antigens rather than one, there may be opportunities to identify antigen pairs that are uniquely expressed on tumor cells.

Dual-target recognition by CARs has been described[9,10] before the work of Roybal *et al.*[1]. But these previous studies used two attenuated, constitutively active CARs. This means that expression of the CARs is not controlled, and partial independent function of each CAR is possible. Moreover, two partially active CARs are unlikely to achieve optimal T-cell signaling.

In the system of Roybal *et al.*[1], T-cell signaling is allowed only by the second CAR, which is not expressed unless the first CAR is activated through ligand binding, creating an all-or-nothing signal (**Fig. 1**). The approach takes advantage of the peculiar mode of signal transduction of the Notch receptor. Upon ligand binding, the Notch receptor's intracellular domain is cleaved and translocates to the nucleus, where it regulates transcription. The authors replaced the Notch extracellular domain with a domain that binds a tumor-associated antigen and the intracellular domain with a tetracycline-controlled transcription transactivator that induces expression of the second CAR with a 4-1BBζ costimulatory domain (driven by the tetracycline response element promoter). The system ensures that T-cell activation occurs only in the simultaneous presence of both tumor-associated antigens.

In a human xenograft tumor model in immune-deficient mice, double-CAR-transduced T cells transferred a few days after cancer cell inoculation prevented cancer cell growth only if the cancer cell expressed both target antigens but not if it expressed only one of them. To assess on-target toxicity, the authors treated mice injected contralaterally with cancer cells expressing either both or only one target antigen (the latter serving as surrogate 'healthy tissue'). The double-CAR-expressing T cells prevented growth of the double, but not single, antigen-positive cancer cells in the same mouse, indicating improved safety.

Although the dual antigen–targeting approach proposed by Roybal *et al.*[1] is attractive, the study has several limitations. The transcriptional activators are derived from bacteria and yeast and are likely to be immunogenic in humans, which could lead to rejection of the therapeutic T cells. Moreover, the tumor-associated antigens that were targeted, CD19 and mesothelin, are unlikely to be co-expressed by cancer cells; experiments with antigen pairs that are truly cancer-specific would have been more informative. The authors suggest that one could combine CARs targeting a tissue-specific antigen and a tumor-associated antigen. But if healthy cells and cancer cells expressing the second antigen are in close proximity, this could lead to bystander elimination of healthy cells, particularly as the half-life of the second, T-cell signaling CAR is 8 hours. The immune-deficient xenograft mouse model is hardly predictive of the human scenario given that human T cells do not function normally in mice. Finally, it remains to be seen whether on-target toxicity is prevented when large established tumors are treated[8] and the second antigen is expressed on healthy tissue.

One way to broaden the repertoire of suitable antigens in the future would be to combine a CAR and a TCR. But as the experience with neoantigens shows, safety is greatest when targets are truly tumor-specific. Medical doctors often say that there is no efficacy without toxicity. Eventually, the field of adoptive T-cell therapy may prove them wrong.

COMPETING FINANCIAL INTERESTS
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Plant immunity switched from bacteria to virus

Artemis Giannakopoulou, Aleksandra Bialas, Sophien Kamoun & Vivianne G A A Vleeshouwers

A plant immune receptor is engineered to recognize viruses rather than bacteria.

Each year, staple crops around the world suffer massive losses in yield owing to the destructive effects of pathogens. Improving the disease resistance of crops by boosting their immunity has been a key objective of agricultural biotech ever since the discovery of plant immune receptors in the 1990s. Nucleotide-binding leucine-rich repeat (NLR) proteins, a family of intracellular immune receptors that recognize pathogen molecules, are promising targets for enhancing pathogen resistance. In a recent paper in *Science*, Kim *et al.*[1] describe a clever twist on this approach in which the host target protein for the pathogen effector is engineered rather than the NLR protein itself (**Fig. 1**).

Several strategies have emerged to improve disease resistance in crop plants (**Fig. 1**). The efficient transfer of *NLR* genes between plant species in the laboratory has suggested opportunities for engineering broad disease resistance in the field[2]. However, the limited specificity of naturally occurring NLR proteins might hinder the success of this approach. NLR variants that recognize a broader range of pathogen effectors have been generated through *in vitro* evolution and rational design (**Fig. 1b**)[3,4]. A related approach is to exploit additional domains found on some NLR proteins that function as effector baits (**Fig. 1c**)[5].

NLR proteins recognize pathogen effectors in two ways, either directly, by binding to them, or indirectly, by sensing host effectors that are modified by pathogens. Kim *et al.*[1] take advantage of this second mechanism by engineering the effector target in the host cell (**Fig. 1d**). In earlier work, they showed that proteolytic cleavage of an *Arabidopsis thaliana* host protein PBS1 by the *Pseudomonas syringae* protease effector AvrPphB activates the NLR protein RPS5 (ref. 6). They have also shown[7] that RPS5 can be bound and activated by a three amino-acid insertion at the cleavage site of PBS1 protein in the absence of any effector. These findings prompted Kim *et al.*[1] to substitute the AvrPphB cleavage site in PBS1 with a 'decoy' sequence that matches the cleavage sites of two different proteases. The PBS1 protein was engineered into two forms that can be cleaved by protease effectors of *Turnip*

Artemis Giannakopoulou, Aleksandra Bialas and Sophien Kamoun are at The Sainsbury Laboratory, Norwich Research Park, Norwich, UK, and Vivianne G.A.A. Vleeshouwers is at Wageningen UR Plant Breeding, Wageningen University and Research Centre, Wageningen, the Netherlands.
e-mail: sophien.kamoun@tsl.ac.uk or vivianne.vleeshouwers@wur.nl

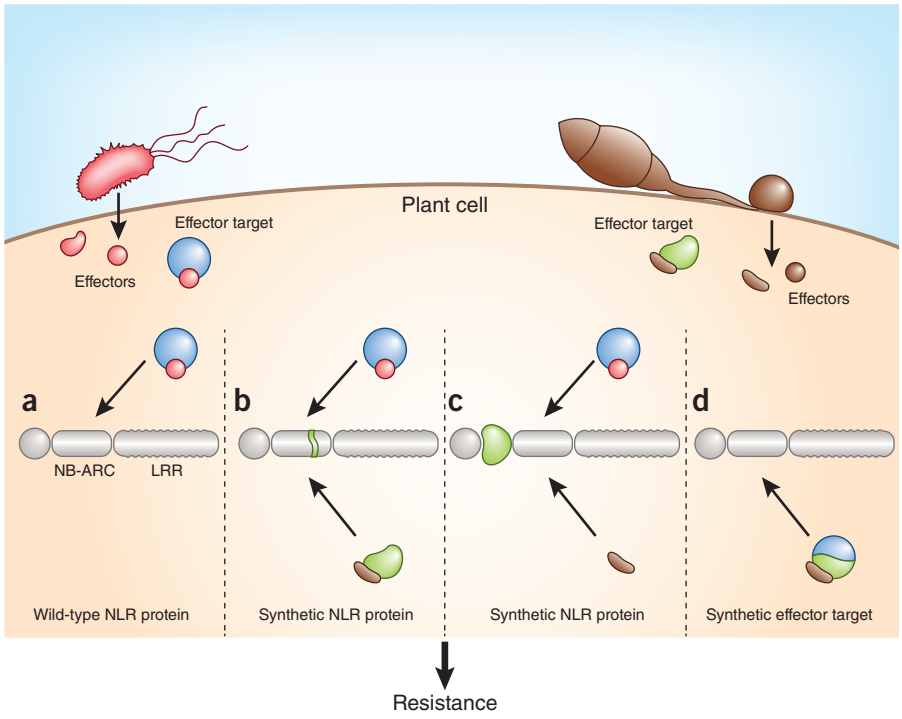

Figure 1 Engineering the plant immune system. Two phylogenetically unrelated pathogens secrete effectors into the plant cell cytoplasm. (**a**) The wild-type NLR protein perceives secreted effectors (red circle) from a pathogen with high specificity. Activation of the NLR protein triggers immune responses that result in plant resistance. Synthetic NLR proteins can be engineered to respond to effectors from different pathogens using two general approaches. (**b**,**c**) Single amino acid changes in the NLR protein (light green line) (**b**) or integration of an effector target domain (light green oval shape) in the NLR protein (**c**). (**d**) Modification of the effector target (dark and light green circle) results in a specificity switch to a different effector (brown shape). Red and brown shapes represent effectors secreted by pathogens. Light and dark green oval shapes represent effector targets in the host.

mosaic virus in addition to a *Pseudomonas syringae* protease effector. This resulted in activation of RPS5 and a specificity switch of RPS5/PBS1 to the different pathogens *in planta*.

The strategy proposed by Kim *et al.*[1] has some limitations. First, PBS1 functions inside plant cells, so it can be engineered to confer resistance only to pathogens that secrete a host-translocated protease, for example, barley powdery mildew fungus BEC1019 (ref. 8). However, most agronomically important pathogens, including oomycetes and rust fungi, are not known to secrete host-translocated proteases. Second, any mechanism of resistance must block pathogen colonization quickly and effectively. Although Kim *et al.*[1] switched RPS5/PBS1 specificity from *Pseudomonas syringae* to *Turnip mosaic virus*, systemic spread of the virus was not prevented, and infection with the virus resulted in a trailing necrosis phenotype[1]. Such a phenotype would preclude commercial applications of the system. Third, the RPS5/PBS1 proteins are present in the plasma membrane and therefore might have to be redirected to other cellular compartments to respond to effectors that have different subcellular localizations. Finally, plant pathogen effectors are typically functionally redundant and tend to be dispensable. Thus, the ability of a pathogen to overcome NLR-protein-mediated resistance is not necessarily dependent on the mechanism by which a particular effector is detected, but is rather a product of the exceptional capacity of these pathogens to carry nimble and rapidly evolving effector repertoires[9]. Owing to these large repertoires, multiple versions of engineered PBS1 might need to be combined to maximize the potential for durable resistance.

To date, plant biotechnologists have focused on engineering broad-specificity immune receptors, in order to keep pace with rapidly evolving plant pathogens. Engineered immune receptors have been developed[3,10] and have become important tools for the development of resistant crops. By manipulating the pathogen targets in the host, Kim *et al.*[1] have substantially expanded the options for engineering synthetic plant immunity. The importance of basic research into plant immunity for devising different approaches to subvert and expand plant immunity should not be underestimated.

COMPETING FINANCIAL INTERESTS
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