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Review

Active specific immunization and adoptive T cell-based immunotherapy for melanoma: Current status and future prospects

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ABSTRACT

Experimental therapies for melanoma with melanoma associated synthetic peptide(s), dendritic cell (DC)-based "vaccines", or with *ex vivo*-expanded tumor infiltrating T lymphocytes (TIL) now have a history of two decades of intense studies. More recently, melanoma epitope specific T cell receptor (TCR)-engineered T cells (Teng) have been incorporated in the field as another potentially useful strategy. These forms of treatment modalities have shown promise as complete and durable responses in some patients have been achieved with all of them. While the result of the individual approaches varies somewhat, the overall rates of useful and durable responses with these therapeutic modalities, in general, remains low. Understandably, these types of experimental therapeutic research have come to a critical crossroads. While a number of strategies (DNA-based vaccines, novel forms of adjuvant, inclusion of reagents that would block the negative signaling pathway(s) in T cells, et cetera) are pursued to improve the outcome, creative new strategies are needed to move the field forward. This review will briefly summarize the results of these forms of experimental melanoma therapies, examine the major obstacles, and present some new ideas for making these forms of immuno-therapies more effective and generally applicable in cancer medicine.

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INTRODUCTION

Three critical sets of observations in tumor immunology provided the rationales for the contemporary exercises at treating cancer with cancer "vaccine" or with the patients' own cytotoxic T lymphocytes (CTL), activated and expanded *ex vivo*. The first set of observation was that cancer patients do harbor T cells that are capable of recognizing the same patients' cancer cells [1, 2], the second was the observation that T cells grown and expanded, *in vitro*, from tumor tissues are capable of exhibiting anti-tumor effector function [3], and the third was the identification of the antigens and peptide epitopes that the CTL recognize on appropriate MHC molecules [4, 5]. All of these observations were, first, made in human melanoma. As such, human melanoma became the model, par excellence, for *in vivo* clinical trials of active specific or adoptive tumor immunotherapy, and the melanoma model dominated the field of tumor immunology, in general, and tumor immunotherapy, in particular. Hence, this review will use human melanoma as the model for human cancer to examine the current status of such immunotherapy and to consider options for improving results. Here, we will briefly point out the raison d'être underlying these approaches, review what has so far been accomplished, and consider options for making the approaches more effective.

The basic idea

Figure 1 describes the basic idea behind the modern approaches to immunotherapy for cancer with peptide epitope specific vaccines or with *in vitro* activated/expanded cancer antigen-specific T cells. As can be seen, with the peptide-based vaccination approach, the idea is to activate and expand a line of tumor associated epitope-specific T cells, *in vivo*, through immunization. The idea behind adoptive therapy on the other hand is to generate a large number tumor antigen-reactive CTL in *in vitro* cultures preferably from tumor explants and then to inject them back to the same host.

Historically, attempts to treat human cancer with cancer cells -- irradiated or modified by one mechanism or another -- as the "immunogen" were not very successful. Those unsuccessful attempts led to the recognition of the need for a "better understanding" of the immunological defense mechanisms underlying tumor immunity and of the nature of the tumor antigen. Presently, just as the evidence that hosts are capable of mounting cell mediated as well as serological responses against their cancer cells is solid, multiple studies have also revealed the presence of "blocking factors" and "suppressor cells" as negative factors. Although these findings made the literature on the subjects substantial, "better understanding" on the immune mechanisms, especially from the cellular side -- came with the clear demonstration that cancer patients do indeed have T cells capable of responding to the hosts' cancer cells once they are "activated", *in vitro* [1, 2], and that such autologous tumor-reactive T cells could be obtained from peripheral blood as well as from tumor tissues and could be expanded in *in vitro* cultures [1-5].

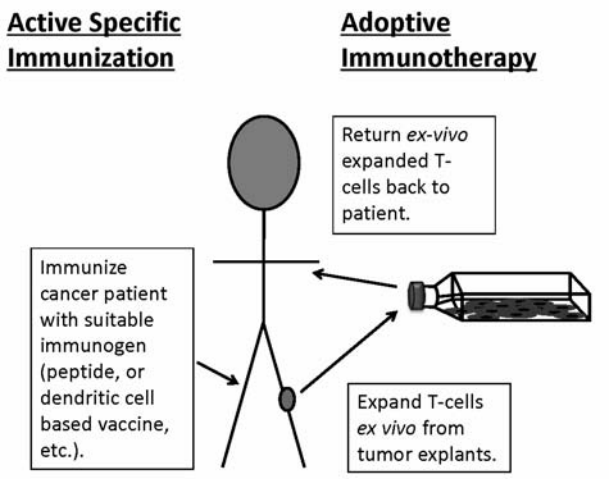

Figure 1. A schematic representation of the ideas behind active specific immunization and T cell-based adoptive therapy for melanoma.

Active specific immunization as treatment for human cancer

A more sound rationale for active specific immunization as a form of immunotherapy for cancer became available with the seminal discovery of the first human cancer associated antigen that is recognized by $CD8^+$ cytotoxic T lymphocytes (CTL) by the Boon group from Brussels [4]. Using molecular techniques and a melanoma reactive CTL clone, Van der Bruggen and colleagues identified the melanoma antigen, named melanoma antigen E-1 or MAGE-1 recognized by the CTL [4]. Shortly, the peptide epitope from the MAGE-1 protein that served as the peptide ligand, presented by HLA-A1, for the CTL was also defined [5]. Although it was not a complete surprise, the nonapeptide epitope, EADPTGHSY, was interestingly found to have no mutation and as such the epitope was, by all definition, a self peptide. The discovery of this HLA-A1-restricted CTL-determined peptide epitope for CTL allowed our group to carry out a clinical study of active specific immunization for HLA-A1 positive melanoma patients with the hypothesis that immunization of such patients with this HLA-A1-restricted peptide loaded on to the patient's monocyte-derived antigen presenting cells might induce a peptide specific T cell response. Interestingly, the hypothesis was found to be correct [6]. Simultaneously, Marchand *et al*. [7] from the Boon group showed that such patients immunized with the peptide alone were also able to induce melanoma reactive immune response, *in vivo*. As more melanoma associated CTL-determined antigens and peptide ligands were identified, it became clear that the MAGE-1 derived EADPTGHSY peptide was not an exception. Other MHC-restricted and CTL-determined peptides are also immunogenic even

though they too were "self" peptide. Clinical trials with a number of HLA-A2-restricted peptides, given alone or injected after loading them onto the patients dendritic cells have also induced a peptide specific T cells response as determined by a significant increase in the number of the peptide specific $CD8^+$ T cells in circulation.

Understandably, these early studies inaugurated the contemporary approaches with active specific immunization in melanoma or in other tumors with defined peptides with or without dendritic cells or with some form of adjuvants. A complete review of the various individual clinical trials of active specific immunotherapy is beyond the scope here and excellent reviews of the subject are available [8-10]. While we will not go into individual reviews of all published reports of active specific immunization in human melanoma, I would like to summarize the results of these studies with the following points:

i) Active specific immunization with peptide alone, peptide injected with some form of adjuvant, or injected after being loaded on to autologous dendritic or non-dendritic APC is capable of inducing evidence of the generation of immune response to the peptide measurable by one form of assay or another.

ii) Active specific immunizations are safe and, at times, they can induce a therapeutic effect with occasional complete tumor regression (complete clinical response) and at times with partial tumor regression (partial clinical response).

iii) Presently, no single assay or test is currently available that can be used as a predictive test for clinical response

iv) While a variety of reasons for failure to immunization has emerged, why some patient responds while others do not, remains unclear.

Needless to mention, concerted efforts are underway at multiple laboratories and clinics around the world to improve the result of active specific immunization and to develop a more effective way to monitor immunological responses. Time will tell whether or not the original expectation could be met, but there is reason to believe that the last word on active specific immunization for cancer therapy has not yet been written.

T cell-based adoptive immunotherapy for human cancer

As mentioned earlier, the rationale for adoptive T cell-based cancer immunotherapy came with the line of observations that melanoma-reactive $CD8^+$ CTL could be activated and expanded, *ex vivo*, from several laboratories. Using *ex vivo* activated and expanded T cells derived from tumor sites, called tumor infiltrating lymphocytes (TIL) and intereleukin-2 (IL-2), Rosenberg's group initiated a series of clinical trials and demonstrated that adoptive therapy with TIL is capable of inducing tumor regressions (total tumor regression or complete clinical responses in a smaller fraction and partial clinical responses in a larger fraction of the treated patients). Rosenberg's group has carried out a number of seminal studies and has demonstrated unequivocal therapeutic potency of T cell based adoptive therapy [10, 11].

The Rosenberg group has also introduced the idea of adoptive immunotherapy for melanoma with T cells engineered to express a set of melanoma epitope specific alpha/beta TCR [12]. They have shown that when a melanoma patient's T cells, transduced with a retroviral vector consisting of the genes encoding the alpha/beta chains of a TCR specific for a melanoma-associated epitope, are injected back to the patient, both tumor regression as well as autoimmune pathologies could be observed [12]. Of note, the strategy of adoptive therapy with TCR-engineered T cells comes with considerable side effects. While the benefits and the side effects from the TCR-engineered T cell-based approach have not been any different with that of TIL therapy, a recent study in a murine model [13] has revealed the potential of more disturbing side effects, including fatality, resulting from the generation of hybrid alpha and beat chains of TCR (i.e., the generation of a set of TCR resulting from anomalous pairing of a transgenic and an endogenous alpha/beta chains possessing far more avidity for certain self epitopes). Needless to say, concerted efforts are underway to develop strategies that would minimize such devastating side effects and improve the benefit. Indeed, given that generating a large pool of T cells expressing a given specificity through TCR engineering is far more easy and predictable than generating large pools of TIL, and as the

scope of treating cancer patients could be substantially enhanced with this strategy, the attractiveness of this approach is understandable. Nonetheless, the adoptive T cell based approach to cancer therapy with TIL or TCR-engineered T cells remains a labor-intensive and elaborate expertise-based methodology. As such, it is not a user-friendly approach. Yet, the strategy remains attractive because the therapeutic results as well as the autoimmune side effects remain impressive. Understandably, investigators are seeking ways to making it more users friendly, to improving the results, and to minimizing the side effects. A thorough review of the results of T cell-based adoptive immunotherapy (with TIL or with TCR-engineered T cells) for melanoma, the pros and cons of the approaches, and areas of potential improvements are beyond the scope of this article. We will examine the current efforts at innovation in this field later. Presently, instead of attempting a thorough review of all published reports of T cell-based adoptive therapy for cancer, we would summarize the present status of this line of cancer immunotherapy with the following generalizations:

i) T cell-based adoptive cell-therapy for melanoma can induce gratifying tumor regressions in a fraction of patients.

ii) These forms of therapies can induce a variety of autoimmune side effects.

iii) While no firm data exists on optimum number of cells and therapeutic response, it does not appear that more will be better.

iv) While the persistence of the transfused cells in general circulation can be documented in a number of treated patients, there seems to be no correlation between persistence and anti-tumor response.

vi) Adoptive cell therapy with TCR-engineered T cells can also induce substantial anti-tumor response as well as autoimmune side effects but presently, such therapy does not show much of an advantage over treatment with TIL.

vii) While a head to head comparison of active specific immunization and adoptive T cell therapy has been conducted and although both forms of therapy are capable of demonstrating tumor regression, the tumor regressions as well as the evidence of autoimmune pathology with T cell-based adoptive therapy seems to be quite impressive.

Constraints against achieving sustained anti-tumor activities with active specific or adoptive T cell-based therapies

We have examined this topic elsewhere [14]. As such, this topic will not be rehashed again here. Nonetheless, in order to provide a synopsis of the current understanding of the constraints, it should be pointed out that the various constraints in the path to obtaining better results with active specific immunization and adoptive T cell-based therapies identified in our earlier review still haunt the field. While additional insights into the major constraints and more mechanistic understanding of how the constraints operate have emerged and continue to emerge, unfortunately, the question of how to handle them -- individually or collectively -- remains elusive. Thus, a diverse family of regulatory T cells (natural T regulatory cells, inducible T regulatory cells, myeloid suppressor cells, etc.) are still being cited as standing in the way, immunosuppressive microenvironment created by tumor cells, themselves, as well as by certain cells within the tumor stroma are still being cited as another obstacle in the way, exhaustion and premature death of the T cells are still being described as another major problem, and the remarkable ability of tumor cells to edit their immunogenic identity so as to escape immune attack continues to frustrate the field.

Current strategies at innovation

While it is now abundantly clear that gratifying clinical benefits can at times be achieved with active specific immunization and adoptive T cell-based melanoma therapies and given that the field has yet to come up with a satisfactory strategy to deal with the various constraints against more successful active specific immunization and adoptive T cell-based melanoma therapy, the general strategy to obtain a better outcome with these two forms of cancer therapy seems to fall under the following two basic thinking:

a) Make the “vaccine” more effective.

b) Make the T cells, to be adoptively transferred, more robust and long lasting.

Making a more effective vaccine

One immediately gets into trouble while contemplating the task of making a more potent "cancer vaccine" as no satisfactory way (a particular assay or a single panel of assays) to measure the efficacy of a cancer vaccine presently exists. Lacking this critical measure of potency as a predictor of efficacy of a "cancer vaccine", the slogan, "*in vivo* veritas", gained popularity as the ultimate test of effectiveness of a cancer vaccine -- perhaps more out of frustration than anything else. However, setting the frustration aside, a number of serious approaches at making a more effective "cancer vaccine", in general, and melanoma vaccine, in particular, are being considered. These approaches may be summarized in the following headings:

a) Find a more appropriate form of "vaccine" or immunogen.

b) Make the vaccine polyvalent.

c) Find a more effective adjuvant.

d) Immunize with an agent that would block negative signals to the T cells.

e) Immunize with another non-immune modality.

f) Develop a more effective way to immunize.

Needless to say, solid theoretical merit exists within each of these approaches and various investigators have been working in the laboratory and in the clinic under these principles. Indeed, in melanoma, the search for an appropriate form of immunogen has begun with modified peptide epitopes, epitopes residing within the oncogenic sequence of the protein, protein antigen (natural proteins or recombinant proteins), DNA, et cetera. Similarly, a series of clinical trials are underway with melanoma vaccines made of multiple peptides derived from the same protein antigen or derived from different protein antigens to make the vaccine polyvalent. Various forms of adjuvant have been and are being evaluated with different types of "vaccines". Anti-CTLA-4-based blocking is also being tested, alone, and with vaccines. Clinical trials with "cancer vaccine" and chemotherapeutic agents are also being tested. And, finally, a variety of mechanism of immunization, in general, and using different types of dendritic antigen presenting cells, in particular, are underway. Time will tell if any of these strategies would improve the results. However, preliminary results, presented at scientific meetings and gatherings, do not appear to suggest that these strategies are going to make a significant difference in the outcome.

Making the T cells, to be adoptively transferred, more robust and long lasting

In some sense, that T cell-based adoptive therapy for melanoma can induce complete regression of bulky metastatic disease lasting for years is reason enough to seek ways to make the T cells, to be adoptively transferred, more robust and durable. Several approaches are pursued to achieve this goal. These approaches fall along the following three lines of thoughts:

i) Expand highly avid T cells (usually tumor infiltrating lymphocytes or TIL).

ii) Engineer T cells with a set of high avidity TCR specific for an epitope of choice.

iii) Enhance the functional properties and the durability of the T cells.

Unfortunately, the task of isolating and expanding TIL exhibiting high avidity for the autologous melanoma cells is not a very practical proposition. To begin with, the chance of generating TIL from a patient's tumor is a 50-50 proposition, at best, and to select TIL exhibiting high avidity for the patients' melanoma cells is essentially an impossible task. Similarly, generating T cell clones exhibiting high avidity for a given patient's melanoma cells is even more impractical proposition. Given these sets of realities, the idea of generating high avidity TIL for adoptive therapy is a non-starter.

As an alternative, the Rosenberg group initiated a series of studies to isolate "high avidity" alpha/beta chains of TCRs with specificity for a melanoma associated epitope of choice and to engineer a given patient's T cells with that TCR, *ex vivo*, for adoptive transfer. They have translated the idea in clinical trial and have shown that such TCR-engineered T cells are also capable of inducing complete regression of metastatic tumors in a fraction of patients as well as capable of inducing partial regressions of metastatic disease in others [12]. These observations have been

duplicated through a similar type of clinical trial conducted at UCLA by a group of investigators including this group as a member of that consortium (Unpublished observation). These studies, therefore, provide a solid proof-of-principle that adoptive therapy with TCR-engineered T cells bear promise. Presently, the initial results of the clinical trials do not suggest that the response rate (complete regressions as well as total response rate) with the TCR-engineered T cell therapy is going to be significantly different than that with TILs. Nonetheless, it should be pointed out that it is too early to make any comparison between TL therapy and therapy with TCR-engineered T cells. Presently, only one set of TCR has been used to engineer T cells in both studies. It is possible, especially given the reach of the technology, that other T cells engineered with other TCRs of different avidity might make a difference. Attempts to engineer a more efficient set of alpha/beta TCR against a melanoma epitope and to engineer T cells with such epitopes are actively pursued.

Unfortunately, adoptive T cell therapy in melanoma has not been cost-free. Patients undergoing adoptive T cell therapy suffer from a number of side effects. Indeed, side effects from adoptive therapy with TCR-engineered T cells following the customary preparative treatments (non-ablative myelodepletion) have been quite substantial. For example, as the epitope, against which the therapy is directed to, is from cells that are involved in pigment biosynthesis, some patients have developed serious ophthalmic complications and some have developed total vitiligo. Of interest, the ophthalmic complications are amenable to appropriate treatment and patients developing vitiligo have recovered pigment production ability later.

More disturbingly, similar strategies tested out in mice by Ton Schumacher's group from The Netherlands have revealed more serious complications, including death [13]. They have found these serious side effects from the generation of hybrid TCRs from anomalous internal pairing of the intrinsic and grafted chains of the TCRs. Our group has also observed similar anomalous pairing between the intrinsic and the grafted alpha/beta chains, *in vitro* (Unpublished observation). In this context, it should be pointed out that while the generation of a set of "undesired" alpha/beta TCR resulting from the current TCR-grafting technique exists, methodology also exists to interfere with such anomalous pairing. In addition, methodology can be incorporated to inactivate and/or delete such T cells, *in vivo*, to limit serious side effects.

Clearly, in the present era of "Molecular Medicine", the idea of using TCR-engineered T cell for adoptive therapy will be pursued to make it more effective as well as more "user friendly". Time will tell if this strategy would take adoptive T cell therapy for melanoma to the next level.

Finally, while investigators continue to explore different strategies in active specific as well as in T cell-based adoptive therapy, attention has also been given to other ways that might make the induced T cells and the adoptively transferred T cell function well and continue to function for an extending length of time so as to extract a sustained and durable response from them. In this context, several leads have emerged. These include ideas like incorporating a procedure that would interfere with activation-induced cell death (AICD), especially with premature AICD, and interfering with "exhaustion" in the T cells. Fortunately, a better understanding of the mechanism(s) underlying AICD in primary T cells [15, 16] and a clearer understanding of the mechanism underneath the induction of exhaustion in T cells [17] are providing opportunities at interfering with these intrinsic physiological processes through pharmacological or biological means. Some of these strategies are being tested in translational melanoma immuno-therapy trials. Again, time will tell, if their inclusion might improve the effectiveness of these forms of therapy.

Novel ideas in the back burner

All scientific progress starts with novel ideas and there is no dearth of novel ideas in cancer immunotherapy. In fact, all established treatments and all strategies that are presently pursued in the field originated from novel ideas. This review could not do adequate justice in the pursuit of novel ideas in the cancer immunotherapy "pipeline".

They will surface in due time from their originators. Instead of attempting to deal with the impossible task of presenting a grab bag of "novel ideas" in the field, the last segment of this review will be devoted to an idea seemingly worthy of consideration in the judgment of the authors. It is the idea of engaging the entire immunological apparatus against a tumor antigen(s) orchestrated through the involvement of antigen specific $CD8^+$ and $CD4^+$ T cells as well as B cells simultaneously. We will discuss how this could be accomplished in the last segment.

Engaging the entire immunological apparatus against melanoma antigen by employing antigen specific $CD8^+$ and $CD4^+$ T cells and B cells

The rationale behind the idea of engaging antigen specific $CD4^+$ T cells in tumor immunity is obvious and not all that novel. A critical role for $CD4^+$ T cells in cellular immune responses has long been recognized and HIV/AIDS has taught us their importance in immune defense. Unfortunately, our contemporary approaches to active specific as well as T cell-based adoptive therapy for melanoma – or for other common solid tumors for that matter – have so far been mostly $CD8^+$ T cell-centric. This, by the way, has not been by choice. This has been essentially dictated by the fact that most non-lymphoid cancer cells only express MHC class I molecules. As such, engaging $CD4^+$ T cells in cancer immunity has been a difficult proposition. However, now that $CD4^+$ T cells engineered to express a set of alpha/beta TCR specific for a MHC class I-restricted epitope can be made to respond to APCs presenting the epitope or made to respond to melanoma cells naturally displaying the epitope [18, 19], we believe that antigen specific $CD4^+$ T cells can be brought to anti-melanoma immunotherapeutic strategies.

Most importantly, by engaging antigen specific $CD4^+$ T cells simultaneously with $CD8^+$ T cells both recognizing the same tumor antigen, the antigen specific $CD4^+$ T cells could: a) expand the effector function repertoire; b) provide "help" to $CD8^+$ T cells making them more robust and durable (i.e., generate memory); c) engage the innate immunological apparatus at the tumor bearing sites; d) "help" B cells to generate a serological response provided they are properly stimulated. Admittedly, this is not altogether a novel idea. Tumor immunologists have talked about it in several different reiterations and the idea of serological responses against defined melanoma or other tumor-associated antigens has been clinically tested [20, 21]. A systematic effort at combining cell mediated (by $CD8^+$ and $CD4^+$ T cells) as well as serological anti-tumor responses, however, is yet to be made. In this context, we believe that the engagement of antigen specific $CD4^+$ T cells could be valuable agent in the process given that, when appropriately engaged, in addition to exhibiting their own effector functions, $CD4^+$ T cells could provide "help" to $CD8^+$ T cells as well as to B cells. In the following section, we will elaborate on the rationale and on a strategy.

An outline of the strategy is shown in Figure 2. As shown, we believe that the idea can be tested by employing $CD4^+$ T cells engineered to express a set of alpha/beta TCR specific for a MHC class I-restricted epitope simultaneously with $CD8^+$ T cells so that the $CD4^+$ could work in concert with the $CD8^+$ T cells, provide "help" to $CD8^+$ T cells to mount a more robust and durable $CD8^+$ T cell response, and also help B cells to mount a serological response. Appropriate TCR-engineered $CD8^+$ and $CD4^+$ T cells could be fairly easily engaged and the serological compartment could be simultaneously stimulated with appropriate antigens and/or "epitopes" so as to generate a response(s) against a given specificity.

A good deal of useful information on the biology of MHC class I-restricted epitope specific TCR engineered $CD4^+$ T cells has emerged in support of parts of the idea [18]. It is now quite clear that $CD4^+$ T cells engineered to express a melanoma epitope specific MHC class I-restricted epitope can be made to recognize the epitope on an APC as well as on the appropriate MHC class I positive melanoma cells and can be made to perform tasks that were once primarily assigned to $CD8^+$ T cells. Additionally, they also seem to provide some form of "help" to $CD8^+$ T cells [19]. While it is unclear if such $CD4^+$ T cells could "help" $CD8^+$ T cells towards the generation of "memory", evidence in support of such function

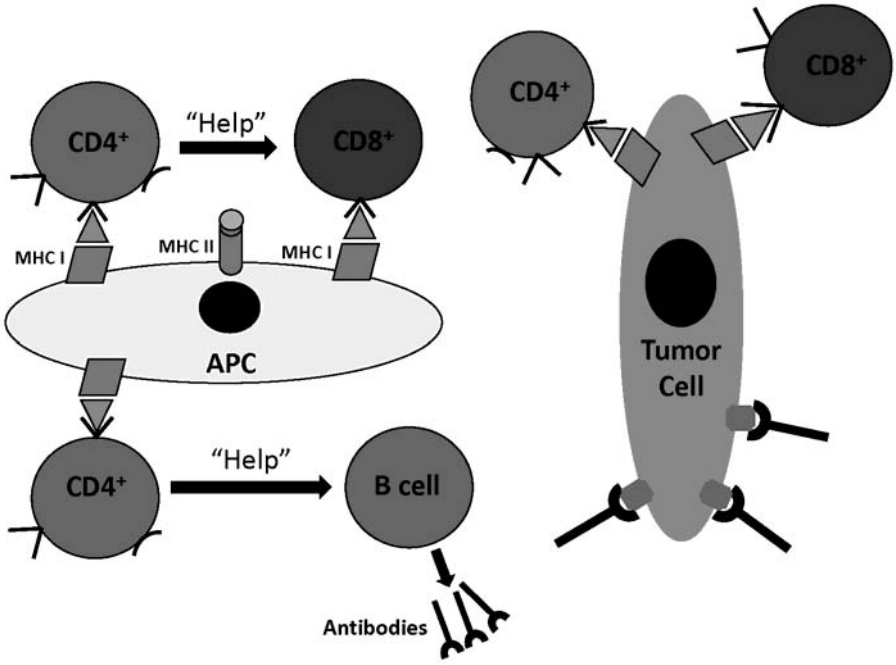

Figure 2. A schematic representation of the proposed idea for engaging both $CD8^+$ and $CD4^+$ T cells as well as engaging the B cell system through immunization and adoptive cell therapy for melanoma.

has already emerged in an animal model [22]. Whether, they could also be made to help B cells mount a serological response against a melanoma antigen remains hypothetical but the hypothesis could surely be tested through existing technologies. In conclusion, we believe that time has come to test whether the promise of tumor immunotherapy could be better met by engaging the entire immune system directed to a tumor antigen of choice.

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NOTE ON BIBILIOGRAPHY

The authors would like to mention that a comprehensive bibliography covering the field is beyond the scope of this review, acknowledge that many groups of investigators have made many contributions in the field, and regret that all those works could not be referred to due to the demand of the space.

REFERENCES

1. Mukherji, B. and MacAlister, T. J. 1983, J. Exp. Med., 158, 240-245.
2. Knuth, A., Danowski, B., Oettgen, H. F., and Old, L. J. 1984, Proc. Natl. Acad. Sci. USA, 81, 3511-3515.
3. Rosenberg, S. A., Spiess, P., and Lafreniere, R., 1986, Science, 233, 1318-1321.
4. van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den Eynde, B., Knuth, A., and Boon, T. 1991, Science, 254, 1643-1647.
5. Traversari, C., van der Bruggen, P., Luescher, I. F., Lurquin, C., Chomez, P., Van Pel, A., De Plaen, E., Amar-Costesec, A., and Boon, T. 1992, J. Exp. Med., 176, 1453-1457.
6. Mukherji, B., Chakraborty, N. G., Yamasaki, S., Okino, T., Yamase, H., Sporn, J. R., Kurtzman, S. K., Ergin, M. T., Ozols, J., Meehan, J., and Mauri, F. 1995, Proc. Natl. Acad. Sci. USA, 92, 8078-8082.
7. Marchand, M., van Baren, N., Weynants, P., Brichard, V., Dreno, B., Tessier, M. H., Rankin, E., Parmiani, G., Arienti, F.,

Humblet, Y., Bourlond, A., Vanwijck, R., Lienard, D., Beauduin, M., Dietrich, P. Y., Russo, V., Kerger, J., Masucci, G., Jager, E., De Greve, J., Atzpodien, J., Brasseur, F., Coulie, P. G., van der Bruggen, P., and Boon, T. 1999, Int. J. Can., 80, 219-230.

8. Boon, T., Coulie, P. G., Van den Eynde, B. J., and van der Bruggen, P. 2006, Annu. Rev. Immunol., 24, 175-208.
9. Rosenberg, S. A., Dudley, M. E., and Restifo, N. P. 2008, N. Engl. J. Med., 359, 1072.
10. Rosenberg, S. A. and Dudley, M. E. 2009, Curr. Opin. Immunol., 21, 233-240.
11. Dudley, M. E., Wunderlich, J. R., Robbins, P. F., Yang, J. C., Hwu, P., Schwartzentruber, D. J., Topalian, S. L., Sherry, R., Restifo, N. P., Hubicki, A. M., Robinson, M. R., Raffeld, M., Duray, P., Seipp, C. A., Rogers-Freezer, L., Morton, K. E., Mavroukakis, S. A., White, D. E., and Rosenberg, S. A. 2002, Science, 298, 850-854.
12. Morgan, R. A., Dudley, M. E., Wunderlich, J. R., Hughes, M. S., Yang, J. C., Sherry, R. M., Royal, R. E., Topalian, S. L., Kammula, U. S., Restifo, N. P., Zheng, Z., Nahvi, A., de Vries, C. R., Rogers-Freezer, L. J., Mavroukakis, S. A., and Rosenberg, S. A. 2006, Science, 314, 126-129.
13. Bendle, G. M., Linnemann, C., Hooijkaas, A. I., Bies, L., de Witte, M. A., Jorritsma, A., Kaiser, A. D., Pouw, N., Debets, R., Kieback, E., Uckert, W., Song, J. Y., Haanen, J. B., and Schumacher, T. N. 2010, Nat. Med., 16, 565-570.
14. Ray, S., Chhabra, A., Mehrotra, S., Chakraborty, N. G., Ribas, A., Economou, J., and Mukherji, B. 2009, Clin. Dermatol., 27, 603-613.
15. Mehrotra, S., Chhabra, A., Chakraborty, A., Chattopadhyay, S., Slowik, M., Stevens, R., Zengou, R., Mathias, C., Butterfield, L. H., Dorsky, D. I., Economou, J. S., Mukherji, B., and Chakraborty, N. G. 2004, Immunology, 113, 472-481.
16. Chhabra, A., Mehrotra, S., Chakraborty, N. G., Dorsky, D. I., and Mukherji, B. 2006, Eu. J. Immunol., 36, 3167-3174.
17. Greenwald, R. J., Freeman, G. J., and Sharpe, A. H. 2005, Annu. Rev. Immunol., 23, 515-548.
18. Chhabra, A., Yang, L., Wang, P., Comin-Anduix, B., Das, R., Chakraborty, N. G., Ray, S., Mehrotra, S., Yang, H., Hardee, C. L., Hollis, R., Dorsky, D. I., Koya, R., Kohn, D. B., Ribas, A., Economou, J. S., Baltimore, D., and Mukherji, B. 2008, J. Immunol., 181, 1063-1070.
19. Ray, S., Chhabra, A., Chakraborty, N. G., Hegde, U., Dorsky, D. I., Chodon, T., von Euw, E., Comin-Anduix, B., Koya, R. C., Ribas, A., Economou, J. S., Rosenberg, S. A., and Mukherji, B. 2010, Clin. Immunol., 136, 338-347.
20. Takahashi, T., Johnson, T. D., Nishinaka, Y., Morton, D. L., and Irie, R. F. 1999, J. Invest. Dermatol., 112, 205-209.
21. Valmori, D., Souleimanian, N. E., Tosello, V., Bhardwaj, N., Adams, S., O'Neill, D., Pavlick, A., Escalon, J. B., Cruz, C. M., Angiulli, A., Angiulli, F., Mears, G., Vogel, S. M., Pan, L., Jungbluth, A. A., Hoffmann, E. W., Venhaus, R., Ritter, G., Old, L. J., and Ayyoub, M. 2007, Proc. Natl. Acad. Sci. USA, 104, 8947-8952.
22. Morris, E. C., Tsallios, A., Bendle, G. M., Xue, S. A., and Stauss, H. J. 2005, Proc. Natl. Acad. Sci. USA, 102, 7934-7939.