This result confirmed that the mechanism for uptake of fusion molecules into the hemocoel is operable in the absence of other viral proteins and with the addition of a foreign protein.

To produce an insecticidal construct, the authors chose a spider-derived, insect-specific peptide toxin (Hv1a) as the cargo to be delivered. The neurotoxin inhibits insect, but not mammalian, voltage-gated calcium channels[8]. A key factor dictating the choice of toxin was the knowledge that in its native form, it is not harmful to insects when ingested. It is toxic only when introduced into the hemocoel by experimental injection or naturally when the spider bites its prey. It follows that only insects with the determinants to transcytose the luteovirid CP-P–toxin fusion into the hemocoel would be affected, thereby limiting collateral damage to nontarget insects.

The authors performed aphid insecticide bioassays with *in vitro*–expressed CP-P–toxin fusion proteins fed to the insects. They documented significant mortality in four aphid species and dose-dependent mortality in the green peach aphid. The aphid species that were selected for the bioassay are agricultural pests that belong to two different tribes, Aphidini and Macrosphini. One might expect that only the two species (*Acyrthosiphon pisum* and *Myzus persicae*) that are natural vectors of the specific luteovirus used would be successfully targeted by an insecticide constructed from the coat protein of this virus. However, the CP-P–toxin fusions were just as orally efficacious at killing the non-vector aphids (*Rhopalosiphum padi* and *Aphis glycines*). This finding supports the hypothesis that the CP-P–toxin could serve as a broad-spectrum aphicide because the gut is not the major barrier for acquisition of luteovirids in general[5]. Furthermore, the authors provide data supporting the aphid-specific nature of the fusion protein. They demonstrate that larvae of the tobacco budworm (order Lepidoptera) are unaffected by ingestion of the fusion protein. More tests of the efficacy of the CP-P–toxin against other hemipteran are needed to confirm the specificity of the approach.

To assess the potential practical utility of their discovery, the authors generated transgenic *Arabidopsis* plants that constitutively express CP-P–toxin proteins. The fusion protein was modified to contain a secretory signal sequence that enhances loading of the protein into the phloem where aphids feed. Second-generation transgenic plants expressing the highest concentration of the CP-P–toxin fusion proteins were assayed for their ability to suppress aphid populations and feeding damage. These plants were compared with transgenic plants expressing one of three negative controls—CP-P fused to green fluorescent protein, CP-P fused to an inactive mutant toxin, and the toxin peptide (with phloem-loading signal sequence) alone. Remarkably, the growth of aphid populations was significantly impaired on the CP-P–toxin transgenics. In contrast, control plants supported aphid populations that increased tenfold by 17 days after exposure to aphids. The CP-P–toxin transgenic plants appeared healthy, and the aphids on them displayed signs of paralysis, consistent with the neurotoxicity of the fusion protein.

The work of Bonning *et al.*[1] has broad implications and opens the door to exciting new prospects. Many vectors of plant viruses are sap-sucking insects that acquire their viral cargo from the plant phloem. Whiteflies and leafhoppers, for instance, transmit viruses in the family *Geminiviridae* that circulate from the insect gut through its body. As an example, Cassava mosaic disease, caused by whitefly-transmitted geminiviruses, is a major food security threat for African subsistence farmers[9]. Like the luteovirus used in this study, geminiviruses encode a coat protein that mediates insect transmissibility. It should be possible to capitalize on the properties of coat proteins of these and other hemipteran-transmitted viruses using an approach similar to that of Bonning *et al.*[1]

Another exciting possibility is the development and use of viral attachment protein–toxin fusion proteins to control viruses that replicate in their insect vectors. For example, *Tomato spotted wilt virus* is one of the most important viruses globally and is transmitted by a tiny insect called thrips (order Thysanoptera). Control of thrips vectors pose many challenges for growers and few chemical controls are effective or sustainable. One protein of *Tomato spotted wilt virus*, G_N, has been shown to bind directly to thrips midguts and inhibit virus acquisition[10]. This viral surface protein provides a good candidate for delivering toxins in an analogous manner to that presented by Bonning *et al.*[1].

The strategy of Bonning *et al.*[1] adds to the toolbox of emerging green biotech approaches. The approach can likely be adapted for use against other insects and with additional toxins that have thus far not been amenable for use in pest control. Importantly, use of these tools could ultimately reduce our reliance on applications of insecticidal sprays over the landscape, and their unique mode of action may minimize the development of insecticide resistance. It will be interesting to follow the application of this technology and its impact on viruses transmitted by aphids. For example, genetic resistance to insect vectors in field crops generally results in reduced secondary spread of viruses transmitted by that target insect. As such, the benefits of using aphicidal transgenics could have far-reaching effects on the ecology of aphids and the viruses they transmit.

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Collateral damage

Robert E W Hancock

Wiser use of antibiotics could help combat the emergence of drug-resistant pathogens.

The efficacy of antibiotics—the most successful medicines in human history—is steadily declining as bacterial strains that are resistant to one or more drugs continue to proliferate. What's more, relatively few novel antibiotics or strategies are under development or entering the clinic[1,2]. In a recent issue of *Science Translational Medicine*, Imamovic *et al.*[3] outline an approach that would allow currently available antibiotics to be used more effectively, thereby slowing the spread of antibiotic resistance.

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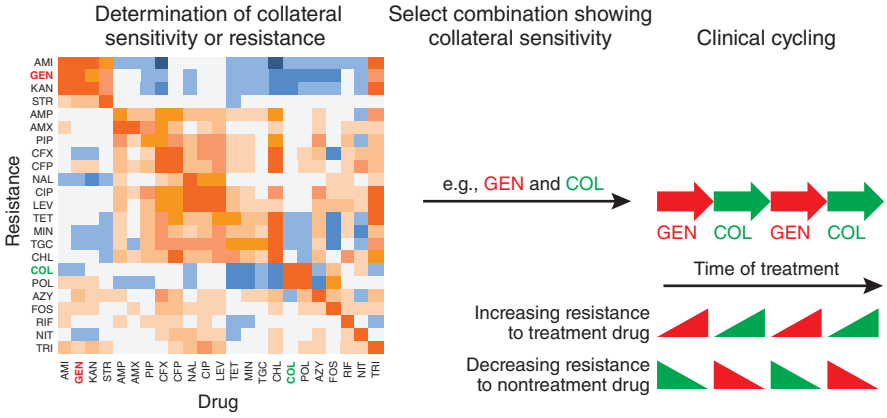

Figure 1 Applying the principle of collateral sensitivity to cycling of antibiotics in the clinic. Collateral sensitivity profiles are determined by first treating bacteria with one antibiotic to select resistant variants (indicated by 'Resistance' on the *y* axis of the heatmap on the left; heatmap is adapted from ref. 3), and then examining the impact of this treatment on susceptibility to each of the other antibiotics (indicated by 'Drug' on the *x* axis of the heatmap). Collateral sensitivity occurs when variants that become resistant to one antibiotic demonstrate increased susceptibility to another (shown as blue in the matrix; collateral resistance is shown as orange). Antibiotics showing reciprocal collateral sensitivity (e.g., gentamicin, GEN, and colistin, COL, in this example) are selected based on this matrix. These antibiotics are then used in an alternating fashion (cycling) in the clinic such that if one antibiotic selects resistant variants these variants will be more susceptible to the second drug, thus eliminating resistance and allowing for the first antibiotic to be reused in the same patient. An alternative possibility is that the drugs can be used together as combinations.

Antibiotics have made a tremendous contribution to life expectancy. Without these drugs, it would not be possible to treat cancer and transplantation patients with immunosuppressive therapies, perform invasive surgical procedures, deliver pre-term births, or protect children and adults from life-threatening infections. One need look no further than under-resourced countries, where infections are the second leading cause of death and are responsible for up to 60% of all childhood deaths (http://www.who.int/whr/2003/chapter1/en/index2.html), to appreciate the benefits of antibiotics.

The risks to public health posed by increasing antibiotic resistance can hardly be overstated. UK Chief Medical Officer Dame Sally Davies has warned of the "catastrophic" potential of resistance combined with the drug discovery void, saying, "This is a growing problem, and if we don't get it right, we wilzl find ourselves in a health system not dissimilar from the early 19th century" (*The Independent*, March 11, 2013). And Janet Woodcock, director of the Center for Drug Evaluation and Research at the US Food and Drug Administration, has said, "We are facing a huge crisis worldwide not having an antibiotics pipeline. It is bad now, and the infectious disease docs are frantic. But what is worse is the thought of where we will be five to ten years from now" (*The New York Times*, June 2, 2013). Confronting this challenge will require a concerted effort to improve both antibiotic drug discovery and treatment regimens.

A major shortcoming of antibiotic discovery programs is that they do not take into account the mechanistic and ecological complexity of antibiotic resistance. In modern niches and situations created by humans—such as healthcare facilities, immunocompromised populations, food chains and global travel—exposure to antibiotics is changing microbial ecology by selecting super-fit clades of bacteria. Resistance can arise in one of three ways: through mutations, through acquisition from other organisms of genetic elements (plasmids, transposons and integrons) and through adaptive resistance wherein the environment to which the organism is exposed (host milieu, subinhibitory antibiotics, growth state such as biofilms) induces altered expression of genes including resistance genes[4]. It is increasingly appreciated that in addition to breakthrough resistance, which leads to insusceptibility to antibiotics used in the clinic, numerous minor mutations can accumulate over time leading to gradual changes in susceptibility (a phenomenon termed creeping baselines); there appears to be a rich panoply of different mutations involved in this process[4]. Understanding this mechanistic and ecological complexity is essential for efficient development of fundamentally new antibiotics. In addition to new methods for screening antibiotic candidates, nontraditional approaches focused on antiresistance and antivirulence strategies, immunomodulation and microbiome manipulation[5–7] are showing promise. But progress will be slow, and the resistance problem is immediate.

The strategy presented by Imamovic *et al.*[3] might be more effective in the shorter term. Specifically, they propose promoting antimicrobial stewardship[8] by exploiting 'collateral sensitivity'. Antimicrobial stewardship aims to minimize inappropriate, ineffectual or excessive use of antibiotics by optimizing the selection, dosing, route and duration of administration. Collateral sensitivity describes a phenomenon, first discovered in the cancer field[9], wherein cells that become resistant to one therapeutic agent also become more susceptible to another agent. Collateral sensitivity could be useful especially in scenarios where doctors cycle through various antibiotics, or use combinations of two or more agents in an attempt to treat a persistent infection in a patient. In the cycling scenario, each antibiotic that succeeds the first treatment would be chosen on the basis of its enhanced efficacy against the resistant organisms that might develop during treatment with the previous antibiotic (**Fig. 1**). In the scenario of combinatorial treatment, combinations of antibiotics would be chosen from those that cause reciprocal collateral sensitivity.

Imamovic *et al.*[3] first treated a laboratory strain of the *Escherichia coli* bacterium with each of 23 antibiotics used in the clinic and found dozens of cases in which bacteria that acquired resistance to one antibiotic became more susceptible to other antibiotics. This phenomenon was especially prevalent among antibiotics that act through different modes of action or were from different chemical classes. They used these observations to map out a collateral sensitivity network (**Fig. 1**), which allowed them to select which antibiotics might be used in which sequence to optimize collateral sensitivity and minimize development of resistance. As predicted by the network, cycling between the aminoglycoside gentamicin (Garamycin, Gentak) and the cephalosporin β-lactam cefuroxime (Ceftin, Zinacef) effectively eradicated antibiotic-resistant *E. coli in vitro*. Finally, the authors verified that this approach also reveals collateral sensitivity patterns for *E. coli* clinical isolates.

Although conceptually exciting, this approach is not without its challenges and limitations. For example, the drug combinations that prevented resistance development in the laboratory *E. coli* strain did not always produce collateral sensitivity when applied to the two *E. coli* clinical isolates. More generally, what works for *E. coli* might not work for other species of pathogens. Complicating matters further, it is increasingly recognized that infections often involve many bacterial species or variants with atypical resistance profiles. It would take a strong international collaborative effort to map collateral sensitivity networks in

multiple individual isolates of a broad range of bacterial species, because the effort involved would be substantial. And a source of funding for such an effort is not immediately obvious. In addition, the mechanistic basis for collateral sensitivity remains unclear. Considerable efforts to provide a firm mechanistic basis for this approach are warranted, as a detailed mechanistic understanding might enable some degree of prediction to supplement laboratory investigations. There will also likely be practical problems when a physician encounters an acute infection requiring immediate treatment before resistance profiles or the identity of the pathogen can be determined. In these cases there may not be time to consider collateral sensitivity cycling, although appropriate combinations are still relevant. Nevertheless, if the studies of Imamovic *et al.*[3] are extended to a much greater range of strains and species of bacterial pathogens, the principle of collateral sensitivity offers a potentially valuable tool for limiting resistance development until such time as new antibiotic therapeutic strategies are developed.

That said, informed cycling of existing antibiotics should not be seen as a permanent fix for the growing problem of antibiotic resistance. Fundamentally new therapeutics will be needed. Achieving this will require much greater commitments from governments, granting agencies and pharmaceutical companies.

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Naiveté of the human pluripotent stem cell

Victoria L Mascetti & Roger A Pedersen

Human pluripotent stem cells can be stabilized in a state resembling the inner cell mass of the blastocyst.

Pluripotency—the ability of a cell to differentiate into any cell type in an organism—remains enigmatic. A central question in the field concerns the striking difference between pluripotent cells derived from mice and humans. Whereas mouse pluripotent stem cells come in two flavors—a 'naive' state corresponding to the inner cell mass (ICM) of the blastocyst and a 'primed' state corresponding to the later epithelial epiblast—until recently stable (transgene-independent) human pluripotent stem cells have all come in the 'primed' flavor. In an intriguing recent report in *Nature*, Gafni *et al.*[1] describe the derivation of stable human pluripotent stem cells of the 'naive' flavor. The novel cells promise otherwise inaccessible benefits in regenerative medicine. Nevertheless, further studies of early human embryogenesis are needed as a benchmark for validating these compelling findings.

Stem cell researchers have puzzled over the origin of the conspicuous differences exhibited by human and mouse pluripotent stem cells. Despite their parallel derivation, whether as embryonic stem cells from the ICM or as induced pluripotent stem cells reprogrammed from differentiated somatic cells, pluripotent stem cells from the two species differ in colony shape, ease of single-cell cloning, growth factor requirements and ability to integrate into the ICM of a mouse blastocyst (**Table 1**). Attempts to reconcile these differences led to the discovery by our laboratory and others of mouse epiblast stem cells, which share properties with human pluripotent stem cells, including flat colony shape and a requirement for Activin and FGF[2,3]. This emphasized that the nature of pluripotency in the embryo changes during development and revealed that pluripotent stem cells can capture the embryo's properties as distinct pluripotent states—the ICM-like state and the epithelial epiblast–like state (**Fig. 1**).

Until now, however, it has remained unclear whether the human genetic background would support stable *in vitro* derivation of ICM-like pluripotent stem cells. Notably, human embryonic stem cells derived under standard conditions acquire an epithelial epiblast–like state[4]. Moreover, previous efforts to achieve human ICM-like pluripotency *in vitro* have relied on expression of transgenes, which could alter the cells' subsequent differentiation or restrict their clinical utility[5].

How were Gafni *et al.*[1] able to achieve the unprecedented? Using a doxycycline-inducible human induced pluripotent stem cell line targeted with the pluripotency-associated reporter OCT4-GFP, they screened for combinations of exogenous factors that could indefinitely stabilize human pluripotent stem cells. These studies were carried out on a basal background known to maintain mouse embryonic stem cells, namely two small-molecule inhibitors (the ERK1/2 inhibitor PD0325901 and the GSK3β inhibitor CHIR99021, together known as '2i') along with the polypeptide growth factor leukemia inhibitory factor (LIF). This led to the identification of an additional two polypeptide growth factors and four small-molecule inhibitors, which, in combination with 2i/LIF, were dubbed naive human stem cell medium (NHSM)[1]. Using the NHSM conditions, the authors generated karyotypically normal 'ICM-like' human pluripotent stem cells by three routes: conversion of conventional embryonic stem cells, reprogramming of fibroblasts to induced pluripotent stem cells and derivation from blastocysts. The resulting ICM-like human pluripotent stem cells had standard features of mouse embryonic stem cells and seemingly fulfilled the definition of naive pluripotent stem cells developed in mouse studies, with the exception of germline chimerism!

The mouse has long been employed as a model system to make assumptions about human development when there is no human equivalent due to the ethical and practical limitations associated with human embryo research. However, using mouse embryonic stem cells as the gold standard for deriving and evaluating ICM-like human pluripotent stem cells may be shoehorning the latter into a mouse-shaped box—this could constrain and misinform studies about human pluripotency. Just as *Drosophila* is not fully representative of

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