

Review Article

Towards an off-the-shelf vaccine therapy targeting shared B-cell tumor idiotypes

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Abstract: The ideal tumor antigen is one expressed selectively by the tumor, present in all cancer patients, essential for tumor survival and nonetheless able to induce both humoral and cellular immune response. The personalized idiotype (Id) of the surface immunoglobulin is a tumor specific antigen in that it is expressed on clonal B-cell tumors, mediates B-cell survival, and induces tumor specific immunity in both human and animal models. With the availability of monoclonal antibodies against B cells, such as rituximab, the cellular immune response mediated by specific T cells has gained more importance as a combination therapy for the complete elimination of residual tumor cells in lymphoma and myeloma.

Keywords: Myeloma, allogeneic T cells, immunotherapy

Introduction

Immunoglobulin molecules are composed of heavy and light chains, which possess highly specific variable regions at their amino termini. The variable regions of heavy and light chains combine to form the unique antigen-recognition site of the Ig protein. Since the clonal B-cell tumor only expresses one type of immunoglobulin, the idiotypic determinants expressed by B-cells malignancies can serve as unique tumor-specific antigens. Previous studies in human and animal models have demonstrated idiotype vaccines can increase the capability of the immune system to fight residual disease and prolong the duration of chemotherapy-induced clinical remission [1-3].

A major limitation of an idiotype vaccine is the requirement for a custom-made product for each patient making the manufacturing of the vaccine expensive, laborious and time consuming. To overcome these difficulties, identification of novel lymphoma-associated antigens that are shared between patients and universally expressed in multiple B-cell malignancies is necessary [4].

This review will update the achievements, advantages, and barriers to the development of

idiotype vaccines as standard of care for lymphoma treatment.

Pre-clinical optimization

As one of the earliest tumor specific antigens discovered in 1970s [1, 5], idiotype protein was demonstrated to induce strong humoral immune responses, as anti-idiotype antibodies could easily be detected in vaccinated animals or patients [2]. Immune responses induced by idiotype vaccines are likely to be polyclonal, directed against multiple epitopes of a candidate tumor antigen and have immunological memory [6].

Because the idiotype protein alone is a weak antigen, in order to induce a strong immunogenic responses, keyhole limpet hemocyanin (KLH) has been used as a carrier. KLH is an oxygen-carrying respiratory protein obtained from a marine mollusk *Megathura crenulata*. KLH has large size and numerous epitopes that can generate a substantial immune response. The abundant lysine residues in KLH allow a high hapten carrier protein ratio to increase the likelihood of generating epitope-specific antibodies. Kwak, working in the laboratory of Levy at Stanford University, observed that

the 38C13 idiotype protein (38C-Id), coupled to KLH and administered with an adjuvant, induce strong antitumor immunity in mice. Animals that were immunized with 38C-Id after 3 weeks recuperation and challenged with 1000 38C13 tumor cells 2 weeks later demonstrated significantly longer survival when compared to control animals which had been immunized with an irrelevant idiotype protein [7]. Anti-38C-Id antibodies, implicated in the mechanism of idiotype induced anti-tumor immunity in this model, were detectable after immunization at both 3 and 5 weeks. Interestingly, however, there was no significant correlation between serum antibody levels and survival of individual mice. Aside from KLH, anti-CD40 antibody and maleimide were also used as carriers with idiotype vaccine to induce a strong immune response in patients [8, 9].

In the late 1990s, there was more and more evidence demonstrating that T-cell mediated cellular immunity played an important role in the clearance of residual tumor cells after standard therapy, and low-dose granulocyte-macrophage colony-stimulating factor (GM-CSF) was added to the idiotype vaccine formulation. GM-CSF is a strong inducer for the recruitment of antigen presenting cells (APCs), including dendritic cells to the tumor site, which stimulate T-cell mediated immunity by cross-presentation. In 1996, Kwak demonstrated that idiotype vaccine, conjugated with KLH, administered together with GM-CSF at the vaccine site, significantly enhanced protective antitumor immunity. This effect was critically dependent upon effector CD4⁺ and CD8⁺ T cells and was not associated with any increased anti-idiotypic antibody production. Lymphocytes from spleens and draining lymph nodes of mice primed with Id-KLH plus GM-CSF, but not with Id-KLH alone demonstrated significant proliferation to Id in vitro without any biased production of interferon gamma or interleukin 4 protein or mRNA. 50% of mice immunized with Id-KLH plus GM-CSF on the same day remained tumor-free at day 80, compared with 17% for Id-KLH alone, when immunization was combined with cyclophosphamide. Taken together, these results demonstrated that GM-CSF could significantly enhance the immunogenicity of a defined self-antigen and that this effect is mediated exclusively by activating the T-cell arm of the immune response [10].

Clinical translation

Translation of the finding from the murine model led to the first human trial conducted in 40 patients with follicular lymphoma using idiotype vaccination [11]. Patients were selected for minimal residual disease or complete remission status after chemotherapy. The immunoglobulin-idiotype protein was conjugated with KLH and the vehicle component of SAF-1 (Syntex adjuvant formulation 1) described by Allison and Byars [12]. This trial demonstrated the efficacy, feasibility and safety of Id-KLH+ emulsion adjuvant to induce immune responses against the autologous tumor Id.

Based on the induction of tumor-specific CD8⁺ T cell in a preclinical study adding GM-CSF as adjuvant, a Phase II clinical trial was conducted at the National Cancer Institute by the Kwak laboratory in which an autologous tumor-derived Id-KLH+GM-CSF vaccine was administered to 20 patients. The vaccine was injected subcutaneously in 5 monthly doses approximately 6 months after chemotherapy to allow time for immunological recovery. No major adverse effects were detected except for a local erythema, induration and pruritus in the area of injection. The results of this phase II clinical trial confirmed and extended the results obtained in the Phase I: 75% of patients (15/20) had anti-idiotypic antibody response and 95% of patients (19/20) had Id-specific and/or tumor specific CD4⁺ and CD8⁺ T-cell responses [13]. Minimal residual disease eradication was observed by monitoring for the t(14;18) chromosomal translocation breakpoints in the peripheral blood which were monitored using a nested PCR assay in a period of time between 8 and 32 months. It was found that 8 of 11 patients with detectable translocations in their primary tumors had cells from the malignant clone detected in their blood by PCR both at diagnosis and after chemotherapy, and converted to undetectable cells in their blood from the malignant clone after vaccination and sustained their molecular remissions. Tumor-specific cytotoxic CD8⁺ and CD4⁺ T cells were uniformly found (19 of 20 patients), whereas antibodies were detected, but apparently were not required for molecular remission. Vaccination was thus associated with clearance of residual tumor cells from blood and long-term disease-free survival. The demon-

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stration of molecular remissions, analysis of cytotoxic T lymphocytes against autologous tumor targets, and the addition of granulocyte-monocyte colony-stimulating factor to the vaccine formulation provide principles relevant to the design of future clinical trials of other cancer vaccines administered in a minimal residual disease setting [14].

Other independent clinical trials confirmed the effects of Id-KLH+GM-CSF vaccination. For example in 25 patients with follicular lymphoma achieving a second complete clinical response, 80% (20/25) of the patients showed a vaccine-induced idiotype- and/or tumor-specific immune response. Thirteen (52%) out of 25 patients showed a specific humoral response mounted after two to four vaccinations. Furthermore, 18 of 25 (72%) patients developed a specific cellular response [15]. Again the finding confirmed the immunogenicity of the Id-KLH+GM-CSF vaccine in a minimal residual disease setting, after chemotherapy in patients with follicular lymphoma. In another Phase II clinical trial, Redfern et al. treated 32 patients with relapsed indolent non-Hodgkin lymphoma to study the efficacy of idiotype vaccine as a single treatment agent. Nine patients have been tested and six of them showed a cellular immune response and 4/20 -20%- responded against their own Id [16].

The availability of monoclonal antibodies has raised the question of whether the idiotype vaccine could be used to complement rituximab-based chemotherapy. Rituximab depletes malignant and normal B cells and thus impairs the generation of antibody due to vaccination. A phase II clinical trial was designed by the Kwak laboratory at NCI to investigate the capability of Id-vaccine to raise tumor-specific immunity despite the severe B-cell depletion due to rituximab-based chemotherapy in 23 previously untreated mantle cell lymphoma patients. Starting from three months after 6 cycles of DA-EPOCH-R, 5 monthly cycles of autologous tumor-derived Id-KLH+GM-CSF vaccination were administered. B-cell repertoire began to recover after 6 months to baseline levels in about 1 year. Although rituximab had depleted the peripheral blood B cells, antibody response against the carrier molecule KLH and Id were detected in 17/23 (74%) and 7/23 (30%) evaluable patients, respectively. Interestingly CD4⁺ and CD8⁺ response against the tumor and against Id were induced in 20/23 (87%) and 23/23 (100%) respectively. These results show

that severe B-cell depletion does not impair T-cell priming in humans. Based on these results, it is justifiable to administer vaccines in the setting of B-cell depletion; however, vaccine boosts after B-cell recovery may be necessary for optimal humoral responses [17].

The successful results from these Phase I/II trials with Id-KLH+GM-CSF formula led to the initiation of three randomized double-blind placebo-controlled multicenter clinical trials to address the question of the clinical benefit induced by idiotype vaccination.

The first phase III clinical trial was started in January 2000 at the NCI and then expanded to 17 centers in the US and Europe under sponsorship of Biovest International. The primary objective of this prospective randomized double-blind controlled trial was to determine whether Id vaccination is able to prolong the disease free survival (DFS) in FL patients in a durable CR/CRu after chemotherapy. 117 patients with follicular lymphoma were randomized; 76 patients - to receive - Id-KLH+GM-CSF (BiovaxId) and 41 patients - not to receive KLH+GM-CSF (control) vaccination. The Id protein was successfully produced and manufactured by heterohybridoma technology in 71 (93%) of 76 patients assigned to receive Id vaccine. Five patients who were assigned to the experimental arm received KLH+GM-CSF because Id protein could not be produced. For the 117 patients who received at least one blinded vaccination, the median disease free survival (DFS) was significantly prolonged in the Id- vaccine arm compared with the control arm. After a median follow-up of 56.6 months, the median DFS was 44.2 months for the Id- vaccine randomized group and 30.6 months for the control (p=0.045). Among patients receiving IgM-Id vaccine the median time to relapse after randomization was 52.9 months versus 28.7 months in IgM tumor isotype control-treated patients. These results demonstrate that the patient-specific Id-protein vaccine significantly prolonged DFS compared with the control vaccine [18].

The Genitope sponsored trial used CVP chemotherapy regimen (cyclophosphamide, vincristine and prednisone), enrolled complete response and partial response patients, and used recombinant DNA technology for production of idiotype protein. Eligible patients were random-

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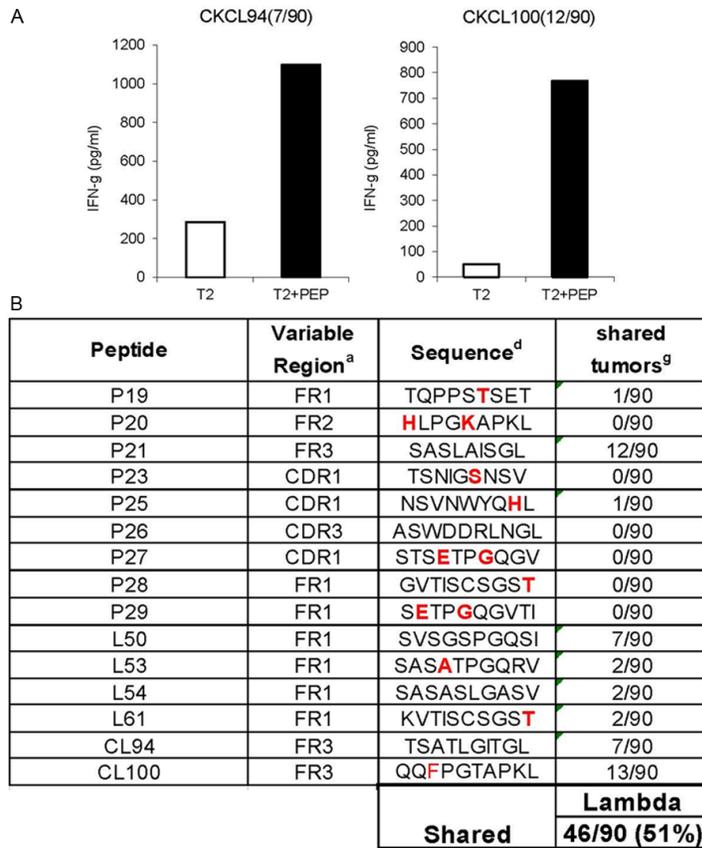


Figure 1. Identification of HLA A2 restricted idiotype shared T-cell epitopes in lambda chain. A. IFN- γ ELISA assay of shared peptide stimulated T cells. B. Summary of shared percentage of HLA A2 restricted idiotype peptides.

ized to receive Id-KLH+GM-CSF or control vaccine (KLH-GM-CSF) in a 2:1 ratio for 6 months after completion of eight cycles of CVP. Seven immunization cycles were administered over 24 weeks. 278 out of 287 randomized patients showed specific anti-Id antibody response. No statistical difference in term of progression free survival was observed between the two arms [19].

The third phase III clinical trial sponsored by Favril investigated the efficacy of recombinant Id-KLH vaccination after treatment with rituximab. Previously untreated and relapsed/refractory FL patients in complete remission, partial response or stable disease 2 months after a standard 4-week course of rituximab were randomized 1:1 to receive Id-KLH plus GM-CSF or control (Placebo+GM-CSF) vaccine. Patients were immunized six times monthly, then six times every other month and finally every 3 months until disease progression. The

primary efficacy endpoint was time to progression. After a median follow-up of 40 months, the Favril-sponsored trial showed no improvement in time to progression for the vaccine when compared with the control arm [1].

There are many differences in the clinical trials described above which account for the difference in results. The Biovest-sponsored trial faithfully reproduced the Phase II design and included only patients in complete remission whereas the Genitope-sponsored trial included patients in partial response, and the Favril-sponsored trial included patients in partial and stable disease. Furthermore, choosing a doxorubicin-containing chemotherapy regimen most likely resulted in a higher chance of having patients achieving complete remission in the Biovaxid trial.

Identification of shared T-cell epitopes in idiotype antigens

The traditional method to manufacture idiotype vaccines involves the use of mouse/human heterohybridoma in order to induce the secretion of a large amount of tumor derived immunoglobulin (idiotype or Id). Supernatant - containing idiotype - is collected and purified by affinity chromatography and conjugated to KLH carrier protein, resulting in a finished protein vaccine that can be shipped and administered to patients. In the Phase III clinical trial, there was a 95% manufacturing success rate of vaccine production. However, this individual preparation of the vaccine is laborious and time consuming. Recently, recombinant DNA technology has been used to generate idiotype proteins with the purpose of shortening the vaccine production time. In the recombinant approach, the variable regions of the heavy (VH) and light (VL) chains of the tumor immunoglobulin are cloned by polymerase chain reaction (PCR) and then inserted into an expression vector for the production of the idiotype protein either in mammalian cells, insect cells, tobacco plants or *Escherichia coli* [4, 20, 21]. This new approach, although faster than the traditional hybridoma

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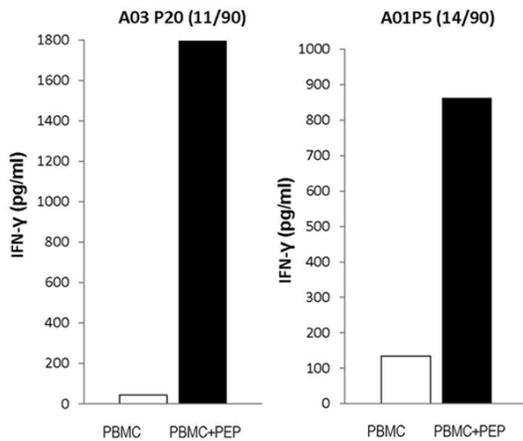


Figure 2. Identification of HLA A3 and HLA A1 restricted idiotype shared T-cell epitopes in lambda chain by IFN- γ ELISA assay.

approach, still takes approximately 2 months to manufacture a vaccine for each patient.

Recently, our group has found that the Ig light chain v-region, as well as the idiotype heavy chain, contain important T cell epitope for T cell stimulation. Moreover, we have found idiotype light chain T cell epitopes that are shared by patients. 14 novel T cell epitopes from lambda light chains of myeloma tumor cells and primary lymphoma tumors could be used to generate 68 CTLs from ten HLA A2+ normal donors, indicating the immunogenicity of lambda light chain peptides. Importantly, about 30% of lymphoma patients with lambda chain express at least one of these 14 epitopes we identified and T cells stimulated by a shared epitope lysed the tumor cells expressing the same epitope and MHC allele, but not tumors expressing irrelevant Ids, or the respective patients' PBMCs, suggesting a novel strategy to overcome the individual preparation of idiotype vaccine [22]. In our continued efforts to find shared epitopes in idiotype light chains we have identified 2 additional HLA A2 restricted T cell epitopes in lambda chain, which have significantly increased the coverage of HLA 2 patients with lambda chain from 30% to 51% (**Figure 1**).

Moreover, according to the literature, HLA A1 and HLA A3 are represented in about 20 to 25% of the Caucasian population [23]. In our effort to characterize shared T cell epitope in idiotype in other HLA alleles, we have identified four HLA A3 and 2 HLA A1 restricted T cell epitopes from idiotype lambda light chain that are shared by 20% and 25% of patients with HLA

A3 or HLA A1 allele, respectively. The T cells stimulated by HLA A3 or HLA A1 peptides secreted a large amount of IFN- γ when incubated with autologous PBMCs loaded with HLA A3 or HLA A1 peptide, indicating the same strategy can be applied to all MHC alleles (**Figure 2**).

Future directions

Adoptive T cell transfer has been found to be an effective immunotherapy in a variety of clinical trials of both solid and hematologic cancers [24-26]. Compared to active immunization, adoptive T cell transfer has several advantages. First: adoptive T cell transfer does not require full integrity of the immune system of the recipients. Second, the adoptive transferred T cells can be selected by function and phenotypes prior to the adoptive transfer. Lastly, the availability of varied cytokines has made possible the in-vitro expansion of T cells to large scale. Recent studies of adoptive transfer with autologous T cells transferred with TCRs against MART-1, gp100, NY-ESO-1, and CEA tumor specific antigens have demonstrated significant tumor growth inhibition in melanoma, prostate carcinoma, colorectal cancer, and kidney cancer, confirming the promising therapeutic effect of this technique [27-33]. Compared to the chimeric antigen receptors (CARs) technology, TCR technology has unique advantages in that it can recognize intracellular tumor antigens, have higher binding affinity to peptide-MHC allele, and retain integral T cell activation signaling pathways [34, 35]. The identification of shared T-cell epitopes in the idiotype protein provides a basis for the future development of either an off-the-shelf vaccine or TCR-based adoptive T cell therapy.

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References

- [1] Sirisinha S and Eisen HN. Autoimmune-like antibodies to the ligand-binding sites of myeloma proteins. *Proc Natl Acad Sci U S A* 1971; 68: 3130-3135.
- [2] Stevenson GT and Stevenson FK. Antibody to a molecularly-defined antigen confined to a tumour cell surface. *Nature* 1975; 254: 714-716.

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- [3] Stevenson GT, Elliott EV and Stevenson FK. Idiotypic determinants on the surface immunoglobulin of neoplastic lymphocytes: a therapeutic target. *Fed Proc* 1977; 36: 2268-2271.
- [4] Weng J, Rawal S, Chu F, Park HJ, Sharma R, Delgado DA, Fayad L, Fanale M, Romaguera J, Luong A, Kwak LW and Neelapu SS. TCL1: a shared tumor-associated antigen for immunotherapy against B-cell lymphomas. *Blood* 2012; 120: 1613-1623.
- [5] Lynch RG, Graff RJ, Sirisinha S, Simms ES and Eisen HN. Myeloma proteins as tumor-specific transplantation antigens. *Proc Natl Acad Sci U S A* 1972; 69: 1540-1544.
- [6] Park HJ and Neelapu SS. Developing idiotype vaccines for lymphoma: from preclinical studies to phase III clinical trials. *Br J Haematol* 2008; 142: 179-191.
- [7] Kwak LW, Campbell M and Levy R. Idiotype vaccination post-bone marrow transplantation for B-cell lymphoma: initial studies in a murine model. *Cancer Detect Prev* 1991; 15: 323-325.
- [8] Carlring J, Szabo MJ, Dickinson R, De Leenheer E and Heath AW. Conjugation of lymphoma idiotype to CD40 antibody enhances lymphoma vaccine immunogenicity and antitumor effects in mice. *Blood* 2012; 119: 2056-2065.
- [9] Kafi K, Betting DJ, Yamada RE, Bacica M, Steward KK and Timmerman JM. Maleimide conjugation markedly enhances the immunogenicity of both human and murine idiotype-KLH vaccines. *Mol Immunol* 2009; 46: 448-456.
- [10] Kwak LW, Young HA, Pennington RW and Weeks SD. Vaccination with syngeneic, lymphoma-derived immunoglobulin idiotype combined with granulocyte/macrophage colony-stimulating factor primes mice for a protective T-cell response. *Proc Natl Acad Sci U S A* 1996; 93: 10972-10977.
- [11] Kwak LW, Campbell MJ, Czerwinski DK, Hart S, Miller RA and Levy R. Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumors. *N Engl J Med* 1992; 327: 1209-1215.
- [12] Allison AC and Byars NE. An adjuvant formulation that selectively elicits the formation of antibodies of protective isotypes and of cell-mediated immunity. *J Immunol Methods* 1986; 95: 157-168.
- [13] Bendandi M, Gocke CD, Kobrin CB, Benko FA, Sternas LA, Pennington R, Watson TM, Reynolds CW, Gause BL, Duffey PL, Jaffe ES, Creekmore SP, Longo DL and Kwak LW. Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. *Nat Med* 1999; 5: 1171-1177.
- [14] Lee MS, Chang KS, Cabanillas F, Freireich EJ, Trujillo JM and Stass SA. Detection of minimal residual cells carrying the t(14;18) by DNA sequence amplification. *Science* 1987; 237: 175-178.
- [15] Inoges S, Rodriguez-Calvillo M, Zabalegui N, Lopez-Diaz de Cerio A, Villanueva H, Soria E, Suarez L, Rodriguez-Caballero A, Pastor F, Garcia-Munoz R, Panizo C, Perez-Calvo J, Melero I, Rocha E, Orfao A and Bendandi M. Clinical benefit associated with idiotypic vaccination in patients with follicular lymphoma. *J Natl Cancer Inst* 2006; 98: 1292-1301.
- [16] Redfern CH, Guthrie TH, Bessudo A, Densmore JJ, Holman PR, Janakiraman N, Leonard JP, Levy RL, Just RG, Smith MR, Rosenfelt FP, Wiernik PH, Carter WD, Gold DP, Melink TJ, Gutheil JC and Bender JF. Phase II trial of idiotype vaccination in previously treated patients with indolent non-Hodgkin's lymphoma resulting in durable clinical responses. *J Clin Oncol* 2006; 24: 3107-3112.
- [17] Neelapu SS, Kwak LW, Kobrin CB, Reynolds CW, Janik JE, Dunleavy K, White T, Harvey L, Pennington R, Stetler-Stevenson M, Jaffe ES, Steinberg SM, Gress R, Hakim F and Wilson WH. Vaccine-induced tumor-specific immunity despite severe B-cell depletion in mantle cell lymphoma. *Nat Med* 2005; 11: 986-991.
- [18] Schuster SJ, Neelapu SS, Gause BL, Janik JE, Muggia FM, Gockerman JP, Winter JN, Flowers CR, Nikcevich DA, Sotomayor EM, McGaughey DS, Jaffe ES, Chong EA, Reynolds CW, Berry DA, Santos CF, Popa MA, McCord AM and Kwak LW. Vaccination with patient-specific tumor-derived antigen in first remission improves disease-free survival in follicular lymphoma. *J Clin Oncol* 2011; 29: 2787-2794.
- [19] Levy R, Robertson M, Leonard J, Vose J and Denney D. Results of a phase 3 trial evaluating safety and efficacy of specific immunotherapy, recombinant idiotype (ID) conjugated to KLH (ID-KLH) with GM-CSF, compared to non-specific immunotherapy, KLH with GM-CSF, in patients with follicular non-Hodgkins lymphoma (FNHL). *Ann Oncology* 2008; 19: 101-101.
- [20] McCormick AA, Kumagai MH, Hanley K, Turpen TH, Hakim I, Grill LK, Tuse D, Levy S and Levy R. Rapid production of specific vaccines for lymphoma by expression of the tumor-derived single-chain Fv epitopes in tobacco plants. *Proc Natl Acad Sci U S A* 1999; 96: 703-708.
- [21] Kanter G, Yang J, Voloshin A, Levy S, Swartz JR and Levy R. Cell-free production of scFv fusion proteins: an efficient approach for personalized lymphoma vaccines. *Blood* 2007; 109: 3393-3399.
- [22] Weng J, Cha SC, Matsueda S, Alatrash G, Popescu MS, Yi Q, Molldrem JJ, Wang M, Neelapu

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- SS and Kwak LW. Targeting human B-cell malignancies through Ig light chain-specific cytotoxic T lymphocytes. *Clin Cancer Res* 2011; 17: 5945-5952.
- [23] Kessler JH, Mommaas B, Mutis T, Huijbers I, Vissers D, Benckhuijsen WE, Schreuder GMT, Offringa R, Goulmy E, Melief CJM, van der Burg SH and Drijfhout JW. Competition-based cellular peptide binding assays for 13 prevalent HLA class I alleles using fluorescein-labeled synthetic peptides. *Hum Immunol* 2003; 64: 245-255.
- [24] Gattinoni L, Powell DJ, Rosenberg SA and Restifo NP. Adoptive immunotherapy for cancer: building on success. *Nat Rev Immunol* 2006; 6: 383-393.
- [25] Rosenberg SA, Restifo NP, Yang JC, Morgan RA and Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. *Nat Rev Cancer* 2008; 8: 299-308.
- [26] Dudley ME and Rosenberg SA. Adoptive-cell-transfer therapy for the treatment of patients with cancer. *Nat Rev Cancer* 2003; 3: 666-675.
- [27] Johnson LA, Heemskerk B, Powell DJ Jr, Cohen CJ, Morgan RA, Dudley ME, Robbins PF and Rosenberg SA. Gene transfer of tumor-reactive TCR confers both high avidity and tumor reactivity to nonreactive peripheral blood mononuclear cells and tumor-infiltrating lymphocytes. *J Immunol* 2006; 177: 6548-6559.
- [28] Schaft N, Dorrie J, Muller I, Beck V, Baumann S, Schunder T, Kampgen E and Schuler G. A new way to generate cytolytic tumor-specific T cells: electroporation of RNA coding for a T cell receptor into T lymphocytes. *Cancer Immunol Immunother* 2006; 55: 1132-1141.
- [29] Wargo JA, Robbins PF, Li Y, Zhao Y, El-Gamil M, Caragacianu D, Zheng Z, Hong JA, Downey S, Schrumpp DS, Rosenberg SA and Morgan RA. Recognition of NY-ESO-1+ tumor cells by engineered lymphocytes is enhanced by improved vector design and epigenetic modulation of tumor antigen expression. *Cancer Immunol Immunother* 2009; 58: 383-394.
- [30] Parkhurst MR, Joo J, Riley JP, Yu Z, Li Y, Robbins PF and Rosenberg SA. Characterization of genetically modified T-cell receptors that recognize the CEA:691-699 peptide in the context of HLA-A2.1 on human colorectal cancer cells. *Clin Cancer Res* 2009; 15: 169-180.
- [31] Abad JD, Wrzensinski C, Overwijk W, De Witte MA, Jorritsma A, Hsu C, Gattinoni L, Cohen CJ, Paulos CM, Palmer DC, Haanen JB, Schumacher TN, Rosenberg SA, Restifo NP and Morgan RA. T-cell receptor gene therapy of established tumors in a murine melanoma model. *J Immunother* 2008; 31: 1-6.
- [32] de Witte MA, Bendle GM, van den Boom MD, Coccoris M, Schell TD, Tevethia SS, van Tinteren H, Mesman EM, Song JY and Schumacher TN. TCR gene therapy of spontaneous prostate carcinoma requires in vivo T cell activation. *J Immunol* 2008; 181: 2563-2571.
- [33] Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, Royal RE, Topalian SL, Kammula US, Restifo NP, Zheng Z, Nahvi A, de Vries CR, Rogers-Freezer LJ, Mavroukakis SA and Rosenberg SA. Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes. *Science* 2006; 314: 126-129.
- [34] June C, Rosenberg SA, Sadelain M and Weber JS. T-cell therapy at the threshold. *Nat Biotechnol* 2012; 30: 611-614.
- [35] Zhang L and Morgan RA. Genetic engineering with T cell receptors. *Adv Drug Deliv Rev* 2012; 64: 756-762.