

The Role of Cytolytic T Lymphocytes in the Eradication of Multiple Myeloma

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ABSTRACT

Multiple myeloma (MM) is a malignancy of terminally-differentiated plasma cells. In spite of recent major advances in therapy, which includes tandem and non-myeloablative haemopoietic stem cell transplantation, as well as the discovery of novel drugs, there is considerable unwanted therapy-related toxicity, and still no cure for MM. More recently, humoral and cellular immunotherapy has emerged as a safer, more specific and less toxic modality of treatment for MM. In this regard, the most important, naturally occurring cell regulating tumour rejection and immunosurveillance in the body is the cytolytic T lymphocyte (CTL). The focus of this review is to provide a greater insight to the practising clinician on the potential use of CTL-based immunotherapy for MM in the future. We briefly discuss the biology of anti-tumour CTL activation, including tumour antigen presentation and recognition. We also discuss the role of cellular immunotherapy, and the strategies for targeting the MM cell, both in the research laboratory as well as in clinical trials. It is hoped that these novel therapies will one day produce the first true and durable cures for MM.

Keywords: adoptive transfer, antigen presentation, antigens, CD40, immunotherapy, lymphocytes, priming

INTRODUCTION

Multiple myeloma (MM) is a malignancy of terminally-differentiated, immunoglobulin (Ig)-secreting plasma cells. Approximately 14400 patients are diagnosed with MM each year in the United States of America.¹ Currently, MM is incurable, even with high-dose chemotherapy or haematopoietic stem cell transplantation (HSCT).^{2,3} Recently, a number of clinical trials using cytolytic T lymphocytes (CTL) as a form of cell-based immunotherapy have been conducted on patients with MM with promising results.^{2,4} In this review, we compare the different approaches to developing CTLs for use in therapeutic strategies in the treatment of MM, and analyse their ease of administration in the clinical setting. Although several of these therapies are still in the early stages of laboratory and clinical development, and conclusive data have not been published, it is hoped that discussion of these topics will provide a greater insight to the practising clinician on the potential use of CTL-based immunotherapy for MM in the future.

TUMOUR ANTIGENS (AG)

The process of tumour recognition by CD8⁺ T lymphocytes involves the presentation of tumour-associated Ags (TAA) as well as tumour-specific Ags (TSA) by Ag-presenting cells (APC). Presentation of both TAAs and TSAs requires the loading of antigenic peptides onto the human leukocyte Ag (HLA) molecules on APCs. Subsequently, engagement of TAA and/or TSA peptide fragments expressed on HLA molecules by CD8⁺ T cells induces tumour-specific cytolytic function in responding CTLs.

Tumour-Associated Ags

Numerous TAAs have previously been described. These include carcinoembryonic Ag (CEA) in colon cancer, alpha-fetoprotein (AFP) in liver cancer and prostate-specific Ag (PSA) in prostate cancer. Tumour associated antigens are presented via the HLA or major histocompatibility complex (MHC) class I pathway to induce tumour-specific CD8⁺ CTLs. Unlike TSAs,

TAAAs are not specific to the malignant cell as they are also expressed in non-malignant cells, albeit frequently at lower levels (1 to 2-log-fold difference).⁵

Tumour-Specific Ags

In contrast to TAAAs, TSAs are theoretically only expressed in malignant cells and not in non-malignant cells. However, this is observed only for anti-idiotypic (Id) antibodies (Ab) in chronic lymphocytic leukaemia and monoclonal immunoglobulins (Igs; M-protein) in MM, which are both highly-specific proteins secreted only by the malignant cells. When the definition of TSAs is more loosely applied, TSAs include tumour Ags that can be found even in normal cells.⁶ It has been widely considered that for true TSAs, the differential expression of tumour Ags in malignant cells is significantly greater than in normal cells, as compared to TAAAs.^{6,7} Hence, it is the pronounced lack of expression of the Ag in normal cells as compared to the relative abundance of expression of the Ag in malignant cells that defines the TSA. As TSAs are produced and/or secreted by the tumour cell that marks the malignant clone, the ability to identify and target true TSAs is important as this may result in effective immunotherapeutic regimens.

OVERVIEW OF IMMUNOTHERAPY

In the purest sense, immunotherapy is a form of therapy that uses the host immune system to eradicate a particular disease. Therapy is directed at either enhancing attenuated immunity or suppressing excessive immunity, rather than at directly eradicating the disease. For example, in immunotherapy, monoclonal antibodies (mAb) may be used to opsonise viral particles (for example, influenza A virus), making these pathogens more readily recognisable to the host immune system, and thereby redirecting specific immunity against the virus.⁸ Cancer immunotherapy (or anti-cancer vaccines) can broadly be considered under two major categories — humoral and cellular. The potential efficacy of existing anti-cancer vaccines may be limited when the therapy is directed at either the cellular or humoral arm of the immune responses at the expense of the other. Ideally, vaccines should be designed to optimally activate both arms of the immune system so as to generate an effective anti-tumour immune response.⁹

Humoral Immunotherapy

Humoral immunotherapy involves the use of non-cellular immunological reagents (the so-called biologics or biopharmaceuticals) to induce host immunological response. This can be further divided into Ig-based therapies (for example, mAbs) and non-Ig-based

therapies (for example, chimeric fusion proteins and small peptides with blocking function). An example of an Ig-based therapy is the use of anti-CD20 mAb immunotherapy (or serotherapy) for the treatment of B cell non-Hodgkin's lymphoma (NHL).¹⁰ The Abelson kinase inhibitor STI571 (Glivec®, Novartis) is a good example of non-Ig based therapy.

Cellular Immunotherapy

Cellular immunotherapy involves harnessing the effector functions of immunocytes and can be broadly divided into immune effector cell-based therapies (for example, CTL vaccines) and APC-based therapies (for example, dendritic cell (DC) vaccines). An example of an effector cell-based therapy is the use of melanoma Ag genes-1 (MAGE-1) peptides to prime tumour-specific CTLs *ex vivo* for eventual use in adoptive immunotherapy against malignant melanoma.¹¹ Dendritic cells have been used as delivery vehicles for exogenous tumour Ags, which is an example of an APC-based therapy, to promote efficient Ag presentation and this has resulted in tumour regression in some patients.¹²

OVERVIEW OF CTLs

Activation of CD8⁺ T Cells in CTL Development

Cytolytic T lymphocytes are the principal effectors of specific cellular immunity against cancer. They kill target cells that express foreign Ags in the form of peptides. Development of CTLs involves the activation of CD8⁺ T cells by primed APCs, and this process requires 2 signals.

Signal 1 — Recognition of Ag by T-Cell Receptor (TCR)

The first signal involves the recognition of non-self or foreign peptide Ags presented via the HLA class I/ Ags complexes expressed on the surface of APCs (especially DCs) by CD8⁺ T cells. The proteolytic degradation of peptides by the proteasome provides for the continual display of the peptide-HLA class I complexes on the surface of the APC. This initial signal is important for the stimulation of CD8⁺ T cells and subsequently for the further development of CTLs. For example, a well-known consequence of the first signal is the stabilisation of the interleukin-2 (IL-2) message in the T cell.

Signal 2 — Activation by Co-Stimulatory Molecules

The second (co-stimulatory) signal involves, for example, the interaction of B7 molecules (B7-1 or B7-2) on the APC with the CD28 molecule

Table 1. *Ex vivo* CTL expansion in MM.

Strategy	Target	Key Features	+ Advantage/- Disadvantage	Reference
MM Id-pulsed DC	MM Id protein	Id is relatively weak Ag	+ autologous Id-specific CTLs	46
MM WCE-pulsed DC	MM peptides	stronger Ag than Id	+ polyclonal MM-specific CTLs	32
MM apoptotic bodies-pulsed DC	MM apoptotic bodies	stronger Ag than WCE	+ autologous, primary MM cell-specific CTLs	33
MM RNA-transduced DCs	MM total RNA-derived peptides	HLA class I restricted	+ polyclonal CTL response against MM - risk of insertional mutagenesis	34
CD40-activated MM cells	MM apoptotic bodies	CD40 ⁺ MM cell restricted	+ anti-MM activity <i>in vivo</i> - not all MM cells CD40 are responsive	35

Table 2. *In vivo* CTL expansion in MM.

Strategy	Target	Key Features	+ Advantage/- Disadvantage	References
Anti-MM Id-protein plus GM-CSF and/or IL-12	MM Id protein	anti-Id immunity induced in some patients; and tumor mass reduced	- not all patients generate Id-specific T cell responses	38
Anti-MM Id-protein-pulsed DCs plus Id-KLH conjugate	MM Id protein	anti-Id and/or anti-KLH immunity induced	- may generate unwanted KLH-specific CTL response	29,30
Anti-MM Id-protein/KLH-pulsed DCs plus GM-CSF and IL-12	MM Id protein	anti-Id immunity induced	+ specific immunity - relapse rate of MM still undetermined	25

on the T cell. Complete activation of T cells occurs when both signals 1 and 2 are present. In the absence of co-stimulation, T cells become anergic and can subsequently become tolerised to the same Ag.¹³ Co-stimulation is also mediated by the interaction of CD40 ligand (CD40L, CD154), expressed on activated T cells, with its receptor, CD40, which is expressed on APCs. CD40-CD40L interaction is required for the generation of memory CTLs, and more importantly, is instrumental in transforming naïve APCs into potent cells which induce CTL-driven immunity.^{14,15} This second signal is important for driving the expansion and proliferation of the antigen-specific CTLs. Most notably, this second signal is necessary for the expression of the IL-2 receptor and response to IL-2 itself.

Mechanisms of CTL-Mediated Cytolysis

Cytolytic T lymphocytes kill target cells by cytolysis through 2 major pathways. The first one involves a process that is mediated by 2 major classes of proteins, perforin and granzymes.¹⁶ Perforin allows the entry of destructive enzymes into the cells and subsequent disruption of the cell's osmotic equilibrium, thereby lysing the cells. Granzymes enter the target cells through perforin pores, activate caspases, and induce cell death by apoptosis. The second pathway involves Fas ligand (FasL) which binds the protein, Fas, on the

target cells and initiates a signalling pathway that results in apoptosis. Both pathways are intimately regulated by the signals through the $\alpha\beta$ TCR.

The Role of CD40 in the Generation of CTLs for Tumour Vaccination

Since tumours have evolved numerous mechanisms to evade immune detection, the use of cytokines to enhance tumour Ag expression would be ideal. It seems possible that autologous, tumour-specific clonal CTL-based vaccines against cancer can be generated using CD40-activated tumour cells as immunogens.^{15,17} CD40 stimulation enhances the host's antitumour immune response by improving Ag presentation, cytokine secretion and CTL activity, while CTLs are able to target and lyse cells.¹⁸

EX VIVO CTL EXPANSION IN MM

Overview

In this section, some approaches used in the generation of anti-MM CTL vaccines via *ex vivo* lymphocyte priming are presented (Table 1 and Fig. 1). The Ag most frequently used for *in vivo* CTL expansion in MM is the MM Id protein, which may be regarded as a true TSA.¹⁹⁻²² As mentioned in the section on TSAs, the idiotype (Id) refers to the specific peptide sequence in the hypervariable region of the Ig molecule that is

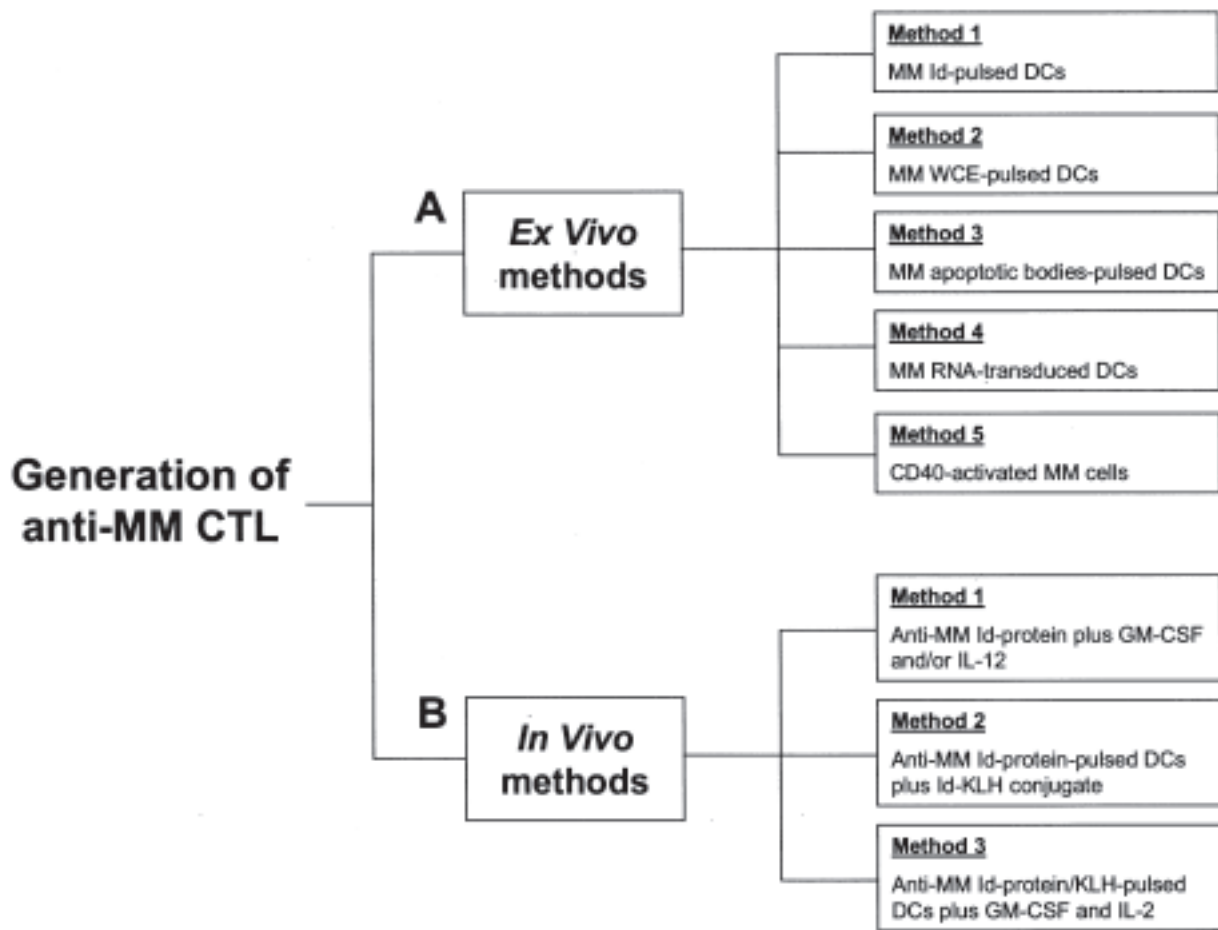


Fig. 1. Summary of approaches used for generating anti-MM CTL responses. The methods used for generating anti-MM CTLs can be divided into those that involve the *ex vivo* expansion of CTLs (A), or those that involve *in vivo* expansion of CTLs (B). In *ex vivo* CTL expansion, T cells in tissue culture are triggered by exposure to Ags borne on APCs (usually DCs) or immunogenic MM cells (A: methods 1 to 5). In contrast, in *in vivo* CTL expansion, an antigenic molecule (usually the Id protein) is first introduced into the patient and T cell triggering occurs within the body (B: methods 1 to 3).

unique to the clonal malignant plasma cell. Immunisation of MM patients with autologous Id protein, followed by augmentation of the immune response using soluble granulocyte macrophage-colony stimulating factor (GM-CSF) effectively induces an anti-Id-specific HLA class I-restricted type I T cell response (Table 2 and Fig. 1).²³ These responses may be further modulated by other more complex factors, like those mediating tumour tolerance and T cell loss, for example, Fas and BCL2 expression.²⁴ Hence, the challenge to anti-cancer immunotherapy would be to not only induce a specific anti-tumour CTL immune response, but also to prevent T cell loss or suppression.²⁵

Method 1 — Anti-MM Id-Protein CTL Vaccine

The Id protein can be used as a target for the development of CTL vaccine in MM.²⁶ Both CD4⁺ and CD8⁺ T cells can be primed using Id-pulsed DCs, and

have been shown to exert anti-MM effects, even in the autologous setting.^{23,27,28} In fact, autologous Id-specific CTLs generated from MM patients have been shown to be efficient in killing primary MM cells from patients, via the perforin-mediated pathway.

Method 2 — Anti-MM Whole Cell Extract (WCE) CTL Vaccine

The MM Id protein is a weak Ag and the outcomes of Id-based immunotherapy trials have not been encouraging, perhaps due to cross-reactivity with normal cells.²⁹⁻³¹ The use of DCs pulsed with MM WCEs to generate polyclonal MM-specific CTLs permits the recognition of a larger repertoire of tumour Ags, potentially producing a more effective anti-MM vaccine.³² Such CTLs have been shown to specifically lyse MM cells but not autologous lymphocytes, suggesting that MM-specificity is preserved. The mechanism of this effect again involves the perforin pathway.

Method 3 — Anti-MM Apoptotic Bodies CTL Vaccine

Similar to WCEs, apoptotic bodies are also sources of whole tumour cell Ags, and can be used to pulse DCs to generate anti-MM-specific CTLs.³³ These CTLs have been shown to exert specific cytotoxicity against autologous primary MM cells, leading to tumour rejection. Although the majority of CTLs are CD8⁺ T cells, CD56⁺ NK cells are also induced by this vaccination strategy.

Method 4 — Anti-MM RNA-Transformed DC CTL Vaccine

Yet another method of introducing MM Ags into DCs for CTL production involves the transfer of total tumour RNA into HLA class I-matched DCs.³⁴ Total RNA which potentially contains the full repertoire of antigenic proteins, including putative TSAs, is ideal for the induction of polyclonal CTL responses against MM Ags. Indeed, RNA-transfected DC-induced CTLs lysed MM cells in an HLA class I-restricted manner.³⁴ However, this strategy is associated with certain risks, including the potential for self- or cross- reactivity, toxicity due to non-specificity, and insertional mutagenesis. Moreover, transgene expression is often transient and may even be lost when the transfected DCs divide.

Method 5 — Anti-MM Induced CD40-Ligand-Primed CTL Vaccine

CD40 ligand is known to restore the Ag presentation function in MM. Introduction of the human CD40L (*bCD40L*) gene into human MM cells via an adenoviral vector has been found to induce anti-MM activity *in vivo*.^{35,36} The mechanisms of the anti-MM effect following engagement of CD40 by CD40L expressed on MM cells include induction apoptosis, maturation of APC, and induction of CTLs that recognise tumour Ags engulfed and processed by the APCs.³⁵ However, not all MM cells express CD40 and respond to CD40 activation.

IN VIVO CTL EXPANSION IN MM

Overview

The immunological theory of cancer argues that cancer develops as a consequence of failed tumour immunosurveillance. Hence, it would seem unlikely that *in vivo* autologous CTL expansion would itself be successful, since the lack of patient CTLs is the precise immunological defect in cancer. In reality, T cell populations are good sources of specific anti-tumour effector cells, and are potentially not exhausted by chronic tumour cell stimulation. Moreover, some MM-

specific T cells require fewer accessory signals to express IL-2 receptor (IL-2 receptor is needed for the expansion of HLA class I- and II- dependent T cells), secrete IL-2, and proliferate upon cross-linking of the CD3/TCR complex.³⁷

Method 1 — Anti-MM Id-Protein Plus GM-CSF and/or IL-12

In this method, MM patients were vaccinated with MM-Id protein, followed by GM-CSF and/or IL-12. Although tumours were reduced in size, only some patients demonstrated Id-specific T-cell responses.³⁸

Method 2 — Anti-MM Id-Protein-Pulsed DCs Plus Id-Keyhole Limpet Haemocyanin (KLH) Conjugate²⁹

In this method, patients with MM who received high dose chemotherapy were intravenously infused with Id-pulsed autologous DCs, followed by adjuvant subcutaneous boosts of Id-KLH conjugate.^{30,39} This resulted in the induction of both Id-specific and/or KLH-specific CTL responses; especially in patients with early stage MM (i.e. when tumour burden is low).³⁹ Similar findings had previously been reported in patients with NHL treated with DC-based Id vaccines.⁴⁰ The longer-term results of this study have not been published.

Method 3 — Anti-MM Id-Protein/KLH-Pulsed DCs Plus GM-CSF and IL-2

Conjugation of MM Id-specific proteins with KLH, has been used as an immunogen to vaccinate MM patients in first remission.²⁵ Both GM-CSF as well as IL-2 were included as immunoadjuvants. Although specific immunity was induced, even after high-dose chemotherapy and HSCT, data with regards to the relapse rate of MM is lacking.⁴¹

CONCLUSION

As mentioned, numerous novel therapeutic strategies are emerging for the treatment of MM, including thalidomide in relapsed/refractory MM.^{42,43} Clearly more research needs to be conducted, especially in studying the induction of tumour Ag expression via CD40 triggering for the purpose of tumour Ag discovery.⁴⁴ The identification of novel target molecules will pave the way for the development of novel anti-MM vaccines, especially polyclonal CTL vaccines, which present the greatest promise of improved patient survival.⁴⁵ In conclusion, the current status of CTL vaccines in MM is at its infancy, but the encouraging results of recent immunotherapy clinical trials provide sufficient hope that cure might one day be possible for MM.

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REFERENCES

- Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1996. *CA Cancer J Clin* 1996; 46:5-27.
- Gupta D, Hideshima T, Anderson KC. Novel biologically based therapeutic strategies in myeloma. *Rev Clin Exp Hematol* 2002; 6:301-24.
- Raje N, Anderson KC. Multiple myeloma. *Curr Treat Options Oncol* 2000; 1:73-82.
- Heiser A, Dahm P, Yancey DR, Maurice MA, Boczkowski D, Nair SK, et al. Human dendritic cells transfected with RNA encoding prostate-specific antigen stimulate prostate-specific CTL responses in vitro. *J Immunol* 2000; 164:5508-14.
- Morris EC, Bendle GM, Stauss HJ. Prospects for immunotherapy of malignant disease. *Clin Exp Immunol* 2003; 131:1-7.
- Bose S, Deininger M, Gora-Tybor J, Goldman JM, Melo JV. The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. *Blood* 1998; 92:3362-7.
- Chiriva-Internati M, Wang Z, Salati E, Wroblewski D, Lim SH. Successful generation of sperm protein 17 (Sp17)-specific cytotoxic T lymphocytes from normal donors: implication for tumour-specific adoptive immunotherapy following allogeneic stem cell transplantation for Sp17-positive multiple myeloma. *Scand J Immunol* 2002; 56:429-33.
- Hartshorn KL, Reid KB, White MR, Jensenius JC, Morris SM, Tauber AI, et al. Neutrophil deactivation by influenza A viruses: mechanisms of protection after viral opsonization with collectins and hemagglutination-inhibiting antibodies. *Blood* 1996; 87:3450-61.
- Reilly RT, Emens LA, Jaffee EM. Humoral and cellular immune responses: independent forces or collaborators in the fight against cancer? *Curr Opin Investig Drugs* 2001; 2:133-5.
- Nadler LM, Stashenko P, Hardy R, Kaplan WD, Button LN, Kufe DW, et al. Serotherapy of a patient with a monoclonal antibody directed against a human lymphoma-associated antigen. *Cancer Res* 1980; 40:3147-54.
- Salgaller ML, Weber JS, Koenig S, Yannelli JR, Rosenberg SA. Generation of specific anti-melanoma reactivity by stimulation of human tumor-infiltrating lymphocytes with MAGE-1 synthetic peptide. *Cancer Immunol Immunother* 1994; 39:105-16.
- Svane IM, Soot ML, Buus S, Johnsen HE. Clinical application of dendritic cells in cancer vaccination therapy. *APMIS* 2003; 111:818-34.
- Mondino A, Jenkins MK. Surface proteins involved in T cell costimulation. *J Leukoc Biol* 1994; 55:805-15.
- Clarke SR. The critical role of CD40/CD40L in the CD4-dependent generation of CD8+ T cell immunity. *J Leukoc Biol* 2000; 67:607-14.
- Diehl L, Den Boer AT, van der Voort EI, Melief CJ, Offringa R, Toes RE. The role of CD40 in peripheral T cell tolerance and immunity. *J Mol Med* 2000; 78:363-71.
- Atkinson EA, Bleackley RC. Mechanisms of lysis by cytotoxic T cells. *Crit Rev Immunol* 1995; 15:359-84.
- Teoh G. The Role of CD40 in the discovery of tumour-specific antigens. *SGH Proceedings* 2001; 9:174-80.
- Sin JI, Kim JJ, Zhang D, Weiner DB. Modulation of cellular responses by plasmid CD40L: CD40L plasmid vectors enhance antigen-specific helper T cell type 1 CD4+ T cell-mediated protective immunity against herpes simplex virus type 2 in vivo. *Hum Gene Ther* 2001; 12:1091-102.
- Massaia M, Attisano C, Peola S, Montacchini L, Omede P, Corradini P, et al. Rapid generation of antiplasma cell activity in the bone marrow of myeloma patients by CD3-activated T cells. *Blood* 1993; 82:1787-97.
- Osterborg A, Masucci M, Bergenbrant S, Holm G, Lefvert AK, Mellstedt H. Generation of T cell clones binding F(ab')₂ fragments of the idiotypic immunoglobulin in patients with monoclonal gammopathy. *Cancer Immunol Immunother* 1991; 34:157-62.
- Yi Q, Osterborg A, Bergenbrant S, Mellstedt H, Holm G, Lefvert AK. Idiotype-reactive T-cell subsets and tumor load in monoclonal gammopathies. *Blood* 1995; 86:3043-9.
- Yi Q, Holm G, Lefvert AK. Idiotype-induced T cell stimulation requires antigen presentation in association with HLA-DR molecules. *Clin Exp Immunol* 1996; 104:359-65.
- Osterborg A, Yi Q, Henriksson L, Fagerberg J, Bergenbrant S, Jeddi-Tehrani M, et al. Idiotype immunization combined with granulocyte-macrophage colony-stimulating factor in myeloma patients induced type I, major histocompatibility complex-restricted, CD8- and CD4-specific T-cell responses. *Blood* 1998; 91:2459-66.
- Massaia M, Borrione P, Attisano C, Barral P, Beggiano E, Montacchini L, et al. Dysregulated Fas and Bcl-2 expression leading to enhanced apoptosis in T cells of multiple myeloma patients. *Blood* 1995; 85:3679-87.
- Massaia M, Borrione P, Battaglio S, Mariani S, Beggiano E, Napoli P, et al. Idiotype vaccination in human myeloma: generation of tumor-specific immune responses after high-dose chemotherapy. *Blood* 1999; 94:673-83.
- Bergenbrant S, Yi Q, Osterborg A, Bjorkholm M, Osby E, Mellstedt H, et al. Modulation of anti-idiotypic immune response by immunization with the autologous M-component protein in multiple myeloma patients. *Br J Haematol* 1996; 92:840-6.
- Dabadghao S, Bergenbrant S, Anton D, He W, Holm G, Yi Q. Anti-idiotypic T-cell activation in multiple myeloma induced by M-component fragments presented by dendritic cells. *Br J Haematol* 1998; 100:647-54.
- Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996; 17:138-46.
- Liso A, Stockerl-Goldstein KE, Auffermann-Gretzinger S, Benike CJ, Reichardt V, van Beckhoven A, et al. Idiotype vaccination using dendritic cells after autologous peripheral blood progenitor cell transplantation for multiple myeloma. *Biol Blood Marrow Transplant* 2000; 6:621-7.
- Reichardt VL, Okada CY, Liso A, Benike CJ, Stockerl-Goldstein KE, Engleman EG, et al. Idiotype vaccination using dendritic cells after autologous peripheral blood stem cell transplantation for multiple myeloma — a feasibility study. *Blood* 1999; 93:2411-9.
- Thurner B, Haendle I, Roder C, Dieckmann D, Keikavoussi P, Jonuleit H, et al. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J Exp Med* 1999; 190:1669-78.

32. Wen YJ, Min R, Tricot G, Barlogie B, Yi Q. Tumor lysate-specific cytotoxic T lymphocytes in multiple myeloma: promising effector cells for immunotherapy. *Blood* 2002; 99:3280-5.
33. Hayashi T, Hideshima T, Akiyama M, Raje N, Richardson P, Chauhan D, et al. Ex vivo induction of multiple myeloma-specific cytotoxic T lymphocytes. *Blood* 2003; 102:1435-42.
34. Milazzo C, Reichardt VL, Muller MR, Grunebach F, Brossart P. Induction of myeloma-specific cytotoxic T cells using dendritic cells transfected with tumor-derived RNA. *Blood* 2003; 101:977-82.
35. Dotti G, Savoldo B, Takahashi S, Goltsova T, Brown M, Rill D, et al. Adenovector-induced expression of human-CD40-ligand (hCD40L) by multiple myeloma cells. A model for immunotherapy. *Exp Hematol* 2001; 29:952-61.
36. Kikuchi T, Crystal RG. Anti-tumor immunity induced by in vivo adenovirus vector-mediated expression of CD40 ligand in tumor cells. *Hum Gene Ther* 1999; 10:1375-87.
37. Massaia M, Bianchi A, Attisano C, Peola S, Redoglia V, Dianzani U, et al. Detection of hyperreactive T cells in multiple myeloma by multivalent cross-linking of the CD3/TCR complex. *Blood* 1991; 78:1770-80.
38. Rasmussen T, Hansson L, Osterborg A, Johnsen HE, Mellstedt H. Idiotype vaccination in multiple myeloma induced a reduction of circulating clonal tumor B cells. *Blood* 2003; 101:4607-10.
39. Liso A, Stockerl-Goldstein KE, Auffermann-Gretzinger S, Benike CJ, Reichardt V, van Beckhoven A, et al. Idiotype vaccination using dendritic cells after autologous peripheral blood progenitor cell transplantation for multiple myeloma. *Biol Blood Marrow Transplant* 2000; 6:621-7.
40. Hsu FJ, Benike C, Fagnoni F, Liles TM, Czerwinski D, Taidi B, et al. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat Med* 1996; 2:52-8.
41. Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. *N Engl J Med* 1996; 335:91-7.
42. Smith ML, Newland AC. Treatment of myeloma. *QJM* 1999; 92:11-4.
43. Clerc D, Fermand JP, Mariette X. Treatment of multiple myeloma. *Joint Bone Spine* 2003; 70:175-86.
44. Shen J, Zhao Y, Poh CK, Tham SC, Au M, Cow G, et al. CD40 Triggering alone without interleukin-4 induces DNA double strand breaks in SGH-MM1 cell line. *Singapore General Hospital Proc* 2003; 12:S83.
45. Raitakari M, Brown RD, Gibson J, Joshua DE. T cells in myeloma. *Hematol Oncol* 2003; 21:33-42.
46. Wen YJ, Barlogie B, Yi Q. Idiotype-specific cytotoxic T lymphocytes in multiple myeloma: evidence for their capacity to lyse autologous primary tumor cells. *Blood* 2001; 97:1750-5.