BiTE® ANTIBODIES
Designed to Bridge T Cells to Cancer Cells
Cytotoxic T-Lymphocytes (CTLs) have an innate ability to target tumor cells, and evidence supports a role for immunosurveillance in tumor targeting. By recognizing and binding to tumor cells, CTLs can lead to tumor cell lysis. To affect tumor cell lysis, CTLs require cell-to-cell contact. However, tumor cells have developed various mechanisms that can prevent an antigen-specific CTL response and evade T cell recognition.

Tumor cells can evade destruction by

- Modification of tumor microenvironment by secretion of immunosuppressive cytokines such as TGF-β
- Modification of immune cell repertoire by stimulation of immunosuppressive response
- Generation of IDO

Suppressive factors within the tumor microenvironment may inhibit effector cells that manage to access the tumor.
Tumor cells can evade destruction by cytotoxic T cells:

- Loss or mutation of MHC class I on tumor cells
- Interference with perforin/granzyme pathway
- Exploitation of immune-checkpoint proteins
- Increased CTLA-4 expression on T cells counteracts CD28
- Increased PD-1 expression on T cells
- Inhibition of kinases involved in T cell activation
- Persistent PD-1 expression causing T cell exhaustive state
- Increased PD-L1 expression on tumor cell surface

Dendritic cells may not recognize antigens as targets for destruction.

Inhibition of T cell priming by interleukins.

**Cytotoxic T Cells**

- Loss or mutation of MHC class I on tumor cells
- Interference with perforin/granzyme pathway
- Exploitation of immune-checkpoint proteins
- Increased CTLA-4 expression on T cells counteracts CD28
- Increased PD-1 expression on T cells
- Inhibition of kinases involved in T cell activation
- Persistent PD-1 expression causing T cell exhaustive state
- Increased PD-L1 expression on tumor cell surface
Recent Approaches in Oncologic Therapies

The aim is to harness and enhance the cytotoxic activity of T cells against tumor cells*

1. Allogeneic HSCT: Tumor cells are eliminated through chemotherapy and graft versus tumor effect\(^6\)\(^–\)\(^9\)

2. Adoptive Cell Therapy: Adoptive cell therapy utilizes autologous antitumor activity of cells such as tumor-infiltrating lymphocytes, to treat cancer\(^10\)

3. Immunovirus: Using a modified virus that has the potential to induce tumor cell lysis through replication within tumor cells and activation of T cells\(^11\)\(^–\)\(^14\)

4. CTLA-4 Checkpoint Inhibitors: Anti-CTLA-4 mAbs augment T cell activation by blocking inhibitor receptors such as CTLA-4\(^5\),\(^15\)

* Some of these mechanisms are still under investigation.
5. PD-1/PD-L1 Checkpoint Inhibitors: Checkpoint inhibitors against PD-1 and its ligand PD-L1 release PD-1 pathway-mediated inhibition of T cell activation\(^5\)

6. Autologous Active Cellular Immunotherapy: Activated antigen-presenting cells are reinfused into patients to direct immune cells against target cancer cells\(^6\)

7. CAR-T Cells: Modified chimeric antigen receptor (CAR)-T cells redirect T cell antigen specificity, activation and further enhance T cell function via costimulation domains in the cytoplasmic tail\(^7\)\(^,\)\(^8\)

8. BiTE\(^\circledast\): BiTE\(^\circledast\) antibody constructs bridge CD3-positive CTLs to cells expressing specific cell surface antigens, resulting in the release of proteolytic substances against the target cell(s)\(^9\)\(^\text{-}\)\(^21\)
Investigational Bispecific T cell engager (BiTE®) Antibodies are designed to bridge cancer cells to CTLs. The BiTE® antibody construct utilizes the binding properties of the variable domains of two monoclonal antibodies. One domain is designed to target an antigen on the surface of a cancer cell whereas the other is designed to engage CD3 on the surface of a T cell. With these two different domains, BiTE® antibodies aim to engage the endogenous cytotoxic potential of CTLs, bypassing MHC/antigen-dependent activation of T cells.
BiTE® Antibodies are Designed to Bridge Cancer Cells and CTLs to Enable Cancer Cell Lysis

Upon binding of both arms of investigational BiTE® antibodies, BiTE®-activated T cells and cancer cells are forced within close proximity of one another. As a result, a cytolytic synapse is created between the T cell and cancer cell, perforin and granzymes are released from the T cells, and target cell lysis ensues. This activation is achieved independently of TCR specificity, costimulation, or peptide antigen presentation.
After target cell destruction, BiTE® antibodies are designed to move through the local environment and target additional tumor cells.\textsuperscript{20} Cytotoxic T cells are not consumed during lysis of target cells. After destruction of one tumor cell, an activated T cell can move on to other tumor cells and initiate additional cell lysis.\textsuperscript{20} Activated T cells may also release proinflammatory cytokines and produce even more perforin and granzyme to support subsequent interactions to engage tumor cells.\textsuperscript{22,23} These mechanisms lead to enhanced effects—a more complete elimination of tumor cell populations combined than with each mechanism alone.
The innate ability of T cells to target tumor cells supports the role for the immune system in recognizing and suppressing tumor growth. Through different mechanisms, tumor cells have the ability to escape T cell immunosurveillance, BiTE® antibodies are designed to bridge CD3+ CTLs to cells expressing cell-surface target antigens. In doing so, BiTE® represents a new investigational approach to target different tumors throughout the body.
After target cell destruction through lysis, BiTE® antibodies are designed to move through the local environment and target additional cells.
References

This booklet contains forward-looking statements that are based on Amgen’s current expectations and beliefs and are subject to a number of risks, uncertainties, and assumptions that could cause actual results to differ materially from those described. All statements, other than statements of historical fact, are statements that could be deemed forward-looking statements. Forward-looking statements involve significant risks and uncertainties, including those more fully described in the Risk Factors found in the most recent Annual Report on Form 10-K and periodic reports on Form 10-Q and Form 8-K filed by Amgen with the U.S. Securities and Exchange Commission, and actual results may vary materially. Except where otherwise indicated, Amgen is providing this information as of November 15, 2015 and does not undertake any obligation to update any forward-looking statements contained in this booklet as a result of new information, future events, or otherwise.