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The Role of Immunotherapy in the Treatment of Mesothelioma

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1. Introduction

Malignant mesothelioma (MM) is an aggressive and incurable malignancy of the mesothelium which is linked largely to previous asbestos exposure. In 2008, 2,249 people in the UK died from MM and median survival is only 6-18 months. The incidence of MM is still rising and not predicted to peak until 2015 in the UK (Hodgson et al., 2005). However, due to the asbestos content of many homes and public buildings, MM will be present for many more decades.

Pemetrexed-cisplatin chemotherapy is the current standard of care, but treatment results in an improvement in median survival of less than 3 months (Vogelzang et al., 2003). Furthermore, due to the age and co-morbidities of patients, many are not eligible for therapeutic intervention. This dictates an urgent need for improved therapies. Pre-clinical and clinical studies conducted over the years have highlighted the sensitivity of MM to immunotherapy. Immunotherapy offers an alternative to conventional therapies, utilising the patients' own immune system to fight the cancer without severe side effects. It may also confer additional benefits such as long-term immunological memory, which protects against future cancer relapse. Furthermore, in a similar manner to the HPV vaccine which is administered in young women to help prevent cervical cancer, some forms of immunotherapy may be offered in a preventative setting in people with known previous asbestos exposure. Most mesothelioma patients are not systemically immunosuppressed (Jasani et al., 2005) thus expected to respond better to immunotherapy than patients with systemically immunosuppressive cancers, such as ovarian cancer.

This chapter will address the complex relationship between MM and the immune system and review the progress of immunotherapy in the treatment of this disease. Furthermore, we will discuss our clinical trial using the targeted vaccine, Trovax®, for the treatment of pleural MM patients.

2. MM inflammation & tumourigenesis

MM has a strong aetiological link with previous asbestos exposure, although genetic factors may also influence susceptibility of individuals to this disease (Weiner & Neragi-Miandoab, 2009). Asbestos exposure causes DNA damage and death of mesothelial cells. One

consequence is the release of the high-mobility group box 1 (HMGB1) protein, a damage-associated molecular pattern molecule which is normally retained in the nucleus by condensed chromatin and is released passively from dead cells. Its release is characteristic of immunogenic cell death (Apetoh et al., 2007) and it actively recruits inflammatory macrophages. HMGB1 and macrophage phagocytosis of asbestos fibres activates the Nalp3 inflammasome and induces secretion of the pro-inflammatory cytokines, interleukin (IL)- 1β and tumour necrosis factor-α (TNF-α) (H. Yang et al., 2010; Dostert et al., 2008). TNF-α is thought to promote malignant transformation of the mesothelium through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB; a protein complex which controls DNA transcription)-dependent mechanism, which allows mesothelial cells with asbestos-induced DNA damage to survive rather than die (H. Yang et al., 2006). This localised inflammation alters the permeability of the mesothelial membrane and facilitates the process of pleural effusion whereby proteins and immune cells from the vascular compartment mobilise to the pleural space, establishing a tumour-associated immune environment.

3. MM Immunity

The immunosurveillance theory by Burnet in the 1950s proposed that lymphocytes continuously recognise and eliminate newly transformed malignant cells. With increasing understanding of how the immune system works in cancer, it is now accepted that immunosurveillance is part of a more complex interaction between the immune system and cancer, desribed as immunoediting (Dunn et al., 2002). The concept is supported by observations such as the depletion of T cells or interferon (IFN)-γ resulting in increased tumour incidence in wild type mice (Koebel et al., 2007). Immunoediting is also likely to occur in humans, as tumour infiltration with T cells and natural killer (NK) cells (both effector immune cell types, able to destroy tumour cells by cell-cell killing) is associated with better prognosis in several malignancies, such as colon cancer (Galon et al., 2006) and indeed MM (Anraku et al., 2008). Furthermore, in immunosuppressed transplant recipients, malignancy is the third most common cause of death and not only from cancers of viral origin (Rama & Grinyó, 2010). It is likely that the immune system in MM, similarly to that in other solid tumours, plays an important role during cancer progression and response to traditional treatments.

3.1 MM immune-engagement

The theory of immune-recognition of the tumour is that professional antigen presenting cells (APC), such as dendritic cells (DC) internalise antigen from dying tumour cells and home to the tumour draining lymph nodes (TDLN). In the TDLN, DC cross-present antigen on major histocompatibility complex class I (MHC I) molecules and in the presence of adhesion and co-stimulatory molecules, prime naïve $CD8^+$ T cells resulting in their clonal expansion. Activated antigen-specific $CD8^+$ T cells leave the TDLN, enter the circulation and migrate towards the tumour. At the tumour site these effector or cytotoxic T cells (CTL) recognise tumour antigen expressed on the surface of tumour cells on MHC I molecules and specifically kill those cells.

3.1.1 Tumour-associated antigens in MM

The presence of tumour-associated antigens (TAA), absent or weakly expressed on healthy cells, is crucial for the instigation of a tumour-specific immune response. Many antigens

may be expressed by the tumour, however not all will necessarily elicit an immune response (Sommerfeldt et al., 2006). Antigens are largely categorised into 4 groups: i) cancer-testis antigens, e.g. New York-ESO, as the name suggests, are thought to be present selectively on cancer cells or in the testis. ii) Differentiation antigens, e.g. mesothelin, are expressed on both malignant cells and on the normal tissue from which they are derived. iii) Overexpressed antigens, e.g. mucin-1 (Muc-1), Wilms tumor-1 (WT-1), folate receptor-α (FR-α) and survivin, are expressed in a variety of normal tissues, but are overexpressed in tumours. iv) Oncofoetal antigens, e.g. 5T4, are expressed predominantly in the developing foetus and in tumour cells.

Antigen uptake by APC, mainly in the periphery, is crucial for the priming of T cells in the TDLN by these APC. TAA may be taken up when a tumour cell is killed by chemotherapeutic agents or undergoes necrosis in hypoxic regions within the tumour. In MM, antigen uptake may even occur at the time of malignant transformation, when asbestos induces the death of mesothelial cells which share some (e.g. differentiation) antigens with malignant cells. However, as many of these antigens are also 'self antigens' present on normal tissues, they may be subject to self-tolerance, which protects the body from autoimmunity. T cells with high affinity to self antigens are either deleted in the thymus during ontogeny or are regulated by peripheral tolerance mechanisms. However, in tumours a lot of these antigens are overexpressed, which, together with other stimulatory signals, may help to break tolerance, leading to immune recognition.

Several TAA have been identified in MM, all of which are potential immune targets and many have also been exploited for targeted immunotherapies.

Mesothelin is a differentiation antigen that is normally present on mesothelial cells but is highly over-expressed in epithelioid mesothelioma, while absent in sarcomatoid subtypes (Ordóñez, 2003). Its gene encodes a precursor protein which is proteolytically processed into two components; a membrane bound protein, mesothelin, and a secreted protein, megakaryocyte-potentiating factor (Chang & Pastan, 1996). Mesothelin is used as a diagnostic marker in MM, while soluble forms of the protein, the levels of which correlate with clinical stage and tumour burden, can be used as surrogate markers for clinical response to therapy (Creaney et al., 2011). Furthermore, soluble-mesothelin related protein, a member of the mesothelin family of proteins, has been identified as a possible biomarker for MM (Robinson et al., 2005) and has been developed as a commercial assay used in the detection and management of MM (Beyer et al., 2007). The normal biological function of mesothelin is unknown, although it has been implicated in cellular adhesion and mestastatic spread (Hassan et al., 2010). Mesothelin can be targeted on tumour cells by CTL (Yokokawa et al., 2005). Furthermore, mesothelin-specific antibodies were found to be elevated in 39.1% of mesothelioma patients (Ho et al., 2005).

WT-1 is a transcription factor that was originally described as a tumour suppressor gene but is now known to be involved in tumourigenesis. It can positively or negatively regulate the expression of various genes involved in cellular proliferation, differentiation and apoptosis (L. Yang et al., 2007). In normal adult tissue, WT-1 is expressed at low levels on haematopoietic stem cells, myoepithelial progenitor cells, renal podocytes and some cells in the testis and ovary (Mundlos et al., 1993). It is known to be upregulated in epithelioid MM but not in the sarcomatoid subtype and furthermore, its expression does not appear to be of significant prognostic value (Kumar-Singh et al., 1997). However, WT-1 is a relevant immune target in MM (May et al., 2007).

Muc-1, also known as epithelial membrane antigen, is a heavily glycosylated transmembrane protein that is normally found on the apical cell surface of normal glandular epithelium of many tissues, on haematopoietic stem cells and normal mesothelial cells (Baldus et al., 2004). It is overexpressed in the majority of epithelioid MM in an altered glycosylation form (Creaney et al., 2008). While it has been shown that Muc-1$^+$ MM cells are subject to CTL-mediated killing *in vitro* (Roulois et al., 2011), there are currently no Muc-1 targeted therapies under development for MM patients.

Survivin is a member of the inhibitor of apoptosis gene family that is implicated in the control of cell division and apoptotic cell death (Altieri, 2004). It is barely detectable in normal adult tissue but specifically upregulated in tumour cells (Ambrosini et al., 1997). In MM, survivin was observed in 91% of surgical specimens and its knockout with siRNA restored the apoptotic potential of the cells (Zaffaroni et al., 2007). Its expression is a negative prognostic indicator in MM and it may play a role in therapy-refractoriness (GJ Gordon et al., 2007). Survivin is also a target for CTL-mediated killing in patients (Andersen et al., 2001).

FR-α is a glycosyl phosphatidylinositol-anchored glycoprotein that is found on the surface of many epithelial cells (Elnakat & Ratnam, 2004; Weitman et al., 1992). It binds folate at high affinity and mediates its transmembrane transport into the cell cytoplasm for use in purine, pyrimidine and ultimately DNA biosynthesis, a process that is essential for rapidly dividing tumour cells (Antony, 1996; Sierra & Goldman, 1999). FR-α is overexpressed in 72% of MM, regardless of subtypes. Furthermore, the level of expression is 2-4-fold higher in the tumour, compared with normal tissues (Bueno et al., 2001). It has also been shown to be a T cell target (Knutson et al., 2006). While the targeting potential of this antigen has been realised in many studies (Salazar & Ratnam, 2007), it has not been tested in MM.

5T4 is a cell surface oncofoetal glycoprotein (Hole & Stern, 1988). It has restricted expression in normal tissues but is overexpressed in numerous malignancies, such as testicular, breast and colon cancer (Southall et al., 1990). Our studies have identified the presence of this antigen on pleural MM cells and we also demonstrated the presence of 5T4-specific T cells and antibodies in patients (Al-Taei et al., Manuscript). It alters cellular dynamics facilitating metastatic spread (Carsberg et al., 1996; Southgate et al., 2010). Consequently, 5T4-targeted therapies are under development, such as antibody-based therapies, conjugated to cytotoxics such as calicheamicin (Boghaert et al., 2008) or Staphylococcal enterotoxin E (ABR-214936) which proved effective in phase II clinical trials in patients with advanced renal cell carcinoma (Shaw et al., 2007). The cancer vaccine Trovax® (a modified vaccinia Ankara virus encoding 5T4) is also undergoing clinical trials. It has been tested in 500 patients in Phase I, II and III 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 (Harrop et al., 2010; DW Kim et al., 2010).

3.1.2 Antigen presentation by MM cells

Correct functioning of the antigen-processing machinery, transporter for antigen presentation-1 (TAP-1) and MHC I expression by tumour cells is necessary for T cell recognition. One study has shown that in four MM primary cell lines, all cells

homogeneously expressed MHC I and II molecules (Mutti et al., 1998). This is in contrast to two further reports, one of which demonstrated the presence of only MHC I molecules on cell lines (Christmas et al., 1991), while the other also found only MHC I and not II expression in 100% of 44 tissue sections from MM patients (Yamada et al., 2010). The widespread detectable expression of MHC I molecules on MM tumour cells is an important feature for immunotherapy as they are downregulated in many cancers to evade immune cell killing (Romero et al., 2005). Furthermore, there are no reports on TAP-1 downregulation in MM.

3.1.3 Clinical relevance of MM immunity

The prognostic value of immune parameters in MM has been reported by independent research groups. Leigh and Webster were the first to report the significance of T cell infiltration on survival in MM patients, demonstrating a positive correlation between T cells and increased survival (9 months vs. 18 months in patients who showed infiltration) (Leigh & Webster, 1982). Later, phenotypic analysis identified that high frequencies of $CD8^+$ tumour infiltrating T cells correlated with significantly increased proportions of apoptotic tumour cells and better progression-free and overall survival than those in patients with low $CD8^+$ T cell frequencies in the tissue (Anraku et al., 2008). In the same study, increased frequencies of $CD4^+CD25^+$ T cells and $CD45RO^+$ memory T cells tended to be negative prognostic indicators following induction chemotherapy. Yamada et al. studied T cell infiltration in 44 patients by immunohistochemistry and also reported a correlation between higher levels of $CD8^+$ T cell infiltration and clinical outcome (Yamada et al., 2010). Higher frequencies of infiltrating $CD3^+$ T cells correlated with worse overall survival but only in patients with sarcomatoid or biphasic histology (Burt et al., 2011). However, the authors did not elaborate on the different T cell subsets and potential ratios of regulatory T cells (Treg) to $CD8^+$ T cells in these patients. MM tissues typically contain high numbers of myeloid cells (B Davidson et al., 2007). High myeloid cell counts, such as monocytes and macrophages, are negative prognostic indicators in MM with sarcomatoid and biphasic histology (Burt et al., 2011). Conversely, no correlation was noted between macrophage infiltration and prognosis in epithelioid patients. Tumour infiltration by tryptase expressing proinflammatory mast cells has also been identified as a positive prognostic indicator in patients (Alì et al., 2009). In addition, several reports have documented spontaneous regression in MM patients with a possible immunological basis (Robinson et al., 2001; Pilling et al., 2007; Allen, 2007).

The studies highlighted in this section have demonstrated that MM is a sufficiently immunogenic cancer and it induces immune recognition, immune cell infiltration and immune-mediated killing, the extent of which defines disease prognosis.

3.2 MM immune-escape

In a recent review, the hallmarks of tumour development were described incorporating our most recent knowledge about these events. Integral to all these hallmarks is the genomic instability of tumours, which fosters aberrant tumour phenotypes (Hanahan & Weinberg, 2011). Immune evasion has recently been included in this concept. Immune evasion may

arise due to immunological pressure which drives tumour transition from immunosensitive to immunoresistant variants. This was shown in a model where tumours only maintained their immunogenicity in immunodeficient mice (Shankaran et al., 2001).

Localised immune evasion has many different forms. Tumour cells can express immunosuppressive markers and release immunosuppressive soluble factors which may in turn promote the accumulation of regulatory immune cells at the tumour site. Alternatively, the inflammatory tumour environment may non-specifically attract suppressor cells to regulate the level of inflammation (Bunt et al., 2007). The immunosuppressive nature of the tumour environment in MM has been widely reported (DeLong et al., 2005; Hegmans et al., 2006) and will also be addressed in another chapter in this book. Immunosuppressive influences that are relevant to MM immunotherapy are discussed here.

3.2.1 Tumour-mediated immune escape mechanisms

MM tumour cells have been shown to release cytokines and chemokines that cause the preferential accumulation of immunoregulatory cells at the tumour site. Treg are characterised as $CD4^+CD25^+$ T cells that express the transcription factor forkhead box P3 (foxp3). They can inhibit both $CD4^+$ and $CD8^+$ T cells by release of immunosuppressive soluble factors such as IL-10 and transforming growth factor (TGF)-β (Hall et al., 2011). The chemokine CXCL12 was identified in all tissue samples from 6 MM patients and was chemotactic to Treg. MM can therefore actively recruit Treg to the tumour site (Shimizu et al., 2009). Also, cytokines released by tumour cells such as IL-6 and IL-8 have been shown to cause preferential migration of Treg towards the tumour (Eikawa et al., 2010). There are conflicting reports for the role of Treg in the survival of MM patients. One study showed that while patients with a high level of $CD4^+CD25^+$ T cells infiltration demonstrated shorter survival (though not statistically significant), the presence of foxp3, did not affect survival (Anraku et al., 2008). Another study concluded that Treg do not mediate immunosuppression in a MM model (Jackaman et al., 2009), while significant increase in the number of Treg was not seen in MM patients (Meloni et al., 2006). However, in a clinical trial involving 66 patients treated with IL-2, Treg frequency was a significant negative prognostic factor (Alì et al., 2009). The frequencies of infiltrating Treg, rather than those in the periphery, may serve as relevant indicators of disease prognosis.

Normal and malignant mesothelial cells can also release cytokines that are chemotactic for monocytes including macrophage inflammatory protein-1, monocyte chemotactic protein-1, granulocyte-colony stimulating factor and granulocyte-macrophage-colony stimulating factor (GMCSF) (Schmitter et al., 1992). Once monocytes infiltrate the tumour, they can differentiate into tumour-associated macrophages (TAM) that are polarised towards the M2 suppressor phenotype (Sica, 2010).

Myeloid-derived suppressor cells (MDSC) are a phenotypically heterogeneous population of myeloid cells at different stages of maturation. They are well characterised in mouse models but less well studied in humans. MDSC inhibit T cell effector functions through a variety of mechanisms (Ostrand-Rosenberg, 2010). While the significance of these MDSC in MM has not been identified, they have been targeted by immunotherapies with promising results (discussed later).

TGF-β is a homodimeric protein with three isoforms; TGF-β1, TGF-β2 and TGF-β3 (Massagué, 1987). It is secreted by various immune cells such as macrophages, neutrophils, lymphocytes and also by malignant cells, including MM (Kumar-Singh et al., 1999). In cancer, TGF-β is a potent tumour promoter, stimulating angiogenesis and altering the stromal environment and is also a powerful local and systemic immunosuppressor (Mantel & Schmidt-Weber, 2011). TGF-β has been implicated in the cytokine profile shift of T cells which infiltrate the tumour in a mouse model of MM, from pro-inflammatory (interferon; IFN-γ) to anti-inflammatory (IL-4) cytokine-producing cells (Jarnicki et al., 1996).

MM tumours produce vascular endothelial growth factor (VEGF) (Strizzi et al., 2001) that mediates angiogenesis. VEGF production by tumours encourages bulky tumour growth and metastatic spread. High levels of VEGF has also been associated with a resistance to IL-2 immunotherapy (Bonfanti et al., 2000).

3.2.2 Resistance to CTL killing

Tumor cells also develop mechanisms to evade T cell killing, such as suboptimal antigen presentation, resulting in the lack of recognition by CTL (Setiadi et al., 2007). Disregulation of the tumour suppressor, p53, may lead to resistance to apoptotic signals. In MM, merlin is a known regulator of murine double minute 2 degradation (Sekido et al., 1995) with implications on p53 regulation. Cell-cycle checkpoint control defects are frequent in MM (López-Ríos et al., 2006), such as consistent overexpression of checkpoint kinase 1, a DNA damage-induced checkpoint kinase in S and G2/M phases (Romagnoli et al., 2009), serving as another potential CTL resistance mechanism. Upregulation of anti-apoptotic and multi-drug resistance pathways may also impact on CTL sensitivity, such as overexpression of the B-cell CLL/lymphoma 2 family of proteins, especially myeloid cell leukemia sequence 1 (SL O'Kane et al., 2006), and abnormal activation of the Raf/MEK/ERK pathway (de Melo et al., 2006) and the PI3K/AKT pathway (Garland et al., 2007) in MM. CD200 is a potential diagnostic marker of MM (GJ Gordon et al., 2002) with inhibitory effects on mixed lymphocyte reaction and NK-cell cytotoxicity (Wright et al., 2003). B7-H1 (programmed cell death 1 (PD-1) ligand 1) is also expressed on MM (AJ Currie et al., 2009) and negatively regulates the activity of PD-1-expressing T cells (Berthon et al., 2010).

Similar to other solid tumours, MM carries the signs of immunological pressure, as several immune evasion mechanisms can be observed in patients. These not only serve as evidence of engagement between the tumour and the immune system, but also offer potential targets to remove evasion mechanisms and empower the immune system to attack the tumour.

4. Immunological interventions in MM

Immunotherapies can either be non-specific, stimulating the immune system in a general way which may also induce or amplify anti-tumour responses or targeted at a known tumour antigen, generating or boosting specific anti-tumour immune responses. Many immunotherapeutic strategies have been tested in MM.

4.1 Non-specific therapies

The aim of non-specific immunotherapy is to induce general immune stimulation, powerful enough to break immune tolerance induced by the tumour, thereby promoting tumour cell destruction.

4.1.1 Cytokine therapy

Cytokines are best described as messengers of the immune system. They are secreted by both tumour and immune cells and have immunoregulatory roles. IL-2 and IFN-α2b are two cytokines currently approved by the FDA for the treatment of cancer. Both IL-2 and IFN-α2b have demonstrated activity against renal cell carcinoma, melanoma, lymphoma and leukaemia. Cytokines tested in MM are stated in Table 1.

Interferons are a family of cytokines that influence the quality of cellular immune responses and amplify antigen presentation to specific T cells. There are two major classes; IFN-α, IFN-β (the type I interferons secreted by virus infected cells and DC) and IFN-γ (the type II interferon secreted by T cells, NK cells and macrophages). The immunoregulatory effects of IFNs extend to antibody production, NK and T cell activation, macrophage function and MHC antigen expression (Wang et al., 2011). Gene delivery of IFN is the prevalent mode of delivery in patients as it leads to high and prolonged local cytokine concentrations that are sufficient to induce immunogenic tumour cell death, break tolerance and activate anti-tumour immune responses (Vachani et al., 2007).

In preclinical studies IFN-γ has been shown to skew the immunosuppressive M2 phenotype of TAM, converting them into M1-polarised immunostimulatory macrophages (Duluc et al., 2009). IFNs have also been shown to have anti-angiogenic and anti-proliferative effects on the tumour (Rosewicz et al., 2004). The antiproliferative properties of IFN have also been shown in several mesothelioma cell lines (Zeng et al., 1993).

Viral mediated IFN gene-transfer is very popular in IFN immunotherapy. Sterman and colleagues have carried out several clinical trials delivering IFN. Their first trial involved the administration of a single dose of adenovirally encoded IFN-β, where four out of 10 patients showed clinically meaningful responses. Administering two doses rather than one showed no survival benefit due to neutralising antibodies against the viral vector. Subsequently they used a similar adenoviral vector but substituting IFN-β for IFN-α2b and found that to be more potent as six of the nine patients treated displayed clinical responses. Boutin et al. conducted a study involving 89 patients. They showed that there was an overall response rate of 20%. However, this went up to 45% when data from only stage I patients was analysed (Boutin et al., 1991). This trend for better survival in early stage patients was commonly seen with interferon therapy.

Interleukins are another class of cytokines with multiple immunoregulatory properties. **IL-2** is a pro-inflammatory cytokine produced by activated T cells and promotes T cell proliferation, differentiation and survival. It can also enhance NK cells, neutrophil and macrophage function. On the other hand, it can also boost Treg function (Malek, 2008; Foureau et al., 2011).

In MM cell lines, IL-2 was shown to affect the cell cycle, resulting in an accumulation of cells in the G0/G1 phase with subsequent apoptosis (Porta et al., 2000). In the AE17 mouse model, IL-2 markedly enhanced $CD8^+$ CTL activity and decreased tumour vasculature, resulting in tumour regression, with mice remaining tumour free for >2 months (Jackaman et al., 2003). This was mirrored in a clinical trial where IL-2 administered preoperatively induced significantly greater recruitment of $CD8^+$ T cells, tryptase mast cells and also inhibited tumour-associated vasculature (Alì et al., 2009). There have also been reports that

Therapy	Route of Administration	Patients Recruited	Reference
Interferon			
IFN-α2b	Intrapleural via Adenoviral gene transfer	9	(Sterman et al., 2011)
IFN-β Phase I	Intrapleural via Adenoviral gene transfer	10	(Sterman et al., 2010)
IFN-β	Oncolytic vesicular stomatitis virus gene transfer	Murine model	(Willmon et al., 2009)
IFN-β Phase I	Intrapleural via Adenoviral gene transfer	7	(Sterman et al., 2007)
IFN-β	Adenoviral gene transfer	Murine model	(Kruklitis et al., 2004)
IFN-β	Adenoviral gene transfer	Murine model	(Odaka et al., 2002)
IFN-γ	Adenoviral gene transfer	Murine model	(Gattacceca et al., 2002)
IFN-β	Adenoviral gene transfer	Murine model	(Odaka et al., 2001)
IFN-α	Systemic	Murine model	(Bielefeldt-Ohmann et al., 1996)
IFN-α2b Phase II	Systemic	14	(Ardizzoni et al., 1994)
IFN-γ	Intrapleural	89	(Boutin et al., 1994)
IFN-α2a	Systemic	25	(Christmas et al., 1993)
IFN-γ	Intrapleural	22	(Boutin et al., 1991)
IFN-β Phase II	Systemic	14	(Von Hoff et al., 1990)
Interleukin			
IL-2	Pre-operative intrapleural	60	(Alì et al., 2009)
IL-2	Intratumoural	Murine model	(Jackaman et al., 2003)
IL-2	Intrapleural	12	(Porta et al., 2002)
IL-2 Phase II	Combined intravenous and subcutaneous	29	(Mulatero et al., 2001)
IL-2 Phase II	Intrapleural then low dose subcutaneous	31	(Castagneto et al., 2001)
IL-2	Intratumoural via Vaccinia Virus gene transfer	6	(Mukherjee et al., 2000)
IL-2 Phase II	Intrapleural	22	(Astoul et al., 1998)
IL-2	Intrapleural	11	(Astoul et al., 1995)
IL-2 Phase I/II	Intrapleural	23	(Goey et al., 1995)
IL-12	Systemic or intratumoural	Murine model	(Caminschi et al., 1998)
IL-12 Phase I	Intraperitoneal	1	(Lenzi et al., 2002)
GMCSF			
GMCSF	Intratumourally	14	(Davidson et al., 1998)
GMCSF	Intratumourally via Fowlpox gene transfer	Murine model	(Triozzi et al., 2005)

Table 1. Cytokine therapy trials conducted in MM

IL-2 activated non-specific NK-like cells with *in vitro* cytotoxic activity (Astoul et al., 1995). Other effects seen with IL-2 include the resolution of pleural effusion which was observed in 90% of patients receiving intrapleural IL-2 followed by low dose IL-2 maintenance (Castagneto et al., 2001). IL-2 trials in MM have been met with varying successes. Systemic administration was associated with adverse toxicities and suboptimal response rates (Bernsen et al., 1999). More promising results were obtained with localised delivery of IL-2, however it remains more toxic than IFN therapy.

More experimental settings have also been tried, such as the intratumoural administration of IL-2 with a poly-N-acetyl glucosamine-based polymer gel which not only caused slow release of the cytokine, but also triggered inflammation, recruiting inflammatory cells to the tumour site in a mouse model of MM (van Bruggen et al., 2005). A possible reason for the weak responses to IL-2 therapy is that IL-2, like IFN, has a short half-life of around 10 mins and may benefit from viral gene transfer to prolong its effects. Vaccinia virus-mediated gene transfer of IL-2 resulted in direct retardation of tumour growth, as well as the release of IL-2 (Mukherjee et al., 2000; Jackaman & Nelson, 2010). However, as only 6 patients were treated, it is difficult to compare it to recombinant IL-2.

IL-12 is a proinflammatory IL with potent immunoregulatory effects on NK and T cells (Trinchieri, 1995). In a murine model of MM, IL-12 was administered intratumourally, resulting in temporary tumour regression, correlating with the influx of $CD4^+$ and $CD8^+$ T cells. However, tumour regrowth was evident after cessation of treatment indicating that protective memory was not generated (Caminschi et al., 1998). In a mixed patient phase I trial, with one mesothelioma patient included, the MM patient showed a complete response when treated with intraperitoneal IL-12 prior to surgery and remained progression-free at two years after therapy. The response correlated with increased IFN-γ and TNF-α serum levels (Lenzi et al., 2002).

GMCSF enhances the APC activity of DC and macrophages as well as enhancing the tumour cell cytotoxic activity of macrophages (Warren & Weiner, 2000). It also upregulates costimulatory molecules on DC (Larsen et al., 1994). When released by MM cells, in contrast to its immunostimulatory properties, it can increase tumour cell proliferation and recruit suppressor cells (MDSC) to the tumour site (Oshika et al., 1998; Young et al., 1992). In both human and animal studies, GMCSF had very limited anti-tumour efficacy. Intratumoural infusion of GMCSF into 14 patients was associated with systemic toxicity. Tumour necrosis was evident around the catheter in one patient while another showed marked T cell infiltration into the tumour and a partial response. Of the other patients, 10 had progressive disease, and three, including the patient with the necrosis in the tumour, had no response (JA Davidson et al., 1998). The intratumoural administration of GMCSF expressed by fowlpox virus in a mouse model had little effect and GMCSF-treated animals died by day 30 vs. control animals by day 35 (Triozzi et al., 2005). It is not clear whether the tumour-potentiating effects of GMCSF were responsible for this observation. No further GMCSF trials have been pursued in MM patients.

4.1.2 Costimulation strategies

CD40 costimulation is another strategy for immunotherapy. CD40 is a glycoprotein that belongs to the TNF receptor superfamily and is expressed on DC and monocytes

(Banchereau et al., 1994). Its ligand, CD40L, is preferentially expressed on mast cells and $CD4^+$ T cells and has an important role in determining whether the CTL response is initiated or tolerised. Antigen presentation in the absence of CD40 ligation leads to T cell tolerance (Schoenberger et al., 1998). Interaction of CD40 with its ligand during antigen presentation results in production of IL-12 and upregulation of B7-1 and B7-2 costimulatory molecules necessary for generating T cell responses (Schoenberger et al., 1998). Exogenous CD40 ligation has been extensively explored as an immunotherapeutic strategy (Todryk et al., 2001).

In a mouse model of MM, where the CD40L was administered via an adenoviral vector, intratumoural delivery of CD40L resulted in not only the regression of the primary tumour, but also regression at distal sites. This was shown to be mediated by $CD8^+$ T cells (Friedlander et al., 2003). Agonistic CD40 antibody administered intravenously did not generate long-lasting immunological memory and tumour regrowth was evident after cessation of treatment (Stumbles et al., 2004). Agonistic antibody in low doses administered into the tumour-bed resulted in 60% survival rate with most mice cured. In this model, $CD8^+$ T cells were not required for anti-tumour response, but instead B cells were the mediators (Jackaman et al., 2011). No patient trials have been reported for CD40 in MM, however, pre-clinical data sets the precedent for a future trial.

Toll-like receptors (TLR) are mainly expressed on antigen presenting cells and control their activation, maturation and migration. Similarly to CD40, antigen presentation in the absence of TLR ligation may also induce tolerance and so they can alter the context of the immune response. Persistent TLR stimulation has been shown to break tolerance against TAA (Y Yang et al., 2004). In a mouse model of MM, treatment with TLR agonists specific for TLR3, TLR7 and TLR9 resulted in tumour resolution in 40% of mice, with a delay in tumour progression in the other treated mice. The treatment induced type I IFNs and was dependent on the induction of $CD8^+$ T cells at the tumour site (AJ Currie et al., 2008). TLR agonists may represent a promising new agent group to stimulate anti-tumour immune responses, especially in an adjuvant role.

4.1.4 Adoptive cell transfer

Dendritic cell based therapies are an exciting area of research and have shown much promise in MM. These will be discussed in another chapter in this book.

4.1.3 Other strategies

Soluble type II TGF-β receptor, which binds TGF-β1 and TGF-β3, was implemented in three MM mouse models. The AB12 and AC29 models produce large amounts of TGF-β, while the AB1 model does not. Predictably, suppression of tumour growth attributed to the addition of the soluble TGF-β receptor was only evident in the AB12 and AC29 tumours. $CD8^+$ T cells were responsible for tumour cell killing following treatment, but were less efficient in bulky TGF-β secreting tumours. Furthermore, complete regressions were not observed (Suzuki et al., 2004).

SM16 is a small molecule inhibitor of TGF-β type I receptor kinase. When administered locally, it also inhibited tumour growth by a $CD8^+$ T cell-dependent mechanism.

Furthermore, post-surgical administration of SM16 reduced tumour recurrence (Suzuki et al., 2007).

CD26 is a cell surface glycoprotein with dipeptidyl peptidase IV activity in its extracellular domain and is a T cell activation molecule, playing roles in T cell costimulation and signal transduction processes (Ishii et al., 2001; Ohnuma et al., 2004). It is also highly expressed on MM, but not in benign mesothelial tissue, and implicated in tumour growth and metastases (Inamoto et al., 2007). A humanised anti-CD26 antibody was shown to lyse MM cell lines and also prolong survival in a mouse model of MM (Inamoto et al., 2007).

Anti-**CD25** antibody, applied intratumourally to target Treg within the tumour, resulted in 85% depletion of intratumoural Tregs and inhibited tumour growth, which was extended with multiple injections (Needham et al., 2006). These data suggest that removing Treg may contribute to the success of combination therapies.

4.2 Targeted therapy

Tumour-specific markers that have been exploited for targeted therapy either in pre-clinical or clinical settings are described below.

Mesothelin has been shown to be a T cell target as CTL specific for mesothelin peptides can lyse tumour cells (Yokokawa et al., 2005). Furthermore, mesothelin-specific antibodies were elevated in 39.1% of mesothelioma patients (Ho et al., 2005). It is the most widely studied antigen in MM and is at the forefront of many MM-targeted therapies. Antibody-guided targeting of mesothelin accounts for the majority of mesothelin-targeted therapies. In pre-clinical studies, monoclonal antibody induced antibody-dependent cellular cytotoxicity against human MM cell lines in a xenograft model (Inami et al., 2010).

SS1P is a high affinity anti-mesothelin murine antibody which has been genetically combined with a fragment of the potent cytotoxic *Pseudomonas* endotoxin, PE38. A phase I trial established the safe dose of the antibody and showed evidence of modest clinical activity (Kreitman et al., 2009).

MORAb009 (Morphotek Inc.) is a high affinity chimeric (mouse/human) monoclonal antibody. It kills mesothelin positive cell lines via antibody-dependent cellular cytotoxicity (Hassan et al., 2007). A phase I trial administering IV MORAb-009 was carried out in 13 mesothelioma patients and no complete or partial responses were noted (Hassan et al., 2010). Morab009 is currently being evaluated in a phase II trial in patients with unresectable MM, where it is being administered in combination with pemetrexed and cisplatin (ClinicalTrials.gov NCT00738582).

Studies demonstrating mesothelin-specific $CD8^+$ T cell responses in patients (Thomas et al., 2004) have provided a rationale for mesothelin as a tumour vaccine. CRS-207 is a vaccine consisting of a live-attenuated bacterium *Listeria monocytogenes* encoding human mesothelin. A phase I clinical trial of CRS-207 for the treatment of 17 patients with mesothelin-expressing tumours is being conducted.

WT-1 peptide mix, incorporating one CD8 epitope, two CD4 epitopes and one peptide with both CD8 and CD4 epitopes, was developed. The peptides were combined with a Montanide adjuvant before injection and the injection site was primed with GMCSF. Nine MM patients

were treated, eight of which developed disease progression and died. One patient however, remained progression-free for 36 months after the start of the study (Krug et al., 2010).

5T4. We are conducting a phase II clinical trial in pleural MM patients due to start later in the year. Patients will be treated with TroVax® (9 injections) in combination with the standard chemotherapy, pemetrexed and cisplatin (4 cycles) according to the scheme shown in Figure 1. Twenty-six chemotherapy-naïve patients with confirmed MM disease and who have normal baseline haematology will be recruited to the trial. The primary endpoint of the trial will be the safety of the regimen and detection of 5T4-specific immune responses either by T cell stimulation assays or the measurement of anti-5T4 antibodies in the patients' plasma. The secondary endpoint will be progression-free survival and overall survival at 6 & 12 months following treatment initiation.

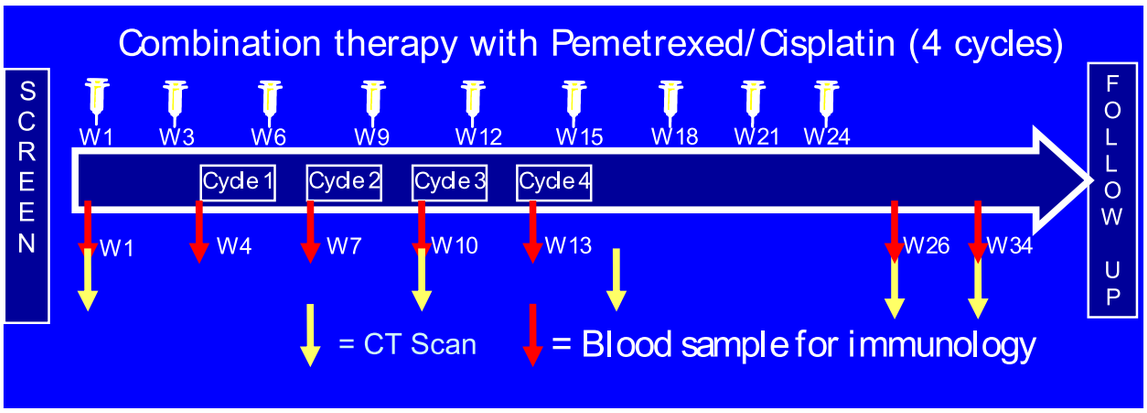

Fig. 1. Schematic diagram of the treatment schedule in the TroVax® trial. Syringes indicate points of vaccine administration. The cycles indicate chemotherapy treatments. W = weeks following trial initiation.

4.3 Combined immunotherapies

As the clinical efficacy of single agent immunotherapies has been limited, combination immunotherapies may induce improved clinical outcomes, particularly if the strategies target both immunosuppressive and immunostimulatory immune mechanisms (Zitvogel et al., 2008). Kim et al. combined SM16, an orally active TGF-β receptor inhibitor, with adenovirally delivered IFN-β in a mouse model of MM and lung cancer. When administered alone, adenoviral IFN-β slowed tumour growth whereas TGF-β inhibition with SM16 alone decreased the size of the tumours and induced one complete regression. However, combination therapy resulted in synergistic effects by shrinking all tumours and inducing complete remissions in four out of five mice. This coincided with significant increases in the frequencies of $CD45^+$ and $CD8^+$ T cells and NK cells (S. Kim et al., 2008).

5. Combination chemo-immunotherapy in MM

Recently, much work has been carried out on the immune-mediated anti-tumour mechanisms of chemotherapy and how chemotherapy could be used to facilitate tumour immunity. Physical destruction of tumour cells by chemotherapy not only makes it a more manageable target for immune attack but also provides tumour antigen for the cross-

presentation pathway. The immune-potentiating effects of chemotherapy have become a new focus in the field of tumour targeted immunotherapy (Lake & Robinson, 2005). On the other hand, improving immune parameters prior to chemotherapy can chemosensitise cells and augment the cytotoxic effects of chemotherapy (Radfar et al., 2009).

5.1 Immunogenic effects of chemotherapy

There is a paradoxical relationship between chemotherapy and the immune system whereby lymphodepletion may be reconciled with the induction of effective anti-tumour immunity. Lymphodepletion is a common side effect of chemotherapy at therapeutically relevant concentrations. Lymphodepletion can be beneficial to tumour immunity as it has been shown to enhance T cell homing into tumour-beds and intra-tumoural proliferation of effector cells (Dudley et al., 2002). Some reports claim that chemotherapy actually spares memory T cells (Coleman et al., 2005). Furthermore, following lymphodepletion, there is a repopulation of naïve T cells which are biased towards self-antigen reactivity which could be exploited as there is decreased competition for cytokines and decreased tumour-derived immunosuppressive effects (Dummer et al., 2002). Many studies have also examined the immunological effects of low-dose chemotherapy, demonstrating immune potentiating effects of chemotherapy, such as increased APC function of DC, a phenomenon known as chemomodulation (Zitvogel et al., 2008; Shurin et al., 2009).

Cyclophosphamide was shown to selectively eliminate Tregs in animal models and patients (Ghiringhelli et al., 2004; Ghiringhelli et al., 2007; van der Most et al., 2009). Cyclophosphamide was also found to deplete 75% of total $CD8^+$ T cells while simultaneously conferring a strong $CD8^+$ T cell-dependent anti-tumour response which culminates in 75-100% cure rate in a MM mouse model. Cyclophosphamide sensitised tumour cells to TRAIL (tumour necrosis factor related apoptosis-inducing ligand)-mediated apoptosis induced by $CD8^+$ T cells, in the absence of their expansion (van der Most et al., 2009).

The cyclooxygenase-2 (COX-2) inhibitor, celecoxib, has been shown to inhibit MDSC in a mouse model of MM, preventing the release of reactive oxygen species from MDSC and inhibiting their expansion. This resulted in a reversal of T cell tolerance (Veltman et al., 2010) and will be addressed in more detail in another chapter in this book. Gemcitabine is another chemotherapeutic agent that has been shown to inhibit MDSC and restore $CD8^+$ T cell function (Le et al., 2009).

Melphalan and mitomycin-C upregulate the expression of T cell co-stimulatory molecules, enabling the tumour cells themselves to actively present tumour antigens to T cells (Sojka et al., 2000). 5,6-dimethylxanthenone-4-acetic acid, a vascular disruptive agent, induces activation of TAM, leading to their tumour infiltration and subsequent influx of $CD8^+$ T cell resulting in tumour cell killing in a MM mouse model. This treatment also generated protective immunity against rechallenge (Jassar et al., 2005).

Some chemotherapies can have both immuno-stimulatory and –inhibitory effects which can cancel each other out. In MM mouse model, zoledronic acid was found to impair myeloid cell differentiation, leading to a reduction in the frequency of TAMs. However, this simultaneously led to increased infiltration by immature myeloid cells, resembling MDSC, which might explain the lack of improvement in overall survival (Veltman et al., 2010).

Immunomodulatory roles for the standard first-line chemotherapy with pemetrexed and cisplatin, have also been identified. Patients treated with pemetrexed-cisplatin have more $CD8^+$ tumour infiltrating T cells which correlate with improved survival when compared to that in patients on cisplatin-vinorelbine (Anraku et al., 2008). This demonstrates that pemetrexed and cisplatin may have immune-potentiating effects. Indeed, a study has shown that pemetrexed sensitises cells to immune cell killing by upregulating the death receptor, DR5, on the surface of tumour cells (Su et al., 2011). DR5 is an important mediator of the extrinsic apoptotic signalling pathway and when presented on the cell surface, it binds to TRAIL expressed on T cells (Whiteside, 2007). Cellular FLICE-inhibitory protein (cFLIP), a protein which inhibits caspase mediated apoptosis, was also downregulated by pemetrexed (Su et al., 2011), suggesting that pemetrexed can sensitise tumour cells to TRAIL mediated apoptosis. These effects of pemetrexed may contribute to the dramatic tumour control in a patient on pemetrexed alone (Fasola et al., 2006).

Pemetrexed has also been shown to induce autophagy. Autophagy refers to the sequestration of cytosolic or cellular components in autophagosomes for subsequent degradation. It plays a part in many cellular processes including cell death and antigen processing for MHC II presentation (Deretic, 2006). Pemetrexed promotes autophagy leading to apoptotic cell death, however, whether this leads to antigen presentation remains to be seen (Bareford et al., 2011). Of particular interest is that COX-2 inhibitors, mentioned above, can enhance the cytotoxic effects of pemetrexed (S. L. O'Kane et al., 2010).

Cisplatin has also increased the sensitivity of tumour cells to cell lysis induced by cytotoxic T cells, though the mechanism was not elucidated (Collins & Kao, 1989). More recently, it was reported that cisplatin activates the Fas-ligand (CD95)-related apoptotic pathway in tumour cells. CD95 is widely expressed on MM cell lines but appears to be dysfunctional (Stewart et al., 2002).

5.2 Combination chemoimmunotherapy

As mentioned above, cisplatin sensitises tumour cells to immune cell- mediated killing and has been exploited in several chemo-immunotherapeutic strategies, the majority of which utilise IFN as the immunotherapeutic component. While IFN-α has had limited effectiveness as a single agent, Sklarin et al. showed that IFN-α was able to increase the therapeutic value of cisplatin and mitomycin (Sklarin et al., 1988). This paved the way for several studies that utilised IFN-α in combination with chemotherapeutic agents. A study by Tansan et al. administered cisplatin and mitomycin with IFN-α2b in 19 patients. There was a non-significant difference in the median survival of patients that were treated with this combination (15 months) compared to the control (8 months) (Tansan et al., 1994). In a similar study utilising the same combination but with IFN-α2a in 43 patients, there was no significant survival benefit in the treated group (11.7 months) vs. the untreated group (7 months) (Metintas et al., 1999). Twenty-six patients treated with cisplatin and IFN-α2a showed a similar median survival time of 12 months vs. 8 months (Soulié et al., 1996). While the median survival was not significant between the treatment groups, the survival times of the responders vs. non-responders were significant. A phase II study utilised doxorubicin and IFN-α and showed that while the combination was more effective than either agent alone, survival was not significantly prolonged (Upham et al., 1993). Cisplatin with doxorubicin (shown to elicit immunogenic cell death) (intravenous) and IFN-α2b

(subcutaneous) was administered in 37 MM patients in a phase II trial. There were considerable toxicities and an overall response rate of 29%. One and two year survival was 45% and 34%, respectively (Parra et al., 2001). Cisplatin alone with IFN-α 2a in 13 MM patients resulted in clinical responses in 11 patients (one with a complete response), but the regimen was again very toxic.

It would seem that although IFN-therapy is relatively well tolerated, in combination with chemotherapy non-negligible toxicities are evident. Furthermore, mono-IFN-therapies utilised adenoviral IFN, but many of the combination therapies with cisplatin utilised recombinant IFN, which may not be optimal to the regimen.

Cisplatin synergistically inhibited proliferation of mesothelioma cell lines when co-applied with HER-2/neu antibody, Trastuzumab (Toma et al., 2002).

Pemetrexed has been combined with the VEGF antibody, bevacizumab, in a MM immunodeficient mouse model. Bevacizumab alone restricted tumour growth, inhibited the formation of large blood vessels and also prevented the development of pleural effusion. Combination with pemetrexed more effectively suppressed pleural effusion and increased survival in mice (Li et al., 2007).

Combining pemetrexed with Treg blockade in another mouse model of MM was also synergistic in prolonging survival. This combination was associated with decreased numbers of Treg in the tumour, increased IL-2 production, DC maturation and increased numbers of tumour-infiltrating IFN-γ-producing $CD8^+$ T cells when compared to either agent alone (Anraku et al., 2010).

Although combination of immunotherapy with pemetrexed and/or cisplatin is in an early phase, it is already providing promising results. It remains to be seen however, whether lowering the dose of chemotherapy in order to prevent high grade toxicity diminishes the clinical benefit.

6. Summary and concluding remarks

The importance of applying immunotherapy in combination with other treatments is becoming increasingly obvious from other cancers, mainly melanoma. It has become apparent that generating a transient immune response with a single specificity is not sufficient for clinical benefit; immunosuppressive effects need to be removed and any ensuing broad-specificity immune response needs to be sustained and enhanced.

It has been proposed recently that successful immunotherapy requires multiple elements, such as suppressing or removing tolerance, generating anti-tumour effector immune cells via vaccination, boosting the immune response by inducing immunogenic cancer cell death, checking and compensating for genetic polymorphism which may cause suboptimal immune responses, and finally, adjuvant treatment may be needed to maintain the immune response (Zitvogel et al., 2008).

Immunotherapies administered to MM patients can be analysed from the point of view of these categories. Removing immunosuppression by targeting Treg cells or blocking VEGF in combination with chemotherapy, the latter generating tumour cell death and providing tumour antigens, resulted in impressive results in mouse models. Immunosuppression may

vary in patients according to histological subtype. Patients with the sarcomatoid variant of MM have a tendency towards more suppressive haematology (ElGendy et al., 2006). Analysing regulatory cell types in the circulation or in the tissue may provide useful information about the requirement of removing immunosuppression before immunotherapy in some patients.

The largest numbers of clinical trials in MM patients were carried out by applying single cytokines. It does not seem surprising now that addressing only the immune cell stimulation step did not translate to clinical success. It would be interesting to see how combining IL-2 or IFN not only with chemotherapy but also with e.g. a cancer vaccine would improve outcome. On the other hand, some viral vectors delivering tumour antigens, such as vaccinia, trigger considerable IFN-α production by infecting DC in the host (Pascutti et al., 2011).

Combination of chemotherapy and immunotherapy in fact has shown synergy in MM patients, as discussed earlier. However, these combinations are often associated with significant toxicities. These maybe alleviated by using less cytotoxic concentrations of chemotherapeutic agents in combination therapies, as demonstrated in *in vitro* studies (Zitvogel et al., 2008; Shurin et al., 2009)

As MM is a relatively rare disease, multi-arm trials with large patient numbers, comparing several treatment modalities, are challenging to organise. On the other hand, information obtained in mouse models does not always translate well to human studies. The model itself influences the results, as observed e.g. in the AB12 and AC29 models which secrete large amounts of TGF-β, whereas the AB1 mouse model does not. AB12 also produce much more GMCSF than AC29 and AC29 is a much slower growing tumour (Triozzi et al., 2005). The models do not always reflect that many patients have large tumours. In the AE17 mouse model of MM the anti-tumour effect of IL-2/anti-CD40 therapy was only mediated by CD4+ or CD8+ T cells when the mice had large tumours, while with a small tumour burden these cells were not crucial. Thus the results obtained in pre-clinical models may not be reproducible in humans.

Immunotherapy may not lead to tumour shrinkage, however, in an incurable cancer such as MM, stabilisation of the disease and palliation of symptoms are just as important. Bevacizumab in combination with pemetrexed have had profound affects on alleviating pleural effusion, thus improving the quality of life of patients (Li et al., 2007). Thus, clinical trials, although designed for immune-mediated tumour control, should be analysed for improving the quality of life of patients and such treatments maybe provided in palliative situations.

Taken together, it is apparent from the varying success of immunotherapies conducted as monotherapies in MM that the next generation of such treatments need to be designed as combination therapies. The right combination of treatments, timing, dose, patient selection needs to be determined. Disease stage, histological subtype, the patients' general health and relevant genetic polymorphisms also need to be considered. In theory, a well designed therapy addressing all the key immunological steps, as discussed earlier, has the potential to lead to tumour control. It remains to be seen whether immunotherapy can be an effective weapon in controlling mesothelioma.

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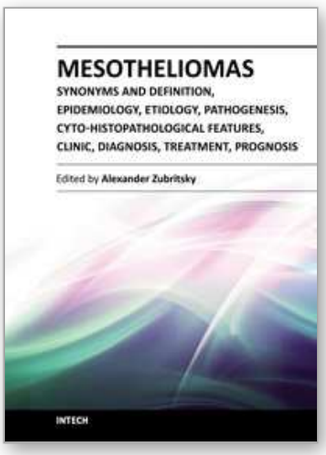

Mesotheliomas - Synonyms and Definition, Epidemiology, Etiology, Pathogenesis, Cyto-Histopathological Features, Clinic, Diagnosis, Treatment, Prognosis

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Mesotheliomas are mysterious mesothelial tumors in that they are relatively rare, difficult to diagnose, with a large number of synonyms, and the etiology and pathogenesis of the disease are still not fully disclosed. This problem attracts the attention of various specialists in the field of medicine and biology every year. In recent years there has been a significant increase of mesothelioma morbidity in most of the countries, due to the further industrialization of society. In this regard, this book has been published with the participation of an international group of experts with rich experience from around the world . The book consists of 14 chapters containing the most advanced achievements of all aspects of the various types of mesotheliomas, both in humans and domestic animals, at a high methodological level. This book is intended for biologists and all health care workers, mostly oncologists of different profiles, as well as students of medical educational institutions engaged or even just interested in the problems of mesotheliomas.

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