Promising investigational drug candidates in phase I and phase II clinical trials for mesothelioma

Guazzelli Alice[1], Bakker Emyr[1], Tian Kun [1], Demonacos Constantinos[2], Krstic-Demonacos Marija[1], Mutti Luciano[1]

1 Biomedical Research Centre, School of Environment and Life Sciences, University of Salford, United Kingdom.

2 School of Health Sciences, University of Manchester, United Kingdom

Electronic address: L.Mutti@salford.ac.uk

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Abstract

Introduction: Malignant mesothelioma is a rare and lethal malignancy primarily affecting the pleura and peritoneum. Mesothelioma incidence is expected to increase worldwide and current treatments remain ineffective, leading to poor prognosis. Within this article potential targets to improve the quality of life of the patients and assessment of further avenues for research are discussed.

Areas covered: This review highlights emerging therapies currently under investigation for malignant mesothelioma with a specific focus on phase I and phase II clinical trials. Three main areas are discussed: immunotherapy (immune checkpoint blockade and cancer vaccines, among others), multitargeted therapy (such as targeting pro-angiogenic genes) and gene therapy (such as suicide gene therapy). For each, clinical trials are described to detail the current or past investigations at phase I and II.

Expert opinion: The approach of applying existing treatments from other cancers does not show any significant benefit, with the most promising outcome being an increase in survival of 2.7 months following combination of chemotherapy with bevacizumab. It is our opinion that the hypoxic microenvironment, the role of the stroma, and the metabolic status of mesothelioma should all be assessed and characterised to aid in the development of new treatments to improve patient outcomes.

Highlights box
• Since the success of immune checkpoint inhibitors and cancer vaccines approved by the FDA for the treatment of cancers such as melanoma, immunotherapy may be evaluated as a third-line therapy for cancer treatment after conventional treatments and targeted agents.
• Mesothelin is one of the main targets in immunotherapeutic approaches but the efficacy of this strategy is still poor.
• CTLA-4 and PD1/L1 blockage are widely investigated in numerous clinical trials as single or combined therapy in mesothelioma patients but the outcome is still unsatisfactory
• Main targeted therapies against EGFR and VEGFA/VEGFR showed no clinical activity in mesothelioma patients and only slightly promising results have been shown in patients treated with bevacizumab in combination with chemotherapy.
• It is our belief that to improve current therapies the role of the hypoxic microenvironment and influence of the stroma in chemoresistance should be further characterised, in addition to uncovering the extent of metabolic reprogramming that occurs in tumours cells.

Keywords: Malignant mesothelioma, immunotherapy, targeted therapy and gene therapy

1. Introduction

Malignant mesothelioma (MM) is a lethal and rare malignancy arising from surface mesothelial cells lining cavities such as pleura (malignant pleural mesothelioma, MPM, approximately 80% of cases), peritoneum (approximately 20% of cases) whilst less than 1% of cases occur in the pericardium and tunica vaginalis [1, 2] ENREF 2. The major common risk factor for MM is asbestos exposure but radiation and simian virus 40 (SV40) have also been implicated as additional risk factors for MM [3]. Recently, it has been reported that BRCA1 associated protein-1 (BAP1) mutation could be a genetic predisposition to MM [4]. MM is characterised by a long latency period (20-40 years) before the symptoms occur. The symptoms themselves are non-specific, which makes this tumour difficult to diagnose and the disease itself has a poor prognosis; the median survival rate is less than twelve months [5] and only 10-20% of cases present life expectancy of 5-years [6]. The worldwide incidence rate is increasing and a peak is expected between 2015-2030. A study on mesothelioma frequency between 1994-2008 reported a global average of 14,200 cases each year [7]. The USA, UK, Australia and Italy all report high incidence rates. In industrialized countries, where asbestos is used illegally, the incidence frequency is expected to increase, thus affecting the global incidence [8]. There is generally a lack of curative treatments for MM patients. Systemic treatment in the form of chemotherapy, radiotherapy and targeted therapy are options as single treatment or in combination in a multimodality regimen [8]. Surgery is debatable and is recommended only in patients with early stage disease and in a good general health. A number of novel therapeutic drugs are under investigation, and may provide further treatment options for MM in the future. In this review, we will summarise current clinical trials and highlight promising agents, giving an overall opinion from a translational research team.

2. Body

There are 256 clinical trials for MM currently reported in ClinicalTrials.gov, including recruiting, completed, active and terminated studies as single intervention or in combination modality. The main categories of agents investigated include immunotherapeutics, gene therapy, and multiple targeted therapies. Different approaches will be discussed in turn throughout this manuscript, in addition to describing phase I and phase II trials for them.

2.1 Immunotherapy

Recently, immunotherapy, including active and passive therapeutic strategies has acquired a significant interest in the field of cancer treatment. Active strategies utilize tumour-specific antigens to trigger a direct immune system response such as cancer vaccines [9]. Passive approaches use an activated immune cell effector component such as immunomodulators

(including cytokines) and tumour-specific antibodies to target tumour antigens without direct stimulation of the immune system [10], whilst adoptive cellular therapy aims to administer genetically modified immune cells directly to patients [11]. For decades immunotherapy has reported clinical failures but recently the development of new molecules such as immune checkpoint inhibitors have shown promising results, leading to the approval of immune checkpoint inhibitors for the treatment of melanoma. Cytotoxic T-lymphocyte antigen 4 (CTLA-4; CD152) is a key immune checkpoint and serves to downregulate immune responses, as does programmed death-1/programmed death ligand-1 (PD-1/PDL-1). Antibodies such as ipilimumab and nivolumab have been developed against CTLA-4 and PD-1 respectively and were approved for the treatment of melanoma in 2011 and 2014 respectively [9]. The most common use of immunotherapy is as part of combination therapy with other conventional and non-conventional approaches which aim to achieve enhanced efficacy with a diminished toxic effect, thus modulating a durable and adaptable cancer control response. Preclinical studies suggest a potential for synergistic effects on tumour response and overall survival in multimodality treatment with agents that target different immune checkpoints [12]. However, combination immunotherapy against multiple checkpoints has also demonstrated a high rate of side-effects, such as one study combining nivolumab and ipilimumab for the treatment of melanoma which showed side-effects at grade three or four in 55% of patients [13]. At present, immunotherapy typically evaluated as a third-line for cancer therapy after conventional treatments and targeted agents.

Active Immunotherapy

2.1.1 Cancer Vaccines

The main aim of cancer vaccination is to educate immune system to recognise cancer cells, which exhibit specific tumour antigens, thereby restoring or promoting the ability of the immune system, mainly by CD8+ cytotoxic T cell activation, to fight cancer cells [14]. Several subcategories of vaccines have been developed, detailed below in the subsequent sections.

2.1.2 Dendritic-Cell-Based Vaccines

A strategy in the use of tumour vaccines is dendritic cells (DCs) loaded with tumour-associated antigens. DCs are a heterogeneous population of antigen-presenting cells (APCs) and are specialized in processing proteins which leads to the subsequent presentation of antigens. Production of autologous DCs is carried out through *ex vivo* differentiation from peripheral blood precursors, followed by maturation of DCs to activate them [15]. Before being injected in the patients DCs are loaded with tumour lysates derived from patients either via viral infection or grown in media containing antigenic peptides [16]. Upon infusion into the patient, the activated DCs generate anti-tumour T-cell responses due to CD4+ T cells

activating CD8+ effector T-cells (Fig 1). In April 2010, the FDA approved Sipuleucel-T (PROVENGE; Dendreon) as the first therapeutic cancer vaccine for use in patients who have metastatic castration-resistant prostate cancer [17]. The success of this vaccine is related to the increase of overall survival, although the disease progression was unchanged, giving an alternative to patients with prostate [18]. However, *ex vivo* therapies such as PROVENGE had a limited practical use in other cancer types and produced an immune response with limited scope [19].

2.1.3 Peptide or Protein-Based Vaccines

Peptide/protein-based vaccines work on the principle of stimulating CD8+ T cells to promote an anti-tumour effect, and consist of five broad categories: mutated antigens expressed only by the tumours; overexpressed normal self-antigens; oncofoetal antigens which are present in foetal tissues as well as some adult tumours; differentiation or lineage antigens; and cancer-testis antigens [20, 21, 22] (Fig 2). Among these, an interesting target is Wilms tumour gene (WT1) protein, which is highly expressed in various tumours, including mesothelioma and is associated with oncogenic function, providing a strong rationale to consider WT1 as an attractive target for cancer immunotherapy [23]. An additional common target is mesothelin, a cell-surface antigen associated with tumour invasion, which is highly expressed in several solid tumours such as mesothelioma and has been targeted in immunotherapy approaches due to the fact that its expression is low in normal mesothelial cells. Cancer vaccines developed against mesothelin use a live attenuated *Listeria monocytogenes*-expressing mesothelin (CRS-207, JNJ-64041757). Numerous antigens with promising potential are under investigation in few cancers [24]. 5T4 vaccine (TroVax®) is targets an oncofoetal antigen called 5T4 which is inserted in a highly attenuated modified vaccinia ankara (MVA) virus. 5T4 is upregulated in cancer with a limited expression in normal tissue and is associated with epithelial mesenchymal transition (EMT), contributing to metastasis of epithelial cancers [24]. Thus, this represents an interesting target for therapeutic development.

2.1.4 Phase I Clinical Trials

A preliminary study on murine malignant mesothelioma model showed that immunotherapy using tumour lysate–pulsed dendritic cells controlled MM outgrowth [25]. Following this a phase I study investigated the safety and immunological response after the administration of tumour lysate-pulsed dendritic cells in MPM patients. The results showed a well-tolerated profile, with the treatment inducing an immunological response to tumour cells in MPM patients (NCT00280982) [26]. Further, to investigate whether the combination of low-dose cyclophosphamide with autologous tumour lysate–pulsed dendritic cells was advantageous compared to monotherapy the effect of this combination on the suppressive function of regulatory T cells was assessed in 10 MPM patients. This approach demonstrated disease control and a well-tolerated profile (NCT01241682) [27].

Another pilot study was designed to assess the safety profile and efficacy of tumour cell vaccines in combination with the chemotherapeutic compound cyclophosphamide and the anti-inflammatory drug celecoxib in patients with mesothelioma. The study has been completed and 10 patients have been enrolled but the data are not available yet (NCT01143545). An additional pilot study investigated the safety and immunogenicity profile of a WT1 vaccine in patients with thoracic neoplasms (mesothelioma and non-small cell lung cancer (NSCLC)) expressing WT1 [28]. Each vaccine dose was prepared by mixing equal volumes of adjuvant Montandine 51 with 200 µg each of four different peptide sequences known to bind MHC molecules and stimulate T cell responses [28]. 200 µg was chosen as it is a known safe and active concentration. Injection sites were pre-stimulated with sargramostim (GM-CSF) and vaccines were administered subcutaneously [28]. The immune response was stimulated in most of the patients, showing the ability of T cells to exert a cytotoxic effect against WT-1 positive cells. Therefore, a randomized II phase trial has been planned [28]. Phase I clinical trials have been recently completed or are still ongoing testing the safety of WT1-based vaccines (NCT00398138) which evaluate WT1 vaccination in conjunction with conventional chemotherapy (NCT02649829). The outcome of completed studies showed the vaccination promoted a tumour-specific immunity (both cellular and humoral) and was safe but a clinical response was not observed [29]. A phase I study tested CRS-207 (vaccine targeting mesothelin which is widely expressed on cancer cells) with chemotherapy in 38 patients. The clinical outcome showed that CRS-207 was well-tolerated and in combination with pemetrexed and cisplatin showed an anti-tumour activity with 59% of patients achieving an objective partial response and median progression free survival (PFS) of 8.5 months (NCT01675765) [30].

2.1.5 Phase II Clinical Trials

A pilot study investigated the clinical effects of cyclophosphamide in combination with autologous or allogeneic tumour cell vaccines to treat patients with various advanced cancers including mesothelioma (NCT00002475). An ongoing phase II study is evaluating treatment with the WT-1 peptide vaccine in combination with Montanide/GM-CSF (granulocyte-macrophage colony-stimulating factor) versus Montanide/GM-CSF alone in patients with MPM who have completed multimodality therapy (NCT01265433).

Table 1: Phase I and II clinical trials for active immunotherapeutic approaches.

ClinicalTrials.gov Identifier	Phase	Treatment	Number of Patients
NCT00280982	I	Autologous dendritic cells	10
NCT01241682	I	Autologous dendritic cells + low-dose	10

		cyclophosphamide	
NCT01143545	I	Tumour cell vaccine in combination with cyclophosphamide and the anti-inflammatory drug celecoxib	10
NCT00398138	I	Wilms' tumour-1 (WT-1) vaccine, with injection sites being pre-stimulated by sargramostim (GM-CSF)	22
NCT02649829	I/II	Autologous dendritic cells loaded with WT-1 antigen in conjunction with conventional chemotherapy	Currently recruiting
NCT01675765	I	Cancer vaccine CRS-207 (attenuated *Listeria monocytogenes*) with or without cyclophosphamide	60
NCT00002475	II	Cyclophosphamide in combination with tumour cell vaccine	40
NCT01265433	II	Either non-specific immunotherapy (montanide and sargramostim) or WT-1 vaccine, montanide and sargramostim	31

Passive Immunotherapy

2.1.6 Antibody-Based Therapies

One extensively investigated approach to overcome the toxicity of currently-used chemotherapies is the use of monoclonal antibodies (mAbs), which have the ability to precisely target malignant cells overexpressing specific surface antigens [31] (Fig 3A) . Rituximab (anti-CD20 mAb) is the first approved mAb for the treatment of cancer, raising the interest in the development of a number of actively pursued antibody (Ab)-based technologies, including immunotoxins, radioimmuno-therapeutics, http://topics.sciencedirect.com/topics/page/Antibody-drug_conjugate and immunoliposomes [32].

The main antibodies tested in clinical trials target mesothelin such as immunotoxins and chimeric antimesothelin antibodies (i.e. amatuximab) [33]. Antimesothelin immunotoxin SS1(dsFv)PE38 (SS1P) is a recombinant molecule with a murine antimesothelin variable antibody fragment (Fv) linked to PE38, a truncated portion of Pseudomonas exotoxin A. In preclinical models, SS1P reduced mesothelin-expressing tumors, both alone and in combination with chemotherapy and radiation therapy [34]. Amatuximab (MORAb-009) is a chimeric monoclonal antibody with a high affinity for human mesothelin (K_D = 1.5 nM) [33, 35]. Preclinical studies showed that amatuximab induces cell-mediated cytotoxicity in tumour cells expressing mesothelin [36]. Moreover, using toxicological analyses it has been demonstrated that amatuximab did not cause any adverse effects in mouse models [36]. GC1008 is a human anti-TGFβ monoclonal antibody that counteracts all isoforms of TGFβ thereby preventing tumour growth and metastasis [37, 38] ENREF 31 . Preclinical data of TGFβ blockade in animal models of MPM and in human MPM cell lines suggest that TGFβ inhibitors can be effective therapeutic agents. In addition, high levels of TGFβ were found in rat and murine MPM cells as well as in tumours and in pleural effusions of MPM patients [39]. However, cost limitations, inadequate pharmacokinetics and tissue accessibility all contribute to the need for further investigation and improvement of this approach [40]. Anetumab ravtansine (BAY 94-9343) is another antibody under investigation in MPM patients overexpressing mesothelin. An *in vitro* study demonstrated that anetumab exerts potent and selective cytotoxicity against mesothelin-expressing cells [41]. A model study reported that anetumab interacts with mesothelin-positive tumours and inhibits tumour growth in xenograft models [41].

2.1.7 Cytokine-Based Therapy

Cytokine-based immunotherapy aims to stimulate a cytotoxic immune response by providing rapid protection from antigens, which involves stimulation of non-specific natural killer cells or highly specific cells such as cytotoxic T cells or Tumour Infiltrating Lymphocytes [42] (Fig 3B). Interleukin-2 (IL-2) is the first cytokine successfully used in clinical therapy but a small proportion of types of cancers can benefit from this approach and a complete clinical response occurs rarely [43]. Among immunotherapeutic cytokines investigated as potential treatments are interferons (α, β, γ), which lead to an immune response with a significant toxic effect against tumour cells [42]. In MPM, INFγ may contribute to a direct cytotoxic effect on mesothelial cells and stimulate NK and macrophages [44] .

2.1.8 Phase I Clinical Trials

A phase I trial of SS1P (which exerts its effects via targeting of mesothelin and Pseudomonas exotoxin A) aimed to verify the side effects and best dose of this immunotoxin approach in treating patients with recurrent unresectable advanced solid tumours, including MPM (NCT00066651) [45]. 24 patients with different cancer types (9 with pleural mesothelioma, 5 with peritoneal mesothelioma and 2 with pleural-peritoneal mesothelioma) were enrolled and the clinical outcome of this single therapy presented a well-tolerated profile up to 25 μg/kg/d ×10 and exhibited a modest clinical activity, with 75% of patients exhibiting immunogenicity and 21% receiving a second cycle. One patient had a partial response, twelve had stable disease, and eleven had progressive disease [45]. Another clinical trial evaluated the safety and tumour response of SS1P in combination with pemetrexed and cisplatin in 24 patients with advanced MPM. SS1P given with pemetrexed and cisplatin had a safe and well tolerated clinical profile and showed significant antitumor activity with a partial response in 60% of patients (NCT01445392) [46]. A phase 1 study was conducted to assess the safest doses of amatuximab in patients with mesothelin-positive cancers (pancreatic, ovarian, mesothelioma and lung) (NCT00325494). Amatuximab exhibited a well-tolerated profile and the maximum tolerated dose (MTD) was chosen at 200 mg/m^2 [47]. Another study investigated the dose-limiting toxicity and estimated MTD involving 17 patients with mesothelin-positive cancer, including mesothelioma patients (NCT01018784). Amatixumab was well-tolerated and MTD was determined to be 200 mg/m^2 in agreement with the study mentioned above [48]. A completed study investigating treatment with INF-α combined with cisplatin, surgery and radiotherapy in 6 MPM patients has also been performed, though results are not yet published (NCT00003263).

2.1.9 Phase II Clinical Trials

An open-label multicentre clinical trial investigated amatuximab plus pemetrexed and cisplatin for the treatment of 89 MPM patients. PFS and overall response (OR) were the endpoints for this study (NCT00738582) [49]. Due to amatuximab demonstrating a favourable safety profile, exploration in other mesothelin-expressing cancers may be justified. A Phase II investigation of GC1008 to assess the overall safety and effectiveness in 13 patients with MPM has also been performed, which demonstrated that the drug was generally well-tolerated in MPM patients (NCT01112293). However, partial or complete radiographic responses were not achieved and stable disease was observed in only 3 subjects. Patients who produced anti-TGFβ antibodies had increased median overall survival (OS) (15 vs 7.5 months, $p < 0.03$) [39].

2.1.10 Adoptive T Cell Therapy

Adoptive immunotherapy uses genetically enhanced T cells to trigger a potent and tumour-specific immune effect that affects small or large tumour burdens. The advantage of this approach is that it in theory avoids side effects and toxicities associated with standard

approaches such as chemotherapy [50]. T cells are engineered to express a tumour antigen by the integration of genes encoding conventional T-cell receptors (TCRs) or chimeric antigen receptors (CARs, receptors which may undertake MHC-independent targeting and thus combine the targeting specificity of antibodies and the cytotoxicity of T cells) [50, 51] (Fig 3C). One of the most attractive CAR T cell applications is targeting overexpressed mesothelin in solid tumours, including mesothelioma [34]. It has been shown that adoptive transfer of engineered antimesothelin human CAR T cells regressed large human MPM xenograft tumors in immunodeficient mice in preclinical models [52]. Alternatively, CAR T cells were used to target non-transformed stromal cells, which promote cancer. CAR T cells were directed against fibroblast activation protein (FAP), which is overexpressed on the surface of reactive tumor-associated fibroblasts [53]. High level of FAP expression in tumour tissue of MPM patients has been reported highlighting the potential of using adoptive T-cell therapy as an effective approach to treat MPM [53].

2.1.11 Phase I Clinical Trials

A clinical trial of CAR T Cell targeting mesothelin is recruiting participants who are diagnosed with metastatic cancers, including mesothelioma. The purpose of this study is to evaluate the safe number of enhanced T cells to infuse and effective doses in an estimated number of 136 subjects (NCT01583686). Another recruiting clinical trial aims to use redirected T cells against FAP to assess the safety of a fixed single dose by direct injection in the pleural effusion for patients (estimated number of six) with MPM not eligible for surgery (NCT01722149).

2.1.12 Immune Checkpoint Treatment

Cytotoxic T-lymphocyte antigen 4 (CTLA-4; CD152) receptor and programmed death-1/programmed death ligand-1 (PD1/PDL-1) are the major immune checkpoints targeted for clinical studies [54, 55] (Fig 3D). The biological role of immune checkpoints is to negatively regulate the immune response by downregulating T-cell function resulting in immune tolerance to self-antigens, and balancing the immune response [56, 57] ENREF 9. CTLA-4 exerts its inhibitory signal through competition with its positive counterpart, CD28, for its ligand B7 [57]. Preclinical studies reported that CTLA-4-deficient mice die due to lethal lymphoproliferation [58]. A strong preclinical rationale justified the development of two main immune checkpoint blockers: ipilimumab (known as MDX-010 or BMS-734016; Yervoy™, Bristol-Myers Squibb, Princeton, NJ), a fully human, IgG1 monoclonal antibody (mAb) which binds CTLA-4. Ipilimumab was the first immune checkpoint blockage therapy approved for treatment of metastatic melanoma in 2011 [59]. Ipilimumab was also approved by the Food and Drug Administration (FDA) as adjuvant for stage III melanoma [59]. Tremelimumab (CP-675 206 or ticilimumab; Pfizer Inc, New York, NY, USA) is a humanized IgG2 mAb against CTLA-4 that has also been used in clinical trials, detailed below [60].

Following the promising results of CTLA-4 blockage, other immune checkpoints such as the PD-1/PD-L1 pathway have been targeted. PD-1 is a negative regulator of T-cells since by interacting with the PD-L1 and the PD-L2 ligands limits their activity. Several therapeutic agents have been developed against PD-1 and PD-L1 but the development of autoimmune diseases has been observed in PD-1–deficient mice in preclinical studies [61]. Nivolumab known also as MDX-1106, BMS-936558, and ONO-4538; Opdivo®, Bristol-Myers Squibb, Princeton, NJ) is a humanized IgG4 mAb. Nivolumab has been approved by FDA for the treatment of Hodgkin lymphoma [62]_ENREF_51, unresectable or metastatic melanoma [63] and metastatic NSCLC [64]. Another therapy targeting PD-1 which has been clinically tested is pembrolizumab (MK-3475, lambrolizumab; Keytruda®, Merck, Whitehouse Station, NJ) which is an IgG4 engineered humanized Ab, approved in 2014 for the treatment of metastatic melanoma [65] and in 2015 for NSCLC [66].

Other drugs targeting PD-L1 such as atezolizumab (MPDL3280A; Tecentriq®, Genentech, Inc, South San Francisco, CA), an IgG1 humanized, engineered mAb, has also been developed. In October 2016 the FDA approved atezolizumab for NSCLC. Avelumab (MSB0010718C,EMD Serono, Rockland, MA) is a fully human IgG1 monoclonal PD-L1 antibody.

Recently, another component of the B7 family, called B7-H3 (CD276), has been shown to regulate the immune response being involved in the inhibition of the signalling of the T-reg cells. B7-H3 is over-expressed in a wide variety of solid tumour types [67]. Enoblituzumab (MGA271,) is an Fc-optimized humanized IgG1 mAb that binds to B7-H3 and has also been used in clinical trials, detailed in subsequent sections. B7-H3 is a negative immune checkpoint and it has been shown that its blockage can promote an anti-tumour immune response [68]

All of these immune checkpoint blockage therapies have been or are under investigation in MPM in phase I or phase II studies as described below.

2.1.13 Phase I

Several phase I clinical trials have been designed to evaluate pembrolizumab in combination with standard chemotherapy, radiotherapy (NCT02959463) or novel strategies such as defactinib (a focal adhesion kinase (FAK) inhibitor) (NCT02419495 and NCT02758587). Some of them are currently recruiting and the main aim is to evaluate the safety of the combination of pembrolizumab in combination with standard chemotherapy (NCT02707666) in particular as a window of opportunity pilot trial of pembrolizumab in patients affected by resectable malignant pleural mesothelioma. One study is investigating the combination of pembrolizumab with an antiangiogenesis agent (nintedanib) since antiangiogenic therapy may synergize with immunotherapy, thus offering a benefit for the quality of life of patients with advanced solid tumours, including MPM (NCT02856425). First considerations about the clinical profile of pembrolizumab has been presented at the AACR Annual Meeting 2015 with a phase Ib study (KEYNOTE-028: NCT02054806) showing safety, tolerance, and robust antitumor responses in patients with malignant pleural mesothelioma.

The PD-L1 inhibitor avelumab is under investigation in a phase I study that is currently recruiting patients with solid tumours including malignant mesothelioma (JAVELIN Solid Tumor: NCT01772004). Investigational studies of combined immunotherapy approaches are currently recruiting patients and are a phase I study of a combination modality of ipilimumab with enoblituzumab in refractory cancers, including MM (NCT02381314). Another phase I study evaluates the safety of enoblituzumab in combination with pembrolizumab in refractory cancers such as malignant mesothelioma (NCT02475213).

2.1.14 Phase II Clinical Trials

The success of ipilimumab and its approval for the treatment of metastatic melanoma, plus the promising clinical efficacy of immunotherapy in combination with other treatments, has led to the investigation of ipilimumab in two phase II studies in combination with nivolumab (NCT03048474 and NCT02716272). The combination treatment with two different immune checkpoint inhibitors may delay tumour progression in patients with unresectable malignant pleural mesothelioma. However, the central immune checkpoint tested in mesothelioma is tremelimumab which has been investigated mainly as single dose in three phase II studies. A phase II study of tremelimumab in patients with chemotherapy-resistant advanced malignant mesothelioma (NCT01649024) has been carried out. Tremelimumab showed an adequate safety and tolerability profile although only 29 patients were enrolled and no patients achieved a complete response and only two patients (7%) exhibited a durable partial response (one of 6 months and one of 18 months) [69]. Another phase II trial assessed the efficacy and safety of an intensified schedule of tremelimumab in patients with unresectable advanced malignant mesothelioma (NCT01655888). In this study, 3% (one patient) achieved a partial response and 38% (11 patients) achieved disease control rate. Gastrointestinal and dermatological effects, as well as fever, were the major adverse effects observed that were related to treatment [70]. The results showed a well-tolerated and safe profile that led to the use of the same intensified schedule in an ongoing randomised, double-blind, placebo-controlled, phase 2b study (NCT01843374). Tremelimumab has been selected for a combined therapy with another immune checkpoint targeting PD-L1 (MEDI4736). Two clinical studies are currently recruiting participants (NCT02588131 and NCT02592551). Nivolumab is also under investigation in an ongoing phase II study as monotherapy in patients previously treated with chemotherapy with mesothelioma (NCT02497508). Recent recruiting clinical studies evaluate the activity of pembrolizumab as a monotherapy in malignant mesothelioma (NCT02399371 and NCT02628067) or in combination with cisplatin/pemetrexed (NCT02784171). Atezolizumab is under assessment in a recruiting phase II study in advanced solid tumours, including mesothelioma (NCT02458638).

Table 2: Phase I and phase II clinical trials for different immunotherapeutic approaches.

ClinicalTrials.gov Identifier	**Phase**	**Treatment**	**Number of Patients**

NCT00066651	I	SS1(dsFv)-PE38 immunotoxin	24
NCT01445392	I	SS1(dsFv)-PE38 in addition to cisplatin and pemetrexed	24
NCT00325494	I	Amatuximab	24
NCT01018784	1	Amatuximab	17
NCT00003263	I	INF-α combined with chemotherapy, radiotherapy and surgery	6
NCT00738582	II	Amatuximab	89
NCT01112293	II	GC1008 (anti-TGF)	14
NCT01583686	I/II	Anti-mesothelin CAR T cells	Currently recruiting
NCT01722149	I	Redirected anti-FAP T cells	Currently recruiting
NCT02959463	I	Adjuvant pembrolizumab after radiation therapy	Not yet recruiting
NCT02419495	I	Numerous (including pembrolizumab and selinexor)	142
NCT02758587	I/II	Defactinib and pembrolizumab	Not yet recruiting
NCT02707666	I	Pembrolizumab in combination with cisplatin and pemetrexed	Currently recruiting
NCT02856425	I	Nintedanib and pembrolizumab	Currently recruiting
NCT02054806	I	Pembrolizumab	477
NCT01772004	I	Avelumab	Currently recruiting
NCT02381314	I	Enoblituzumab in combination with ipilimumab	Currently recruiting

NCT02475213	I	Enoblituzumab in combination with pembrolizumab	Currently recruiting
NCT03048474	II	Nivolumab and ipilimumab	Currently recruiting
NCT02716272	II	Nivolumab monotherapy or nivolumab in conjunction with ipilimumab	125
NCT01649024	II	Tremelimumab	29
NCT01655888	II	Tremelimumab	29
NCT01843374	II	Tremelimumab	658
NCT02588131	II	Tremelimumab in combination with MEDI4736 (anti-PD-L1)	Currently recruiting
NCT02592551	II	MEDI4736 or MEDI4736 in combination with tremelimumab	Currently recruiting
NCT02497508	II	Nivolumab	33
NCT02399371	II	Pembrolizumab	Currently recruiting
NCT02628067	II	Pembrolizumab	Currently recruiting
NCT02784171	II	Pembrolizumab, cisplatin, pemetrexed	Currently recruiting
NCT02458638	II	Atezolizumab	Currently recruiting

2.2 Multiple Targeted Therapies: Small Molecule and Antibody Approaches

The increased understanding of molecular pathways involved in tumorigenesis allows for the development of valid rationales to specifically target malignant cells in solid tumours, including MPM. It is well-known that proliferative signalling promotes tumour progression by

releasing growth factors such as epidermal growth factor receptor (EGFR) which plays a pivotal role in proliferation and mediates cell growth by activating specific downstream pathways contributing to survival, differentiation, migration and adhesion. EGFR is part of the family of transmembrane tyrosine kinase receptors (TKRs), including platelet-derived growth factor receptors (PDGRs), fibroblast growth factor receptors (FGFRs) and vascular endothelial growth factor receptors (VEGFRs) [71]. The discovery of the role of this family prompted the development of tyrosine-kinase inhibitors (TKIs), small molecules able to target the intracellular tyrosine kinase residue such as gefitinib and erlotinib which target EGFR (Fig 4).

In MPM, studies revealed that EGFR was overexpressed at the protein level in around 52% of MPM patients [72, 73, 74]_ENREF_62. The role of vascular endothelial growth factor (VEGF) and its receptor VEGFR has been identified in MPM, showing high levels of both molecules in tissue specimens [75]. Numerous inhibitors have been designed and tested in MM for these targets such as vatalanib, sorafenib, nintedanib, axitinib and cediranib and have shown limited or absent levels of activity, resulting in a lack of clinical benefits [76, 77]. The most promising treatment to interact with VEFG signalling is bevacizumab (Avastin), a humanized monoclonal antibody against VEGFA which was approved in the EU in 2005 for the treatment of many solid cancers such as NSCLC, colorectal carcinoma, renal cell, and ovarian [78, 79, 80, 81] (Fig 4).

Moreover several small molecules acting as inhibitors have been developed for different pathways, including against targets involved in epigenetic regulation of tumour suppressor genes, such as histone deacetylase (HDAC) inhibitors. The main agents tested in clinical trials are belinostat and vorinostat. Another strategy uses asparagine–glycine–arginine–human tumour necrosis factor α (NGR-hTNF) which is TNF-alpha fused with NGR. NGR is able to bind specifically an aminopeptidase N isoform overexpressed on tumour blood system [82, 83] . FAK represents an encouraging target for MPM in particular due to its involvement in cancer stem cell (CSC) renewal [84]. Recently reported targeted therapies include RNA as a therapeutic target using ranpirnase, which promotes impaired protein synthesis and cell cycle arrest, leading to an antitumor activity [85]. Moreover, it is possible to list a variety of inhibitors for example; heat shock protein 90 (HSP90) inhibitor (ganetespib), enhancer of zeste homolog 2 (EZH2) inhibitor (tazemetostat), inhibitor of the MET receptor tyrosine kinase (tivantinib), CSC inhibitor (napabucasin/BBI608) and TargomiRs (a mimic microRNA treatment).

2.2.1 Phase I Clinical Trials

A phase I study investigated the clinical profile of vorinostat in advanced tumours, including 13 patients with MPM [86]. The results of this study demonstrated there was no improved survival with the use of this drug. Although vorinostat has been tested in a phase III study as a second-line therapy, there was no improvement in OS and it cannot be suggested as an option for treatment for MPM patients in advanced stage [87].

2.2.2 Phase II Clinical Trials

Gefinitib (ZD1839, Iressa) is the main TKI studied in a clinical trial involving 40 malignant mesothelioma patients with unresectable disease but did not show any clinical benefit despite the fact that 97% of patients overexpressed EGFR (NCT00025207) [88]. Erlotinib, another TKI, did not improve survival in a study when used as a single agent in untreated patients [89]. Promising results have been reported from a study of erlotinib in combination with bevacizumab in pre-treated patients, showing a stable disease in 50% of patients, a PFS of 2.2 months and a median survival of 5.8 months (NCT00137826) [90]. Bevacizumab, one of the most promising anti-VEGF therapeutic agents, has been tested in combination with cisplatin plus gemcitabine in a multicentre study involving 108 chemo-naïve mesothelioma patients. PFS was 6.9 months for the bevacizumab arm versus 6 months for the placebo and OS was improved by only 1 month (15.6mo vs 14.7mo) (NCT00027703). From these data, the conclusion is that bevacizumab does not improve PFS and OS [91]. However, an additional phase III study showed that bevacizumab in combination with pemetrexed and cisplatin improved overall survival by 2.7 months relative to pemetrexed and cisplatin alone [92]. A study of belinostat has been performed in patients with relapsed MPM but did not show activity in patients (NCT00365053) [93]. Defactinib (VS-6063) is a potent selective FAK inhibitor. A phase II randomized multicenter trial (COMMAND) of defactinib in previously treated MPM was initiated but the study has been terminated due to the lack of evidence that it is efficient (NCT01870609).

2.3 Gene Therapy

Another therapeutic approach under investigation for MPM is gene therapy which consists of the transfer of genetic material in cells for therapeutic purposes. In MPM, several genes have been detected to be interesting targets for gene therapy and different types of delivery systems have been clinically investigated to assess safety and activity in MPM patients [94]. MPM represents a promising target for gene therapy since the MPM tumour lining to the pleural cavity makes it easy to reach using *in vivo* gene delivery [95]. Although these trials have demonstrated good safety results, there has been relatively limited efficacy [96].

2.3.1 Suicide Gene Therapy

Suicide gene therapy uses viruses to deliver a transgene which encode for a specific enzyme that is able to transform a prodrug into toxic metabolites, leading to tumour cell death or "suicide" [97]. The most commonly investigated approach is herpes simplex virus-1 thymidine kinase (HSVtk) gene which sensitises transduced cells to the nucleoside nontoxic antiviral drug ganciclovir [94] (Fig 5A).

2.3.2 Cytokine Gene Therapy

Cytokine gene therapy is a promising treatment since it has the ability to activate systemic, intrapleural, and intratumoral immune effector cells. Cytokine gene therapy is an improved technique to express increased amount of cytokines (such as IL-2, IL-12, TNF or INF -α, β, or γ) using a viral vector with the advantage to reduce toxicity and increase the local concentration [98] (Fig 5B).

2.3.3 Phase I Clinical Trials

Clinical trials assessing gene therapy for mesothelioma have been designed. One study tested transgene expression and clinical profiles in MPM patients receiving high-dose adenovirus HSVtk/ganciclovir suicide gene therapy [99]. The intrapleural administration was safe and well-tolerated in 34 patients but in only 2 long-term durable responses to the treatment were evident. Another study assessed the gene transfer, immune response profile and tumour response of single-dose intrapleural IFN-β gene transfer using an adenoviral vector (Ad.IFN-β) in 10 mesothelioma patients, the results of which showed immune responses at a high rate [100]. A clinical trial testing adenoviral-mediated IFN-β gene as a monotherapy for MPM patients is ongoing (NCT00299962). The first one is investigating two doses of the treatment whilst the second is a dose-escalation study. Currently an ongoing study "Autologous Redirected RNA Meso-CIR T Cells" is evaluating the safety and potential of Meso-CIR T cells (autologous chimeric immune receptor T cells that have been transfected with an anti-mesothelin mRNA) in 18 patients (NCT01355965).

Table 3: Phase I and II clinical trials for targeted and gene therapies.

ClinicalTrials.gov Identifier	**Phase**	**Treatment**	**Number of Patients**
NCT00025207	II	Gefitinib (EGFR inhibitor)	40
NCT00137826	II	Bevacizumab (VEGF inhibitor) and erlotinib (EGFR inhibitor)	37
NCT00027703	II	Chemotherapy with or without bevacizumab	106
NCT00365053	II	Belinostat (HDAC inhibitor)	13
NCT01870609	II	Defactinib (FAK inhibitor)	344
NCT00299962	I	Adenoviral-mediated	18 (estimated,

		IFNβ	study ongoing)
NCT01355965	I	Redirected RNA Meso-CIR Autologous T Cells	18

3. Expert Opinion

There is no doubt that in the last few years more effort has been placed in developing and testing new therapeutic options for mesothelioma. Unfortunately the major weakness of this (welcome) interest has been the idea that therapies with some activity for other tumours could be applied "tout court" to mesothelioma. It is clear that this approach is not based on a solid scientific background and is unlikely to achieve significant results.

Among others factors, it is our opinion that taking the hypoxic microenvironment of mesothelioma into account can assist in the development of much more "mesothelioma tailored" therapies [101]. With regard to what the clinical trials for mesothelioma so far have shown as the "best result" underpinning the front line therapy for this neoplasm is based on a trial that allows a gain of survival of 2.7 months and on a preclinical study published by our group sixteen years ago [92, 102].

Hence rather than discussing what the most promising results of treatment for this tumour have been so far we should ask ourselves why we have not achieved more significant steps forward yet. In our understanding a more precise focus on mesothelioma biology (encompassing genetics, metabolomics and functional studies) conducted on cell models closer to the *in vivo* events in primary tumour cell lines and 3D studies could foster our clinical impact. It is clear that surrogate end points such as progression-free survival do not mirror the real clinical effect of any novel treatment in oncology [103]. As opposite our purpose should be that of extending mesothelioma patient survival by longer than three months.

This is particularly true for immunotherapy as recently demonstrated by the failure of clinical trials for mesothelioma with OS as a primary end point (in spite of some efficacy of the phase II trials) and, more generally, by the low number of patients who can truly benefit from immune checkpoint inhibitors. Moreover, the purely "genetic" approach to cancer treatment is currently being discussed and evaluated. This approach is even more contentious when one looks at the low mutational load of MPM [103, 104].

We believe that only a more integrated and balanced approach to MPM will allow us to get ahead and achieve more significant clinical results. This should encompass multiple disciplines such as genetics, biochemistry and immunology that are already applied to cancer research and be even more focused on MPM than done so far. A more precise understanding of how the stroma may affect the response to therapy is also an important area and offers a novel therapeutic target [53, 105]. The effect of microenvironment and the subsequent MPM metabolic reprogramming offers a unique scenario with potentially significant therapeutic implications for this orphan disease (manuscript in preparation).

Ultimately magic bullets do not exist in oncology but we are confident that an unbiased multidisciplinary approach to this tumour could allow us to achieve significant results sooner than expected. Metabolic reprogramming of tumour cells, the hypoxic microenvironment and the stroma are all areas that offer promising points of interest for therapeutic development.

Conflict of Interest Statement

All authors have nothing to disclose.

Figure 1

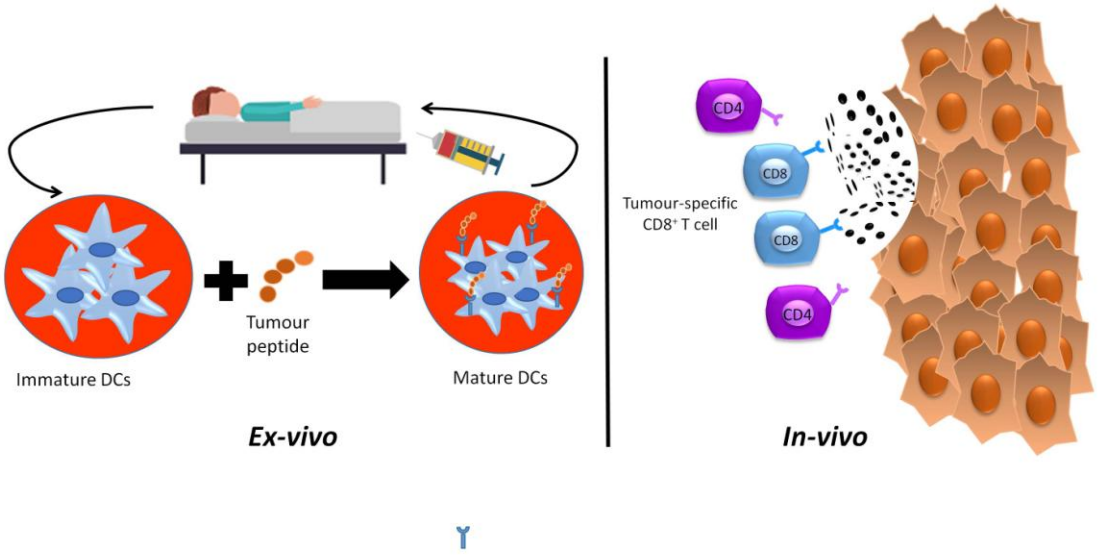

Figure 1. Passive Immunotherapy: Dendritic cell-based vaccines are developed by isolating immature dendritic cells from the patient and incubating them ex- vivo with tumour lysate. Mature DCs are injected back to the same patient to induce a tumour-specific immune response by activating tumour-specific CD8+ T cells.

Figure 2

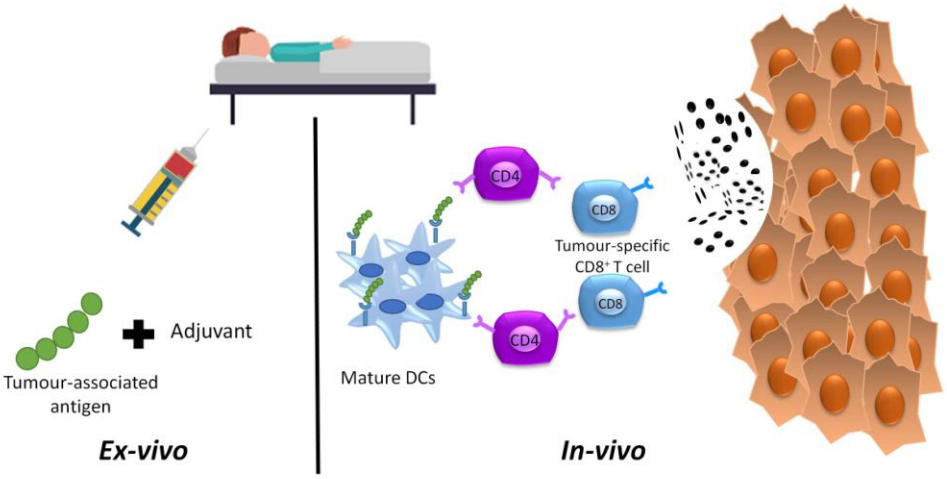

Figure 2. Peptide-based vaccines are single peptides or a cocktail of peptides obtained from cancer cells of patients and are amplified ex-vivo. These peptides are conjugated to an adjuvant and are injected back to the patient. DCs endocytose these peptides and present them and activate CD4+ cells which subsequently induce CD8+ CTL specific anti-tumour immune response.

Figure 3

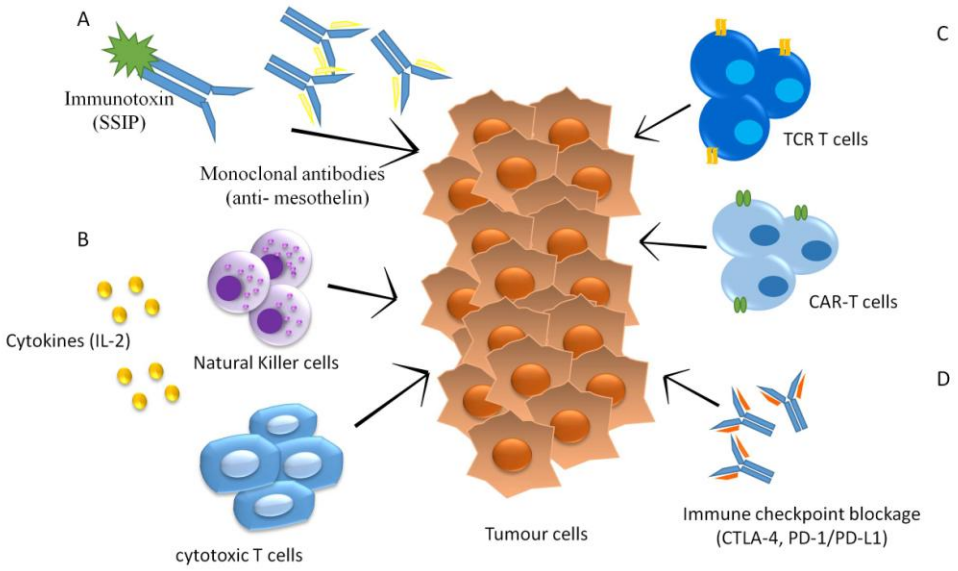

Figure 3. Schematic representation of passive immunotherapeutic strategies. A) antibody-based strategy using monoclonal antibodies or immunotoxins against cancer cells (mesothelin is the main target for mesothelioma). B) Cytokine-based therapy provides cytotoxic immune response by activating natural killer (NK) cells or tumour-specific cytotoxic T cells. C) Adoptive T cells therapy uses engineered T cells expressing T-cell receptor (TCR) or a chimeric antigen receptor (CAR) to interact with cancer cells; D) In immune checkpoint blockade approach monoclonal antibodies specifically recognizing receptors such as the cytotoxic T-lymphocyte antigen 4 (CTLA-4) receptor or the programmed death-1/programmed death ligand-1 (PD1/PDL-1) are used to block the function of these receptors.

Figure 4

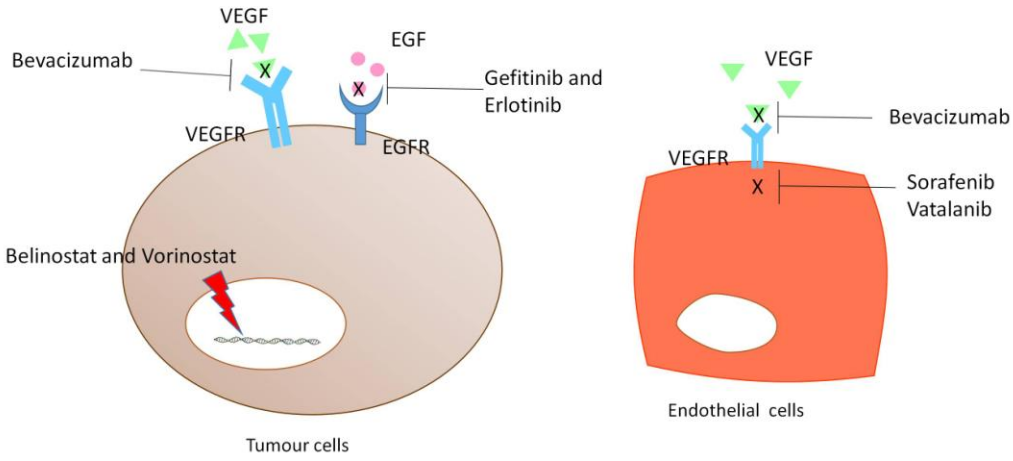

Figure 4. Graphic representation of anticancer multiple targeted approaches in MM. Belinostat and vorinostat induce DNA damage whereas the antibodies bevacizumab, gefitinib and erlotinib prevent the binding of VEGF and EGF respectively to their receptors thereby inhibiting their intracellular signalling. Bevacizumab functions extracellularly whereas the small molecules sorafenib and vatalanib inhibit the VEGFR intracellular signalling.

Figure 5

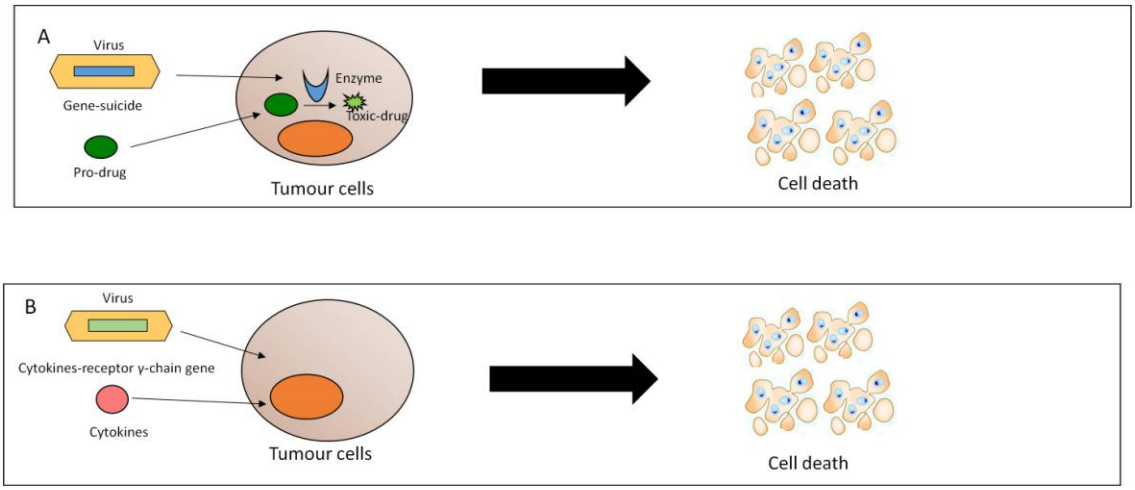

Figure 5. Summary of gene therapies for MM treatment. A) Suicide gene therapy: tumour cells modified to express a specific enzyme to metabolise a prodrug into a cytotoxic product to induce tumour cell death; B) Cytokine gene therapy: expression of cytokines to activate a more effective immune response.

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