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Role of Immune Response in Breast Cancer Vaccines

BY BRIAN J. CZERNIECKI, MD, PHD

Over the past several years, it has become increasingly clear that the immune response plays a significant role in determining response to therapy as well as long-term survival in patients with breast cancer.

Studies supporting this trend have focused on lymphocyte infiltration of the tumor; however, recent evidence also exists that suggests peripheral anti-tumor CD4 Th1 responses are critical to effective immune-based therapy approaches (*Cell* 2017;168(3):487-502).

Central anti-oncogene CD4 Th1 responses are progressively lost during breast tumorigenesis (*Oncol Immunology* 2015; doi:10.1080/2162402X.2, *Ann Surg Oncol* 2017;24:407-417). Anti-CD4 Th1 responses predict response to neoadjuvant therapy in those patients with pathologic complete response restore, at least partially, the anti-CD4 Th1 response

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How We Treat Systemic Mastocytosis

BY PRITHVIRAJ
BOSE, MD, & SRDAN
VERSTOVSEK, MD, PHD

Systemic mastocytosis refers to a heterogeneous group of uncommon clonal disorders characterized by the expansion and accumulation of neoplastic mast cells (MCs) in various organs and tissues (e.g., skin, bone marrow, liver, spleen, GI tract, etc.). The disease is usually limited to the skin in children (cutaneous mastocytosis) and often resolves spontaneously, while adults typically have systemic mastocytosis (SM), with or without cutaneous involvement. Within SM, five major entities are recognized: indolent SM (ISM), smoldering SM (SSM), SM with an associated hematologic neoplasm (SM-AHN), aggressive systemic mastocytosis (ASM), and mast cell leukemia (MCL).

Diagnostic Criteria & Disease Entities

The diagnosis of SM requires the presence of either the major criterion and one minor criterion, or three minor criteria. The major criterion is the presence of multifocal, dense infiltrates of MCs (>15 in aggregate) in sections of bone marrow and/or another extracutaneous organ. The minor criteria include the following:

- In biopsy sections of the bone marrow or another extracutaneous organ, >25 percent of the MCs in the infiltrate are spindle-shaped or have atypical morphology; or, of all the MCs in the bone marrow aspirate smears, >25 percent are immature or atypical (mostly spindle-shaped).
- Presence of an activating point mutation at residue D816 of *KIT* in bone marrow, peripheral blood, or another extracutaneous organ.

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Evaluation of Pathogen-Enhanced Adoptive Cell Transfer Against Solid Tumors

BY RICHARD SIMONEAUX

Adoptive cell transfer (ACT), which utilizes genetically-modified T cells for cancer immunotherapy, has been successfully applied for the treatment of a

number of hematologic B-cell malignancies. This technique, also known as chimeric antigen receptor (CAR) therapy, has proven less successful against those cancers having solid tumors.

A number of factors contribute to this technique's poor applicability in a solid tumor setting, including modest immunogenicity of tumor-associated antigens (TAAs), poor expansion of genetically-modified T cells, heterogeneity of the TAAs within a genetically-diverse solid tumor, impaired migration of the modified T cells to within the body of the tumor, and immunosuppression in the tumor microenvironment (TME).

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To address these issues, Weiguo Cui, PhD, and colleagues at the Blood Research Institute, BloodCenter of Wisconsin, Milwaukee, undertook a pre-clinical study to evaluate a modified ACT technique whereby the genetically-modified CD8 T cells bore T-cell receptors (TCRs) for both a TAA and an antigen present on a recombinant *Listeria monocytogenes* (LM) organism (PNAS 2017;114(4):740-745).

This methodology, described as reenergized ACT (ReACT), was evaluated using a murine B16-F10 melanoma model. In this model, the dual specific CD8 cells would be selectively directed to the cancer site by intratumoral (i.t.) injection of the recombinant LM pathogen.

Previous Research

As previously stated, ACT has met with good success against some B-cell malignancies. CD19 chimeric antigen receptor (CAR) T cells were utilized in clinical settings for these conditions. Of these, the greatest clinical success has been noted for acute lymphoblastic leukemia (ALL).

In one phase I clinical trial, 16 B-cell ALL patients with relapsed/refractory disease or CR1 status were treated with CD19-28z CAR T cells (*Sci Transl Med* 2014;6(224):224ra25). This therapy yielded a complete response rate of 88 percent, and of these patients, 86 percent were minimum residual disease negative.

Promising results have also been obtained with CD19 CAR T cells against other B-cell malignancies, such as non-Hodgkin lymphoma and chronic lymphocytic leukemia, however, those findings have not met with the same success found for ALL. Clinical trials have also been performed for the following solid tumor cancers: neuroblastoma (GD2-CAR T cells), glioblastoma (IL13R α 2-CAR T cells), and sarcoma (HER2-CAR T cells).

The results obtained for these cancers have not matched those obtained for CD19-CAR T-cell-based therapies. Among the reasons cited for the lack of efficacy of CAR therapies in solid tumor settings are immunosuppressive TME, lack of an appropriate single tumor antigen to target, lack of modified T-cell expansion, and lack of tumor penetration by T cells.

Bifunctional T cells

To address the poor expression and activity of CAR T cells, Kershaw, et al., sought to utilize bifunctionally modified T cells (*Nat Biotechnol* 2002;20(12):1221-1227). Theoretically, the CAR would confer specificity to the cancerous cells while another TCR could function as an immunogenicity enhancer.

For these studies, the investigators utilized alloreactive T cells that were then modified with an extracellular folate binding protein TAA, a known human ovarian cancer antigen coupled with a γ -chain human Fc receptor complex-derived intracellular signaling domain. To enhance the expansion and activity of the bifunctional T cells, the allogeneic major histocompatibility complex (MHC) found on the surface of donor cells was utilized as an immunogen.

The allogeneic antigen was chosen based on its ability to quickly stimulate vigorous alloreactive T-cell expansion and activity. The results of this study showed that there was an expansion of the dual-functionalized T cells, which in all likelihood did contribute to the antitumor effects observed.

BCG & LM Immunotherapy

Another method that has been utilized to stimulate a cancer patient's immune system is therapy with pathogenic organisms. In the 1890s William Coley, a pioneer in the field of cancer immunotherapy, injected terminally ill cancer patients with streptococcal organisms in the hopes their immune systems would be activated and reduce tumor size. The bacteria and bacterial products used over the next 40 years in more than 1,000 patients were collectively referred to as Coley's toxins. This nascent form of immunotherapy was typically applied to those patients having soft tissue sarcomas or bone cancer.

Since the 1980s, an attenuated live strain of *Mycobacterium bovis*, bacillus Calmette-Guérin (BCG) has been used primarily for immunostimulation in the treatment of bladder cancer and, in some instances, melanoma. While this form of therapy has shown some success, the immune responses obtained to the tumors were often non-specific and transient.

Another method to obtain tumor-specific immune responses in a pathogenic setting involves the use of recombinant LM. In this therapy, tumor-specific T-cell responses are obtained when genetically-modified LM expressing TAAs are given to a patient. LM is particularly suitable as a bacterial vector, as antigen presenting cells are selectively infected, with the TAAs being efficiently delivered to MHC class I and II antigen presentation pathways.

Additionally, this methodology is amenable to a variety of TAAs, showing positive results in clinical trials of pancreatic and breast cancer patients. However, for LM-based therapies utilizing a single expressed TAA, complete and durable regression of the cancer has been challenging. This is primarily due to the heterogeneous nature of tumors. Those tumor cells not expressing the TAA targeted in the therapy can avoid elimination by the patient's immune system, and when they reproduce, afford a new population of tumor cells that are not vulnerable to that particular therapy. This makes it critical to initiate a vigorous T-cell response that is able to target a variety of TAAs that may be present in a genetically diverse tumor.

ReACT Methodology

When asked to describe the modified ReACT protocol, Cui stated, "In this study, we used dual-specific CD8 T cells, which had TCRs for both a TAA and a bacterial antigen. We used pmel-1 CD8 T cells functionalized with a TCR that recognized the glycopeptide 100 (GP100) epitope for murine melanoma; subsequently, the CD8 cells were genetically engineered with the OT-I TCR that recognized ovalbumin (OVA) residues 257-264, which served as our bacterial antigen surrogate."

The OT-I TCR was added to these CD8 cells via in vitro retroviral transduction. For this study, the LM organism used (LM-OVA) was genetically engineered to express the OVA residues recognized by the OT-I TCR. When asked about the choice of organism, Cui replied, "We chose the LM organism because its use has been well-documented, especially in a clinical oncology setting; it shows good amenability to functionalization with a variety of TAAs."

The principle of this method is similar to that used by Kershaw, et al., however, whereas that methodology relied on allogeneic T-cell expansion, the ReACT methodology relies on the pathogen utilized to enhance T-cell response. Additionally, by injection of the LM-OVA intratumorally, it was hoped that doing so would enhance the migration of the dual-functionalized T cells to within the tumor as well as break the immunosuppressive nature of the TME.

In Vitro & In Vivo Tests

Once obtained, in vitro tests were performed for both monofunctionalized GP100-recognizing TCR CD8 T cells and the dual-specific cells recognizing both the TAA and the OVA₂₅₇₋₂₆₄ peptide segment. Activity was confirmed via IFN- γ production for the monospecific CD8 cells when challenged with gp100; however, these cells did not produce IFN- γ when challenged with the OVA₂₅₇₋₂₆₄ peptide motif. The dual-specific cells showed IFN- γ production when in the presence of both the gp100 or OVA₂₅₇₋₂₆₄ peptides.

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Learning Objective for This Month's CME Activity: After participating in this CME activity, readers should be able to discuss the findings of several studies on the uses of reenergized adoptive cell transfer (ReACT) methodology.

ADOPTIVE CELL TRANSFER

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Modified CD8 cells were evaluated in vivo by adoptive transfer of $\sim 5 \times 10^5$ cells in C57BL/6 mice bearing well-established s.c. B16-F10 melanoma tumors. As with previous studies, ACT failed to reduce tumor growth for both mono- and dual-functionalized CD8 cells; however, administration of low dose LM-OVA with the dual-specific CD8 T cells did show pronounced tumor regression in all of the mice, with seven of the 10 showing complete tumor regression.

It is notable that a strong tumor response was only observed with administration of both LM-OVA and the dual-specific CD8 T cells. Mice receiving the monospecific CD8 T cells and LM-OVA only showed modest and transient tumor growth suppression.

Bystander Antibacterial Effect Test

To show the antitumor effects were not an artifact of a bystander antibacterial effect, bacteria-specific OT-I CD8 cells were injected alone or in combination with gp-100-specific pmel-1 CD8 cells into mice bearing B16-F10 melanoma tumors followed by i.t. injection of LM-OVA.

For the mice receiving OT-I + LM-OVA, no obvious therapeutic benefit was noted. In the presence of the pmel-1 tumor-specific cells, only moderate transient adjuvant effects were obtained, failing to result in tumor elimination.

Generating Polyclonal TCRs

“Since TAAs are typically poorly defined for human tumors and there are considerable advantages to using patient-derived tumor-infiltrating lymphocytes, which may recognize numerous TAAs, we attempted to generate polyclonal CD8 T cells, which recognized a number of TAAs as well as the immunostimulating LM-OVA pathogen,” Cui explained regarding his group’s attempts to address tumor heterogeneity.

In this procedure, bone marrow cells isolated from C57BL/6 mice were cultured for a period of time to create dendritic cells (DCs). These DCs were then incubated with freeze-thawed tumor lysate (containing TAAs), providing mature DCs that were then used to activate naïve CD8 T cells. Cui noted that, “In order to develop sensitivity to the TAAs, it is necessary for those antigens to be presented to the appropriate naïve CD8 T cells by the DCs.” Once the tumor-activated CD8 T cells were obtained, they were then submitted to retroviral transduction for functionalization with the OT-I TCRs.

In Vivo Results

In line with the earlier in vivo studies, administration of the tumor-specific or polyclonal dual-specific T cells in the absence of LM-OVA produced no therapeutic responses.

Transfer of the monospecific T cells with LM-OVA produced tumor eradication in one of the 11 mice used. Conversely, 11 of the 16 mice receiving both LM-OVA and the polyclonal dual-specific T cells showed complete tumor elimination. Moreover, treatment with the dual-specific

T cells and LM-OVA appeared to confer immunological memory to the mice studied.

When those mice were treated with a lower dosage of B16-F10 cells and a cancer cell line to which they had no previous exposure (E0771 breast cancer cells), seven of the 10 mice tested showed resistance to the melanoma cells but none to the breast cancer cells. Rather unsurprisingly, treatment-naïve mice showed no resistance to both cell lines. This experiment did show that some degree

of long-term immunoprotection may be afforded to those mice receiving the ReACT therapy.

Tumor Infiltration Studies

Given the robust tumor responses observed with the ReACT protocol, the migration and expansion of the dual specific—cells were investigated. As expected, the levels of dual-specific CD8 cells found in tumors were highest in those mice receiving i.t. LM-OVA.

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ADOPTIVE CELL
TRANSFER

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Interestingly, the numbers of CD8 T cells recruited to the tumor sites was inversely proportional to tumor size for this and other therapeutic groups. Importantly, the dual-specific T cells displayed phenotypes indicative of activation (CD44^{hi}, granzyme B^{hi}, and

KLRG-1^{hi}) as well as enhanced expression of the CXCR3 chemokine receptor. This last marker has been associated with increased T-cell migration to tumor sites.

Additionally, only those mice receiving LM-OVA and the dual-specific T cells showed significant numbers of TNF α - and IFN- γ -producing multipotent CD8 T cells. These data would seem to show the bacterial infection affords robust T-cell expansion, gain of effector function, and migration to the tumor site.

Tumor Microenvironment
Changes

To assess how the ReACT therapy affected the TME in these mice, two major classes of immunosuppressive cells were studied: regulatory T cells (Tregs) and myeloid-derived suppressive cells (MDSCs). LM-OVA infection was shown to significantly decrease the numbers of certain Tregs (specifically CD4⁺, Foxp3⁺, and CD25⁺) independent of the type of CD8 T cell utilized (mono- or polyclonal dual-functionalized). An important point

to note here is that the number of Tregs associated with a tumor showed a positive correlation with the tumor's size. The ratio of effector T cells/Tregs was shown to increase only in those mice receiving both dual-specific CD8 T cells and i.t. LM-OVA. Importantly, the effector/Tregs ratio showed inverse proportionality with respect to tumor size.

In addition to Tregs, LM-OVA infection also clearly had an impact on reducing certain MDSCs (CD11b⁺Gr1⁺ cells).

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Patient Self Checks Are Critical to Avoid Melanoma Recurrence

Recurrences of early stage (stage II) melanoma are more often detected by patients and their physicians than by routine imaging tests (*J Am Coll Surg* 2016; doi: <http://dx.doi.org/10.1016/j.jamcollsurg.2016.12.038>).

More than 87,000 people are diagnosed every year, and nearly 10,000 people die from the disease, according to the American Cancer Society. Deadly melanoma has a 95 percent cure rate if caught and treated early. Still, some research findings suggest the recurrence rate for melanoma is as high as 50 percent.

“We are most concerned about patients who have stage II melanoma,” said study coauthor Adam C. Berger, MD, FACS, Professor of Surgery at Thomas Jefferson University, Philadelphia. “They have more advanced primary melanomas and, on average, between 20 percent and 45 percent of these patients will die within 5 years. In the past, we didn’t have good therapies for this type of melanoma, but new therapies mean survival continues to improve.”

Finding Melanoma Recurrence

In this new study, which covered the years from 1996 to 2015, investigators from Thomas Jefferson University and the University of North Carolina analyzed data from a multi-institution database on 581 patients with stage II melanoma and at least 1 year of follow-up. Of those, 171 patients with early stage melanoma developed a recurrence (29.4%). Male sex, ulceration, and stage were significant predictors of recurrence.

“We wanted to get a break down on how we are discovering recurring melanomas,” Berger said. The question was whether it was a change a patient observed that warranted a trip to the doctor, a symptom a physician identified during a scheduled visit, or something detected with routine imaging. Study data included place of first

recurrence and how recurrence was detected—patient symptom, physician exam, or routine surveillance imaging.

The analysis revealed patient symptoms were the most frequent mode of detection, with 40 percent of recurrences spotted by patients, whether it was a suspicious change they felt on their skin or a symptom such as coughing blood or seizures.

Further, 30 percent of recurrent melanomas were identified by scheduled physician exam and 26 percent by surveillance imaging. One reason melanoma is lethal is that it can spread rapidly to other organs like the liver or lungs. Regional nodes were the most common site of recurrence (30%), followed by lung (27%), and in-transit metastases (18%).

Detection Patterns

“The fact that imaging picked up 26 percent of patients with recurrence is notable because it is a little higher than what we’ve seen in the past, which I think reflects the current trend to do more imaging in general,” Berger said. “There is a move to use CT scans and other imaging techniques as an important strategy in early recurrence detection.”

Very few studies have specifically analyzed recurrence and detection patterns for patients with stage II melanoma. Researchers expect the findings from this study to help reshape protocols for people diagnosed with melanoma. “Our hope is to have some impact on future follow-up guidelines and how we think about screening for recurrent melanoma. Every study like this helps contribute to framing those guidelines,” Berger said.

Importantly, people who have been previously diagnosed with early stage melanoma need to be on the alert for symptoms of recurrence. But the takeaway message from this research is that two heads are better than one.

“Patients need to be aware of all of their symptoms and their body. But it’s also important for physicians to educate patients as to what to look for and what symptoms are a cause for concern,” Berger said. “Patients should examine their skin and the area where lymph nodes would be on a monthly basis. And if they have a symptom that doesn’t go away after 2 or 3 weeks, it should be brought to a physician’s attention, because that is an indicator that the melanoma has come back.” **OT**



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ADOPTIVE CELL TRANSFER

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Listeria organisms have been documented to infect MDSCs, making them more susceptible to T-cell-mediated cytotoxicity. The i.t. delivery of LM-OVA was also shown to diminish the levels of Arg-1, a marker for CD11b⁺Gr1⁺ MDSCs.

Further evidence that this phenotypic change correlated with reduced immunosuppression was obtained when CD11b⁺ cells, isolated from LM-OVA infected tumors, were co-cultured with activated CD8 cells (in vitro). Those CD11b⁺ cells from the LM-OVA-infected tumors were less immunosuppressive than those CD11b⁺ cells isolated from uninfected tumors.

An important observation was made when considering the inhibitory receptors present on the mono- and dual-specific CD8 T cells utilized in this study. The dual-specific CD8 T cells used in the ReACT protocol displayed lower expression levels for the Tim3, CTLA-4, LAG-3, and PD-1 inhibitory receptors relative to their monospecific counterparts. This further suggests the dual-specific T cells may have elevated antitumor activity.

When asked to comment on the state of the project, Cui said, “We have been very encouraged by the results we have seen to date; in

many instances, we have seen complete tumor eradication using this technique.

“We wanted to circumvent issues of T-cell expansion, activation, and migration to the tumor site, tumor antigen heterogeneity as well as the immunosuppressive TME, and, to a large degree, we have addressed these concerns,” he continued. “Additionally, we have also shown that in some instances immunological memory was attained for these melanomas, thus preventing tumor growth when re-challenged with the same cell line.”

When asked to highlight how their procedure differed from earlier attempts at dual-specific T-cell therapy, Cui replied, “One important distinction of dual-specific T-cell generation in our approach is that we take tumor reactive T-cells and retrovirally transduce a pathogen-specific TCR onto them. This is opposite from previous work that modifies pathogen-(EBV, CMV, and Influenza virus) or alloreactive T cells with a single TAA-specific TCR. Our approach allows us to generate polyclonal dual-specific T-cells targeting multiple TAAs to increase the ability of tumor control.”

When asked about future directions for the ReACT technology, Cui explained, “Clinically, bladder cancer may be a good candidate for this protocol, as it is often characterized by relapses; additionally, there is a history with immunotherapy for this cancer using the BCG organism to trigger the patient’s immune system.” **OT**

Richard Simoneaux is a contributing writer.