Targeted T-cell Therapy in Stage IV Breast Cancer: A Phase I Clinical Trial

Lawrence G. Lum1,2,3, Archana Thakur1, Zaid Al-Kadhimi1,2, Gerald A. Colvin4, Francis J. Cummings5, Robert D. Legare6, Don S. Dizon7, Nicola Koultab6, Abby Maizel6, William Colaiacce6, Qin Liu10, and Ritesh Rathore5

Abstract

Purpose: This study reports a phase I immunotherapy trial in 23 women with metastatic breast cancer (MBC) in 5 years. Although most patients experience objective responses to chemotherapy or hormonal therapies, progression is inevitable (1–3). Overexpression of HER2/neu (HER2) in breast, ovarian, lung, gastric, head and neck, and prostate cancers makes it an ideal target for antitumor agents (4, 5). Furthermore, recent studies suggest that the anti-HER2 reagents may be effective against cancer stem like cells in tumors that are HER2-negative (6).

Experimental Design: ATC were expanded from leukapheresis product using IL2 and anti-CD3 monoclonal antibody and armed with HER2Bi. In 3+3 dose escalation design, groups of 3 patients received 5, 10, 20, or 40 × 10^9 armed ATC (aATC) per infusion.

Results: There were no dose-limiting toxicities and the MTD was not defined. It was technically feasible to grow 160 × 10^9 ATC from a single leukapheresis. aATC persisted in the blood for weeks and trafficked to tumors. Infusions of aATC induced anti-breast cancer responses and increased in immunokines. At 14.5 weeks after enrollment, 13 of 22 (59.1%) evaluable patients had stable disease and 9 of 22 (40.9%) had progressive disease. The median OS was 36.2 months for all patients, 57.4 months for HER2+ patients, and 27.4 months for HER2− patients.

Conclusions: Targeting HER2+ and HER2− tumors with aATC infusions induced antitumor responses, increases in Th1 cytokines, and IL12 serum levels that suggest that aATC infusions vaccinated patients against their own tumors. These results provide a strong rationale for conducting phase II trials.

Introduction

In women who present with localized breast cancer, approximately 10% develop metastatic breast cancer (MBC) in 5 years. Although most patients experience objective responses to chemotherapy or hormonal therapies, progression is inevitable (1–3). Overexpression of HER2/neu (HER2) in breast, ovarian, lung, gastric, head and neck, and prostate cancers makes it an ideal target for antitumor agents (4, 5). Furthermore, recent studies suggest that the anti-HER2 reagents may be effective against HER2− cancer stem like cells in tumors that are HER2-negative (6).

Materials and Methods

Clinical protocol

Patients with progressive HER2+ MBC, HER2-targeted agents such as trastuzumab, pertuzumab, trastuzumab–maytansine, (7–10) lapatinib, neratinib, and afatinib (11–13) have improved progression-free survival (PFS). However, these agents are not effective for patients with MBC with HER2-negative disease. Nontoxic targeted approaches are needed for these patients.

Activated T cells (ATC) armed with anti-CD3 × anti-HER2 bispecific antibody (HER2Bi) exhibit high levels of specific cytotoxicity directed at both high and low HER2-expressing breast cancer cell lines (14). Arming ATC with HER2Bi redirects the non-MHC–restricted cytotoxicity of ATC to HER2-specific targets (14). HER2Bi-armed ATC (aATC) repeatedly kill, proliferate, and release Th1 cytokines, RANTES, and MIP-1α when cocultured with HER2-negative cell lines (15). In murine studies, infusions of aATC completely prevented tumor development in coinjection assays and inhibited established HER2+ PC-3 tumors in SCID/Beige mice (16, 17).

In this study, we used combination immunotherapy consisting of HER2Bi aATC infusions, IL2, and granulocyte macrophage colony-stimulating factor (GM-CSF). GM-CSF was empirically chosen because it is known as a potent immune adjuvant and approved for human use. Our data show that aATC infusions were safe and feasible, persist in patients’ blood, and induce cytotoxic responses to breast cancer cells and elevations of serum immunokines.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Authors: Lawrence G. Lum, Wayne State University and Karmanos Cancer Institute, Detroit, Michigan. Gerald A. Colvin, Harvard Medical School, Boston, Massachusetts. Nicola Koultab, Department of Medicine, Wistar Institute, Philadelphia, Pennsylvania.

doi: 10.1158/1078-0432.CCR-14-2280

©2015 American Association for Cancer Research.

www.aacrjournals.org

Published OnlineFirst February 16, 2015; DOI: 10.1158/1078-0432.CCR-14-2280

Cancer Therapy: Clinical

Clinical Cancer Research

Targeted T-cell Therapy in Stage IV Breast Cancer: A Phase I Clinical Trial

Lawrence G. Lum1,2,3, Archana Thakur1, Zaid Al-Kadhimi1,2, Gerald A. Colvin4, Francis J. Cummings5, Robert D. Legare6, Don S. Dizon7, Nicola Koultab6, Abby Maizel6, William Colaiacce6, Qin Liu10, and Ritesh Rathore5

Abstract

Purpose: This study reports a phase I immunotherapy trial in 23 women with metastatic breast cancer (MBC) in 5 years. Although most patients experience objective responses to chemotherapy or hormonal therapies, progression is inevitable (1–3). Overexpression of HER2/neu (HER2) in breast, ovarian, lung, gastric, head and neck, and prostate cancers makes it an ideal target for antitumor agents (4, 5). Furthermore, recent studies suggest that the anti-HER2 reagents may be effective against cancer stem like cells in tumors that are HER2-negative (6).

Experimental Design: ATC were expanded from leukapheresis product using IL2 and anti-CD3 monoclonal antibody and armed with HER2Bi. In 3+3 dose escalation design, groups of 3 patients received 5, 10, 20, or 40 × 10^9 armed ATC (aATC) per infusion.

Results: There were no dose-limiting toxicities and the MTD was not defined. It was technically feasible to grow 160 × 10^9 ATC from a single leukapheresis. aATC persisted in the blood for weeks and trafficked to tumors. Infusions of aATC induced anti-breast cancer responses and increased in immunokines. At 14.5 weeks after enrollment, 13 of 22 (59.1%) evaluable patients had stable disease and 9 of 22 (40.9%) had progressive disease. The median OS was 36.2 months for all patients, 57.4 months for HER2+ patients, and 27.4 months for HER2− patients.

Conclusions: Targeting HER2+ and HER2− tumors with aATC infusions induced antitumor responses, increases in Th1 cytokines, and IL12 serum levels that suggest that aATC infusions vaccinated patients against their own tumors. These results provide a strong rationale for conducting phase II trials.

Introduction

In women who present with localized breast cancer, approximately 10% develop metastatic breast cancer (MBC) in 5 years. Although most patients experience objective responses to chemotherapy or hormonal therapies, progression is inevitable (1–3). Overexpression of HER2/neu (HER2) in breast, ovarian, lung, gastric, head and neck, and prostate cancers makes it an ideal target for antitumor agents (4, 5). Furthermore, recent studies suggest that the anti-HER2 reagents may be effective against HER2− cancer stem like cells in tumors that are HER2-negative (6).

Materials and Methods

Clinical protocol

Patients with progressive HER2+ MBC, HER2-targeted agents such as trastuzumab, pertuzumab, trastuzumab–maytansine, (7–10) lapatinib, neratinib, and afatinib (11–13) have improved progression-free survival (PFS). However, these agents are not effective for patients with MBC with HER2-negative disease. Nontoxic targeted approaches are needed for these patients.

Activated T cells (ATC) armed with anti-CD3 × anti-HER2 bispecific antibody (HER2Bi) exhibit high levels of specific cytotoxicity directed at both high and low HER2-expressing breast cancer cell lines (14). Arming ATC with HER2Bi redirects the non-MHC–restricted cytotoxicity of ATC to HER2-specific targets (14). HER2Bi-armed ATC (aATC) repeatedly kill, proliferate, and release Th1 cytokines, RANTES, and MIP-1α when cocultured with HER2-negative cell lines (15). In murine studies, infusions of aATC completely prevented tumor development in coinjection assays and inhibited established HER2+ PC-3 tumors in SCID/Beige mice (16, 17).

In this study, we used combination immunotherapy consisting of HER2Bi aATC infusions, IL2, and granulocyte macrophage colony-stimulating factor (GM-CSF). GM-CSF was empirically chosen because it is known as a potent immune adjuvant and approved for human use. Our data show that aATC infusions were safe and feasible, persist in patients’ blood, and induce cytotoxic responses to breast cancer cells and elevations of serum immunokines.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Authors: Lawrence G. Lum, Wayne State University and Karmanos Cancer Institute, Detroit, Michigan. Gerald A. Colvin, Harvard Medical School, Boston, Massachusetts. Nicola Koultab, Department of Medicine, Wistar Institute, Philadelphia, Pennsylvania.

do: 10.1158/1078-0432.CCR-14-2280

©2015 American Association for Cancer Research.

www.aacrjournals.org

Published OnlineFirst February 16, 2015; DOI: 10.1158/1078-0432.CCR-14-2280
Translational Relevance

Infusions of anti-HER2 × anti-CD3 bispecific antibody (HER2Bi) armed activated T cells (aATC) are feasible, safe, and did not cause dose-limiting toxicities. Our phase I clinical trial using HER2Bi aATC to target HER2+ and HER2+ metastatic breast cancer in combination with IL2 and granulocyte macrophage colony-stimulating factor stabilized disease in 5 women (5/22 evaluable patients, 22.7%) at 14.5 weeks. Given the number of patients is small, the median OS of 36.2 months for all patients, 57.4 months for HER2 3+ patients, and 27.4 months for HER2 0–2+ patients is encouraging. aATC infusions also polarized the immune system to a Th1/Type 1 cytokine profile with remarkable increases in IL12 production. These results provide the rationale for the design of phase II clinical trials in solid tumors.

Production of clinical HER2Bi

Trastuzumab (Herceptin, Genentech) was heteroconjugated to anti-CD3 (OKT3, Centocor, Ortho-Biotech) to produce HER2Bi under cGMP conditions (14).

Phase I clinical trial design

The primary endpoint was to determine the safety and maximum tolerated dose (MTD) of aATC in a standard 3 + 3 dose escalation trial with dose levels of 5, 10, 20, and 40 billion aATC per infusion (2 infusions/week for 4 weeks) for total doses of 40, 80, 160, and 320 × 10^9 aATC. aATC were given with IL2 (300,000 IU/m^2/day) and GM-CSF (250 μg/m^2/twice weekly) beginning 3 days before the first infusion and ending 1 week after the last aATC infusion. Fig. 1A shows the treatment schema. Tumor evaluations were performed 14.5 weeks after chemotherapy or hormonal therapy (time for lymphoid recovery, ATC production, 4.5 weeks after immunotherapy, and 4 weeks of observation). All patients with MBC (HER2 0–3+) who met enrollment criteria were eligible.

Eligibility criteria

Women 18 years of age or older with histologically documented metastatic infiltrating ductal or lobular breast carcinoma with 0–3+ Her2 expression, Karnofsky score of ≥ 70%, ECOG 0–2, and life expectancy of >3 months with good organ function were eligible (See Supplementary Information). Women with no measurable disease were eligible if the tumor or metastatic disease was removed or successfully treated before enrollment in the study. No serious medical or psychiatric illness that prevents informed consent or intensive treatment were allowed. Minor changes from these guidelines would have been allowed at the discretion of the attending team under special circumstances. The reasons for exceptions would have been documented. HER2/neu, estrogen, and progesterone receptor positivity were recorded. Details of patient characteristics are presented in Supplementary Table S1.

Leukopheresis, T-cell expansion, and production of aATC

ATC were produced as described (18). After 10–14 days, ATC were harvested and armed with 50 ng of HER2Bi/10^6 ATC, and cryopreserved (18). Aliquots were tested for bacteria and fungus, endotoxin, mycoplasma, phenotype, and cytotoxicity.

Dose modification and toxicity scoring (NCI Toxicity Criteria, v2, June 1, 1999).

Patients were accrued to each dose level based on the dose-escalation schema (Supplementary Table S2). If there was one patient with persistent grade 3 nonhematologic toxicity or grade 4 toxicity was encountered in the first 3 patients or 2 out of the first 6 patients, the dose would not have been escalated. Patients with grade 4 nonhematologic toxicity were removed from protocol. As cardiac toxicity associated with Herceptin treatment was a concern (19), patients were removed if aATC infusions decreased the ejection fraction from the baseline MUGA by >10%. Treatment was held for persistent grade 3 toxicity until toxicity decreased to ≤ grade 2. If grade 3 toxicity occurred again, subsequent doses of aATC were washed to eliminate DMSO. If toxicity persisted, then the next dose of aATC was resumed at a 50% reduction. If the toxicities continued at the reduced dose of aATC, the IL2 would be stopped and the aATC infusions continued at the reduced dose. If grade 3 toxicity occurred again, the ATC infusions would be stopped. Toxicities were assessed for 7 days after each infusion and weekly for unresolved toxicities.

Non-MHC-restricted cytotoxicity

Specific cytotoxicity was performed with fresh PBMC from patients (14) using SK-BR-3 (breast specific target) and K562 cells (NK cell target) in ^51Cr release assay (20). ^51Cr release and IFNγ EliSpot assays were used to assess immediate cytotoxicity of fresh endogenous PBMC directed at breast “tumor antigens” to immediately lyse tumor targets or T cells ability to secrete IFNγ without in vitro restimulation. The cytotoxicity or IFNγ EliSpots exhibited by PBMC would represent the development of endogenous immune responses to unknown tumor associated antigens on SK-BR-3 targets.

Cytokine profiles

Serum cytokines were detected by multiplex cytokine array as described previously (18) using the Bio-Plex system (Bio-Rad Laboratory).

Immunohistochemistry

Tissues samples were sectioned, deparaffinized, stained, with hematoxylin and eosin and characterized for tumor content by a pathologist. Adjacent sections were stained with anti-CD3 to detect T cells using the Catalyzed Signal Amplification (CSA Peroxidase System, DAKO) after target retrieval and endogenous biotin/avidin and peroxidase quenching with the CSA Ancillary System (DAKO). Anti-CD3 antibody (1 µg/mL) was diluted in background reducing components (CSA Ancillary System) and incubated with tissue samples for 30 minutes at room temperature. Primary antibody was detected by incubating for 15 minutes...
with biotinylated goat anti-mouse immunoglobulins, and the signal was amplified and visualized by diaminobenzidine precipitation at the antigen site. In parallel, adjacent sections were stained with biotinylated goat anti-mouse IgG2a followed by streptavidin-FITC to detect the goat anti-mouse-IgG2a. Images acquired using fluorescent filters were overlayed upon images acquired by light microscopy creating composite images to evaluate colocalization of staining.

Detection of aATC in patients

Goat anti-mouse IgG2a directed at the OKT3 part of the BiAb was used to detect aATC in the peripheral blood by flow cytometry and in biopsy or surgical samples by immunohistochemistry.

Statistical analysis

The primary endpoint of the study was to determine safety and MTD of aATC. The secondary endpoints were to assess response rates (complete response (CR), stable disease (SD), partial response (PR), and no evidence of disease (NED)), time to progression (TTP), and OS. TTP and OS were measured from the date of enrollment. Kaplan–Meier estimates were performed for OS and TTP. Descriptive statistical analyses were performed for immune monitoring using Prism (GraphPad, Version 5.0).

Results

Patient characteristics

Table 1 and Supplementary Table S1 (Supplemental Information) show the clinical characteristics, prior therapy, number of lines, aATC doses, sites of metastases, disease status at enrollment and at 14.5 weeks, OS for 23 women, and TTP for 22 women. Median age was 48 years (range: 31–68 years). All of the HER2 3+ patients except one (patient #16) received Herceptin®/C210 (patient #16 was randomized to receive no trastuzumab on CALTB49909). All patients received chemotherapy except one (patient #8). One patient (patient #12) was treated twice but counted once for dose escalation and survival analysis. One patient had T cells expanded but was not treated due to disease progression (23 of 24 enrolled were treated).

Phenotype and cytotoxicity of aATC

One patient underwent a second leukapheresis to obtain the T cells necessary to meet the required dose and one patient underwent a second leukapheresis to regrow product due to
specific cytotoxicity of armed ATC was highest in CD8⁺ ATC, lowest in CD4⁺ ATC and intermediate with unfractionated T cells.

**Phase I evaluation of MTD**

The highest dose level completed was 20 × 10⁹ aATC per infusion (160 × 10⁹ total dose of aATC). We accrued one patient at the dose level of 40 × 10⁹ aATC per infusion (320 × 10⁹ total dose), but it was not technically feasible to achieve the 320 × 10⁹ total dose with a single leukapheresis. The technically feasible dose was 160 × 10⁹, and the MTD was not reached.

**Phase I evaluation of toxicities**

The most frequent side effect (SE) was grade 3 chills. Grade 3 headaches emerged as the second most common SE. Table 2 shows the frequency of side effects as a function of dose level (NCI Immunotherapy Protocol Toxicity Table). By episode per infusion, the incidence of chills was 8.6%, 20.8%, and 43.1% at dose levels 1, 2, and 3, respectively. The incidence of headaches was 3.1%, 8.3%, and 19.6% at dose levels 1, 2, and 3, respectively. All patients with grade 3 chills responded to meperidine. Patient #13 at dose level 3 experienced a grade 4 headache and hypertension and was removed from the study after 3 infusions (65.7 × 10⁹ total aATC). The patient had developed a subdural hematoma that was evacuated without neurologic deficits or complications. Three additional patients were added to dose level 3 without any DLTs. One patient achieved dose level 4 of 40 billion/infusion dose for a total of 320 billion. One patient (#2) died of digoxin toxicity related congestive heart failure and the autopsy showed no myocardial T-cell infiltrates. Patients #8 and #14 were admitted for management of hypotension, nausea, vomiting, and dehydration; these infusions were resumed and completed after resolution of their SEs. There were no DLTs attributed to aATC.

**Clinical responses**

Twenty two of 23 patients were clinically evaluable at 14.5 weeks. Patient #2 who died of digoxin toxicity was NED at time of death. Although she was not evaluable for response, she was included in the survival analysis. In the evaluable patients at 14.5 weeks, one patient had NED, one patient had a PR, 11 patients had specific cytotoxicity of armed ATC was highest in CD8⁺ ATC, lowest in CD4⁺ ATC and intermediate with unfractionated T cells.

**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Category</th>
<th>Adverse reaction</th>
<th>Number of patients affected (% at dose level 1)</th>
<th>Total # of episodes by grade at dose level 1</th>
<th>Number of patients affected (% at dose level 2)</th>
<th>Total # of episodes by grade at dose level 2</th>
<th>Number of patients affected (% at dose level 3)</th>
<th>Total # of episodes by grade at dose level 3</th>
<th>Number of Patients affected (% at dose level 4)</th>
<th>Total # of episodes by grade at dose level 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>General disorder</td>
<td>Chills</td>
<td>5 (62.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td></td>
<td>Fever</td>
<td>2 (25)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td></td>
<td>Malaise</td>
<td>2 (33.3)</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td>3 (50)</td>
</tr>
<tr>
<td></td>
<td>Pain</td>
<td>2 (33.3)</td>
<td>0</td>
<td>2 (33.3)</td>
<td>0</td>
<td>2 (33.3)</td>
<td>0</td>
<td>2 (33.3)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Back pain</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Weight gain</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Hypotension</td>
<td>1 (12.5)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
<td>1 (12.5)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cardiac</td>
<td>Atrial rhythm</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Tachycardia</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Nausea/vomiting</td>
<td>3 (37.5)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>1 (16.7)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Dyspnea</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neurological</td>
<td>Headache</td>
<td>2 (25)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Allergy</td>
<td>Nasal congestion</td>
<td>1 (25.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Publication OnlineFirst February 16, 2015; DOI: 10.1158/1078-0432.CCR-14-2280
SD, and 9 patients had PD. Patient #9 (ER+, PR−, HER2−) who was progressing on letrozole developed a PR after aATC treatment that continued beyond 7 months. She had two well-defined liver metastases on a PET/CT scan (2.5 × 1.7 cm and 2.5 × 1.3 cm as shown in Fig. 1B, "before") that decreased in size after immunotherapy (Fig. 1B, "after"). The sum of longest diameters was decreased by 30% at 14.5 weeks and by >70% at 7 months. At 14.5 weeks, 59.1% (13/22) of patients had SD or better (NED, PR, or SD) and 40.9% (9/22) of patients had PD. Five of 22 (22.7%) patients with PD before immunotherapy achieved SD after aATC infusions.

Overall survival and time to progression

Supplementary Table S1 shows the disease status (most patients with visceral disease) before therapy, status at 14.5 weeks, TTP, and OS. Fig. 1C, left shows the Kaplan–Meier curve. The median OS for 23 patients is 36.2 months, 57.4 months for the HER2 3+ group, and 27.4 months for the HER2 0–2+ group. Fig. 1C, right shows the Kaplan–Meier curve for 22 patients who were evaluable for TTP. The median TTP after enrollment was 4.2 months for the entire group, 7.9 months for the HER2 3+ group, and 3.7 months for the HER2 0–2+ group. Supplementary Table S4 shows clinical responses in MBC patients who had SD at 14.5 weeks compared with patients who had PD. The median OS is 40 and 57.9 months for the HER2 0–2+ and HER2 3+ patients with SD, respectively, and 21.3 and 36.6 months for the HER2 0–2+ and HER2 3+ patients with PD, respectively. The proportion of patients (Supplementary Table S5) who had SD or better was 56.5% and the proportion of patients with PD was 39.1% at 14.5 weeks (n = 23). Five of 7 (71.4%) HER2 3+ patients had SD or better disease, whereas 7 of 14 (50.0%) HER2 0–2+ patients had SD or better disease. Patient #14 with an unknown HER2 status had SD (included in the HER2 0–2+ group in the Kaplan–Meier).

Immune responses

Non-MHC–restricted cytotoxic T-cell responses and IFNγ EliSpots. Cytotoxicity exhibited by PBMC without stimulation directed at SK-BR-3 and K562 targets was performed to evaluate the development of immune responses by endogenous lymphocytes. Figure 2A shows anti-SK-BR-3 cytotoxicity mediated by fresh PBMC of 12 patients along with their corresponding HER2Bi aATC dose levels, OS, and TTP. There were no correlations found between aATC dose and OS (r² = 0.0732), aATC dose and TTP (r² = 0.3011), cytotoxicity and OS (r² = 0.0932), or cytotoxicity and TTP (r² = 0.0862). Figure 2B (top left) shows anti-SK-BR-3 cytotoxicity before immunotherapy (preimmunotherapy), during (between infusion #4 and #5) immunotherapy, and 4 weeks postimmunotherapy. Cytotoxicity directed at SK-BR-3 increased significantly (P < 0.003) during immunotherapy compared with preimmunotherapy levels. The top right panel of Fig. 2B shows the increased anti-K562 (NK activity) responses at midimmunotherapy and postimmunotherapy compared with preimmunotherapy, but it was not significantly higher.
Antitumor immunokines. Serum cytokine levels were tested in preimmunotherapy serum (preimmunotherapy), after aATC infusion #4 (midimmunotherapy) and after completion of aATC infusions (1 week after completion of all infusions, postimmunotherapy) to determine whether immunotherapy induced changes in serum profiles (n = 13). There were significant increases in IL12 (P < 0.0005), IL2 (P < 0.002), GM-CSF (P < 0.05), and IL10 (P < 0.01) levels during aATC infusions (midimmunotherapy) compared with baseline (preimmunotherapy). The same trend was seen for IFNγ and TNFα, but there were no statistical differences (Fig. 3). The bottom right panel of Fig. 3 shows the mean Th1/Th2 ratio = [IL2 + IFNγ]/[IL4 + IL10] at the pre-, mid-, and postimmunotherapy time points.

Tumor markers. In 14 of 22 evaluable patients, 4 had a reduction in CEA (2 of 4 had a >50% decrease and 2 of 4 had a 15%–50% decrease), 5 had a reduction in CA 27.29 (2 of 5 had a >50% decrease and 3 of 5 had a 15%–50% decrease), and 5 had a reduction in soluble serum HER2 receptor (2 of 5 had >50% decrease and 3 of 5 had a 15%–50% decrease) levels (Supplemental Table S6).

Kinetics and survival of infused aATC. Phenotyping of PBMC for IgG2a+ cells at pre- and postinfusion time points showed transient increase in IgG2a+ cells up to 50% of the circulating T cells.
after 2- to 4-hour postinfusion in one patient (Fig. 4A, top left). Phenotyping in 4 patients at postinfusion #1, 5, 8, and 1 week postinfusion showed accumulation and persistence of aATC up to 1 week after the last infusion in dose level 1 patients (Fig. 4A, top right).

**Traficking and clearance of aATC.** Infusions of $^{111}$In-labeled aATC localized to the lungs, liver, and spleen, and eventually bone marrow within 4 hours of injection in a HER2$^+$ patient (Fig. 4B, left). However, the $^{111}$In-labeled aATC could not be seen in the lung metastasis of a patient who was HER2$^-$. After 24 hours, aATC had cleared from the lungs but persisted in the bone marrow, liver, and spleen for up to 96 hours (4 days) postinfusion. Blood was sampled and counted to determine the clearance of armed aATC from the blood. The clearance of $^{111}$In-labeled aATC (right). Heparinized blood (1 mL aliquots) were drawn at 0, 0.7, 1, 6, 8, 27, and 96 hours after infusion and counted for gamma irradiation. Results are presented as % radioactivity in serum (cpm) of injected dose. C, the sternal tumor biopsy 1 week (left) and 1 month (right) postimmunotherapy showing poorly differentiated mammary ductal carcinoma. Composite immunofluorescence staining detected HER2Bi bispecific antibody using anti-mouse IgG-FITC overlaying IHC for detection of T cells using anti-CD3. HER2Bi detection colocalized with T cells infiltrating connective tissue surrounding nests of tumor cells. D, the HAMA response before each aATC infusion and post infusions at indicated time points. HAMA antibody response (n = 11) could not be detected more than 10 ng/mL at any time point.

**Localization of aATC to tumors.** Formalin-fixed, paraffin-embedded samples were prepared from a chest wall nodule excision and a sternal tumor biopsy and stained for IgG2a on aATC. IgG2a$^+$ aATC could be detected at 1 week (left) and 1 month (right) after treatment, respectively (Fig. 4C).

**Antibody responses to mouse monoclonal antibody.** Patient sera were tested for human anti-mouse antibodies (HAMA) directed at murine IgG2a (OKT3). Human antibody responses to mouse IgG2a (OKT3) were very low (<10 ng/mL) and were not clinically significant (Fig. 4D).
Discussion

The results from this phase I trial of HER2Bi aATC infusions in women with MBC are clinically encouraging. Multiple infusions of aATC in combination with IL-2 and GM-CSF were safe and technically feasible with persistence of the infused aATC in the circulation for 1 week. The median OS is 36.2 months for all 23 patients (22 evaluable and 1 nonevaluable). The median OS is 57.4 months for the HER2+ patients, and 27.4 months for the HER2 0–2+ patients. Immunotherapy induced endogenous cytotoxic T-cell and immunokine responses that persisted up to 4 months (15).

A highlight of available therapies in patients with progressive disease (regardless of HER2 status) after treatment with anthracyclines and taxanes shows OS ranging from 4 to 18.1 months (21–26). Median OS for MBC was 18.6 months after first-line capcitabine therapy, between 5 and 15 months after second-line therapy, and 8 months after third-line therapy (21–26). For HER2+ locally advanced or MBC patients, sorafenib in combination with capcitabine for first-line therapy resulted in a median OS of 22.2 months (27). Second-line bevacizumab containing therapy for TNBC (RIBBON-2) showed an OS of 17.9 months (28), and combination of cetuximab with cisplatin resulted in an OS of 12.9 months (29). Use of several third-generation aromatase inhibitors given after tamoxifen failure as first- or second-line therapy in postmenopausal women with MBC reported OS up to 26–28 months (30).

In this phase I study, most patients were treated with ≥3 lines of therapy (14/23, 61%) and many had visceral disease (17/23, 74%; Supplementary Table S1). Infusions of aATC stabilized disease in 5 women (5/22, 22.7%) including a very good partial remission in patient #9. There were no DLTs observed. The major side effects were chills, fever, headache, fatigue, and hypotension. Cytokine "flurries" were observed but not life-threatening cytokine "storm". Several patients had their aATC washed to reduce side effects, but no one had their dose of aATC reduced. Three patients were hospitalized for cell-based toxicities, resolved their side effects, and completed immunotherapy without recurrent DLTs. Only patient #13 stopped therapy due to a subdural hematoma most likely due to hypertension, and patient #2 died of digoxin toxicity not related to aATC infusions. The remaining patients received their infusions as outpatients, and there have been over 115 patients to date who have received armed T-cell infusions without DLTs. There were 15 patients who received anti-CD3 × anti-CD20 aATC after high-dose chemotherapy and stem cell transplantation without DLTs (18, 31).

The development of CTL and IFNγ Elispots directed at SK-BR-3 provides immunologic evidence for the development of an endogenous cellular immune response. Consistent with our previously reported data (15), these data also show persistence of CTL up to 4 months after aATC infusions. High anti-SK-BR-3 cytotoxicity levels cannot be attributed to the infused aATC as they would make up only 1% of the endogenous lymphocyte population after dilution (~1 × 10^12 cells). Furthermore, our earlier aATC depletion experiment showed that endogenous cells had developed cytotoxicity directed at SK-BR-3 targets (15).

Increases in serum Th1 cytokine levels leading to high Th1/Th2 ratios and increase in IL-12 that developed mid immunotherapy and persisted for weeks after immunotherapy show that the immunologic milieu and tumor microenvironment were shifted towards an antitumor environment. These results are corroborated by our recent study showing that aATC targeting the triple-negative cell line MDA-MB-231 in Matrigel not only inhibited the growth of tumor cells but also inhibited the growth of immune suppressor cells (32) and generated a Th1 cytokine rich microenvironment. These preclinical and clinical findings support the concept of in situ vaccination with infusions of aATC.

The expansion of T cells resulted in >90% of the T cells becoming memory phenotype of CR45RO+ with more than 50% CD4+ T cells. HER2Bi aATC showed cytotoxicity to SK-BR-3 with consistent increases in cytokotoxicity as the proportion of CD8+ T cells increased in the product.

There are major differences between chimeric antibody receptors (CAR) transduced anti-CD3/anti-CD28 activated T cells (CART) and our approach of using the anti-CD3/IL2 activated T cells armed with bispecific antibodies. CARTs rapidly expand and develop an antitumor effect upon tumor engagement. On the other hand, armed TCM mediate immediate cytotoxicity, undergo short-term proliferation, and release Th1 cytokines/chemokines in the tumor microenvironment (15). The repeated infusions of armed aATC may overcome the tumor immunosuppressive factors to recruit endogenous immune cells leading to in situ vaccination. Treating solid tumors with CAR or armed TCM approaches remains a challenge due to tumor microenvironmental factors.

In summary, aATC were not only feasible and safe but also induced endogenous cytotoxicity and cytokine responses in women with MBC with a possible survival benefit. Our findings show that cellular immune responses develop and may augment immune based killing of tumors even in patients who are progressing. These results provide the rationale for the design of phase II clinical trials using armed activated T cells in solid tumors.

Disclosure of Potential Conflicts of Interest

L.G. Lum is founder of and has ownership interest (including patents) in Transtarget, Inc. D.S. Dizon is an employee of UpToDate. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: L.G. Lum, F.J. Cummings, R.D. Legare, R. Rathore

Development of methodology: A. Thakur, L.G. Lum, F.J. Cummings, D.S. Dizon, N. Kouttab, A. Maizel

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Thakur, L.G. Lum, Z. Al-Kadhimi, G.A. Colvin, F.J. Cummings, R.D. Legare, D.S. Dizon, R. Rathore

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Thakur, L.G. Lum, F.J. Cummings, A. Maizel, Q. Liu

Writing, review, and/or revision of the manuscript: A. Thakur, L.G. Lum, Z. Al-Kadhimi, G.A. Colvin, F.J. Cummings, D.S. Dizon, N. Kouttab, Q. Liu, R. Rathore

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Thakur, L.G. Lum, N. Kouttab, A. Maizel, R. Rathore

Study supervision: A. Thakur, L.G. Lum, Z. Al-Kadhimi, G.A. Colvin, D.S. Dizon, A. Maizel, R. Rathore

Other (imaging interpretation): W. Colaiacce

Acknowledgments

The authors thank the nurse clinical coordinators: Wendy Young, Annette Olson, Lori Hall, Janet McIntyre, Patricia Steele, and Karen Myers who provided scheduling, informational, and emotional support to the women who participated in this clinical trial. The immunotherapy team acknowledges the special efforts of all of the members of the Immunotherapy Program at Roger Williams Hospital and the BMJ/Immunotherapy Program at KCI that have provided support and infrastructure for the compassionate care of the women with MBC.
The authors also appreciate the careful reading and suggestions made by Drs. Abhinav Deol and Ulka Vaishampayan.

Grant Support

This study was supported by the National Cancer Institute of the NIH under award numbers CA92344 (to L.G. Lunn) and CA140314 (to L.G. Lunn). The Microscopy, Imaging and Cytometry Resources Core is supported, in part, by NIH Center grant P30CA22453 to The Karmanos Cancer Institute, Wayne State University and the Perinatology Research Branch of the National Institutes of Child Health and Development, Wayne State University. The studies were also supported by the Young Family Foundation and the Raymond Neag Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Published OnlineFirst February 16, 2015; DOI: 10.1158/1078-0432.CCR-14-2280


Clinical Cancer Research

Targeted T-cell Therapy in Stage IV Breast Cancer: A Phase I Clinical Trial

Lawrence G. Lum, Archana Thakur, Zaid Al-Kadhimi, et al.

*Clin Cancer Res* Published OnlineFirst February 16, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-14-2280

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2015/02/17/1078-0432.CCR-14-2280.DC1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.