

Effective double whammy targets DNA synthesis in leukemia

Inhibition of DNA synthesis is a keystone of cancer therapy. The purine and pyrimidine precursors of DNA are obtained either by the de novo pathway (DNP) or the nucleotide salvage pathway (NSP). The DNP generates nucleotides that are reduced to the corresponding deoxynucleotide by ribonucleotide reductase (RNR). Thymidine, a potent inhibitor of de novo synthesis, acts by specific inhibition of RNR. The NSP salvages circulating deoxynucleosides released from dead cells or from the liver and converts them to deoxynucleotides, a process that is catalyzed by deoxycytidine kinase (dCK) and thymidine kinase 1 (TK1).

In this issue, Nathanson et al. provide an elegant dissection of the roles of the DNP and NSP in leukemic cells and propose that the combined inhibition of both pathways offers a new therapeutic approach. Using multiple tumor models, including human-to-mouse xenografts and mouse models, they showed that the NSP is required to generate deoxycytidine triphosphate (dCTP) to compensate when de novo dCTP synthesis is



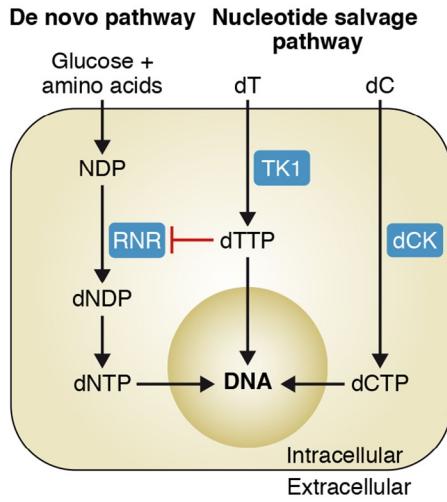
Insight from
Bruce Chabner

blocked using thymidine, which may explain why using thymidine alone to target the DNP has shown limited efficacy in clinical trials. The availability of a fluorinated analogue of deoxycytidine allowed the authors, in prior work, to track the effect of DI-39—a potent small molecule inhibitor of dCK activity—in vivo using positron emission tomography. Crystallographic studies of DI-39 bound to dCK showed that it inhibits dCK activity by binding to the nucleoside-binding site. Using DI-39 in combination with thymidine (to inhibit de novo synthesis) profoundly decreased dCTP levels, leading to regression of T and B cell acute lymphoblastic leukemia in mice, with no significant toxicity to normal hematopoiesis. Although thymidine was the chosen inhibitor for de novo synthesis in this study, there may be better compounds to inhibit RNR, specifically hydroxyurea.

Could this strategy work in humans? This study offers important insights into how cells generate dCTP and convincingly demonstrates the clinical potential of a dual inhibition strategy for cancer therapy that targets both de novo dCTP synthesis and the salvage pathway. Further work will establish the basic pharmacology (kinetics, bioavailability, and toxicology) of dCK inhibitors and identify which human tumors are most sensitive to this strategy of combined inhibition.

Nathanson, D.A., et al. 2014. *J. Exp. Med.* <http://dx.doi.org/10.1084/jem.20131738>.

IMAGE ENHANCED BY SIMON FEWICK



Deoxycytidine triphosphate can be produced by both the de novo pathway and the nucleotide salvage pathway.

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Unexpectedly uneven: posttransplant skeletal distribution of hematopoietic stem cell clones



Insight from Elliott Hagedorn (left) and Leonard Zon

A new study by Gerald de Haan, Leonid Bystrykh, and colleagues suggests that the skeletal distribution of transplanted hematopoietic stem cell (HSC) clones is surprisingly nonuniform.

The extent to which HSCs engraft in different bones and subsequently move between bone marrow niches is poorly understood. Much of what we do know comes from experiments using parabiotic rodents with shared circulation. These and other studies suggest that HSCs egress continuously from their niche into the circulation. Under these conditions, one would expect individual HSC clones to eventually be represented equally in all bones of the body. This new study, however, suggests that some transplanted HSC clones preferentially expand in certain skeletal sites with limited migration to others. Other clones were more indiscriminant.

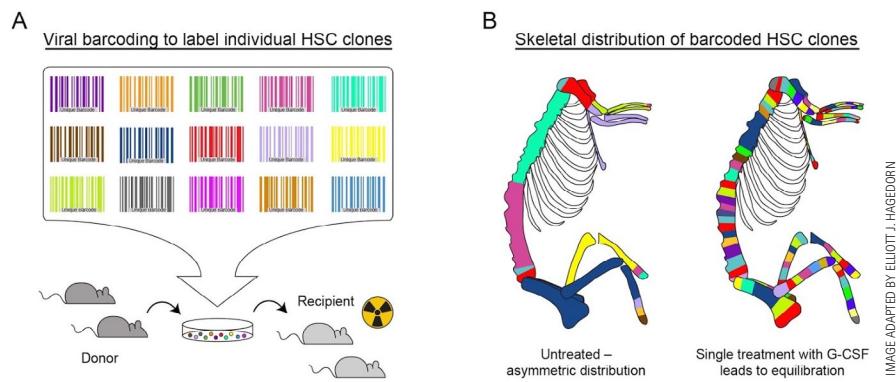
Using viral barcoding to monitor transplanted mouse HSC clones, Verovskaya et al. uncovered a highly skewed representation within different bones up to 11 months posttransplantation. Clonal differences were observed with both young and old HSCs and were maintained when clones from particular bones were transplanted into secondary recipients. Strikingly, just a single treatment with G-CSF equilibrated the skeletal distribution of all clones.

This work has significant implications for both clinical practice and experimental research. It may be important, for example, to take marrow biopsies from more than one site (rather than a single site as is the current practice) to fully assess hematopoietic clonality

after transplantation. This issue should also be addressed in future mouse barcoding studies, as the routine practice of using one limb for secondary transplants might exclude clones that preferentially reside in other skeletal sites.

In the long term, this paper should provide the groundwork for mechanistic studies addressing why HSCs expand in some skeletal sites and not others, how G-CSF treatment leads to rapid clonal redistribution, and what role preconditioning irradiation might play in these processes.

Verovskaya, E., et al. 2014. *J. Exp. Med.* <http://dx.doi.org/10.1084/jem.20131804>.



(A) Verovskaya et al. used a barcoding strategy to track individual HSC clones. (B) At 11 months posttransplantation, recipients displayed highly skewed clonal representation within different bones (left). A single challenge with G-CSF, however, led to the equilibration of all HSC clones across the skeleton (right).

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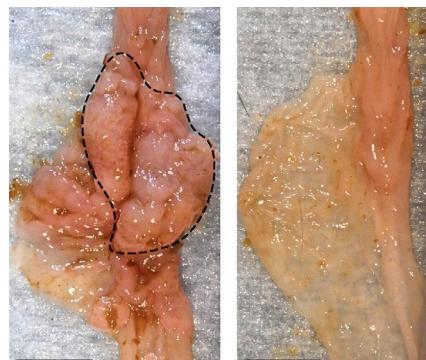
Do bugs define cancer geography?

Although genetic factors are essential to the development of colorectal cancer (CRC), the intestinal microbiota were recently recognized as an important environmental contributor. In this issue, Gerold Bongers, Sergio Lira, and colleagues demonstrate for the first time that the microbiota influence site-specific development of tumors in a mouse model of CRC.

HBUS mice express two oncogenic transgenes throughout their intestines—cytomegalovirus chemokine receptor US28 and heparin-binding EGF-like growth factor—but they develop tumors (serrated polyps or SPs) only in the cecum. When the authors treated *HBUS* mice with broad-spectrum antibiotics they noticed that development of cecal SPs was virtually ablated. Importantly, changing the *HBUS* microbiome by transferring *HBUS* embryos into female mice obtained from a different animal vendor also attenuated the development of SPs. Microbiome analysis indicated that antibiotic-mediated attenuation of SPs was correlated with reduced invasion of cecal tissue by bacteria belonging to the Clostridiales/Lachnospiraceae family. Together, these findings link bacterial topology and intestinal barrier defects with site-specific tumor development.

The work of Bongers et al. raises questions about the relationship between bacteria and site-specific cancer development. First, longitudinal analysis of the microbiota in *HBUS* mice would help define the geographical establishment of microbial niches in relation to tumor development. Similarly, longitudinal assessment of intestinal barrier integrity in *HBUS* mice would determine whether the barrier defect is the consequence of neoplasia, as observed in a mouse model of colon adenoma, or due to an intrinsic host malfunction that bacteria exploit to promote tumorigenesis.

Interestingly, enrichment of the mucin-degrading bacterium *Akkermansia muciniphila* in SPs of *HBUS* mice may strip the mucosa of its protective layer, thereby creating favorable conditions for Clostridiales/Lachnospiraceae invasion into the mucosal tissues with ensuing inflammatory host responses. In the context of dysregulated EGFR signaling in *HBUS* mice, this situation favors tumor promotion. Experiments using germ-free *HBUS* mice would help assess this possibility as well as establish the functional interaction between various microbial communities and tumor locations.



Development of serrated polyps (SPs, hatched line) in *HBUS* mice is attenuated (right panel) when embryos are rederived into foster females from a different animal vendor.

In summary, this study adds additional layers of complexity to cancer etiology by highlighting the interplay between host genetics, microbial location, and tumor geography. Whether microbial niche localization influences SP development in humans requires further investigation.

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