Immunotherapy

Three is a charm for an antibody to fight cancer

Alfred L. Garfall & Carl H. June

Immunotherapy approaches seek to boost immune responses against cancer. A single antibody engineered to recognize three targets shows promise, when tested in animals, in improving the ability of T cells to target cancer.

Antibodies with specificity for one target — called monoclonal antibodies — were the first cancer immunotherapy to achieve widespread clinical use. The therapeutic potency of antibodies can be amplified by engineering them to recognize two distinct molecular targets (termed antigens). These bispecific antibodies can simultaneously bind to cancer cells and immune cells called T cells, and this dual binding directs the T cell to unleash its cell-killing power towards the cancer cell. Writing in *Nature Cancer*, Wu *et al.*[1] now report the development of a trispecific antibody, one that has three targets: a cancer cell, a receptor that activates T cells, and a T-cell protein that promotes long-lasting T-cell activity against the cancer cell (Fig. 1).

The mammalian immune system generates an immense diversity of antibodies, and antibodies can also be engineered to recognize therapeutic targets. Antibodies usually recognize a single antigen, which might be part of a disease-causing agent or an abnormal version of a protein or sugar. Such monospecific antibodies against targets on cancer cells can recruit immune cells, including neutrophils, natural killer cells and macrophages, to kill or ingest the cancer cells.

Antibodies can also be engineered to block or stimulate the function of the proteins to which they bind. For example, there are regulatory receptors that inhibit T-cell function, and antibodies that have been engineered to block these receptors provide a clinical strategy known as checkpoint blockade, which boosts T-cell function. These inhibitory receptors govern T-cell exhaustion, a non-functional T-cell state that protects against autoimmunity and that can occur in the tumour microenvironment as cancers evade antitumour responses mediated by T cells. Checkpoint-blockade treatment can awaken exhausted antitumour T cells to great clinical benefit, but it also risks causing autoimmune toxicity. The antibody developed by Wu and colleagues takes a similar approach to promote T-cell activity against cancer cells. However, their method stimulates the function of receptors that positively boost T-cell function, rather than blocking the function of inhibitory receptors.

The human antibody developed by Wu *et al.* builds on bispecific-antibody technology that reconfigures the antigen-recognition domains of two different antibodies into one bispecific molecule. Bispecific antibodies usually target one antigen on the cancer cell's surface and one on a protein complex on T cells called CD3. CD3 is part of the T-cell receptor (TCR) complex. The TCR also includes antigen-recognition domains and delivers an activating signal to the T cell when an antigen binds. Engagement of CD3 by the antibody also generates an activating signal. Such a bispecific antibody therefore activates T cells, brings them into close proximity to cancer cells — irrespective of the T cell's natural antigen specificity — and redirects their killing capabilities towards the cancer cells.

This concept has proved to be clinically effective for the bispecific antibody blinatumomab, which targets CD3 and the protein CD19 on cancer cells. Blinatumomab treatment doubles the remission rate and survival among people with an advanced stage of a cancer called B-cell acute lymphoblastic leukaemia (B-ALL)[2], and it is being tested as part of the initial therapy for B-ALL, with promising early results[3].

Wu and colleagues devised a clever strategy to simultaneously boost T-cell activation and enhance the targeting of cancer cells in relation to multiple myeloma, which is a cancer of plasma cells in the blood. The authors developed a trispecific antibody that was engineered to have three antigen-binding sites, rather than two. This trispecific antibody targets CD3 plus the proteins CD38 (on cancer cells) and CD28 (on T cells). The CD38-targeting antibody daratumumab is clinically effective in treating this disease[4], and CD38 is also a potential target in other cancers, such as acute lymphoid leukaemia and acute myeloid leukaemia.

CD28 belongs to a class of protein called co-stimulatory receptors, which positively regulate T-cell activation. When a T cell recognizes its target antigen through the TCR, the extra engagement of a co-stimulatory receptor such as CD28 is needed to achieve the sustained T-cell proliferation required for

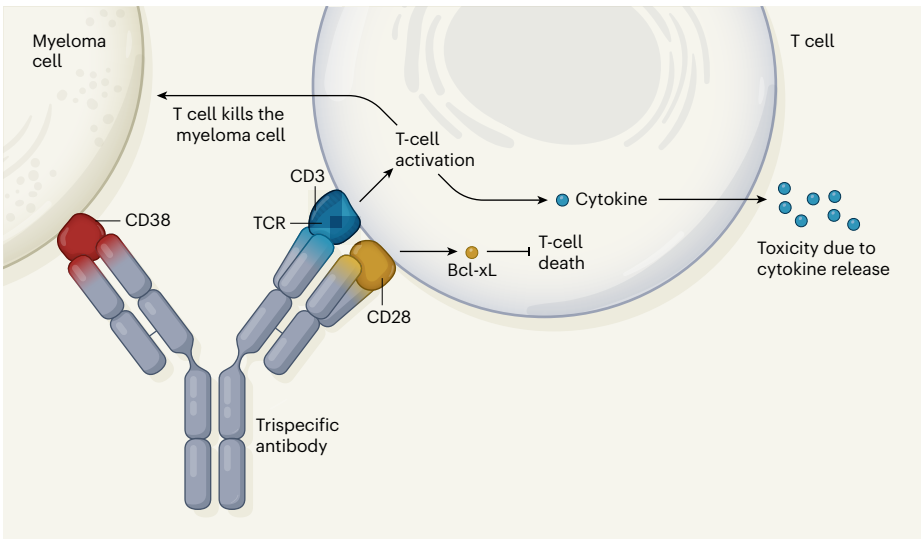

Figure 1 | An antibody that helps immune cells to target cancer cells. Wu *et al.*[1] report the development of a human antibody that is engineered to bring an immune cell called a T cell into close proximity with a type of cancer cell called a myeloma cell and to boost the T cell's anticancer response. This trispecific antibody binds three targets: the protein CD38 on a myeloma cell, and the protein CD28 and the protein complex CD3 on a T cell (the antibody's target-binding domains are shown in red, blue and yellow, respectively). CD3 is part of the T-cell receptor (TCR), which recognizes abnormal cells by binding molecules called antigens. The binding of CD3 by the antibody drives T-cell activation (without requiring antigen recognition by the TCR), which leads to the killing of the myeloma cell and the production and release of toxic cytokine molecules. Binding of CD28 by the antibody drives expression of the protein Bcl-xL. Bcl-xL blocks T-cell death, which might otherwise occur if there was prolonged TCR activation in the absence of CD28 stimulation by the antibody.

an effective immune response. In the absence of co-stimulation, activation through the TCR can lead to a state of T-cell non-responsiveness called anergy, or to the related state of exhaustion. Prolonged activation of the TCR without co-stimulation can lead the T cell to undergo a form of programmed cell death called apoptosis.

The addition of a co-stimulatory signal such as CD28 is notable because this signal has also been incorporated into another type of immunotherapy called chimaeric-antigen receptor T cell (CAR-T) therapy[5], in which a receptor is engineered to both recognize a cancer-cell antigen and include T-cell activation domains such as CD3 and CD28. The main reason for including a CD28-binding domain in the trispecific antibody is T-cell co-stimulation. However, CD28 is also frequently expressed by multiple myeloma cells, so this might increase the antibody's affinity for the myeloma cells, and thus enable it to bind to cells in which CD38 is low, absent or masked by previous daratumumab therapy.

To confirm that the CD28-binding domain augmented the trispecific antibody's activity, the authors made versions of the antibody in which different combinations of the three binding domains were mutated. They tested these versions in 'humanized' model mice, which had human T cells and human myeloma cells. A functional CD28-targeting domain boosted T-cell activation above that observed using antibodies lacking this domain. This augmented T-cell activation drove T-cell proliferation and the expression of the anti-apoptotic protein Bcl-xL in T cells, supporting the authors' hypothesis that having a co-stimulatory signal would prevent T-cell apoptosis. The presence of the CD28-targeting domain on the antibody boosted the ability of T cells to kill different myeloma cell lines *in vitro* and in the humanized mouse model, even at the lowest antibody dose tested.

The main limitation of this study is that the risk of a side effect called cytokine release syndrome (CRS), which can occur if the immune system is highly stimulated, is unknown. In CRS, the simultaneous activation of many T cells causes excessive release of signalling molecules called cytokines from cells of the immune system, which drives inflammation. CRS can occur with bispecific antibodies and with CAR-T. It typically manifests as fever, but can progress to fatal multi-organ failure in severe cases[6].

The authors report cytokine-related toxicities with their trispecific antibody when administered to monkeys by intravenous injection, but toxicity was less if it was delivered under the skin (subcutaneously) instead, leading to a more gradual exposure to the antibody. It is reassuring that the inclusion of the CD28-targeting domain did not lead to overwhelming CRS in these tests. However, a key caveat is that the amount of CD38 in monkeys is much less than in people with multiple myeloma, and the higher amount of CD38, and thus of antibody-mediated T-cell activation, would probably increase the risk of CRS in humans. But in terms of possible negative effects of the antibody on healthy non-cancerous cells, it is reassuring that only transient decreases in the number of normal white blood cells that express CD38, such as lymphocytes and myeloid cells, were observed in monkeys treated with the antibody. Another limitation of the study is that the authors did not assess whether this trispecific antibody format might trigger an immune response against the antibody and cause its rapid destruction.

"Targeting cancer using a trispecific antibody is an important conceptual advance."

Targeting cancer using a trispecific antibody is an important conceptual advance, building on previous work by this group[7] on a trispecific antibody that targets HIV. For multiple myeloma, fresh therapeutic approaches are needed, because even the most potent emerging therapies, including a CAR-T that targets an antigen called BCMA, are only temporarily effective for most people[8–10]. A trispecific antibody is a flexible platform that might offer a way to deliver precise combinations of immunomodulatory signals (for example, a co-stimulatory signal and a checkpoint blocker) specifically in the tumour microenvironment, which might be safer and more effective than the systemic administration of combinations of individual, single-specificity immunomodulatory antibodies. Such efforts to make immunotherapy more precise and potent than it is at present might be necessary to broaden the reach of immunotherapy to include the many types of cancer that have so far proved difficult to target.

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Microbiology

Microbial clues to a liver disease

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Treatment options are limited for alcoholic hepatitis, a liver disease associated with high alcohol intake. Studies in mice reveal that the microorganisms responsible for this condition can be tackled by a viral treatment. **See p.505**

In 1984, the microbiologist Barry Marshall notoriously used himself as an experimental subject for his research, and drank the contents of a flask containing the bacterium *Helicobacter pylori* as part of his efforts to demonstrate that bacteria cause stomach ulcers[1]. On page 505, Duan *et al.*[2] do not report taking such drastic action to investigate a bacterial connection to disease. Nevertheless, their careful analysis of a liver disease called alcoholic hepatitis, in studies of mice and analysis of samples from people who have the disease, also provide attention-grabbing evidence for the involvement of a suspected bacterial culprit.

Alcoholic hepatitis is a poorly understood condition related to high alcohol intake, and is difficult to treat. Previous experiments in mice have hinted that the gut-dwelling bacterium *Enterococcus faecalis* might be involved[3]. However, *E. faecalis* is usually thought of as an old friend that inhabits the guts of many animals across the evolutionary tree, from humans to nematode worms[4]. This species usually represents less than 0.1% of all the bacteria in faecal samples from healthy people[5]. However, after antibiotic treatment, bacteria of the genus *Enterococcus* increase in prevalence to become one of the most common types of microbe in the gut[6]. *E. faecalis* can infect the