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The predictive role of serum VEGF in an advanced malignant mesothelioma patient cohort treated with thalidomide alone or combined with cisplatin/gemcitabine

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ABSTRACT

There is a need for new treatment strategies and prognostic markers for the management of malignant mesothelioma (MM). The activity of thalidomide/cisplatin/gemcitabine (arm A) or thalidomide alone (arm B) was investigated in two parallel phase II studies in patients with advanced MM, using 6 month progression free survival (PFS) as the principal end-point. The predictive role of pre-treatment and 8 week follow-up serum C-reactive protein (CRP), interlukin-6 (IL-6), interlukin-6 soluble receptor (sIL-6R), mesothelin (SMRP) and vascular endothelial growth factor (VEGF) was also assessed. The proportion of patients with stable disease for >6 months was similar in both studies (arm A 35%, arm B 29%) and toxicity was mainly grade I/II. In univariate analyses only pre-treatment VEGF and CRP were correlated with survival. At 8 weeks post treatment, increased survival was found with low (<median) VEGF and CRP compared with high (>median) VEGF and CRP ($P < 0.05$). Change in VEGF over the first 8 weeks of treatment was also predictive for survival ($P < 0.05$). When pre-treatment VEGF was >median, decreasing VEGF was associated with increased survival ($P < 0.05$). In conclusion, thalidomide alone, or in combination with cisplatin/gemcitabine, controlled disease for >6 months in ~30% of patients. Patients with decreasing VEGF during treatment had longest survival. Pre-treatment VEGF or CRP and early change in VEGF on treatment may predict treatment benefit and should be examined in future studies.

1. Introduction

Malignant pleural mesothelioma (MM) is a usually fatal malignancy associated with exposure to asbestos decades earlier [1]. Most patients present with inoperable disease and therapy is given with palliative intent. Prognosis in patients with advanced disease undergoing chemotherapy is associated with clinical and disease related factors such as age, performance status, epithelial histologic subtype, and blood markers such as white cell count, hemoglobin and platelet count [2]. Platinum doublet chemotherapy was shown in 1999 to have promising activity [3] and subsequent Phase III trials have shown that platinum and anti-folate drugs have further improved survival. However further improvements in treatments are still needed.

Increased microvessel density and high levels of tumor and blood vascular endothelial growth factor (VEGF) have been associated with a poor prognosis in MM patients [4]. Consequently recent therapeutic approaches in MM have involved exploration of anti-angiogenic therapy. In vitro evidence of synergy between anti-angiogenic therapy and chemotherapy has been demonstrated with multiple agents [5]. The concept of low dose continuous chemotherapy ("metronomic") has been hypothesized to potentially overcome cytotoxic drug resistance by targeting tumor vasculature [6]. Thalidomide was one of the first anti-angiogenic agents to be explored following the demonstration that it could inhibit VEGF mediated angiogenesis in vitro [7]. Subsequent clinical trials have confirmed its greatest efficacy in myeloma [8]. However the mechanisms behind its anti-tumor effects have not been fully elucidated and there is evidence to suggest that it can modulate interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), both of

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which are suggested to play a role in the pathogenesis and disease progression of MM [9].

Although many biomarkers have been analyzed in MM, none has been firmly established as definitively diagnostic, prognostic or predictive of treatment response. Selected serum/plasma markers that have been studied include VEGF [10] and VEGF Receptor-3 (VEGFR-3) [11], IL-6 [12], tissue polypeptide antigen (TPA) [13], cancer antigen 125 (CA 125) [13], Hyaluronic acid or Hyaluronan [13,14], Cytokeratin 19 fragment (CYFRA 21-1) [15] and soluble mesothelin related protein (SMRP) [16].

Tissue VEGF has been shown to correlate with microvessel density [10] and mesothelioma patients expressing high levels of this cytokine in the tumor have shown poor prognosis [17]. C-reactive protein (CRP), an acute phase reactant has been shown to predict for poor prognosis in some cancers [18]. IL-6 has both pro -and anti-inflammatory properties [12] and is known to be produced by mesothelioma cell lines. It also interacts with several target cells, leading to the production of acute phase reactants like CRP [19]. A significant component of the regulation of IL-6 responses is the soluble IL-6 receptor (sIL-6R). It forms a ligand/receptor complex with IL-6 which is capable of stimulating a number of cellular processes [20].

Recent reports in the literature have suggested that serum SMRP (circulating product of mesothelin) is a potential diagnostic marker for MM. Mesothelin is a cell surface glycoprotein protein expressed by mesothelial cells [21,22].

Two parallel non-randomized Phase II studies in patients with advanced MM were undertaken to explore the activity of (1) thalidomide in combination with cisplatin and gemcitabine in chemotherapy naïve patients (Arm A), and (2) thalidomide alone in patients having previously received chemotherapy or considered unsuitable for first line chemotherapy (Arm B). Within this patient cohort, blood biomarkers were analyzed to explore their potential prognostic role.

2. Materials and methods

Two parallel single arm Phase II studies were conducted in two Australian centres (Royal North Shore Hospital, Sydney and the Prince Charles Hospital, Brisbane). All participating patients gave written informed consent under the protocol approved by the respective Institutional Human Ethics committees. Patients were enrolled in arm A (thalidomide in combination with cisplatin (25 mg/m^2 iv; Day 1, 8, 15; Q 28 days) and gemcitabine (800 mg/m^2 iv; Day 1, 8, 15; Q 28 days) or arm B (thalidomide alone). Thalidomide was commenced in both arms at 100 mg nocte escalating weekly to a maximum dose of 500 mg nocte, limited by toxicity and patient tolerance.

Eligible patients had to have histologically confirmed inoperable pleural MM with measurable disease; adequate renal and hepatic function; adequate bone marrow reserve; life expectancy greater than 8 weeks. Patients were enrolled in arm A if they had not received prior chemotherapy or arm B if they received prior chemotherapy or were deemed by investigators to be unsuitable to receive chemotherapy. Patients were treated for a maximum of 6 cycles in arm A and with thalidomide until progression in both studies.

Baseline and treatment assessments included a complete history and physical examination, as well as bloods weekly for patients in arm A while receiving chemotherapy and monthly if on thalidomide alone. Quality of life (QoL) was evaluated monthly during treatment using the Lung Cancer Symptom Scale (LCSS) until progression [23]. Radiologic disease status was assessed at baseline and every 8 weeks by computed tomography (CT), using the modified RECIST criteria [24]. Independent tumor assessment was undertaken (G.M. and M.C.) to confirm investigator response. This caused significant delay as imaging was not performed centrally and required retrospective collection from the treating physicians, hence the delayed final reporting of the study. Blood in serum tubes was collected at baseline and every month for biomarker analysis. Collected blood was spun at 2500 × *g* for 10 min and separated serum was centrally stored at −80 °C.

The primary endpoint of both arms was 6 month progression free survival (PFS) with stable QoL (QoL score defined as ±20% from baseline) [23]. The secondary endpoints were response, overall survival (OS), toxicity (using the NCI Common Toxicity Criteria) and exploratory biomarker analysis (protocol pre-specified for VEGF). A two-stage Simon design [25] was used for both studies with 2/15 patients in stage 1 required to have demonstrated response or stable disease (and QoL score) for progression to stage 2 (n = 35 total/arm). PFS was defined as the time from enrolment to progression or death. The trial design assumed a 6 month PFS probability ≥ 20% for the treatment(s) to be considered active with a standard error of <10%. The Kaplan–Meier method was used to evaluate survival statistics. Univariate analyses were performed on OS and the log rank test was used to compare Kaplan–Meier survival curves where indicated (Graphpad Prism Version 4.03, San Diego, CA). To determine if the pre-treatment biomarker levels were prognostic of survival, patients were divided into two groups for the analysis of each marker; those with levels above the median level (high) and those with levels equal to or below the median level (low). Significant factors in the univariate analysis were entered into the Cox regression model (SPSS 17.0) to determine their independent prognostic effect. Sample t-test was used to examine if a difference existed in the biomarker level for the primary end-point (<6 months PFS vs. ≥6 months PFS with stable QoL).

The European Organisation for Research and Treatment of Cancer (EORTC) prognostic index is a validated prognostic tool in MM [26]. This index used Eastern Cooperative Oncology Group (ECOG) ≥1, white cell count > 8.3 × 10^9/l, male gender, sarcomatoid subtype and probable or possible pathological diagnosis as poor prognostic factors. Poor prognosis was defined by the presence of three or more of these factors.

2.1. Biomarker analyses

All biomarkers were measured by ELISA using commercially available kits and in accordance to the manufacturer's instructions, shortly after the conclusion of the study. Serum SMRP was determined using the MESOMARK kit (Fujirebio Diagnostics, Inc., Malvern, PA, USA), serum VEGF by the Quantikine kit (R&D Systems, Minneapolis, MN, USA) and serum CRP by the Alpha Diagnostic International kit (San Antonio, TX, USA). Serum IL-6 and serum sIL-6R were measured by the duo kit (R&D Systems) using Nunc C96 Maxisorp plates (Nunc, Denmark) and TMB Blue Substrate Chromagen (Dako, Sydney, Australia). All samples were assayed in duplicate and the relevant absorbance was measured using a Synergy HT spectrophotometric multiwell plate reader (Bio Tek, Winooski, VT). Analyte concentrations were calculated from a polynomial regression curve of the assay standards fitted using KC4 software (Bio Tek, Winooski, VT).

3. Results

Between April 2001 and August 2003, 34 patients were enrolled in arm A and 29 in arm B. Both studies were closed before achieving the target accrual when pemetrexed became available in Australia. Patient characteristics are shown in Table 1 and were similar in both studies. In arm B, 20 patients had no prior chemotherapy at the time of enrolment, but were considered unsuitable for concomi-