Fully Functional Protein Microarray for Predictive Toxicology Studies

**Technology background**

Recombinant protein expression is a fundamental technique that underpins clinical diagnostics, drug discovery and screening, vaccine development and pure research for elucidating mechanisms of disease development and progression. However, high-throughput production of correctly folded and functional, full-length human proteins has a very high failure rate. Protein folding is a highly complex process requiring a combination of an aqueous environment, chaperones, post-translational modifications and the formation of multimeric structures held together by covalent bonds. Any deviation from the correct sequence of events can result in a misfolded protein. Loss of protein function is directly linked to misfolding. Use of misfolded proteins in downstream assays and interactions studies can result in identification of false positive biomarkers.

The Sengenics KREX™ technology utilises the biotin carboxyl carrier protein (BCCP) as a folding marker and solubility enhancer which results in high-throughput expression of full-length, correctly folded and functional proteins. BCCP-protein fusions are capable of being biotinylated either in vivo or in vitro, allowing the use of highly specific biotin-streptavidin interaction for surface capture. As biotinylated proteins bound to a streptavidin-coated surface show negligible dissociation, this interaction therefore provides a vastly superior means for tethering proteins to a planar surface and is ideal for applications such as protein microarrays, glass micro-titer plates, SPR and bead-based assays.

**Introduction**

Very often, the effectiveness of administered drugs varies from patient to patient due to the complex nature of most diseases. In fact, some patients, depending on their physiological and immunological makeup, may suffer from immune-related adverse events (irAE) from therapies. Leveraging the KREX™ technology, we have developed a fully quantitative protein microarray platform which affords the simultaneous screening of over thousands of functional proteins for autoantibody-based immunotoxicity profiling. All arrayed proteins are assayed simultaneously under identical conditions resulting in quantitative and genuinely comparative data. It is a highly reproducible, miniaturised assay platform for systematic, high-throughput studies of protein function. This platform has been successfully used to:

- predict the severity of adverse effects that could manifest in patients,
- predict the right dosage and/or combination of administered drugs,
- predict overall response of patients to the drugs.

We believe that our approach using patients’ autoantibody profile to investigate the effectiveness of treatment, adverse effects and the order of use of drugs could be invaluable in lessening the effect of irAE in patients.

**TECHNICAL PERFORMANCE**

- Expression of correctly folded and functional proteins with a 98% success rate.
- Fully quantitative clinical-grade assay metrics. Dynamic range is linear up to five orders of magnitude.
- Excellent reproducibility and precision with a mean CV% below 4% between replica spots.
- Highly sensitive with a limit of detection of approximately 1:1,000,000 serum dilution and autoantibody titer of 190 pg/mL.
- Exceptional specificity and accuracy: non-specific binding eliminated as all proteins are immobilised as functional and correctly folded BCCP-fusions.

**Case studies**

1. **Checkpoint Inhibitor (Beeton-Kempen N, et al., 2014)**

A miniaturised version of the Sengenics protein array platform covering a total of 123 tumour associated antigens (TAA) which utilises the Sengenics KREX™ technology was used to monitor treatment responses of metastatic melanoma patients. The autoantibody assay protocol used for this study is summarised in Figure 2.

**Study Design**

The study aimed to explore responses to an experimental therapy, NY-ESO-1, in 46 stage III and IV malignant melanoma patients. Serum samples were collected at time points before receiving vaccination (Day 0) and then 2 weeks after further vaccination boosters (e.g. Days 70 and 154). The autoantibody profiles of melanoma patients were also compared to that of healthy normal individuals.

**Figure 1. The BCCP folding marker acts as a marker for correctly folded proteins. Proteins will be immobilised on the array only when they are properly folded and biotinylated on the BCCP folding marker.**

**Figure 2. Summary of the autoantibody biomarker discovery protocol.**

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correlation between mutation in the tyrosine kinase domain of EGFR and susceptibility to the drug was also observed.

Figure 3. A representation of data from all 46 patient sera samples exhibiting an immune response to one or more of the 13 antigens of interest (with the exception of NY-ESO-1 and CTAG2).

Results
As expected, the NY-ESO-1 antigen on the array showed the most significant overall autoantibody binding intensities in approximately 61% of patients. Further observations were made for all 46 patient sera samples exhibiting an immune response to one or more of the 13 antigens of interest (with the exception of NY-ESO-1 and CTAG2). It was seen that for a given patient, these responses were found to be relatively consistent at different time points, but are shown randomly distributed between different patients as summarised in Figure 3.

Conclusion
Results obtained from this study suggest the potential application of these identified autoantibody biomarkers for vaccine response and cancer prognosis. Furthermore, it also highlights the platform’s capability allowing for patient stratification and personalised medicine to improve the development of effective therapies and predicting treatment responses and disease progression in patients.

2. Off Target Drug Screening (Blackburn JM & Shoko A, 2011)
Protein kinases represent the largest enzyme gene superfamily in the human genome. Changes in the activity of protein kinases play a significant role in the development of various diseases. As a result, human protein kinases have been heavily utilised to evaluate the selectivity of potential drug candidates by pharma and research institutions for:

- the estimation of inhibitor concentrations needed to inhibit phosphorylation,
- the assessment of inhibitor selectivity, and
- the comparison of inhibitors of different kinases.

Figure 4 shows a graphical representation of how a protein kinase array may be utilised to produce small molecule kinase inhibitor data.

Study Design
A protein microarray consisting of 150 correctly folded and functional protein kinases was used to assay a universal fluorescent kinase ligand in the presence and absence of two small molecule kinase inhibitors; Iressa (a specific kinase inhibitor) and Staurosporine (a broad-spectrum kinase inhibitor).

Results
The universal fluorescent kinase ligand bound to only 48 out of the 150 protein kinases on the array. Figure 5 below shows the graphical representation of the results depicting the percentage inhibition for each kinase inhibitor for these 48 kinases.

Conclusion
Iressa has many off-target kinase inhibition events. It is only effective in approximately 10% of lung cancer patients. Furthermore, a

Figure 4. Summary of small molecule kinase inhibition assay using Sengenics human protein kinase array.

References

Terms and conditions
Patented KSAF functional proteomics technology which allows the BCP-FP labelling method for the production of full-length, correctly folded and functional proteins. Protected by the following patents: US6030332, US6080183, US6351916, US6420570, US6620620, US6922461, US7099987, US7137740, CA2418657, EP1968651, CA191697, EP1456086, A2110632, A2121810, A2139062. Trademarked in the United Kingdom 2005000842758 under classes 12, 10 and 16. Grants as otherwise agreed to by us in writing, the purchase of Products from us only entitles you to the non-transferable right for you to use the quantity of Products purchased in compliance with any applicable limited use statement or limited label licence, as detailed in our catalogues, on our website, or in the label or other documentation accompanying the goods (all such statements or licences being incorporated herein reference as if set forth herein in their entirety). Unless otherwise indicated by us in writing, Products purchased from us may not be resold, resold for, or used to manufacture commercial products. All products and results from services are supplied / handed over to you on the condition that they may only be used by you above and no other third party for and/or on your behalf as intended and detailed in writing by Sengenics, or your own internal, non-commercial and non-revenue and non-free generating research purposes only. They are not in any circumstances to be used for drug or diagnostic purposes, nor for any purpose intended for use in or on humans. By accepting delivery of our products or services, you are expressly agreeing to use our products or services for internal, non-commercial and non-revenue and non-free generating research purposes only as specified in this paragraph. Products are not to be repackaged or resold, or results from services are not to be used for any purpose apart from the research purposes specified in this paragraph. Any non-research use requires an SENG licence, the cost of such licence is based upon the type of application of any Sengenics technologies, products or services for any purpose other than the internal, non-commercial and non-revenue and non-free generating research purposes specified in this paragraph. You represent and warrant to us that the Products will be sold to you (i) will not be used only for your own internal research, (ii) will only be used in compliance with any applicable limited use statement or limited label licence or applicable law and (iii) will not be resold or otherwise transferred or conveyed to any third party. No licence or immunity under any patent in either granted or reported by the sum of any of our Products except to the extent expressly granted in any respective label licence or limited use statement (all such statements or licence being incorporated herein reference as if set forth herein in their entirety). You should indicate whether your use of Products purchased from Sengenics requires permission or license from any third party. You should seek written permission or license from the appropriate person or body, as may be necessary. The absence of a written statement herein that you will not infringe the rights of any third party should not be construed as a warranty that such use will not constitute a violation of the rights of any third party. You are responsible for any infringement claims resulting from your use of our Products. You may only use our Products beyond that granted under any label licence or statement applicable to the Products, (i) as granting you the right to be supplied with goods or components thereof beyond those detailed by us in accordance with these Terms, or (ii) as a licence or grant of any right to you to manufacture or to have manufactured the Products.

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