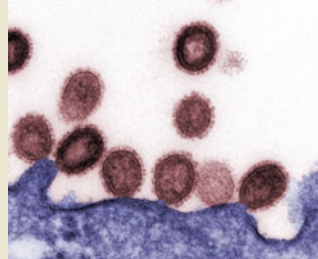


Anatomy of a pandemic flu threat

The emergence of highly pathogenic H5N1 avian influenza virus strains has raised concerns that such strains might adapt to bind receptors in the human respiratory tract, thereby facilitating human-to-human transmission.



C. Goldsmith/CDC

The observation that hemagglutinins of most avian flu viruses bind to α 2-3 sialylated glycans on the host cell surface, whereas human influenza viruses preferentially bind sialic acids with α 2-6 linkages, has suggested that H5N1 strains would need to acquire specificity for α 2-6 receptors to become established in the human population. Now, Sasisekharan and colleagues redefine the criteria for humanization of influenza viruses, including H5N1, by showing that the conformation of the flexible glycan chains—and not the type of linkage *per se*—is the key determinant of viral tropism. Lectin staining of human respiratory tissues, mass spectrometry, and analysis of glycan array and existing structure data point to the conclusion that human-adapted viruses bind long α 2-6 sialylated glycans with the topology of an open umbrella. In contrast, glycans bound by avian viruses assume a cone-like conformation conferred by α 2-3 linkages or short α 2-6 sialylated oligosaccharides. This insight into the criteria that must be met for influenza viruses to cross the species barrier has implications for developing new vaccines and therapies, as well as for the surveillance and diagnosis of seasonal and potentially pandemic influenza strains. [Letters, p. 107; News and Views, p. 60]

PH

iPS cells without oncogenes

In several recent papers, Yamanaka and coworkers have pioneered a simple method for reprogramming fibroblasts into cells that resemble embryonic stem cells. Fibroblasts are transduced with viral vectors expressing four pluripotency-associated genes (*Oct3/4*, *Sox2*, *Klf4* and *c-Myc*), and the rare reprogrammed cells are isolated by drug selection. Thus far, however, the four genes have included the oncogene *c-Myc*, a risk factor for tumor formation that precludes applications of iPS cells in the clinic. Working with adult mouse and human fibroblasts, the Yamanaka group now demonstrates a reprogramming method that dispenses with *c-Myc*. Compared with the initial protocol, the critical difference is simply to extend the time between viral transduction of the reprogramming genes and the initiation of drug selection. To assess the consequences of excluding *c-Myc* on tumorigenesis, the

Written by Kathy Aschheim, Laura DeFrancesco, Michael Francisco, Peter Hare & Jan-Willem Theunissen

authors study chimeric mice made by injecting iPS cells into mouse blastocysts. At the last 100-day time point, none of the 26 animals generated from *c-Myc*⁻ iPS cells has died of tumours, whereas 6 of 37 animals derived from *c-Myc*⁺ iPS cells have, indicating that lack of *c-Myc* reduces tumor incidence. The authors intend to examine tumor formation at later time points and using other assays. [Letters, p. 101; News and Views, p. 59]

KA

Assessing GM crop allergenicity

Introducing a new food into the market brings the risk of introducing a new allergen. Mindful of that, several groups have attempted to devise schemes to identify this risk before marketing, when genes or traits are inserted into food crops. Goodman and colleagues present a summary of the current guidelines for determining the allergenicity of new crop varieties. They discuss several tests, some that have been validated and others that have not. Case studies reveal that on those occasions when authorities have requested unvalidated tests, the results have been of little value, while at the same time the tests have consumed resources and created a climate of uncertainty. [Perspective, p. 73]

LD

Quantitative profiling of kinase inhibitors

Development of novel kinase inhibitors requires insight into kinase specificity. Zarrinkar and colleagues have analyzed the activity of 38 kinase inhibitors against a panel of 287 distinct kinases, representing more than half of the predicted human kinome (and including three lipid kinases and 27 disease-relevant mutant variants). They derive quantitative dissociation constants with their previously developed *in vitro* competition binding assay in which the binding of each small molecule



is measured against the ATP-binding sites of all 317 kinases. Twenty-one tyrosine kinase inhibitors, 15 serine-threonine kinase inhibitors and staurosporine are assayed, and 3,100 binding interactions with $K_d < \sim 1-3 \mu\text{M}$ are identified. Aside from depicting these small molecule-kinase interactions graphically, the authors also introduce several selectivity scores to quantify and differentiate the binding data. For example, when the authors calculate high-affinity selectivity scores for each compound by dividing the number of kinases bound with a $K_d < 100 \text{ nM}$ by the total number of distinct kinases, the data reveal that most inhibitors bind only a small number of targets with high affinity. The authors indicate that surveying the activity of large small-molecule libraries against a large number of kinases with standardized assay platforms should facilitate targeted and high-throughput discovery and development of novel inhibitors and combination regimens. [Resource, p. 127]

JWT

Peptide homes antisense to tumors

Non-steroid hormone receptor transcription factors are considered undruggable using small-molecule DNA-binding inhibitors, and antisense oligonucleotides are not sufficiently efficacious to have a therapeutic effect. To overcome the poor pharmacokinetic properties of antisense molecules, Benezra and colleagues covalently couple an antisense oligonucleotide against the transcriptional regulator of angiogenesis Id1 to a peptide—fragment F3 of the high mobility group protein (HMG)N2—that homes specifically to tumor endothelial cells. As Id1 is downstream of multiple pro-angiogenic factors, such as vascular endothelial growth factor and basic fibroblast growth factor, inhibition of Id1 might not be susceptible to the emergence of resistance associated with an anti-angiogenic drug against a single growth factor. After demonstrating that this peptide-conjugated antisense oligonucleotide (Id1-PCAO) is specifically internalized by endothelial cells and downregulates Id1 in tissue culture cells, the authors show that systemically administered Id1-PCAO homes to the tumor endothelium. In addition, continuous delivery of Id1-PCAO with osmotic pumps in two different tumor models triggers enhanced hemorrhage and hypoxia and inhibited tumor growth. The antitumor effect in one of the tumor models is substantially enhanced by adding the Hsp90 inhibitor 17-(allylamino)-17-demethoxygeldamycin to the treatment regimen. Future experiments are required to determine whether other peptides with *in vivo* homing properties can be used to render antisense oligonucleotides therapeutically active. [Articles, p. 91] *JWT*

Nanoparticles boost Raman imaging

Colloidal gold nanoparticles can amplify Raman scattering efficiencies by as much as 14 to 15 orders of magnitude. Nie and colleagues show that this surface-enhanced Raman scattering (SERS) property of colloidal gold can be used effectively for *in vivo* molecular imaging. The thiol-polyethylene glycol (PEG)-coated gold nanoparticles the authors synthesize are extremely stable, nontoxic and substantially brighter than near-infrared-emitting quantum dots and other toxic particles. Usually, nanoparticles accumulate preferentially in xenografts through an enhanced permeability and retention effect. When the authors coat the PEG layer with a single-chain Fv antibody fragment against epidermal growth factor receptor (EGFR) using a heterofunctional PEG, the targeted gold particles not only accumulate in EGFR-positive xenograft tumors one order of magnitude more than nontargeted particles, but also detected small xenograft tumors in mice at a penetration depth of 1–2 cm. The biodistribution experiments also indicate that the particles accumulate in the spleen and liver, but no toxicity is observed 2–3 months after particle injection. Future experiments are required to look at short-term toxicity and determine whether these relatively large nanoparticles—with a diameter of 80 nm—can be used for imaging of other tissues. [Articles, p. 83] *JWT*

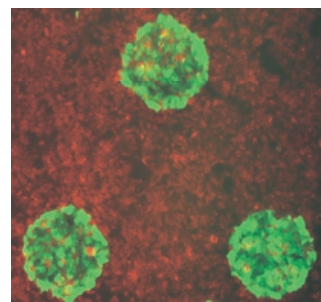
Patent roundup

'Reach-through' claims to drug targets have been the subject of considerable attention and controversy. Robert Bohrer argues that interpreting patent law in a way that makes reach-through claims difficult to obtain may not be in the best interests of public health. [Patent Article, p. 55] *MF*

Recent patent applications in antibodies. [New Patents, p. 57] *MF*

Liver cell microarrays

Drug toxicity often manifests itself in the liver, yet assays for testing the effects of candidate drugs on human liver function leave much to be desired. Human liver cells and slices are viable in culture for only a short time, and immortalized hepatocytes or hepatocarcinoma cell lines show abnormal phenotypes. Bhatia and Khetani describe a microarray-based coculture system that allows viable human hepatocytes to be maintained *in vitro* for several weeks. Micron-scale collagen islands are patterned on a polystyrene surface through the holes of an elastomeric stencil, the stencil is removed, hepatocytes are adhered selectively to the collagen and mouse fibroblasts are placed around the hepatocytes. The authors demonstrate maintenance of human hepatocyte function by analysis of gene expression profiles, phase I/II metabolism functions, canalicular transport, secreted products and the effects of hepatotoxins. [Letters, p. 120] *KA*



Humanized mouse for testing antithrombotics

Current platelet therapies can trigger bleeding complications. To expedite discovery and development of novel antithrombotics, Diacovo and colleagues have developed a mouse model of arterial thrombosis in which human platelets can form blood clots. The authors show that mouse von Willebrand factor (VWF), a large plasma glycoprotein that initiates clot formation by interacting with the platelet receptor glycoprotein Ib α (GPIb α), cannot support human platelet adhesion. After analyzing murine-murine and murine-human VWF-GPIb α crystal structure-based models, they show that a single amino acid change (P1326H) in the murine A1 domain of VWF (VWF-A1) is sufficient to support human platelet adhesion at levels comparable to human VWF-A1. To test the *in vivo* effects of the R1326H mutation and generate an *in vivo* model, the authors modify mice to express the VWF^{R1326F} mutation. Whereas endogenous murine platelets do not support platelet formation in VWF^{R1326F} mice, infused human platelets in these mutant mice interact with the humanized VWF-A1 and restore bleeding times to levels observed in the wild-type controls. The ability of both clopidogrel (Plavix; an inhibitor of platelet activation) and an antibody fragment against human integrin α IIB (the platelet integrin that ultimately supports thrombus growth) to substantially reduce human thrombus formation in VWF^{R1326F} mice suggests that this system could have utility for preclinical testing of novel human platelet therapies. [Letters, p. 114; News and Views, p. 62] *JWT*

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