Antigen-based protein microarrays

Advantages including:

Â High throughput, much increased capacity for multiplexing detection of a range of specific AABs.

Â Reduced requirements for TAAs, serum and reagents;

Â Increased assay robustness; better sensitivity and specificity than that achievable by ELISA-based assays.
**Pilot study**

**Serum samples:** sera from 3 different cohorts; 200 sera from Pittsburgh, USA (100 CRC and 100 controls), 42 sera (21 CRC and 21 matched controls) from New York, USA, and 20 sera from Dundee, UK (10 CRC and 10 controls) were tested.

**A panel of multiple tumour-associated antigens (TAAs)** using an optimised multiplex microarray system. Tumour-associated antigen selection: a panel of 32 TAAs (non-glycosylated recombinant proteins expressed in E.coli) were included: P53, SOX2, NY-ESO-1, GBU, MAGE A4, HuD, AFP, Gankyrin, GRP78, HCC1, HDGF, H-Ras1, IMP, p62, RalA, MUC1, CEA, Annexin A1, rhUteroglobulin (CCSP1), K-Ras, APC1, APC2 blocking peptide, SDCCAG8 (NY-CO-8), TDRD6 (NY-CO-45), vWFA2 (CCSP2), ErbB2, RAF1, SCGB1A1, CA19-9, UTP14A (NY-CO-16), K-RAS-Q61H and APC-N.
Results
1- Quality assessment of the antigens

A) Antigen Quality

A representative analysis of a selection of TAAs by Western blot (WB) and Silver stain.

B) Printing New Antigens

Evaluation of the optimal concentration to print the new antigens
2- Internal QC measures on Microarray

Serial dilution of purified human IgG to verify function of the detection system and provide a standard curve of human IgG against which AAb responses could be calibrated.
3- Cohort results associated with TAA responses:
4- Sensitivity and Specificity of the assay.

<table>
<thead>
<tr>
<th>Antigen/Panel</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>27</td>
<td>95</td>
</tr>
<tr>
<td>P53</td>
<td>26</td>
<td>95</td>
</tr>
<tr>
<td>KRas</td>
<td>27</td>
<td>96</td>
</tr>
<tr>
<td>NY-CO-16</td>
<td>41</td>
<td>95</td>
</tr>
<tr>
<td>RAF1</td>
<td>18</td>
<td>95</td>
</tr>
<tr>
<td>Annexin</td>
<td>29</td>
<td>94</td>
</tr>
<tr>
<td>A panel of the 6 TAAs</td>
<td>61.1%</td>
<td>80.9%</td>
</tr>
</tbody>
</table>

Sensitivity and specificity of individual TAAs and panels of TAAs were calculated to establish the best combinations of this test set of TAAs that would provide good discrimination between cancer-positive and normal serum samples.
5- Sensitivity of CRC sample set by stage (A) and site (B).

The sensitivity is not a stage dependent. The data also show no difference in AAb detection by CRC location (76% sensitivity for left side CRCs and 72% for right side CRCs).
Human blood autoantibodies in the detection of colorectal cancer


PLOS one- June 2016
3- Breast Cancer
Pilot Study for Early Detection of breast cancer

- Initial proof of principle studies have been run to confirm our hypothesis.

- Breast cancer serum samples included 300 invasive BC, (approx. 50% being stage 1/2) and 300 age and gender matched controls.

- The preliminary data confirmed that BC also induces autoantibodies (AAbs) against the small number of specific tumour associated antigens (TAAs) used in this pilot study.

- These pilot results showed positive autoantibody signal was detectable across all age ranges (ie <50yrs, 50-70 years and >70 years) and that the positive tests were as frequent in early stage cancer (stages 1 & 2) as in late stage disease. The results showed that 90% of positive samples for the autoantibody test were hormone receptor positive (HR +ve).
Reactivity of selected TAAs to sera from breast cancer patients and normal controls

Plots of reactivity to four individual TAAs: BRCA1 (A), EPCAM (B), MUC1 (C) and MAGE A4 (D) probed with breast cancer (BC) and normal control (Con) sera. Each graph shows side-by-side comparisons of AAb responses to the TAA antigens for clinically confirmed BC (white circles) and control (grey circles).
Proven preventative treatments: potential to prevent ~35% of all breast cancer cases

70% of all breast cancers are stimulated to grow by estrogen

50% Prevented by drugs like tamoxifen

= ~35% of cases could be prevented

Blood test to identify at early stage
What will be achieved

Early detection of all types of solid cancers will mean:

- More people have a greater chance of survival
- Improved survival rates and life expectancy
- Less aggressive treatments
- Less money spent on treatment.
- Worldwide impact, applicable to low & medium income countries too

Within 5 years

- Can get 3 common cancers (eg breast, colon & primary liver cancer), through to market and available for clinical use in the UK.
- Tests for other cancers would be at various stages of development.
HCC needs explaining
Kennedy Alan, 12/10/2015
Acknowledgment

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