between the inner and outer sides of the coil. As the wood dried, the cells all shrank, but those on the inner surface shrank more than those on the outer edge. This led to the shape change that made the whole structure rotate. Having understood the physical principles underlying this hygromorphic behaviour, Luo *et al*. designed a simple process that involved boiling the veneer in a chemical bath to improve mouldability, while maintaining the stiffness required for drilling into the soil.

The versatility of the design is particularly appealing; it comes from the authors' thorough understanding of the material. Using a combination of computational modelling and physical experiments, they tailored their seed carriers to deliver seeds with masses ranging from 52 to 75 milligrams. The mechanism remained the same for all seeds: moisture caused the cells to swell and the coil to unwind, drilling the payload down; the coil then wound up again as it dried, pushing the payload deeper underground.

Although these wooden robots have potential, they are not flawless. The manufacturing process involves positioning the veneer on the delicate 3D-printed mould and then carefully gluing the tails together. This manual process will need to be refined if it is to be scaled up in a way that could make the technology truly useful. Luo *et al*. used white oak because it is reasonably stiff, but this could be replaced with other types of wood if the fabrication process were adapted to involve stiffening (see, for example, ref. 7).

The authors' tests also seem to indicate that stormy weather severely compromises the efficiency of the device. Further work is needed to ensure that the robots can function in the potentially harsh, variable and remote environments for which they are intended.

There has been much talk of soft robotics using flexible materials[8], but hygromorphic wood does not quite fit this description. Actuators of this type fall into the realm of 'firm' robotics — devices made from relatively stiff materials that have the shape-changing behaviour of soft robots. These devices have the advantage of interacting with resistive substrates (such as soil) more easily than do soft robots. Thinking beyond seed carriers, the insight presented in this work could enable a range of hygromorphic wooden actuators, or contribute to developments in passively responsive architecture.

Luo and colleagues' bioinspired solution to the inefficiency of aerial seeding is a prime example of problem-led engineering. The authors' ability to tailor their seed carriers for different payloads and forces is impressive and shows that wood is still a state-of-the-art material. It also highlights the potential for natural materials to be highly controllable and functional, as well as sustainable.

Samuel E. Mason and **Naomi Nakayama** are in the Department of Bioengineering, Imperial College London, London SW7 2AZ, UK.
e-mail: n.nakayama@imperial.ac.uk

1. Reyssat, E. & Mahadevan, L. *J. R. Soc. Interface* **6**, 951–957 (2009).
2. Luo, D. *et al*. *Nature* **614**, 463–470 (2023).
3. Vovchenko, N., Novikov, A., Sokolov, S. & Tishchenko, E. *IOP Conf. Ser.: Earth Environ. Sci.* **595**, 012009 (2020).
4. Lee, S.-W., Prosser, J. H., Purohit, P. K. & Lee, D. *ACS Macro Lett.* **2**, 960–965 (2013).
5. Krapež Tomec, D., Straže, A., Haider, A. & Kariž, M. *Polymers* **13**, 3209 (2021).
6. Joosten, S., Radaelli, G. & Vallery, H. *Smart Mater. Struct.* **30**, 025008 (2021).
7. Song, J. *et al*. *Nature* **554**, 224–228 (2018).
8. Coyle, S., Majidia, C., LeDuc, P. & Hsia, K. J. *Extreme Mech. Lett.* **22**, 51–59 (2018).

The authors declare no competing interests.

Medical research

For antibodies, sometimes less is more

Christoph Wülfing & Simon J. Dovedi

Antibodies that activate stimulatory or inhibitory receptors are of great therapeutic interest for the treatment of cancer or autoimmune diseases. It emerges that such antibodies work better if they don't bind to receptors too tightly. **See p.539**

Antibodies have a remarkable capacity to recognize specific parts of proteins called antigens, particularly those associated with disease or infection. B cells of the immune system make antibodies, and they can be generated to target any protein, engineered to tune their properties and produced at scale *in vitro*. Thus, they have become widely used therapeutics. Yu *et al*.[1] report on page 539 how antigen-binding strength determines the effectiveness of three antibodies used to treat tumours and autoimmune diseases, and the authors suggest that less is more.

In a therapeutic context, antibodies that target stimulatory or inhibitory receptors are used to regulate the function of immune cells (Fig. 1). Those that bind to a receptor and thereby impede the receptor's interaction with its usual binding partner (ligand) are called blocking antibodies. Antibodies that block the inhibitory receptors PD-1 and CTLA-4 have revolutionized cancer therapy by offering a way to activate tumour-targeting immune cells. By contrast, 'agonist' antibodies can function as synthetic ligands to activate a receptor.

Both types of antibody hold interest as clinical tools. Although the immune system can eliminate tumour cells, the tumour microenvironment is often immunosuppressive, with high expression of inhibitory receptors and ligands, and a lack of ligands for stimulatory receptors. Some of the current developments for cancer treatment are focused on antibodies that activate stimulatory immune receptors[2]. Yu and colleagues investigated clinically relevant agonist antibodies that target CD40, a stimulatory receptor on certain types of immune cell (myeloid and B cells), and CD137, which is also known as 4-1BB, a stimulatory receptor on T cells of the immune system.

Another area of therapeutic development focuses on self-destructive immune responses that cause autoimmune disease. To examine antibody-mediated suppression of autoimmunity, the authors investigated an antibody that stimulates inhibitory signalling in immune cells through PD-1.

The immune system generates high-affinity antibodies — their binding, as assessed by a measurement called a dissociation constant, is often in the picomolar range. This ensures long-lasting binding, which is important if blocking antibodies are to fulfil their role. However, the binding affinities of natural receptor–ligand interactions are weak and are often in the low micromolar range. Work on the main activating receptor of T cells, called the T-cell receptor, suggests that low affinities are optimal for receptor function[3]. Such low binding affinities enable a ligand to bind for long enough to enable the receptor to initiate a consecutive series of signalling steps, yet for a short enough length of time to enable one ligand molecule to subsequently engage many receptors to amplify the signal[3].

Yu and colleagues investigated whether an optimum intermediate affinity exists for agonist antibodies. The authors generated a range of agonist antibody variants that target CD40 with a large nanomolar range of binding affinities. The authors assessed these variants using mouse and human immune cells. They examined the activation of B cells and immune cells called dendritic cells, monitored the activation-associated proliferation of T cells

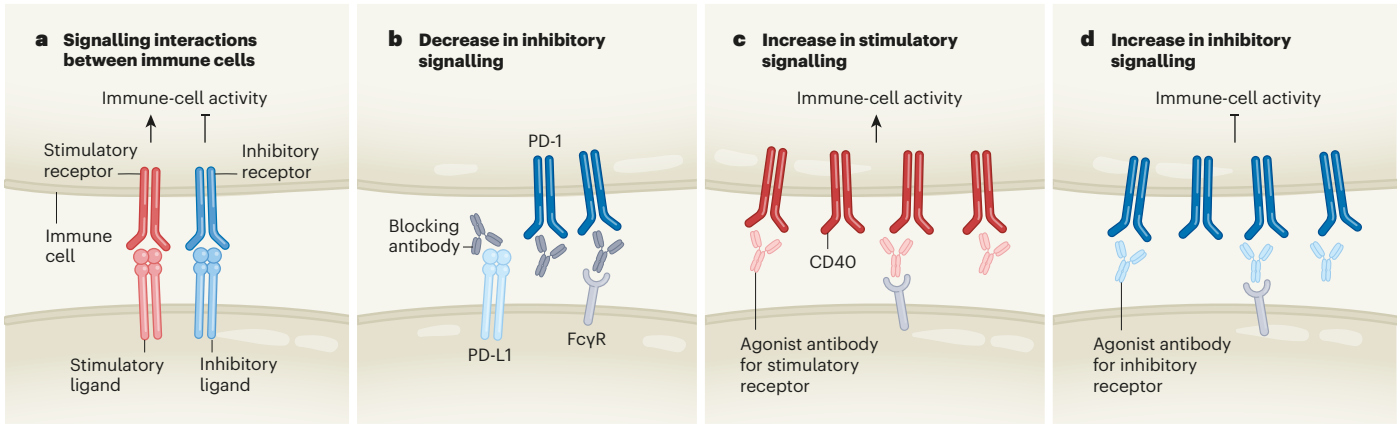

Figure 1 | Antibody use to control immune-cell function. Yu *et al.*[1] studied how the receptor-binding strength of antibodies used in clinical treatments influences antibody effectiveness. **a**, Immune-cell function is commonly regulated through the interaction of stimulatory and inhibitory receptors and their binding partners (ligands) at the cellular interface between two immune cells. **b**, A type of antibody called a blocking antibody can bind and thereby prevent the interactions between an inhibitory receptor (such as PD-1) and its ligand (such as PD-L1). Antibodies used to target such proteins dampen inhibitory signalling, which helps the immune cells to target tumour cells. The antibodies might bind to Fcγ receptors (FcγR), which can influence their receptor-binding affinity. **c**, Agonist antibodies bind to and activate receptor targets. If these antibodies target a stimulatory receptor such as CD40, this boosts immune-cell targeting of tumours. **d**, Agonist antibodies that bind to inhibitory receptors, such as PD-1, can suppress immune cells in the context of autoimmune disease. The authors report that agonist antibodies of intermediate affinity in their receptor-binding capacity are more effective than are those of high or low affinity.

in vivo and tracked the generation of an anti-tumour immune response. Yu *et al.* noted a bell-shaped response curve, with an optimal dissociation constant around an intermediate value of 50 nM. A similar binding-affinity range of agonist antibody variants against CD137 and PD-1 gave results with a comparable bell-shaped response curve.

These data show that the optimal antibody binding affinity depends on its intended application. For blocking antibodies, a high affinity is most effective. However, for agonist antibodies, the results show that an intermediate affinity works best.

Why are intermediate-affinity antibodies good agonists? This question can be broken down into two parts. How does receptor activation by the antibodies compare with what happens with the receptor's usual (endogenous) ligand? And which elements of the mechanism of antibody action show a bell-shaped curve in terms of their dependence on antibody affinity?

A ligand can activate receptors through a variety of processes. These include driving conformational change of the receptor, enabling the small- or large-scale clustering of multiple receptors and slowing receptor diffusion across cell membranes. Moreover, these endogenous ligands might also induce receptor internalization into the cell, either for continued signalling in an organelle called the endosome or for receptor degradation. The ligand can act sequentially in such a manner on more than one receptor molecule.

Agonist antibodies don't necessarily bind to the same part of the receptor's surface as the endogenous ligand does. Antibodies have two binding regions on separate 'arms', and such a bivalent structure can trigger receptor clustering, in particular when binding to receptors such as CD40 and CD137. These receptors require three receptor proteins to form a defined complex for signalling. Antibodies bound to a receptor target might also be bound to Fcγ receptors (Fig. 1). This constrains antibody dissociation in the limited space between the two interacting cells, with an accompanying increase in the apparent binding affinity[4]. Given this multitude of options, the mechanisms of receptor activation are often difficult to determine. They might differ between agonist antibodies and the endogenous ligands.

In Yu and colleagues' findings, the intermediate-affinity antibodies were particularly effective at trapping targeted receptors at the interface between two immune cells, and could do so more effectively than the endogenous ligand and the high-affinity antibody variants. This might indicate that intermediate-affinity antibodies have an enhanced ability to cluster receptors compared with the endogenous ligand or high-affinity antibodies.

However, previous work[5] modelling receptor clustering indicated that increasing the ligand's binding affinity for the receptor exponentially enhances receptor clustering, and those results did not display a bell-shaped response curve. Yet when antibodies are used in excess over their antigen target, as was the case for Yu and colleagues' work, high-affinity antibodies might bind to the receptor using just one of their two binding regions (monovalently) and, thus, might be less capable of clustering receptors than lower-affinity variants are. This is because, for receptor clustering, one antibody needs to bind to two receptors at the same time for a short period of time and then repeat this process continually. With an excess of high-affinity antibodies, most receptors will be stably bound to a single antibody arm and there would be few free receptors available for binding to the second arm of a receptor-bound antibody.

The effects of the agonist antibodies on receptor diffusion, internalization or ability to sequentially engage multiple receptors might also contribute to the high effectiveness of intermediate-affinity antibodies. Further investigations of agonist antibodies promise to bring insights into the mechanisms of receptor activation.

The determination of an optimum affinity for agonist antibodies will drive therapeutic development. So far, clinical responses for cancer treatment using agonist antibodies that target members of the tumour-necrosis factor receptor (TNFR) family have been disappointing. Although multiple contributing factors might be involved in the case of TNFR, many of these approaches use high-affinity antibodies, raising the possibility of improving responses through affinity optimization.

The concept of an optimum intermediate affinity might also apply to antibodies and synthetic therapeutics containing antibody fragments that bind to a T cell and a tumour cell. In those bispecific reagents, one binding region of the antibody can activate a T cell by engaging a part of the T-cell receptor called the CD3 protein, with the other binding region engaging a tumour-associated antigen, usually with relatively high affinity.

A lower CD3-binding affinity might diminish T-cell production of inflammatory signalling molecules called cytokines, while T cells retain their capacity for tumour-cell-killing activity, thus reducing the toxicity of these treatments that is caused by excessive cytokine action[6,7]. Moreover, emerging data for bispecific antibodies that target the T-cell receptor CD28

provides an example of antibodies that use binding domains that have intermediate affinities, highlighting the potential effect of affinity for maximizing the responses generated with agonist antibodies[8].

Christoph Wülfing is at the School of Cellular and Molecular Medicine, University of Bristol, Bristol BS8 1TD, UK. **Simon J. Dovedi** is in Oncology R&D, AstraZeneca, Cambridge CB21 6GH, UK. e-mails: christoph.wuelfing@bristol.ac.uk; simon.dovedi@astrazeneca.com

1. Yu, X. *et al. Nature* **614**, 539–547 (2023).
2. Mayes, P. A., Hance, K. W. & Hoos, A. *Nature Rev. Drug Discov.* **17**, 509–527 (2018).
3. Valitutti, S. *Front. Immunol.* **3**, 272 (2012).
4. Zhu, D.-M., Dustin, M. L., Cairo, C. W. & Golan, D. E. *Biophys. J.* **92**, 1022–1034 (2007).
5. Nandi, S. K., Österle, D., Heidenreich, M., Levy, E. D. & Safran, S. A. *Phys. Rev. Lett.* **129**, 128102 (2022).
6. Faroudi, M. *et al. Proc. Natl Acad. Sci. USA* **100**, 14145–14150 (2003).
7. Malik-Chaudhry, H. K. *et al. mAbs* **13**, 1890411 (2021).
8. Wei, J. *et al. Sci. Transl. Med.* **14**, eabn1082 (2022).

S.J.D. declares competing interests. See go.nature.com/3jmwj4p for details.
This article was published online on 1 February 2023.

Genetics

Contribution of rare variants to complex traits

Luke M. Evans & Pamela N. Romero Villela

An analysis of rare genetic variants reveals that they influence human traits through similar biological pathways to common ones. The work deepens our understanding of how this type of variant affects complex traits. **See p.492**

Our understanding of the genetic mutations that affect complex human traits — such as height, smoking-related behaviour or the risk of diabetes — has been vastly broadened by genome-wide association studies (GWASs). But such research has focused largely on associations between traits of interest and variants that are common in the human population. Rare variants pose challenges to GWASs, because they can be studied using only large samples and in-depth genetic information, and can be more strongly confounded by non-genetic factors than can common variants, increasing the chance of spurious findings[1]. Until now, therefore, researchers have been unable to assess accurately whether rare variants contribute substantially to complex traits. On page 492, Weiner *et al.*[2] introduce a new approach to address this question. They find that, although rare variants account for a much smaller proportion of heritability than do common variants, they act through the same genes and biological pathways.

GWASs can reveal the 'single nucleotide polymorphism (SNP) heritability' of a given trait — that is, the portion of variability in the trait that is attributable to genetic variation (with many small effects from individual common variants acting cumulatively). For example, data indicate that the SNP heritability for human height is around 50–60% among people of European descent[3]. GWAS data can be further analysed by partitioning how different functional categories — such as enhancer elements that regulate gene expression, or genes that are specifically expressed in connective tissues — contribute to this heritability[4], and assessing to what extent genes influence multiple traits (a phenomenon called genetic correlation)[5]. Such analyses can tell us much about the biology underlying the disease or trait being studied, from finding relevant molecular pathways and cell types to revealing the role of natural selection in shaping the trait.

Rare variants are often assessed through burden tests, which give each individual a burden score on the basis of how many rare, protein-altering variants that person carries in a gene. The burden score is then related to the risk of developing a given disease[6]. Burden tests can reveal the degree to which rare variants in each gene of interest alter disease risk, but Weiner *et al.* took a different tack, developing a genome-wide burden test. This test uses a statistical approach called a regression-based framework to estimate the total proportion of trait variance attributable to rare variants — a value that the authors called burden heritability.

The authors used their framework, named burden heritability regression (BHR), to analyse the contribution of rare variants to 22 complex traits, including height, alcohol consumption and cholesterol levels. They analysed nearly 400,000 exomes (the protein-encoding regions of the genome) from people whose data had been deposited in a large biomedical repository called the UK Biobank. They found that, on average, the burden heritability was only 1.3% — significantly less than SNP heritability (at a median of 13%). Most of this heritability was due to ultra-rare variants (those with frequencies of less than 0.001%) that prevent a gene from functioning.

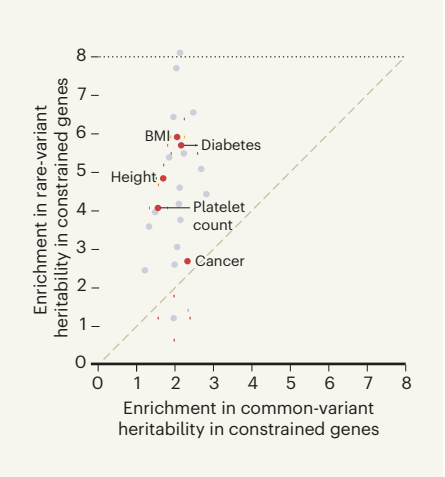

Figure 1 | How different types of genetic mutation contribute to complex traits. A combination of rare and common genetic variants act together to affect complex traits, such as height or risk of diabetes. Weiner *et al.*[2] developed a statistical analysis specifically to assess the total proportion of trait variance explained by rare protein-coding variants for 22 complex traits across the human genome. They found that, compared with common variants, rare variants associated with the 22 traits were more strongly enriched in evolutionarily constrained genes (in which mutations are more likely to prevent function than in less-constrained genes). Numbers indicate how much more of a trait's heritability is attributable to variants in constrained genes than expected on the basis of their frequency in the genome (nearly six times more for rare variants affecting body-mass index (BMI), for instance, and only about two times more for common variants affecting BMI). (Adapted from Fig. 4b of ref. 2.)

Next, Weiner and colleagues extended their analysis to partition the burden heritability into functional categories and to assess the genetic correlation between rare and common variants and between traits. They found that rare and common variation affect the same cell types and pathways. Furthermore, genetic correlations between traits are similar for both rare and common variants. However, the authors found that burden heritability is concentrated in fewer genes than is common-variant SNP heritability. Furthermore, these genes were more strongly evolutionarily constrained (less tolerant to disruption of function) than were those in which common variants cluster (Fig. 1). These findings suggest a fundamental difference between common and rare variation — the effects of common variants are spread throughout the genome, but rare variants that affect these traits are