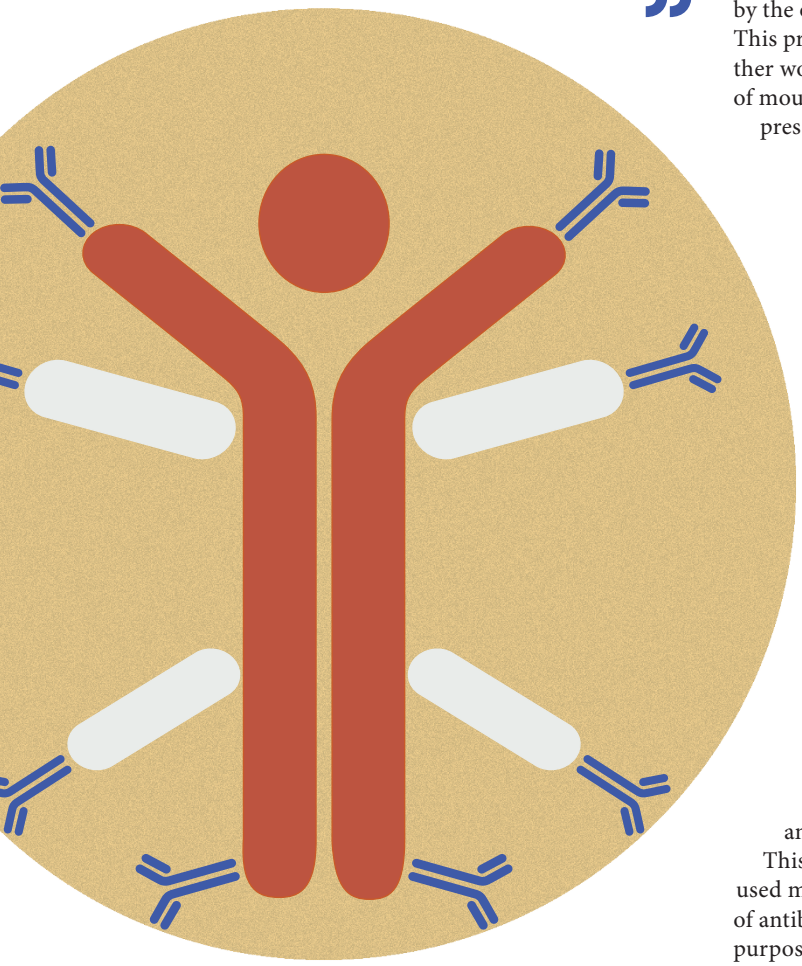
MILESTONE 12

To humans, and beyond!

Today, we may take for granted that monoclonal antibodies are central to the present and future of precision medicine (MILESTONE 9). Whether in highly specific diagnostics or as targeted therapeutics—from chimeric antigen receptors to antibody-drug conjugates—antibodies enable not only the discovery of important biology but also the application of this knowledge to treat disease. (MILESTONES 11, 13, 14). Engineering these versatile, robust and highly specific tools for use in humans required tackling issues of immunogenicity, biodistribution, and mass production.

> "the first chimeric antibodies [were made] by replacing the variable regions of a human antibody with those of a mouse one"



Advances in antibody engineering, such as the development of humanized antibodies, have been vital for improving the safety and efficacy of antibody-based drugs in the clinic. Image credit: P. Guha/Nature Publishing Group.

In the mid-1980s, when it became evident that monoclonal antibodies had great potential for use in humans, it was rapidly recognized that antibodies generated in rodents would be immunogenic to humans. One solution to this problem came from Vernon Oi and colleagues, who made the first chimeric antibodies by replacing the variable regions of a human antibody with those of a mouse one. The product was functional and bound the antigen recognized by the original mouse antibody. This promising result spurred further work to minimize the regions of mouse protein that had to be present.

Could the framework regions also be of human origin? Whether the structure of the complementarity-determining regions (CDRs)—and, notably, their ability to bind antigen—was independent of the context of the framework regions was not clear. To solve this, Greg Winter and his team, in work that led to the first humanized antibodies, used available three-dimensional immunoglobulin structures to carefully map the CDRs. They then grafted CDRs of a mouse antibody on a human scaffold. This continues to be a widely used method for the development of antibodies for therapeutic purposes and defining the CDR boundaries is key to success.

Fragment antigen-binding (Fab) antibodies—advantageous particularly because of their small size, which decreases half-life and improves tissue penetration—had thus far been generated by proteolysis. However, the preparation has low yields and the product is not homogeneous, containing low-molecular-weight peptide products of degradation. An alternative, still used today, came from enlisting bacteria to produce the antibodies. Expressing the variable (V) and constant (C_H1 and C_k) regions that constitute the Fab along with a secretion signal peptide allowed the production of Fab without enzymatic digestion.

Unfortunately, this was not a 'cure-all', as not every Fab was so easily generated in bacteria. However, a clever way to get around the problem was soon discovered: a linker connecting the V_H and V_L would enable expression of the variable fragment as a single chain. The idea was that the framework regions would mediate proper folding of the domains, as long as they were linked by a peptide of the right length and sequence. The scientists predicted that these single-chain 'Fv' antibodies would have substantial advantages and could be used in conjugation with drugs and toxins for imaging and therapy. Time would soon prove them right.

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