Therefore, the authors next focused on the role of nuclear PKR. Phospho(threonine 451)-PKR (p-PKR), indicative of activated PKR, was observed mainly in the nucleus of $CD34^+$ cells isolated from AML patients as previously reported, suggestive of the specific role of nuclear PKR in AML. Eventually, the authors found that PKR has a novel nuclear function to inhibit DNA damage response signaling and double-strand break repair. Mechanistically, they found that nuclear PKR activates protein phosphatase 2A (PP2A), which consists of a dimeric core enzyme composed of the structural A and catalytic C subunits and a regulatory B subunit (B55α), by promoting nuclear localization of B55α. Activated PP2A in turn antagonizes autophosphorylation and activation of ataxia-telangiectasia mutated (ATM) and its association with downstream targets. Thus, inhibition of PKR expression or activity promotes ATM activation and promotes more rapid and complete repair of double-strand breaks (see figure).

The authors then validated this new function of nuclear PKR by crossing PKR-transgenic mice or PKR knockout mice with the NUP98-HOXD13 (NHD13) MDS mouse model to produce NHD13-TgPKR and NHD13-PKRKO mice, respectively. PKR-transgenic but not PKR-null mice demonstrate a mutator phenotype that leads to more rapid MDS evolution to acute leukemia. Furthermore, the age-associated accumulation of somatic mutations that occurs in the NHD13 mouse model was significantly elevated by coexpression of a PKR transgene whereas knockout of PKR expression or treatment with a PKR inhibitor reduced the frequency of spontaneous mutations in vivo.

Taken together, these results establish that increased nuclear PKR has an oncogenic function that promotes the accumulation of deleterious mutations by inhibiting DNA damage response and double-strand break repair. Thus, PKR inhibition may represent a novel therapeutic strategy to prevent leukemia progression and potentially tumorigenesis of other cancers.

Finally, several important questions remain unsolved. How does PKR become upregulated and activated, and then shuttle into the nucleus in AML and other cancers? As mentioned above, PKR becomes activated in response to multiple stimuli, such as cytokines, bacterial and viral infection, and DNA damage. Chronic inflammatory stress or DNA damage signals could activate PKR, but there might be unknown pathways. Enhanced shuttling of activated PKR into the nucleus might also be regulated by some AML-related signals. In addition, the precise mechanism of activation of PP2A by PKR remains undetermined. Addressing these questions would promote our understanding of the complex functions of PKR and provide more therapeutic options targeting the PKR signaling pathway in AML.

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Deguchi et al, page 1595

Does AC stand for acylcarnitine, anticoagulant, or both?

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It has long been appreciated that hemostatic systems represent complicated dynamics, involving multiple factors, which work in concert to regulate the balance between coagulation and bleeding in both health and disease. In this issue of *Blood*, Deguchi et al present evidence to support a novel role for acylcarnitines as anticoagulants.[1] These findings were arrived at starting with an untargeted metabolomics approach, which identified 10:1 and 16:1 acylcarnitines as decreased in plasma from patients with venous thrombus and pulmonary embolism (VTE) compared with a control group. A follow-up targeted approach was used as a means of method validation. Importantly, the addition of acylcarnitines to clotting assays demonstrated a direct effect through inhibition of factor Xa. Moreover, the chain length of the acylcarnitines was important, with carbon lengths of over 14 having the highest activity. Finally, mapping studies were carried out to analyze binding sites of acylcarnitines with factor Xa.

Philosophers of science have long made the distinction between the logic of discovery and the logic of justification. At the end of the day, many (although not all) philosophers of science have abandoned the program to define a distinct logic of the process of discovery. In contrast, the logic of justification, the process by which scientific claims are evaluated, continues to mature in a highly meaningful way. In many ways, the advent of "omics"-type approaches to discover new knowledge has rejuvenated issues of the logic of discovery and, in doing so, both generated new capacities and resurrected old problems. Mill's method of comparing what variables are common to one state (eg, a disease) but different in another (eg, health) and of attributing a potentially causal role to observed

differences is essentially the basis of discovery-level untargeted metabolomics.[2] Because omics cannot assess all possible variables, one cannot go as far as Mill claimed and assess causality, but only correlation. A more rigorous evaluation of causality requires isolation of a single variable, either through study design or interventional experimentation (with the removal of 1 variable, only 1 variable, and without changing anything else; a high threshold for success indeed). Given both the technical and ethical restrictions of human studies, such is not a possibility; however, Deguchi et al take additional measures to address the correlation/causality issue.

First, additional targeted and quantitative metabolomics were carried out as a means of validating the accuracy and precision of the antecedent and unbiased discovery-based untargeted approach. Second, having accomplished the discovery phase goal of identifying acylcarnitines as candidate molecules for playing a causal role, the investigators tested a prediction deduced from their new acylcarnitine hypothesis; in particular, that acylcarnitines would have a functional effect on coagulation itself. Indeed, using controlled assays of coagulation, it was observed that acylcarnitines had a hitherto unappreciated activity of inhibiting factor Xa-dependent coagulation assays. Thus, both verification of the untargeted analyte screen was carried out and the hypotheticodeductive process was explored, testing predictions from the hypothesis that acylcarnitines play a role in VTE (eg, that acylcarnitines would have activity in coagulation assays). Together, these data provide provocative evidence of a new pathway, which may yield insight into both the biology of coagulation and a potential target for therapeutic intervention.

As a general property of omics-type explorations of natural phenomena, several essential next steps are required. The first is a mindful consideration that traditional statistics and metrics for significance (eg, *P* values with a .05 cutoff) are not meaningful in the context of many omics-based approaches. By traditional metrics, a type I error will be made in 1 of every 20 studies by chance alone (measuring a single variable). However, omics-type approaches measure hundreds of variables on each specimen, and for every 100 variables, 5 will be significant by chance alone. Statistics has evolved to test the deviation of *P* values from this predicted normal distribution, giving rise to more stringent metrics of significance (ie, *q* values)[3]; however, although a low *q* value indicates likely significance, a higher *q* value does not rule it out. Moreover, such statistical considerations assume that each variable is independent of each other variable, which is clearly not the case for metabolic pathways, and thus the nature of statistical predictions changes in omics-type studies. A second concern is what is meant by "verification." A targeted analysis of the same samples on which the untargeted approach was carried out is a form of method verification and tests the accuracy and precision of the untargeted semiquantitative data. However, although this verification addresses whether the observed correlations actually occurred in the cohort studied, it does not verify that such correlations did not happen by chance alone. To evaluate this latter question, a new cohort, distinct from the group analyzed to generate the initial observation, must be analyzed. If the same correlation is observed in a separate cohort, then this will provide much confidence.

The above cautions notwithstanding, the data put forth by Deguchi et al demonstrate a distinct biological basis for how acylcarnitines might play a causal role in VTE pathogenesis. These findings represent a new view of coagulation regulation, involving a class of compounds not previously appreciated to be intricate to this process. The previously ubiquitous and unnecessarily pejorative descriptor of "fishing expedition" has recently been replaced with the somewhat euphemistic label of "hypothesis-generating studies." In this case, Deguchi et al have caught a very nice fish and generated a provocative and potentially seminal hypothesis.

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● ● ● TRANSPLANTATION

Comment on Gartlan et al, page 1609

All pain, no gain: Tc17 phantoms in GVHD

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Elusive $CD8^+$ T cells that transiently secrete interleukin (IL)-17 cause graft-versus-host disease (GVHD) but do not contribute to beneficial graft-versus-leukemia (GVL) responses, as reported by Gartlan et al in this issue of *Blood*.[1] GVHD remains a lethal and morbid complication of allogeneic bone marrow transplantation, but GVHD is tightly linked to beneficial GVL effects, and removal of donor T cells that cause GVHD also diminish GVL, leading to greater relapse after bone marrow transplantation (BMT). This elegant paper from the laboratory of Geoffrey Hill has identified a rare "night fury" T-cell subset that causes much pain with no gain, a finding that may take us one large step closer to the long sought after goal of separating GVL and GVHD.

Cytolytic T cells (CTLs) that mediate GVL effects are predominantly $CD8^+$, and therefore, elimination of the entire $CD8^+$ T-cell subset usually leads to greater relapse. Current GVHD prophylaxis efforts, such as calcineurin inhibitors or antithymocyte globulin, are nonspecific and target all T cells. It has not been clear which donor T-cell subpopulations could be eliminated without impairing GVL effects or damaging reconstitution of the patient's immune system after BMT. The Hill group had previously reported that IL-17, an inflammatory cytokine that is important in autoimmune disease, also mediates GVHD in experimental models.[2] Here they identified a weakly cytolytic but