

Synopsis of Research Articles

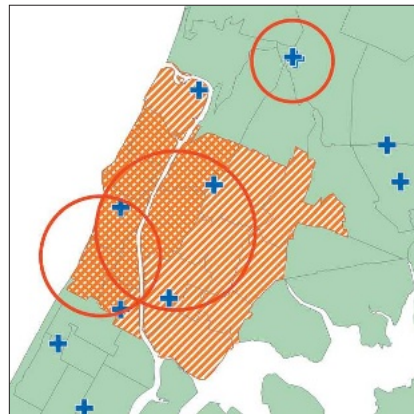
Early Detection of Disease Outbreaks

DOI: 10.1371/journal.pmed.0020065

For disease outbreak detection, the public-health community has historically relied on the watchful eyes of doctors and other health-care workers, who have reported individual cases or clusters of cases of particular diseases to health-care and other authorities. The increased availability of electronic health-care data, however, raises the possibility of more automated and earlier outbreak detection and subsequent intervention. Besides diagnoses of known diseases, pre-diagnostic syndromic indicators—such as the primary complaints of patients coming to the emergency room or calling a nurse hotline—are being collected in electronic formats and could be analyzed if suitable methods existed. Martin Kulldorff and colleagues have been developing such methods, and now report a new and very flexible approach for prospective infectious disease outbreak surveillance.

Their method, which they call the “space–time permutation scan statistic,” is an extension of a method called scan statistic. All previously developed scan statistics require either (i) a uniform population at risk (with the same number of expected disease cases in every square kilometer), (ii) a control group (such as emergency visits not due to the disease of interest), or (iii) other data that provide information about the geographical and temporal distribution of the underlying population at risk, such as census numbers. The new method, because of a different probability model, can be used for the early detection of

disease outbreaks when only the number of cases is available. It also corrects for missing data and makes minimal assumptions about the spatiotemporal characteristics of an outbreak. To make it



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Disease surveillance in New York City

widely accessible, the method has been implemented as a feature of the freely available SaTScan software.

In their article, Kulldorff and colleagues illustrate the utility of the new method by applying it to data collected from hospital emergency departments in New York City. The researchers analyzed diarrhea records from 2002, and did both a “residential analysis” (based on the home address of the patients) and a “hospital analysis” (based on hospital locations). The former has more detailed geographical information, the latter may be better able to detect outbreaks

not primarily related to place of residence but, for example, school or workplace. They found four highly unusual clusters of diarrhea cases, three of which heralded citywide gastrointestinal outbreaks due to rotavirus and norovirus.

Since November 2003, the space–time permutation scan statistic has been used daily to analyze emergency department data in New York City in parallel with other methods, and it seems to perform well. As the authors discuss, as any other surveillance method, theirs has limitations. Because it adjusts for purely temporal clusters, the method can only detect outbreaks if they start locally (not simultaneously across the entire surveillance area). The less geographically compact an outbreak is, the less power there is to detect it. And some outbreaks, for example, those caused by exposure to an infectious agent in the subway, will be hard to cluster by place of residence or choice of emergency department.

In the present study, Kulldorff and colleagues have applied their method to infectious disease surveillance in a metropolitan area in the United States. As they state, however, “the ability to perform disease surveillance without population-at-risk data is especially important in developing countries, where these data may be hard to obtain.”

Kulldorff M, Heffernan R, Hartman J, Assunção R, Mostashari F (2005) A space–time permutation scan statistic for disease outbreak detection. DOI: 10.1371/journal.pmed.0020059

How Tumor Cells Acquire Resistance to Kinase Inhibitors

DOI: 10.1371/journal.pmed.0020074

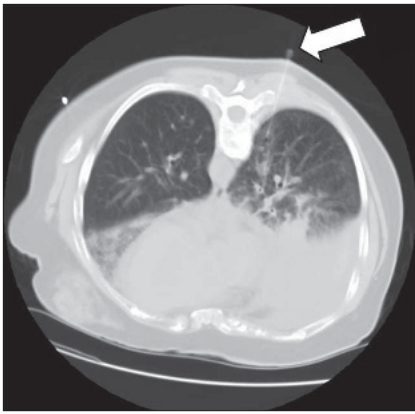
Acquired resistance to chemotherapy is a major obstacle to successful cancer treatment. Understanding the mechanisms by which tumors become resistant to a particular agent is key to identifying new drugs or combination regimens.

Kinases are signaling molecules that control many aspects of cell behavior, including cell proliferation, i.e., whether and how fast cells divide. Abnormally active kinases promoting tumor growth

are found in many cancers and are a focus of rational cancer drug design. One target for kinase inhibitors is the epidermal growth factor receptor (EGFR). Two EGFR inhibitors, gefitinib and erlotinib, showed therapeutic benefits in a subset of patients with non-small cell lung cancer. Recent work has helped us understand why some patients respond and some don't: responsive tumors usually harbor activating mutations in the EGFR gene, which somehow make the

tumors sensitive to treatment. Nearly all patients whose tumors initially respond to EGFR inhibitors, however, eventually become resistant to the drugs and progress despite continued therapy.

William Pao and colleagues examined tumors from six patients with non-small cell lung cancer who initially responded to gefitinib or erlotinib but subsequently relapsed. Tumors from all six patients carried activating mutations in the EGFR gene. In addition, in three out of the six



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Re-biopsy of progressing lung lesion

cases, the resistant tumor cells carried an identical second mutation in the EGFR gene. Whereas the activating mutation was present in tumor cells before

treatment with erlotinib or gefitinib, the second mutation was not found in pre-treatment biopsies from these patients, nor in over 150 lung cancer samples from patients who had not been treated with either drug. Additional cell culture studies supported the notion that the secondary mutation causes resistance to gefitinib or erlotinib. It is clear, though, that this is only one mechanism of resistance, because in the three other cases resistance occurred in the absence of the second mutation. What caused the resistance in those tumors is not known.

All kinases share some common features, and a resistance mutation very similar to the one identified here has also been found in other kinase genes from tumors with acquired resistance to imatinib, another kinase inhibitor. As Gary Gilliland and colleagues point

out in an accompanying Perspective (DOI: 10.1371/journal.pmed.0020075), the initial identification three years ago of resistance mutations against imatinib led to the rapid development of alternative kinase inhibitors that work even against tumors with the resistance mutation. Similarly, the results by Pao and colleagues should help researchers develop second generation drugs for lung cancer.

Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, et al. (2005) Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. DOI: 10.1371/journal.pmed.0020073

Cats and Allergies

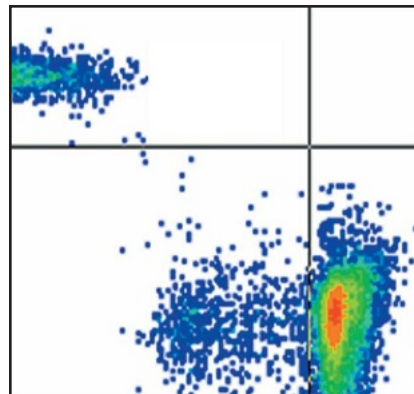
DOI: 10.1371/journal.pmed.0020094

The prevalence of asthma and allergy has risen in all industrialized countries during recent decades, and there is much debate about exposure to pets in early life and later development of asthma and allergy. Some studies have suggested that keeping pets actually protects against later allergy—i.e., that early exposure may somehow modify an individual's immune system to tolerate specific antigens. What might be the mechanism for such protection against allergy? One theory of how allergies arise is that an imbalance in T helper cell subtypes tips the body's immune response towards overreacting to a particular antigen. There is some evidence that early exposure to high natural levels of cat allergens can prevent such an inappropriate immune response. Other researchers have suggested that normally immune responses are kept under control by another group of T cells—regulatory T cells. The two mechanisms may be linked, since exposure to high levels of cat allergens may induce regulatory T cells.

Various attempts to modify aberrant immune responses to specific allergens, such as those to cat dander, have been made. Investigators have treated patients with related molecules, either peptides derived from the allergen itself, or much smaller peptides produced synthetically. Although therapy with peptides seems to reduce allergic responses, the mechanism

of the response to treatment has not been clear, in particular, exactly which cells, cell surface markers, and cytokines are involved in modifying the immune response.

In a paper in this month's *PLoS Medicine*, Mark Larché and colleagues



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Identifying the T cells that suppress proliferation to allergens after immunotherapy

have attempted to dissect out this pathway in a group of individuals with asthma and allergy to cats. They treated the individuals with short synthetic peptides derived from the sequence of the major cat allergen, *Felis domesticus* allergen 1, and then measured the

clinical and immunological response to allergen. They found that treatment with the peptides led to the induction of a population of T cells that were capable of suppressing the proliferation of allergen-reactive T cells in vitro. Peptide treatment also resulted in increased levels of a molecule called CD5 on the surface of blood T cells—CD5 has recently been associated with suppressing T cell sensitivity to stimulation. Finally, the authors found that the degree of suppression was not related to the amount of peptide given to the patients.

Where does this finding leave patients who might wonder about exposure to cats and the development of allergy? The simple answer is that we do not know exactly how exposure to antigen triggers either an immune reaction or tolerance. Once triggered, an immune reaction to a cat may be hard—though not impossible—to reverse, but how or why a specific individual becomes sensitized is as yet far from clear.

Verhoef A, Alexander C, Kay AB, Larché M (2005) T cell epitope immunotherapy induces a CD4⁺ T cell Population with regulatory activity. DOI: 10.1371/journal.pmed.0020078

Uric Acid and the Heart

DOI: 10.1371/journal.pmed.0020090

What is the level of medical evidence that should be used to inform medical practice? At the bottom of the hierarchy of evidence are anecdotes, expert opinion, case reports, and case series, and at the top is the systematic review of published (and sometimes unpublished) evidence. By necessity, systematic reviews come many years after hypotheses are first raised, and in the interim recommendations for practice may sway back and forth. One example of this is the debate over the role of uric acid in heart disease, which has been going on for more than 50 years. It started with a paper published in 1951 in the *Annals of Internal Medicine* that found higher serum uric acid concentrations in patients with coronary heart disease (CHD) compared with controls. Since then, measurement of serum uric acid has been suggested as a predictor of CHD. But many of the studies on serum uric acid are epidemiologic studies—somewhere in the middle of the hierarchy of evidence—and have come to different conclusions about how useful measurement of uric acid is.

Dissecting out the role of uric acid is further complicated by three

things: high levels of uric acid are associated with hypertension and being overweight (other risk factors for CHD); levels of uric acid can be altered by drugs such as diuretics that people with CHD often take; and finally, alteration of renal function can affect uric acid levels. Another problem is the type of studies that have been used to address the question of uric acid's role in CHD. Retrospective studies may be unable to control adequately for other risk factors—hence prospective, ideally population-based, studies would be the best to answer the question of whether there really is an association between high uric acid and CHD.

In this month's *PLoS Medicine*, John Danesh and colleagues from the University of Cambridge, along with investigators from the Icelandic Heart Association, report the single largest prospective study addressing the role of uric acid in heart disease. Further, their systematic review combines their findings with those of 15 previously published prospective studies of serum uric acid—9,458 cases of CHD and 155,084 controls in all..

The paper answers the question of the role of uric acid in prediction of CHD clearly: the risk ratio for prediction of disease was 1.13 (1.07–1.20), but it was only 1.02 (0.91–1.14) in the eight studies that had the most complete adjustment for possible confounders. What this paper does not do is directly address the question of whether or not serum uric acid is involved in causing CHD through intermediates; however, it does suggest that serum uric acid levels are unlikely to be a major determinant of CHD.

Where does such a result leave patients? Well, it is likely that improving diet, losing weight, and controlling blood pressure may all contribute to reducing both one's risk of CHD and one's serum levels of uric acid. The role of uric acid in CHD is now likely to be of interest only to those studying basic science; for now, the clinical question seems closed.

Wheeler JG, Juzwishin KDM, Eiriksdottir G, Gudnason V, Danesh J (2005) Serum uric acid and coronary heart disease in 9,458 incident cases and 155,084 controls: Prospective study and meta-analysis. DOI: 10.1371/journal.pmed.0020076

Using Integrins for Tumor Imaging

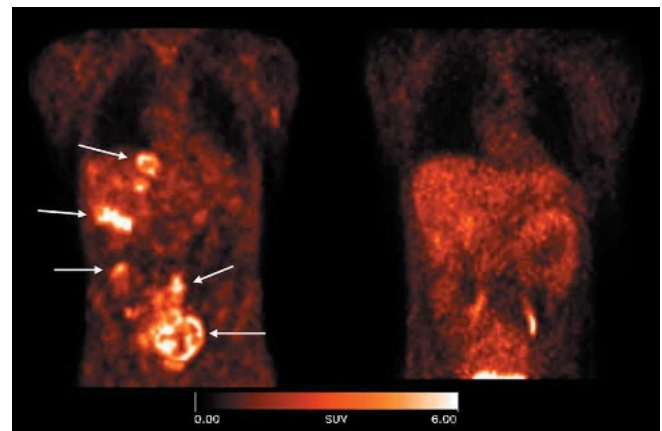
DOI: 10.1371/journal.pmed.0020091

What holds cells together or connects them with the extracellular matrix—and what happens when these interactions break down—is one of the keys to determining how tumors metastasize. One group of compounds—integrins—are a central part of these interactions. Not only do integrins play a part in cell–cell and cell–matrix adhesion, but they also are involved in signal transduction (the method by which a cell relays information from receptor binding to cellular response) and in triggering cell death by linking to other molecules. One such member of this receptor family is the $\alpha\beta3$ integrin, which is expressed on both the tumor cells and the new vasculature of various tumors, including melanomas. $\alpha\beta3$ integrin has a role in cell migration and extravasation, which occurs during metastasis, and also in angiogenesis—the development of new blood vessels that are essential for the growth of tumors. These blood vessels are the target for one class of anti-cancer drugs—angiogenesis inhibitors. Molecules that bind to $\alpha\beta3$ integrin have also been used to target therapeutic compounds to tumors: compounds that antagonize this integrin can lead to apoptosis (programmed cell death) of cells that express it.

Haubner and colleagues, the authors of a paper in this month's *PLoS Medicine*, have previously developed a fluorine-labeled peptide, [^{18}F]Galacto-RGD, that has a high affinity for $\alpha\beta3$ integrin. [^{18}F]Galacto-RGD has many of the features essential for a

tracer: it is specifically accumulated by tumors that express $\alpha\beta3$ integrin, it is efficiently eliminated by the kidneys, and it is stable in vitro and in vivo.

In the research paper in *PLoS Medicine*, Haubner and colleagues take the development of the compound further towards clinical application. First, in a mouse with human



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$\alpha\beta3$ integrin expression in tumors

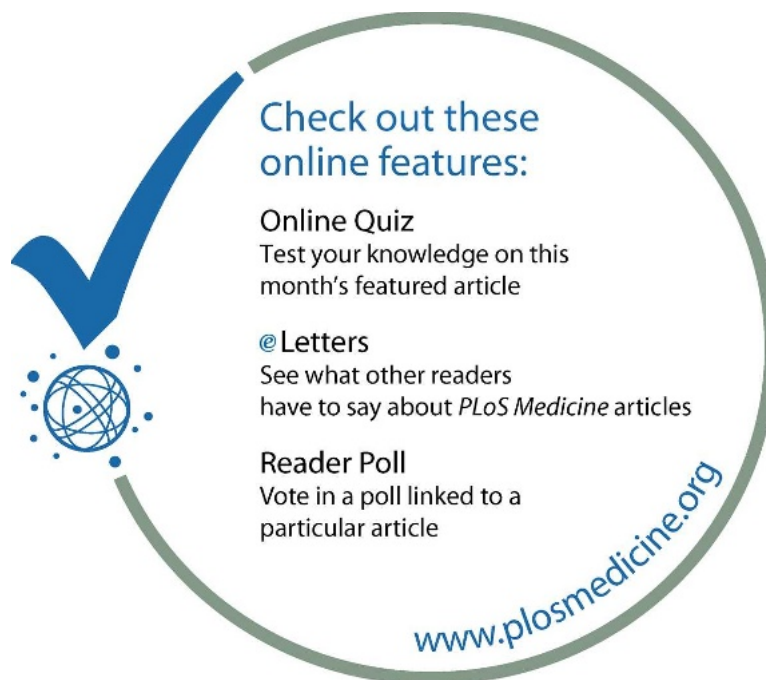
melanoma they used highly sensitive positron emission tomography (PET) scanning to show not only that the level of uptake of integrin was specific for the tumor, but also that the uptake was in direct proportion to the amount of $\alpha v \beta 3$ expressed, thus potentially allowing quantification of receptor expression; however, larger tumors showed a poorer correlation, possibly because of the presence of necrotic areas that do not express the integrin.

In humans, this picture was a little less clear; in a small study of patients with tumors including melanoma, the authors found a good deal of difference between patients in the uptake of the marker by tumor cells and the corresponding tumor vasculature. However, there was good correlation between the tracer uptake and conventional staining for the integrin by

immunohistochemistry—again suggesting that the marker is truly reflecting the *in vivo* level of the integrin.

What do these results mean for clinical applications? As well as identifying tumors that express this marker, this approach might also offer a noninvasive way to assess the degree of new vessel formation in tumors. The approach could provide important information for planning and monitoring anti-angiogenic therapies targeting this integrin and could reveal the involvement and role of this integrin in metastatic and angiogenic processes in various diseases.

Haubner R, Weber WA, Beer AJ, Vabuliene E, Reim D, et al. (2005) Noninvasive visualization of the activated $\alpha v \beta 3$ integrin in cancer patients by positron emission tomography and [^{18}F]Galacto-RGD. DOI: 10.1371/journal.pmed.0020070



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