MAGE A4 is a member of the MAGE gene family of cancer/testis antigens and its over-expression has been reported in a number of cancers including lung cancer. Autoantibodies to recombinant forms of this antigen have recently been described.

**METHODS**

**Protein purification:** Specific MAGE A4 cDNA was subcloned, with addition of a small tag, into the pET21b expression vector. BL21(DE3) E. coli competent cells, transformed with pET21-MAGE A4 plasmid, were cultured overnight in LB at 37°C and used to seed 20L of Power Broth media. Purification of the MAGE A4 protein was by immobilised metal affinity chromatography (IMAC) with (830-MAG-04p) and without (830-MAG-08p) a further size-exclusion chromatography (SEC) purification step to separate aggregated from less native protein (Figure 1).

**Immuo-assay:** Serum from patients with lung cancer (n=122) as well as age, sex and smoking matched normal control sera (n=122), were investigated, by ELISA, in 2 separate studies for the presence of autoantibodies to the MAGE A4 proteins. The presence of AAbs was evaluated using a semi-automated ELISA method where optical densities (OD) were converted to calibrated reference units (RU). Full assay details are described elsewhere.

**RESULTS**

Figure 1: Analytical SEC analysis Superdex 200 10/300 GL column (GE Healthcare) 0.5ml sample injection run 0.5ml/min on AKTA Purifier (GE Healthcare).

Blue trace: MAGE4-BiRA 830-MAG-04p purified by quantitative SEC after IMAC (21mg 3.5% recovery)

Pink trace: MAGE4-BiRA 830-MAG-08p purified only by IMAC, no quantitative SEC (543mg)

Further purification of the MAGE A4 protein resulted in separation of aggregated material from dimeric forms of the antigen, and resulted in the production of a purer protein.

Figure 2: Silver-staining and Western-blots SDS-PAGE analysis of both MAGE4-BiRA samples

1. MAGE4-BiRA 830-MAG-04p SEC purified dimers
2. MAGE4-BiRA 830-MAG-08p no SEC

**REFERENCES**