Strategic synergy

By Tracey Baas, Senior Editor

Researchers from the **New York University School of Medicine** have shown that induction of NKG2D ligands on poorly immunogenic tumor cells is a key molecular mechanism that contributes to the synergy of radiotherapy plus anti-CTLA-4 mAbs.¹ The findings could be used to identify patients most likely to respond to anti-CTLA-4 treatments such as **Bristol-Myers Squibb Co.**'s melanoma drug Yervoy ipilimumab and could help pinpoint radiation regimens that enhance the treatment to potentially provide a new standard of care in the disease.

CTLA-4 (CD152)-mediated inhibition of T cell activation can prevent the development of antitumor T cell responses. Although there is a clear rationale for blocking CTLA-4, monotherapy with such agents is more successful in treating intrinsically immunogenic tumors than poorly immunogenic ones.

Researchers have been trying to increase the priming of antitumor T cells and establish greater anti-CTLA-4 mAb-mediated antitumor immunity in poorly immunogenic tumors. The three main routes of promoting a more immunogenic environment are vaccination, chemotherapy and radiotherapy.

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—Sandra Demaria,
New York University School of
Medicine

Figure 1. Nkg2d ligands contribute to the synergy of radiotherapy and anti-CTLA-4 mAbs. In mice with poorly immunogenic murine carcinoma cells treated with an anti-CTLA-4 (CD152) antibody, infiltrating cytolytic T cells did not form stable interactions with tumor cells (I). Radiotherapy promoted stable interactions and tumor cell elimination by inducing retinoic acid early inducible-1 (Rae-1) on tumor cells (II). The interaction of Rae-1 on tumor cells with Nkg2d (killer cell lectin-like receptor subfamily K member 1; Klrk1; Cd314) on infiltrating T cells was required for antitumor activity in mice treated with the anti-CTLA-4 antibody.

Of those three options, the NYU researchers have been focused on using local radiation in combination with immunotherapy. In 2005, the team first showed in mice that local radiotherapy converted an unresponsive tumor into a tumor responsive to an anti-CTLA-4 mAb.² In 2009, they showed that metastases located away from the site of local irradiation also responded to anti-CTLA-4 mAb treatment, a phenomenon dubbed an abscopal effect.³

More recently, three pilot studies, each highlighting one patient with metastatic melanoma, demonstrated that patients receiving radiotherapy plus Yervoy showed an abscopal effect and underwent complete remission.^{4,5}

These studies led to clinical trials of the combination in melanoma by **Stanford University** and in prostate cancer by Bristol-Myers.

Bristol-Myers markets Yervoy as a monotherapy to treat melanoma. The drug is in Phase III testing for prostate cancer and Phase II testing as a monotherapy for lung cancer, pancreatic cancer and solid tumors.

Other anti-CTLA-4 mAbs in the clinic include tremelimumab from **Pfizer Inc.** and **AstraZeneca plc.** Tremelimumab is in Phase II trials for liver cancer and solid tumors and in Phase I testing for melanoma and prostate cancer.

Now the NYU team has determined the mechanism by which CTLA-4 blockade plus radiotherapy leads to a greater antitumor response than CTLA-4 alone.

The team used its own breast carcinoma mouse model to visualize tumor-infiltrating CD8⁺ T cells during monotherapy or combination therapy to uncover mechanistic clues.

In mice with poorly immunogenic murine carcinoma cells, an anti-CTLA-4 mAb plus ionizing radiation increased intratumoral T cell infiltration compared with either treatment alone and led to tumor regression. Tumor-infiltrating lymphocytes are traditionally predictive of a positive outcome in cancers.

Animals receiving anti-CTLA-4 mAb monotherapy had infiltrating T cells that moved faster and longer distances than those in untreated mice. The T cells showed randomized patterns of long-distance migration and stopping within the tumor.

In mice treated with the combination therapy, the increased migration seen during monotherapy was abrogated and T cells showed a significantly increased arrest coefficient compared with T cells from mice given monotherapy ($p < 0.0001$), indicating greater contact time with tumor cells.

The data suggest the anti-CTLA-4 mAb disrupts the long-lasting interactions between T cells and tumor cells that are required for antitumor effector functions and that radiation induces changes that restore the interactions.

To tease out the precise mechanism, the researchers looked at levels of tumor cell receptors and found that retinoic acid early inducible-1 (Rae-1), a ligand for Nkg2d (killer cell lectin-like receptor subfamily K member 1; Klrk1; Cd314), showed greater expression on cells from irradiated tumors than on cells from nonirradiated tumors. About 60% of infiltrating T cells—regardless

of irradiation status—expressed Nkg2d, supporting the idea that T cell arrest and tumor cell recognition might rely on NKG2D ligands.

To validate this theory, the team showed that an anti-NKG2D mAb prevented T cells from stopping and interacting with tumor cells in mice treated with the combination therapy and also reversed the immune-mediated antitumor effect of the combination therapy. These data suggest that the NKG2D ligand–NKG2D interaction is a critical step leading to ionizing radiation synergy with anti-CTLA-4 treatment (see Figure 1, “Nkg2d ligands contribute to the synergy of radiotherapy and anti-CTLA-4 mAbs”).

Results were published in *The Journal of Clinical Investigation*.

What next?

“We think that our findings have at least two important implications for the treatment of cancer patients,” said Sandra Demaria, associate professor of pathology at the New York University School of Medicine and lead principal investigator on the study.

First, she said, “NKG2D ligand expression could be used as a marker to predict tumor responsiveness to anti-CTLA-4 treatment, as well as immune-mediated toxicity in normal tissues.”

She added, “NKG2D ligand induction may also provide a marker that could be used to guide selection of radiation strategies that optimally synergize with anti-CTLA-4 treatment. We have previously shown in two other mouse tumor models that not all radiation regimens are equally effective in

inducing antitumor immunity in combination with anti-CTLA-4.”

“We will be conducting two separate clinical trials,” she continued. “One trial has just opened at NYU, comparing the effect of ipilimumab alone or with radiotherapy, and is led by [Silvia] Formenti, a coauthor on the *JCI* paper. Another trial that will test the effect of different radiation regimens and ipilimumab on progression is expected to open at the end of the month and is supported by Bristol-Myers Squibb.”

Going forward, Demaria said the group is “most interested in determining whether the anti-CTLA-4 human mAbs used in the clinic have the same effects on human CD8⁺ T cell motility as we have seen in the mouse system. Michael Dustin’s lab is currently working on this.”

Dustin, an author on the *JCI* paper, is professor of molecular immunology and pathology at the **New York University Langone Medical Center**.

“We are also beginning experiments to determine if the requirement for NKG2D engagement to achieve a stable interaction between CD8⁺ T cells and their tumor targets depends on the affinity of the T cell receptor for a given tumor antigen,” said Demaria.

“Their preclinical work adds an interesting mechanism for understanding the synergistic effects of combination therapy,” said Ignacio Melero, professor of immunology and consultant in the Department of Oncology at the **University of Navarra**.

“It is likely that local radiotherapy would enhance NKG2D ligands on human tumor cells,” said Melero. “This might be determined from tumor samples obtained from patients that received radiotherapy prior to surgery.”

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Glenn Dranoff, professor of medicine at the **Dana-Farber Cancer Institute, Brigham and Women's Hospital** and **Harvard Medical School**, said the work suggests "treatments that target the NKG2D pathway might also be combined effectively with anti-CTLA-4 mAbs."

Melero cautioned that any therapies directed against the NKG2D pathway will have to be tightly regulated. "Rapid induction of NKG2D ligands provides stimulation to natural killer and T lymphocytes, but chronic expression and release from tumor cells may desensitize this receptor-ligand system," he said.

"Chronic NKG2D ligand expression and/or shedding from the tumor surface can indeed trigger NKG2D downregulation," said Dranoff. "Treatments would need to enhance NKG2D function. One approach would be to administer antibodies against the soluble NKG2D ligands being shed."

The work from NYU is not patented or licensed.

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Contact: Sandra Demaria, New York University School of Medicine, New York, N.Y.
e-mail: sandra.demaria@nyumc.org
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COMPANIES AND INSTITUTIONS MENTIONED

AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.

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New York University School of Medicine, New York, N.Y.

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Stanford University, Stanford, Calif.

University of Navarra, Pamplona, Spain