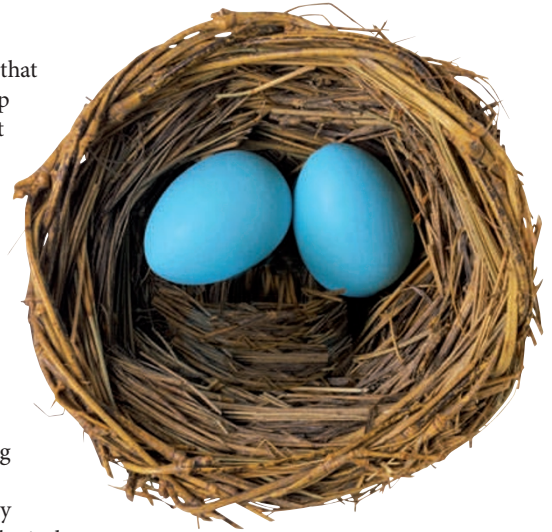⊡ **ANTICANCER DRUGS**

Two for one

Therapeutic monoclonal antibodies that are used to treat cancer and other diseases selectively target one antigen. Although it is thought that antibodies might evolve specificity for more than one antigen, this phenomenon has only been observed for antibodies that recognize small haptens. Germaine Fuh and colleagues have isolated a variant of the monoclonal antibody trastuzumab (Herceptin) that can bind two distinct cancer-related proteins with high affinity.

Trastuzumab binds to its target ERBB2 primarily through its heavy chain complementarity-determining regions (CDRs). Based on the hypothesis that preservation of the heavy chain CDRs might maintain specificity for ERBB2 binding, Bostrom *et al.* generated a library of trastuzumab variants by mutating the light chain CDRs. The light chain variant library was then screened for the ability to bind to the therapeutic targets vascular endothelial growth factor A (VEGFA) or death receptor 5 (DR5, also known as TNFRSF10B), as well as maintain ERBB2 binding. The antibody with the highest dual affinity was bH1, which bound VEGFA and ERBB2.

How is bH1 able to bind two unrelated protein antigens? Analyses of the crystal structures of the bH1 antigen binding fragment (Fab) bound to the extracellular domain of ERBB2 or the receptor-binding domain of VEGFA showed that there was significant overlap in the bH1 amino acids that were in close contact with either the ERBB2 or VEGFA epitopes. However, mutagenesis of the CDR residues showed that, energetically, bH1 light chain CDRs are largely responsible for mediating binding to VEGFA and heavy chain CDRs for mediating binding to ERBB2.

Does this dual-specificity antibody also have dual biological effects? The authors tested bH1 and two affinity-improved variants for their ability to inhibit the growth of cell lines. All three antibodies blocked VEGFA-induced proliferation of human umbilical vein endothelial cells (HUVECs) and BT474 breast cancer cells, which overexpress ERBB2. The variant with the highest affinity for ERBB2 and VEGFA, bH1-44, had a similar potency to trastuzumab or bevacizumab (Avastin; which blocks VEGFA). bH1-44 blocked the growth of Colo205 human colorectal cancer cell xenografts in a manner similar to the anti-VEGFA antibody B20-4.1 (a surrogate of bevacizumab), whereas trastuzumab had no effect, indicating that the inhibition of tumour growth was a result of VEGFA inhibition. Similarly, bH1-44 and trastuzumab blocked the growth of BT474M1 human breast cancer cell xenografts, but B20-4.1 did not.

Although bH1-44 is able to inhibit tumour xenograft growth to a similar extent as anti-VEGFA or anti-ERBB2 specific antibodies, the authors note that more studies in different tumour models are required to determine whether bH1-44 is as effective as these antibodies. In addition, whether dual targeting of VEGFA and ERBB2 might be beneficial for cancer patients is still being evaluated.

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ORIGINAL RESEARCH PAPERS Bostrom, J. *et al.* Variants of the antibody Herceptin that interact with HER2 and VEGF at the antigen binding site. *Science* **323**, 1610–1614 (2009)