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ProteoSep® Microarrays

AN INTRODUCTION TO A NEXT GENERATION BIOMARKER DISCOVERY AND DRUG DEVELOPMENT TECHNOLOGY

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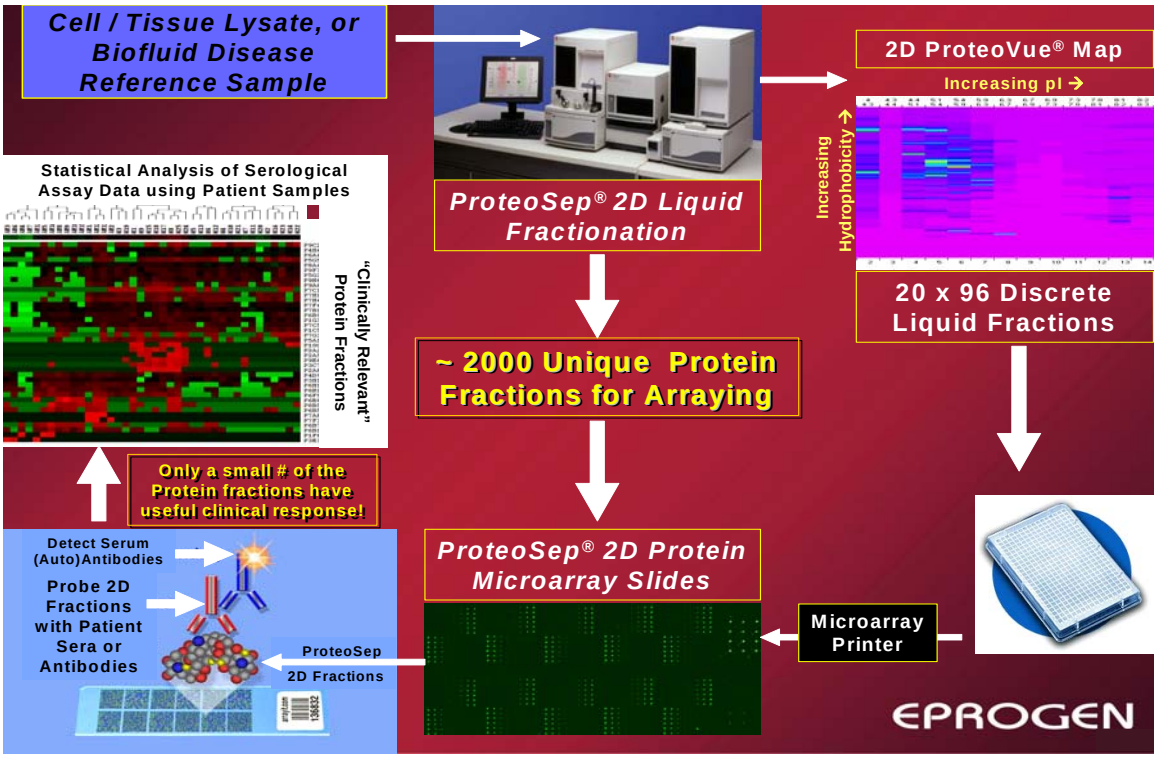
Eprogen's "Bedside-to-Bench" Approach to Biomarker Discovery

Eprogen's approach is grounded in the established notion that surveying a patient's immune response can serve as a sentinel for over- or under-expression of groups of proteins particular to a disease and its expression[1]. The goal is to use easily obtained, well defined patient cohorts [sera or other biofluids) much more effectively in the search for new biomarkers. These cohorts will provide the "biological relevance" to the choice of the potential biomarkers identified by analysis of antibody responses that effectively stratifies the patient cohorts into their respective groups.

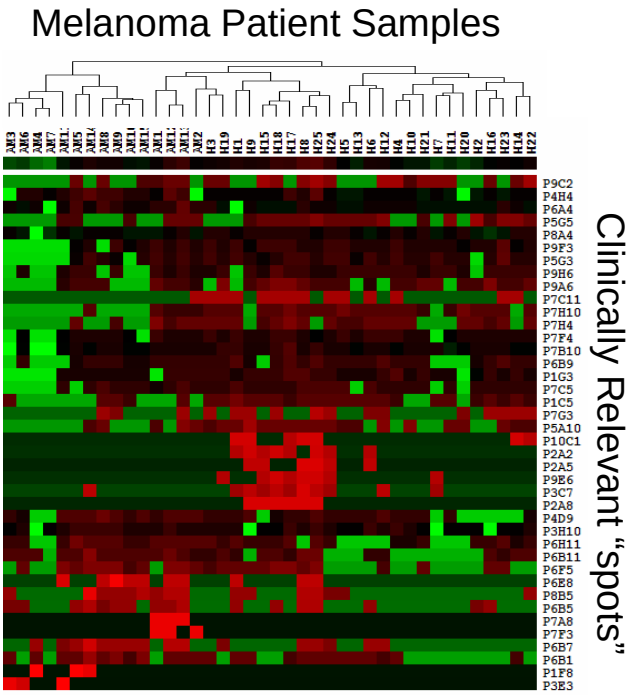
To do this, however, requires a significant reduction in the complexity of potential protein antigens (markers) that might be involved in the patient up- or down-regulated antibody response. To this end, we take one or more reference cell lines (lysates) specific to a disease of interest and, using 2D HPLC, fractionate them to produce many hundreds of well-defined subsets of intact (natural) protein antigens that can serve as "bait" for



patient antibodies (or known antibodies). These intact protein antigen subsets (liquid fractions) obtained from the reference lysates are then "spotted" onto coated glass slides to produce 100's to 1000's of identical protein microarrays for analysis. This fractionation process, in essence, takes one large "Haystack" and breaks it down into a large number of smaller "Haystacks" for easy search of the "needles" that are active in the antibody response of a patient cohort design for the disease of interest. Moreover, the individual liquid fractions used to prepare the microarrays are stored for later access and in-depth analysis (e.g. LC MS/MS) of the proteins they contain if they are found to be "biologically relevant to the disease stratification.

1. Anderson, K.S., & LaBaer, J., *J. Proteome Res*., 2005; 4(4): 1123-1133

To establish which fractions contain relevant antigens, statistical analysis of the protein "spots" reactive to autoantibodies present in the patient cohort sera [typically measured using fluorescence] is performed. The "spot" signatures are established for the particular reference lysate which identifies the "biologically relevant" protein fractions containing the antigens of interest that readily classify patients into their respective populations or subpopulations. Using normal control patients along with benign or non-related disease patients, the same serum samples can be screened across many different reference cell line microarrays to generate a global patient profile with relative ease.



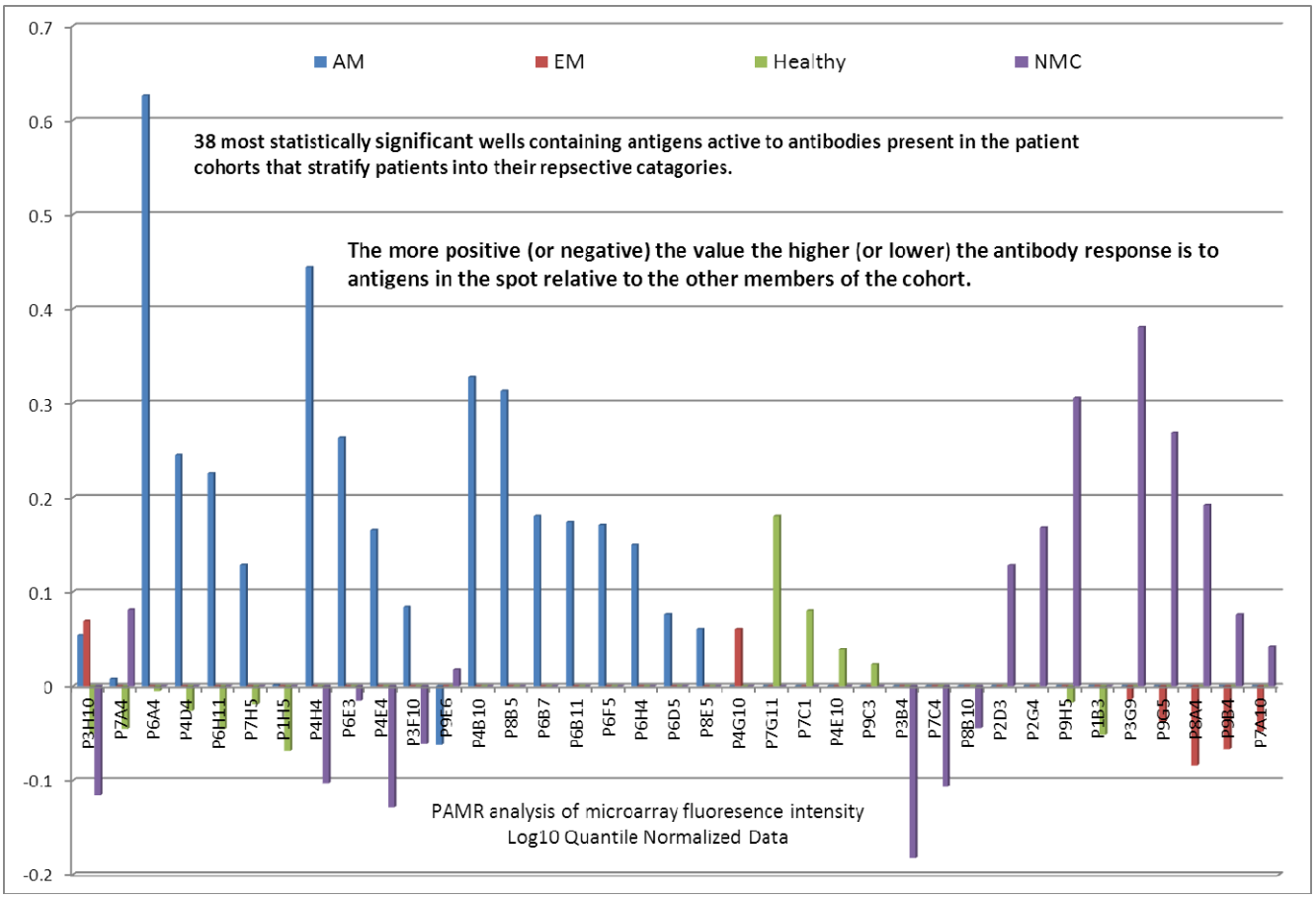
ProteoSep 2D Microarray Analysis Protocol

1. **Reference Cell Line: Primary Melanoma cells from patient lymph node.**
2. **Patient Cohort:**
   - 25 Healthy Volunteers (H)
   - 50 early stage
   - 15 advanced stage (AM)
   - 25 non-melanoma skin Ca
3. **Prepared 864 spot 2D Microarrays and assayed Humoral Response (HR) profiles using fluorescence.**
4. **Statistical Methods Cluster Advanced patients from Healthy using 42/864 "spots" on the microarray (<5% of the total) [> 95% confidence limit].**
5. **"Prioritized" protein fractions sent to MS to ID HR candidates for AM Patients.**

Once the protein fractions (spots) that differentiate the patient populations of interest are identified (typically < 5% of the total number of spots on the microarray), the liquid fraction used to print the spot on the microarray can be readily accessed for more detailed analysis of the proteins present for validation and further research. This approach in reality "prioritizes" the spots containing the proteins of interest. Now, biologically important candidate biomarkers are pinpointed before resorting to more sophisticated techniques (like MS) to characterize the exact nature of the proteins expressed. Having a sound clinical basis at the outset to pursue a protein or group of proteins as biologically significant is of great value.

These characteristic reference antigen (autoantibody) profiles serve not only to highlight active biological pathways related to the disease onset or progression, but also help to "discover what we do not know" about these active pathways as they are expressed in the patient. These immune response profiles of patient samples (Bedside) are now used to generate new, or improve on existing strategies of drug and biomarker development (Bench) by first discerning the relevant proteins that are truly associated with the disease as measured by real patient response.

A clear and much more detailed picture is now possible using a simple analysis of biofluids against a fractionated reference cell line. This now details how many classes in the cohort relate to each other from analysis of the antibodies present.



The unique aspect this "Bedside-to-Bench" approach offers and the efficiencies this will introduce in both time and cost can have a significant impact on how protein biomarker and drug discovery research as well as clinical trials are performed in the future. If the same process can also be used in both drug development efforts and to monitor patients before, during and after the drug treatment, it would save enormous amounts of time, effort and money in drug and biomarker development and validation costs. It would also serve as a significant aid to clinicians dealing with patients throughout the course of diagnosis and treatment.

ProteoSep Microarray Strategies: Make More Effective Use of Clinical Samples!

Cell Line or Tissue Screening/Profiling

Western Blot alternative:

- 1D & 2D liquid multi-well fractions contain intact proteins for direct probing with antibodies.
- Screening multiple arrays produced from the same sample with many different antibodies!
- Antibody qualification and coverage assays.

PTM analyses – Phospho-, glyco-protein detection and coverage.

Serological (biofluid) assays:

- Drug treated vs. Control studies with patients – clinical trial monitoring
- Autoantibody signatures or profiles – Patient (sub)classification
- Disease progression analysis
- New Biomarker discovery studies

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