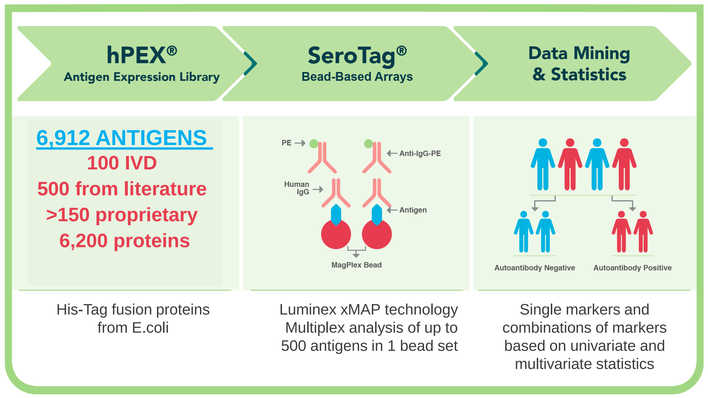
Antigens	Measurement	TEST	SLE	SSC (lim.)	SSC (diff.)	RA
Histone			30-80			10-50
Sm			10-30			
PCNA			<1			
SS-A (Ro50, 60)		x	30-60			
SS-B			10-20			
U1-snRNP (U1-70k)		x	30-40	15		< 5
Topoisomerase I				10	30	
Centromere				50	2	
PM-Scl		x		5	5	
Fibrillarin				5	5	
RNA polymerases				25	2	
Th / To				5	5	
tRNA synthetases						
SRP						
Mi-2						
ß2-Glykoprotein			40			
ANCA						25
Ribosomal proteins			10-40			
MAP-2		x	77			
M2				25		

Figure 1: SeroTag® CTD array composition and its application. Right side measurement: In blue, AAB reactivity profile of one SLE reference serum; in red: median signal intensity measured in healthy controls.

Material and Methods

In-depth analysis of the CTD AAB repertoire yielded novel autoantigen candidates, which were combined with diagnostic antigens to build a novel 400-plex CTD array. The array was tested on 110 SLE, 100 SSc and 100 RA patients and control sera.

Figure 2: The SeroTag® process.
The Luminex xMAP® technology is applied to measure the reactivity of autoantibodies against up to 7,000 antigens in serum samples.



Results

AABs hold the potential for the early and sensitive detection of CTDs such as RA, SLE, and SSc. We combined novel and well-established antigens to a 400-plex CTD array, which enables simultaneous testing of a wide spectrum of different CTDs. Replicate measurements of 4 control sera (n=90) yielded good test performance towards recombinant antigens. To visualize the AAB repertoire of RA, SLE and SSc patients, significantly increased AAB ($p \leq 0.05$) were grouped using a hierarchical cluster algorithm. Fig. 3 shows that RA, SLE and SSc patient sera contain distinct as well as overlapping patterns of AAB reactivity. The multiplexing approach can now be utilized to construct biomarker panels for specific patient subgroups.

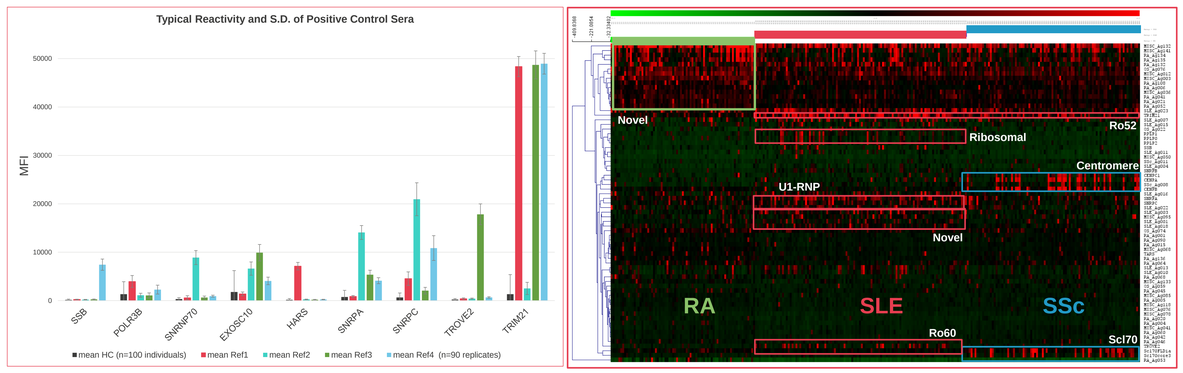


Figure 3: A) Control Sera and B) heat map of autoantibody profiles in individual RA, SLE and SSc patients.

Conclusions

- The SeroTag® CTD array reveals the breadth of AAB responses in CTD patients
- Novel antigens can be evaluated in a single step measurement
- The wide spectrum of AABs is used to define subsets of patients
- The multiplex approach allows miniaturization and high sample throughput
- The flexible array design allows to integrate emerging biomarkers and to develop customized disease-specific arrays
- Protagen is open to working with partners interested in leveraging the CTD array for developing diagnostic (Dx) and companion diagnostic (CDx) tests