Reviewer's report

**Title:** Serological identification of Tektin5 as a cancer/testis antigen and its immunogenicity

**Version:** 2  **Date:** 21 August 2012

**Reviewer:** Karen Pulford

**Reviewer's report:**

The authors used the SEREX technique to identify tumour-associated antigens in colon cancer. Two genes with testis specific expression were identified. The authors have previously described AKAP3 so the TEKT5 antigen was chosen for further study. Quantitative PCR results showed TEKT5 to have the distribution similar to that of another cancer testis antigen NY-ESO-1 with limited expression in normal tissues (except testis) but more highly expressed in a variety of solid tumours. Antibodies to TEKT5 were also detected in some sera from patients with liver, head and neck tumours as well as in colon cancer. No antibodies were found in sera from patients with lung cancer or from normal subjects.

**General comments.**

The manuscript describes interesting findings but would benefit from additional information being provided.

**Major compulsory revisions.**

1. The authors use the whole coding region of the TEKT5 gene to produce recombinant protein for the ELISA assay. A figure should be shown to demonstrate whether there is a high degree of homology between different members of the TEKTIN family – if so then it is possible that the autoantibodies detected may not be specific for TEKT5 but may also cross react with other TEKTIN proteins. In this case the authors must use a region of TEKT5 that is specific for TEKT5 only and use this to make recombinant protein for the ELISA.

2. The paper would benefit from NY-ESO-1 expression studies being performed in the same tumour tissues to enable a proper comparison to be made between these two antigens in malignant as well as the normal tissues. These results should be of value in further evaluating the potential of TEKT5.

3. Error bars should be provided for the results in Figure 2.

4. The authors mention that they will now go on to look at a T-cell response to TEKT5. However, this may not be the appropriate next step. Surely this should be the investigation of TEKT5 expression at the protein level? There are antibodies commercially available to human TEKT5. The specificity of these reagents should be checked and then use to study protein expression of TEKT5 in normal and malignant tissues.

**Minor essential revisions**
1. Authors cite that 11 positive clones were identified in screening a normal testicular library with colon cancer serum. However Table 2 shows 10.
2. Also do the authors mean WDSUB1 rather than WDSAM1 mentioned?
3. The authors state that TEKT5 appears to have a ‘high immunogenic potential’. This statement should be modified or explained in the context of other studies using autoantibodies since only a subset of patients had autoantibodies to TEKT5.
4. There is a mixture of roman and Arabic numerals for the Table numbers.

Discretionary revisions
The manuscript would benefit from more discussion on the type of cancer testis antigen that TEKT5 probably represents and additional information on the use of such antigens as immunotherapeutic targets.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests’.