Overexpression and potential targeting of the oncofoetal antigen 5T4 in malignant pleural mesothelioma

Saly Al-Taei a, Josephine Salimu a, Jason F. Lester b, Seamus Linnane c, d, Madusha Goonewardena e, Richard Harrop e, Malcolm D. Mason a, Zsuzsanna Tabi a, b, *

a Department of Oncology, School of Medicine, Cardiff University, Velindre Cancer Centre, Velindre Road, Whitchurch, Cardiff CF14 2TL, UK
b Velindre NHS Trust, Velindre Cancer Centre, Velindre Road, Whitchurch, Cardiff CF14 2TL, UK
c Cardiff and Vale NHS Trust, Llandough Hospital, Penlan Road, Llandough, Penarth CF64 2XX, UK
d Blackrock Clinic, Suite 13, Rock Road, Blackrock, Co Dublin, Ireland
e Oxford BioMedica UK Ltd., The Medawar Centre, Robert Robinson Avenue, Oxford Science Park, Oxford OX4 4GA, UK

A R T I C L E   I N F O

Article history:
Received 10 January 2012
Received in revised form 2 March 2012
Accepted 14 March 2012

Keywords:
Malignant pleural mesothelioma
Tumour-associated antigen
Novel target
5T4
Cancer vaccine
T-cell killing

A B S T R A C T

Malignant pleural mesothelioma (MPM) is resistant to conventional treatments. Novel, targeted treatments are hampered by the relative lack of MPM-associated tumour antigens. The aim of this study was to evaluate the level of expression and the relevance of 5T4 as a tumour-associated antigen in MPM. 5T4 expression was assessed by Western blotting, flow cytometry, immuno-cytchemistry and -histochemistry in 11 mesothelioma cell lines, 21 tumour biopsies, and ex vivo tumour cells obtained from the pleural fluid (PF) of 10 patients. 5T4 antibody levels were also determined in the plasma of patients and healthy donors. The susceptibility of MPM cells to 5T4-specific T-cell-mediated killing was determined using an HLA-A2+, CD8+ T-cell line, developed against the ST4 17-23 peptide. We report here that cell surface 5T4 expression was detected in all mesothelioma cell lines and PF cell samples. Mesothelin and CD200, a suggested mesothelioma marker, were co-expressed with 5T4 on tumour cells in PF. Immunochemistry confirmed overexpression of 5T4, similar to mesothelin, on tumour cells but not on reactive stroma in all tissue sections tested. Median 5T4 antibody levels were 46% higher in patients than in healthy donor plasma, indicating immune recognition. Importantly, 5T4-specific CD8+ T-cells were able to kill four out of six HLA-A2+ MPM cell lines but not an HLA-A2- cell line, demonstrating immune recognition of MPM-associated 5T4 antigen at the effector T-cell level. We conclude that 5T4 is a potential new antigen for targeted therapies such as immunotherapy in MPM, as it is overexpressed on mesothelioma cells and recognised by 5T4-specific cytotoxic T-cells. Our findings have been translated into a Phase II clinical trial applying 5T4-targeted therapies in MPM patients.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Malignant pleural mesothelioma (MPM) is an incurable malignancy of the pleural membranes. In 2008, 2249 people in the UK died from MPM and median survival is only 6–18 months. Pemetrexed–cisplatin chemotherapy is the current standard of care, but treatment results in median survival advantage of less than 3 months [1], dictating an urgent need for the development of new therapies.

The impetus to pursue immunotherapy in MPM stems from various observations. Tumour-infiltrating CD8+ T-cells correlate with favourable prognosis in MPM [2,3], whilst a rare case of regression has correlated with positive immune parameters [4]. Furthermore, mesothelioma models respond to immunotherapeutic strategies [5]. For example, intrapleural interferon-α triggered local immune responses, detectable even at distant sites with clinical benefit [6].

In patients, a trial utilised autologous tumour cell lysate loaded on patients’ dendritic cells (DC) in order to trigger anti-tumour immune responses with positive results [7].

Targeted immunotherapy relies on the overexpression of tumour-associated antigens (TAA). Mesothelin, a differentiation antigen normally present on mesothelial cells but overexpressed in malignancy, is currently the main target antigen [8], but its overexpression is restricted to epithelioid mesothelioma [9]. In this study, we aimed to determine the expression profile of 5T4 and its relevance for immunological targeting in MPM. 5T4 is a 72 kDa oncofoetal glycoprotein with cell surface expression [10]. It has restricted expression in normal tissues but is overexpressed in numerous malignancies, including testicular, breast and colon cancer [11]. It alters cellular dynamics facilitating metastatic spread.
2.3. Plasma UK).

2.4. TAP-deficient Welsh determined plasma NP-40, 2 mM Na-deoxycholate, an II and II clinical trials in advanced colorectal, renal and prostate cancer. The vaccine was well tolerated and a positive association was observed between the level of vaccine-induced antibody responses and clinical outcome [17].

In this study, ST4 expression was assessed in mesothelioma cell lines, tumour tissue and pleural fluid (PF) cells, representing the three disease subtypes; epithelioid, sarcomatoid and biphasic. ST4-specific antibody responses were studied in patient plasma, and the susceptibility of mesothelioma cells to ST4-specific T-cell killing was established in cytotoxicity assays.

2. Materials and methods

2.1. Tumour tissue

Ethical approval for obtaining human-derived material was granted by the South East Wales Research Ethics Committee and informed consent was obtained from all subjects. Biopsy specimens were snap-frozen and stored in liquid-nitrogen vapour. Formalin-fixed paraffin-embedded (FFPE) tissue sections were obtained from the Histopathology Department at Llandough Hospital (Cardiff, UK).

2.2. Peripheral blood mononuclear cells (PBMC), PF cells and plasma

PF cells were isolated by centrifugation from PF collected via indwelling catheters. Venous blood of healthy donors or the pelleted PF cells were subjected to density gradient centrifugation on Histopaque® (Sigma, UK) then the cells (PBMC or PF cells respectively) were cryopreserved in RPMI-1640 media (Lonza, UK) supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine, 25 mM HEPES, 1 mM Na-pyruvate (sRPMI) plus 10% DMSO and 20% foetal bovine serum (FBS; PAA, UK) were added. Plasma collected from the uppermost fraction of separated blood was frozen at −20 °C.

2.3. Cell lines and culture

Mesothelioma cell lines were generated by long term culture of explanted tumour biopsies and maintained by regular passage in sRPMI 5% FBS. PF-derived tumour cell lines were obtained by long-term culture of adherent PF-cells. Tumour subtype was determined at the time of surgery. Cell lines were used between passages 6–12. B lymphocytic cell lines (BLCL) were generated from PBMC by standard EBV-immortalisation. LNCaP human prostate adenocarcinoma cell line (ECACC, UK) and T2 cells (HLA-A*2+ human TAP-deficient hybridoma; Cardiff University, MRC Cooperative) were grown in sRPMI 10% FBS. Cell lines were HLA-typed by the Welsh Blood Service and underwent regular mycoplasma testing using Vector® GeM mycoplasma detection kit (Cambio Ltd., UK).

2.4. Western blotting

Lysates were extracted from cell lines using lysis buffer (2% NP-40, Sigma). Snap frozen tumour tissue was thawed in ice-cold RIPA buffer (50 mM Tris–HCl pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 1 mM EDTA and 150 mM NaCl) then crushed. Protein concentration was determined using micro BCA™ protein assay kit (Thermo Fisher, UK). ST4 protein was included (Oxford BioMedica, UK). Broad-spectrum protein markers (Biorad, UK) were used. Lysates were separated on a 12% polyacrylamide gel (30% acrylamide/bis-acrylamide; Sigma) by SDS-PAGE under non-reducing conditions, transferred to Hybond™-P PVDF membrane (GE Healthcare, UK), blocked in PBS/Tween 20 (Sigma) with 5% non-fat dry milk and probed with anti-ST4 (Oxford Biomedica) [10]. Horseradish-peroxidase-conjugated secondary antibody was used (Insight Biotechnology Ltd., UK). Bands were developed by ECL+ on film (GE Healthcare, UK) and visualised using the Xograph Compact x4 X-ray film processor (Xograph Healthcare Ltd., UK). For calcitinin, the transfer was carried out using the iBlot™ Dry Blotting System with PVDF gel-transfer stacks (InVitrogen, UK). After probing for calcitinin (BD, UK), the Western Dot™ 625 Western blot secondary detection kit was used (InVitrogen). Membranes were visualised using MiniBIS-Pro with GelCapture software (Berthold Technologies UK Ltd., UK).

2.5. Flow cytometry

The ST4 antibody (Oxford Biomedica) was conjugated to Alexa-488 (InVitrogen). Cells were blocked with FcR blocking reagent (Miltenyi Biotec, UK), labelled with ST4-Alexa-488 mesothelin (Rockland Immunoc hemicals, USA) followed by Alexa-488-conjugated secondary antibody, or isotype controls. PF cells were labelled with 7–AAD (eBioscience), CD14-APC-Cy7, CD200-PE, CD3-APC (BD), ST4-Alexa-488 and mesothelin-APC (R&D, UK). Flow cytometry was performed on a FACSCanto flow cytometer (BD) using FACSDiva software.

2.6. Immunocytochemistry

Mesothelioma cells (10⁴) were seeded onto coverslips. Once 60% confluent, cells were fixed in 4% paraformaldehyde (Sigma), permeabilised in 0.05% Triton-X100 (Sigma) and blocked in 0.1% mouse serum (Gibco) before labelling with ST4 (Oxford Biomedica) or isotype antibody (Dako, UK), followed by Alexa-488-conjugated secondary antibody (InVitrogen). Coverslips were mounted using Vectashield mounting media with DAPI (Vector Laboratories, UK). Imaging was performed on Zeiss Axiovert 40 CFL epifluorescence microscope, connected to Cannon Powershot S45 digital camera and acquired with RemoteCapture software.

2.7. Immunohistochemistry

FFPE sections were de-waxed in xylene (Genta Medical Ltd., UK) then rehydrated in 100%–92%–70% ethanol-gradient. Antigen retrieval was performed by heating slides in retrieval solution (Dako) at 95 °C then at room temperature and washing in distilled water. Sections were stained for ST4 (R&D), isotype (eBioscience) or mesothelin (R&D) using a signal amplification kit (Dako). Sections were counterstained with haematoxylin (Thermo-Fisher), dehydrated in 70%–92%–100% ethanol-gradient then in xylene and mounted with DPX (Thermo-Fisher). Slides were imaged on Mirax Scanner (Zeiss, UK) and analysed using Pannoramic Viewer software (3DHistech, Hungary).

2.8. ST4 antibody levels

Plasma ST4-specific antibody levels were determined using a validated semi-quantitative ELISA as described previously [18]. AB serum (Sigma), pooled from 200 individuals, was used as a control.
2.9. Generation of a 5T4 peptide-specific T-cell line

A CD8+ T-cell line was developed from a HLA-A2+ healthy donor by stimulation of non-adherent PBMC with autologous monocyte-derived day-six DC, generated from adherent PBMC with 500 ng/ml GM-CSF (Prospect-Tany Technogene Ltd., Israel) and 500 U/ml IL-4 (Peprotech, UK). DC were loaded with 2 μg/ml ST4[17–25] peptide (RLARALVLSQ; 90.4% purity; Prolimmune, UK)[19] and treated simultaneously with 5 ng/ml LPS for 1 h. DC were washed and plated out in 24-well trays at 2 × 10^5 cells/well with 4 × 10^6 non-adherent PBMC in 2 ml. Cultures were supplemented with 1000 U/ml IL-6 and 5 ng/ml IL-12 [20] and grown for eight days. Peptide-specific stimulation was repeated weekly with peptide-loaded autologous DC as above, supplemented with 5 ng/ml IL-7 and 10 U/ml IL-2 [20]. After 24 days, peptide-specific cells were separated using IFN-γ cell enrichment (Miltenyi Biotech, UK). Separated T-cells (1–5 × 10^5) were expanded weekly with a mixture of 5 × 10^6 peptide-pulsed autologous BLCI irradiated with 4000 rad; 5 × 10^7 allogeneic PBMC mixed from 2 to 3 donors and irradiated with 3000 rad; 50 U/ml IL-2 and 1 μl/ml OKT3 hybridoma supernatant (MRC Cooperative, Cardiff University) in 50 ml sRPMI 10% FBS and 1% AB-serum (Sigma) in a T75 flask. Half the media was replaced at day four with fresh media containing cytokines. CD8+ T-cell separation was carried out after seven days using the EasySep CD8+ T-cell enrichment kit (StemCell Technologies, UK). Cytotoxicity assay was carried out after 7–9 days in expansion.

2.10. Cytotoxicity assay

T-cell cytotoxic activity was tested on peptide-pulsed or unpulsed T2 cells labelled with 51Cr. Target cells (3 × 10^4)/100 μl/well and increasing numbers of T-cells in 100 μl, at Effector:Target (E:T) ratios ranging from 80:1 to 2:5:1, were plated in triplicates in a 96-well tray. Control wells contained target cells only with 100 μl media or 100 μl 5% Triton-X100. Supernatant (50 μl) was collected from each well 4 h later and 51Cr-release was measured on a Microbeta-3 (Perkin-Elmer, UK) counter. Tumour cell killing was tested the same way as above, on six HLA-A2+ and one HLA-A2- mesothelioma line (3 × 10^3 tumour cells/well) in a 5 h 51Cr-release assay at 20:1 E:T ratio. Percent specific lysis was calculated as described [21].

2.11. Statistical analysis

Statistical analyses were carried out using Student’s t-test (GraphPad Prism 5), as indicated. Differences were considered statistically significant at p < 0.05.

3. Results

3.1. Characterisation of mesothelioma cell lines for 5T4 expression

In this study cell lysates from five biphasic (M2, 18, 19, 26, 36), five epithelioid (M1, 15, 30, 38, 43) and one sarcomatoid (M34) tumour cell lines and LNCaP cells were subjected to Western blotting to assess total 5T4 expression. All mesothelioma lines were positive for 5T4, including the sarcomatoid line M34 (Fig. 1A), whilst LNCaP cells were negative. The observed 5T4 band co-migrated with purified 5T4 protein. Flow cytometry revealed 5T4 surface expression on all mesothelioma lines but not on LNCaP cells (Fig. 1B). To confirm that the cell lines were true mesothelioma cells, expression of mesothelin and calretinin, both diagnostic markers for MPM [9], was assessed. All epithelioid cell lines were positive for either mesothelin (Fig. 1C) or calretinin (Fig. 1A) or both.

Fig. 1. Characterisation of mesothelioma cell lines for 5T4 expression. (A) Western blot of total 5T4 expression. Equal amounts of cell-lysate protein (30 μg) from LNCaP or mesothelioma cells or purified 5T4 protein (100 ng) were probed for 5T4 (10 μg/ml; top) and cell-lysates also for calretinin (1 μg/ml; bottom). The numbers on top of the gels represent the sample IDs. (B) Flow cytometry analysis of 5T4 cell surface expression on LNCaP and mesothelioma cell lines. Cells were labelled with either IgG1-Alexa488 isotype (black bar) or ST4-Alexa488 (10 μg/ml; grey bar) conjugated antibodies. Means and SD of mfi (mean fluorescence intensity) are shown, calculated from two independent experiments. (C) Flow cytometry analysis of mesothelin surface expression. Cells were labelled with either rat IgG2a isotype (black bar) or mesothelin antibody (2 μg/ml; grey bar), followed by a goat anti-mouse-Alexa-488 antibody. Means and SD of mfi are shown, calculated from two independent experiments. (D) ST4 cellular distribution in M15 (i), M38 (ii), M43 (iii) and M19 (iv) mesothelioma cell lines by epifluorescence microscopy. Cells were stained with 5T4 primary antibody (10 μg/ml) followed by Alexa-488-conjugated anti-mouse secondary antibody. Cells were imaged at 100× magnification.
The cellular distribution of ST4 was analysed by immunocytochemistry in four cell lines (Fig. 1D). The pattern of ST4 was similar in three of four cell lines, characterised by prominent plasma membrane, diffuse cytoplasmic and dense perinuclear labelling, whilst M19 cells (Fig. 1D(iv)) labelled predominantly in a punctate cytoplasmic manner.

3.2. ST4 expression on tumour cells in PF

We assessed ST4 surface expression on tumour cells present in PF. The tumour cell gate was pre-determined based on the forward and side scatter profiles of cultured tumour cells mixed with PBMC (Fig. 2A, top panel). PF cells from all 10 donors expressed ST4 (Fig. 2A). To confirm that ST4 was expressed on tumour cells, 7-AAD negative (live) PF cells from three patients were labelled with ST4, mesothelin and CD200. There was a distinct population of CD200+ cells present, and 73%, 83% and 83% of these cells, respectively from M2.10, M2.13 and M2.21, expressed ST4 (Fig. 2B(ii) vs (i)). Mesothelin was also highly expressed on ST4+CD200+ cells (mfi 1068, 792 and 1669) but not CD200−/ST4− cells (81, 63 and 52 respectively; Fig. 2B(iii) vs (iv)), confirming that the majority of ST4+ cells in the PF are tumour cells. Cells with the triple-positive phenotype were not detectable in PBMC (data not shown).

3.3. ST4 expression and distribution in tumour tissue

To further verify ST4 expression in MPM and rule out the possibility that ST4 is upregulated during extended in vitro culture of cell lines, ST4 expression was analysed in fresh-frozen tumour tissues by Western blotting (Fig. 3A). A 75 kDa band, consistent with the typical ST4 size, was observed in all samples. Bands were also observed at ~50 kDa, consistent with an immature form of ST4. A 155 kDa band, not evident in cell lysates, was present in most samples and suggested ST4 dimerisation, the relevance of which is not known.

We also assessed ST4 expression and distribution in FFPE tissue sections (Fig. 3B). There was strong labelling for ST4 and mesothelin, compared to isotype-control, and similar distribution of the two antigens. The highest magnification (Fig. 3B(iii)) confirms that ST4 expression is associated with nucleated tumour cells. ST4 and mesothelin expression were confirmed in tissue sections obtained from seven more patients (Supplementary Fig. 1). In contrast, there was little staining of normal pleura for ST4 and mesothelin above that of isotype control (Fig. 3C). To confirm that ST4 expression is tumour-specific, images of non-tumour structures are highlighted. A blood vessel wall (Fig. 3D(i)) is ST4− and is adjacent to a ST4+ tumour mass. An area of tumour surrounded by reactive stroma reveals a distinct pattern of ST4+ stromal cells and ST4+ tumour cells (Fig. 3D(ii)). Pseudo-capillary structures characterised by reactive stroma (ST4−) encased in malignant mesothelioma (ST4+) (Fig. 3D(iii)). Gland-like structures surrounded by malignant mesothelioma show ST4 positivity of the tumour cells only (Fig. 3D(iv)).

3.4. ST4-specific antibodies in plasma

Antibody responses to ST4 were identified in the plasma of MPM patients (n = 17) and healthy donors (n = 7; Fig. 4). The median ST4 antibody level was 46% higher in the patient plasma than in healthy plasma but was not statistically significant (p = 0.472). AB-serum had a low level of ST4 antibody.

3.5. Tumour cell killing by a ST4-specific CTL line

HLA-A2+ CD8+ T-cells, with demonstrable specificity for the ST417-25 peptide (Fig. 5A) were tested for their ability to kill
Fig. 3. 5T4 protein in tumour tissue (A) Western blot of 5T4 expression in tumour tissue. Protein samples (60 µg) from tissue homogenates were probed for 5T4. Numbers on top of the blots indicate sample IDs, and the observed molecular weights are shown to the right of the blot. (B) A representative sample with H&E (highlighting the tissue structure), isotype-specific, 5T4-specific (25 µg/ml) and mesothelin-specific (10 µg/ml) staining of an FFPE section as indicated above each column. White boxes indicate the area magnified and shown in the row below. Scale bars shown are 5000 µm (i), 500 µm (ii) and 50 µm (iii). (C) A normal tissue section stained for H&E, isotype, 5T4 and mesothelin, respectively, as indicated. The black box indicates the area magnified and shown in the row below. The scale bars represent 5000 µm (i) and 500 µm (ii), apart from the 5T4 stained section in (ii) which is 200 µm. Arrow indicates layer of normal mesothelial cells. (D) Tissue sections from different donors highlighting 5T4-structures. (i) The asterisk indicates the wall of a blood vessel. (ii) The black box indicates the area magnified and shown in the row below. The scale bars represent 5000 µm (i) and 500 µm (ii), apart from the 5T4 stained section in (ii) which is 200 µm. Arrow indicates layer of normal mesothelial cells. (iv) The asterisk is placed next to a gland-like structure which is surrounded by a layer of malignant mesothelium.

HLA-A2+ and HLA-A2− mesothelioma cell lines, expressing 5T4 (Fig. 5B). Four of the six A2+ MPM cell lines (M18, 24, 34, 36) were killed at a range of 16–27% specific lysis at 20:1 E:T ratio in a 5 h CTL assay (Fig. 5B). The lysis of these cells was significantly higher than that of the A2− cell line, M38. Two cell lines (M24 and M34) sensitive to the killing were sarcomatous, indicating that 5T4-targeting of mesothelioma is not restricted to the epithelial subtype. The lysis was <10% for two of six A2+ MPM lines (M15 and M40). This resistance to killing was not related to their expression level of 5T4 at the time of the assay (Fig. 5C).

4. Discussion

In this study we aimed to establish whether 5T4 is a relevant antigen for potential targeted therapies in MPM. 5T4 expression has been observed on all cell lines, tumour and PF samples from...
Fig. 4. 5T4 antibody levels in patient and healthy donor plasma. Plasma from MPM patients (n = 17), healthy controls (n = 7) and AB-serum were tested for levels of 5T4-specific antibody using an ELISA test. The medians of antibody levels (healthy donor: 8.23 U/ml and MPM: 12.04 RU/ml) are indicated by black bars. Statistics were performed using the Mann–Whitney U-test.

42 patients. Expression was seen in 100% of samples, regardless of subtype. 5T4 expression has been described in most adenocarcinomas with ~70% frequency [11,22,23] and is associated with poor prognosis likely due to increased metastatic activity [13]. Its presence has not been reported in MPM before and it may contribute to the aggressive nature of the disease by mechanisms other than metastatic spread.

5T4 expression was membrane-associated in all mesothelioma cell lines and PF tumour cells studied. In one cell line (M19) we observed relatively low expression of membrane-associated 5T4. In non-small-cell lung cancer, membranous 5T4 expression was observed on poorly differentiated tumour cells and associated with shorter survival time and shorter time to recurrence [24]. The cellular fraction of PF also contained 5T4 positive cells co-expressing CD200 and mesothelin, which strongly indicates that these cells are tumour cells that entered the tumour–associated fluid. As our histology results from FFPE biopsies reveal that non-malignant mesothelium, reactive stroma and non-malignant structures in the pleura do not express 5T4, 5T4 detection in patient samples may have diagnostic as well as prognostic value. Immune-engagement with 5T4 was also studied. 5T4-specific antibodies were observed both in MPM patients and healthy donors, with higher levels in patients and similar to levels observed in other solid cancers [17]. In our study, the difference did not reach statistical significance, probably due to the relatively small number of samples and one high healthy responder. Furthermore, 5T4 antibody levels (without vaccination) had no prognostic value in prostate or colorectal carcinoma [17] and its significance in MPM is also unknown.

Finally, we demonstrated that 5T4 peptide-specific T-cells can recognise and kill mesothelioma cells in a HLA-A2-restricted manner. 5T4-specific CD8+ T-cells have been generated against 5T4 sequences before [25]. High affinity T-cell receptor binding of the 17–25 amino-acid sequence of 5T4 has been demonstrated (ED50 1.5 × 10⁻⁷ M), and strong ELISPOT responses were elicited against this peptide in a patient vaccinated with TroVax® [19]. We found that mesothelioma cells even with low 5T4 expression were killed by T-cells effectively. On the other hand, two HLA-A2+ mesothelioma cell lines expressing medium-high level of 5T4 were not killed in a 5 h CTL assay. There are several possible explanations as to why this is the case. The first is low HLA-A2 expression, although

![Graph showing 5T4 antibody levels in patient and healthy donor plasma.](image)

Fig. 5. MPM cell killing by a 5T4-specific T-cell line. (A) T2 cells pulsed with the peptide (black circles) or unpulsed (white circles) were exposed to increasing numbers of T-cells (E:T ratio shown on x axis). The symbols represent the means and SD of the percent specific lysis of target cells in a 4 h ⁵¹Cr-assay. (B) HLA-A2+ mesothelioma cell lines (M15–M40) and an HLA-A2- cell line (M38) were pulsed with ⁵¹Cr and served as targets of T-cell killing by the 5T417–25 T-cell line in a 5 h ⁵¹Cr-release assay at 20:1 E:T ratio. The bars represent the means and SD of % specific lysis from triplicate samples. (*p < 0.05; **p < 0.01; Student’s t-test). (C) 5T4 surface expression on the target cells of the CTL assay, determined by flow cytometry. The bars represent mfi of 5T4 expression (grey) or isotype control (black columns).
A2 expression was tested on M15 cells (not shown) and its expression was not downregulated. Another possibility is that the 5h assay was not sufficiently long; it has been shown that HPV-specific T-cells only kill tumour targets after 20h co-culture [26]. Other mechanisms, such as p53 inactivation or pSTAT3 induction may also be responsible for suppressing CTL responses [27,28]. However, CD8+ T-cells can exert tumour control not only via cytotoxic but also cytostatic effects, as shown in a mouse model of melanoma [29]. Further studies will be carried out to investigate the nature of resistance to killing by CTL in these mesothelioma cell lines.

A Phase II clinical trial, starting this year, will study the immunological and clinical effects of Trovax®, administered to MPN patients in combination with pemetrexed and cisplatin (SKOPOS trial, Velindre Cancer Centre, Cardiff, UK). We have previously shown that the majority of MPN patients have normal haematological profiles and immune responses [30], so are likely to respond better to the vaccine than end-stage renal cancer patients. Targeting ST4 positive cells also has the advantage of eliminating not only tumour cells but also cells with tumour-initiating potential [24].

5. Conclusion

To conclude, ST4 is widely expressed in mesothelioma tissues and cell lines and serves as a target for immune-mediated killing. These observations have been translated into a Phase II trial applying Trovax® in MPN patients.

Conflict of interest statement

Dr. Richard Harrop is employed by and has shares in an organisation which have patent rights to ST4-based therapies.

Acknowledgements

We thank Mr. Mike Saunders and Dr. Thomas Hockey (Histopathology Department at Llandough Hospital), for providing FFPE slides and advice. Also, Lynda Churchill for technical assistance and Dr. Lisa Spary for helpful discussions.

The study was funded by the June Hancock Mesothelioma Research Fund (Leeds, UK) and the British Lung Foundation. The sponsors had no additional involvement in the study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.jlungcan.2012.03.008.

References