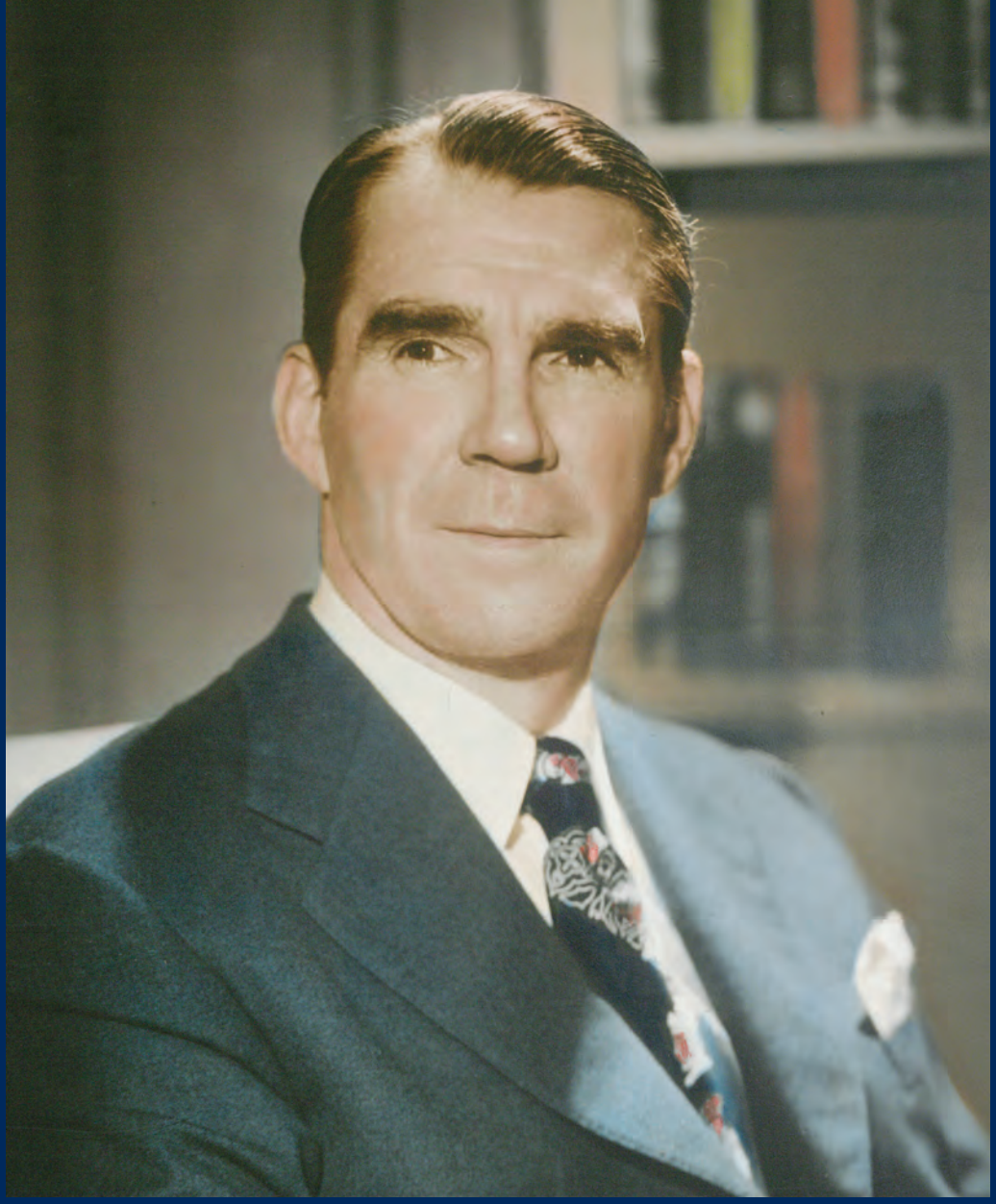


September 2024

Ludwig Link

LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

Welcome

Unmesh Kher
Editorial Director

The Ludwig Institute is so special that its founders gave it not just one, but two birthdays.

We kid thee not. Although Daniel Keith Ludwig had established the Cancer Research Institute Ltd. in 1971, the Ludwig Institute for Cancer Research we know and love today actually came into being three years later. That was when Ludwig transferred an immense pile of his commercial assets to the Institute and, on December 17, 1974, tweaked its articles of incorporation to ensure the proceeds from those businesses would forever be applied exclusively to its charitable cause. Only then, and only upon the vociferous insistence of his advisors, did Ludwig lend his name to the cancer research organization he had created.

This means the Ludwig Institute as we know it today turns 50 this year. (You might recall, through the haze of the pandemic years, that we celebrated our first 50th anniversary through 2021.) This explains both the cover of this Ludwig Link and the brief biography of Ludwig we're running in lieu of our usual Q&A (page 9). We find Ludwig the man fascinating and are betting you will too.

Those more interested in the here and now, meanwhile, will find plenty to entertain them in our research news section (page 18). Our briefs cover discoveries from Ludwig Branches and Centers on everything from an exploitable metabolic dependency shared by tumor cells and infiltrating T cells to a targetable checkpoint the former impose on the latter to the mechanisms by which radiotherapy promotes cancer metastasis.

We also share news of awards and honors received by Ludwig researchers (page 5) and tidings of big changes at the helm of the Ludwig Institute (page 8).

Read on to find out more. And happy reading!

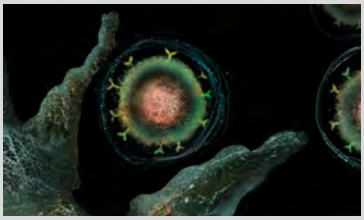
Sincerely,

Unmesh Kher
Editorial Director

On the cover
Daniel Keith Ludwig, 1897-1992.
STORY ON PAGE 9

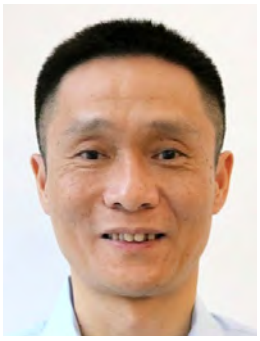
In this issue

FEATURED RESEARCH



Ludwig Stanford's Crystal Mackall and her colleagues reported that administering engineered anti-tumor T cells together with antibodies that block the CD47–SiRPα axis leads in animal models to rapid clearance of the therapeutic T cells by macrophages. **PAGE 20**


Eileen White


Chuan He


Kornelia Polyak


Arlene Sharpe

Four Ludwig researchers elected to the American Academy of Arts & Sciences

Four Ludwig researchers were elected to the American Academy of Arts & Sciences (AAAS) in April. Established in 1780, the AAAS, in its own words, "honors excellence and convenes leaders from every field of human endeavor to examine new ideas, address issues of importance to the nation and the world and work together 'to cultivate every art and science which may tend to advance the interest, honor, dignity, and happiness of a free, independent, and virtuous people.'" Suffice it to say that membership to this Academy is an especially high honor. Its members have included such legends as Benjamin Franklin, Martin Luther King, Jr., Charles Darwin and Margaret Mead. This year, Ludwig Princeton Associate Director Eileen White was elected to the Cellular and Developmental Biology section of their Class II – Biological Sciences division. Joining her in the 2024 class of the AAAS were Ludwig Chicago's Chuan He, in the Biochemistry, Biophysics and Molecular Biology section, and Ludwig Harvard's Kornelia Polyak and Arlene Sharpe, elected to the Medical Sciences and Microbiology and Immunology sections, respectively. Our congratulations to them all on this well-deserved recognition of their work and their many contributions to cancer biology.

Ludwig Institute's Jingjing Zhu received the Baillet Latour Biomedical Award 2024

We extend our warmest congratulations to Jingjing Zhu of the Brussels laboratories of the Ludwig Institute for Cancer Research on being named a laureate of the prestigious Baillet Latour Biomedical Award. The Award was formally presented to her by Queen Mathilde of Belgium during an official ceremony at the Academy Palace in Brussels on April 29, 2024. Given each year to just one researcher in Belgium, the award provides €1 million over 5 years to support a research project in one of five biomedical domains selected by the Baillet Latour Fund. This year's domain was cancer and Jingjing was selected for the prestigious award by a jury of international experts. She will use the funding to build on a study she led with Ludwig Institute's Benoît Van den Eynde and published in *Nature* last year showing that agonists of the alpha2-adrenergic receptor stimulate anti-tumor immunity through their effect on myeloid cells of the innate immune system. This means such drugs, which are already sold as anti-hypertensive medications, could be repurposed and tested as potential boosters of cancer immunotherapy. The proposal Jingjing submitted to win this very competitive prize—the cancer domain comes up only once every five years—will explore the mechanistic underpinnings of that effect.

Queen Mathilde of Belgium with Jingjing Zhu of the Ludwig Institute's Brussels lab.

Ludwig Oxford's Yang Shi elected Fellow of the Royal Society and to the U.S. National Academy of Sciences

In May, Ludwig Oxford's Yang Shi was elected Fellow of the Royal Society, the UK's storied and perhaps most prestigious academy of science, as well as the U.S. National Academy of Sciences, a private, nonprofit organization that promotes outstanding science and provides advice to policymakers on critical issues. Yang is best known for his identification and characterization in 2004 of an enzyme, LSD1, that erases methyl marks from histones—a discovery that upended a 40-year dogma about the reversibility of such modifications and has led to the development of new cancer therapies. His lab has since identified and characterized many other histone demethylases and, more recently, identified several enzymes that methylate RNA. Yang and his team today focus mainly on two cancers, acute myeloid leukemia and diffuse intrinsic pontine glioma, in which epigenetics has been shown to play a crucial role. These honors are a testament to his significant contributions to the field of epigenetics. The Royal Society counts among its Fellows and Foreign Members scores of Nobel laureates, including Ludwig Oxford's Sir Peter Ratcliffe. Ludwig Oxford Director Xin Lu and Ludwig Lausanne's Douglas Hanahan are also members of the Society. Our congratulations to Yang on both these well-deserved honors.

Yang Shi

Ludwig Lausanne's Johanna Joyce named president-elect of EACR

Our congratulations to Ludwig Lausanne's Johanna Joyce, who in June was named president-elect of the European Association for Cancer Research and appointed to its Board of Directors at the organization's 2024 Congress in Rotterdam, The Netherlands. Johanna is an authority on the tumor microenvironment. Her lab has developed groundbreaking technologies to analyze the cellular geography and heterogeneity of tumors, making these tools freely available to the research community. Her team's research has identified key differences between immune cells in primary and metastatic brain tumors and examined how they are biochemically manipulated and functionally altered in the tumor microenvironment. It has explored how these phenomena, along with unique properties of the brain tumor vasculature, might be harnessed to develop new therapies and strategies to effectively treat diverse brain cancers. A noted advocate for women in biomedical research, Johanna recently co-authored a commentary, published in *Cell*, that graphically illustrated in a striking scissor-shaped curve how gender disparities widen over successive stages of the scientific and academic career trajectory, with representation of women declining with rising seniority. Johanna also discussed many of these issues with us in our *Women in Science* publication, where she highlighted the benefit of dedicated mentorship by women for women, and the cultivation of a network of advocates for women in science.

Johanna Joyce


Edward McDermott


John Notter


Chi Van Dang

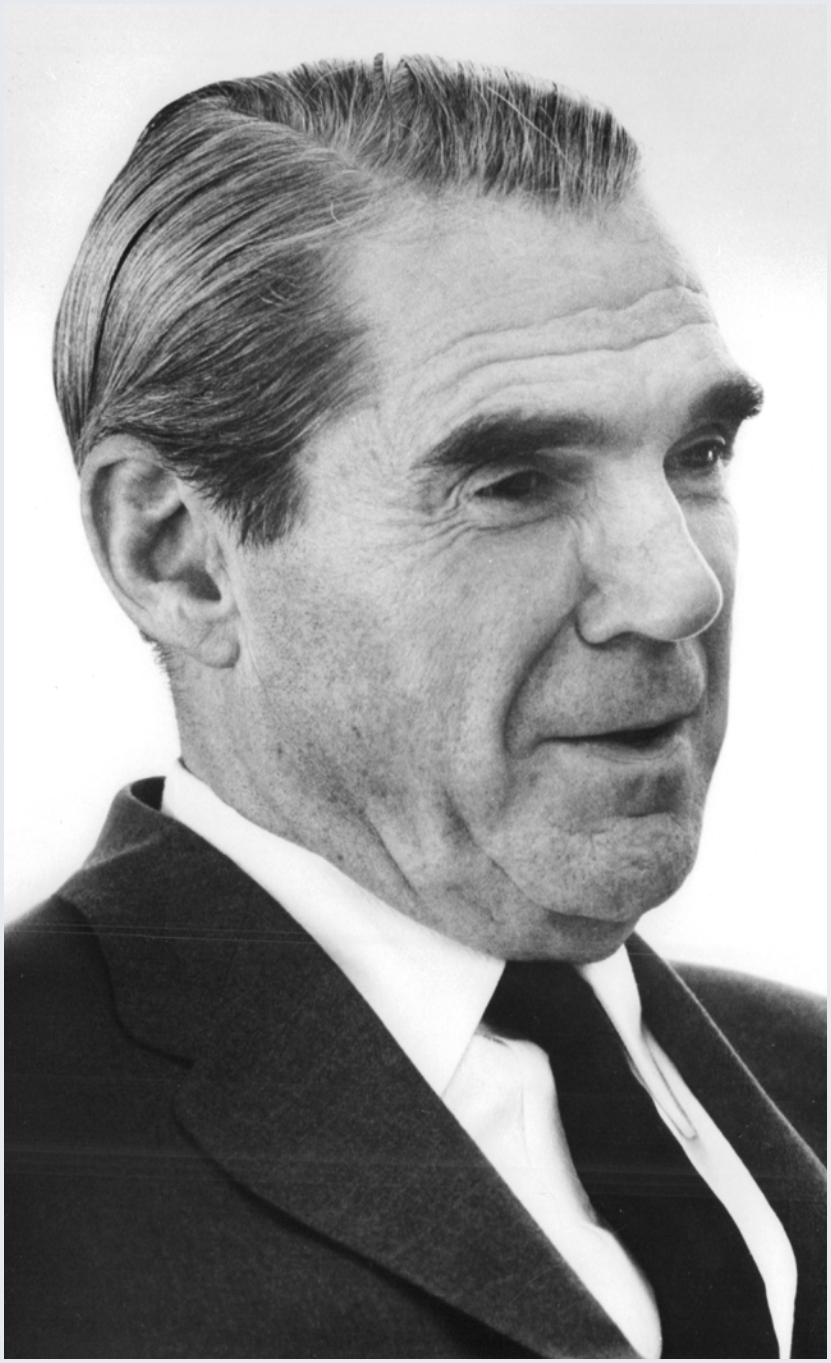

Jonathan Skipper

The Ludwig Institute for Cancer Research announces changes in its executive leadership team

The Ludwig Institute for Cancer Research (LICR) announced significant changes in its executive leadership team. Edward McDermott, until recently the CEO and president of LICR, retired from his post on July 1. Ed, who has been with the Ludwig organization since 1988, has played a central role in the financing, administration and strategic planning of the Institute for over three decades. He steered the Institute through the global financial crisis of 2008, oversaw its subsequent restructuring and helped guide the launch of new Branches to create the Ludwig Institute of today. Ed's retirement, however, does not end his association with Ludwig Cancer Research: He will continue to serve at the helm of the Ludwig Institute as chairman of its Board of Directors. The current Chairman, John Notter—one of the founders of the Institute—remains a director. The Institute's Scientific Director Chi Van Dang has taken on the additional role of CEO, while former Executive Vice President for Technology Development Jonathan Skipper has been named president of LICR. The changes in leadership, occasioned by Ed's retirement, ensure continuity in the Institute's administration and execution of its research strategy. Ed's journey to the Ludwig Institute began when he was working at a law firm that represented various legal interests of LICR and was recruited by its chairman to help oversee the variegated businesses that funded its operations at the time. He quickly became involved in the Institute itself after he was named secretary to its Board of Directors in 1989 and then president of the organization in 1995. Ed's steady hand has been an invaluable asset to the Ludwig Institute, and we look forward to working with him in the years ahead in his new role as our chairman.

The making of a magnate

An excerpt from *A Vision Realized: The story of Ludwig Cancer Research*

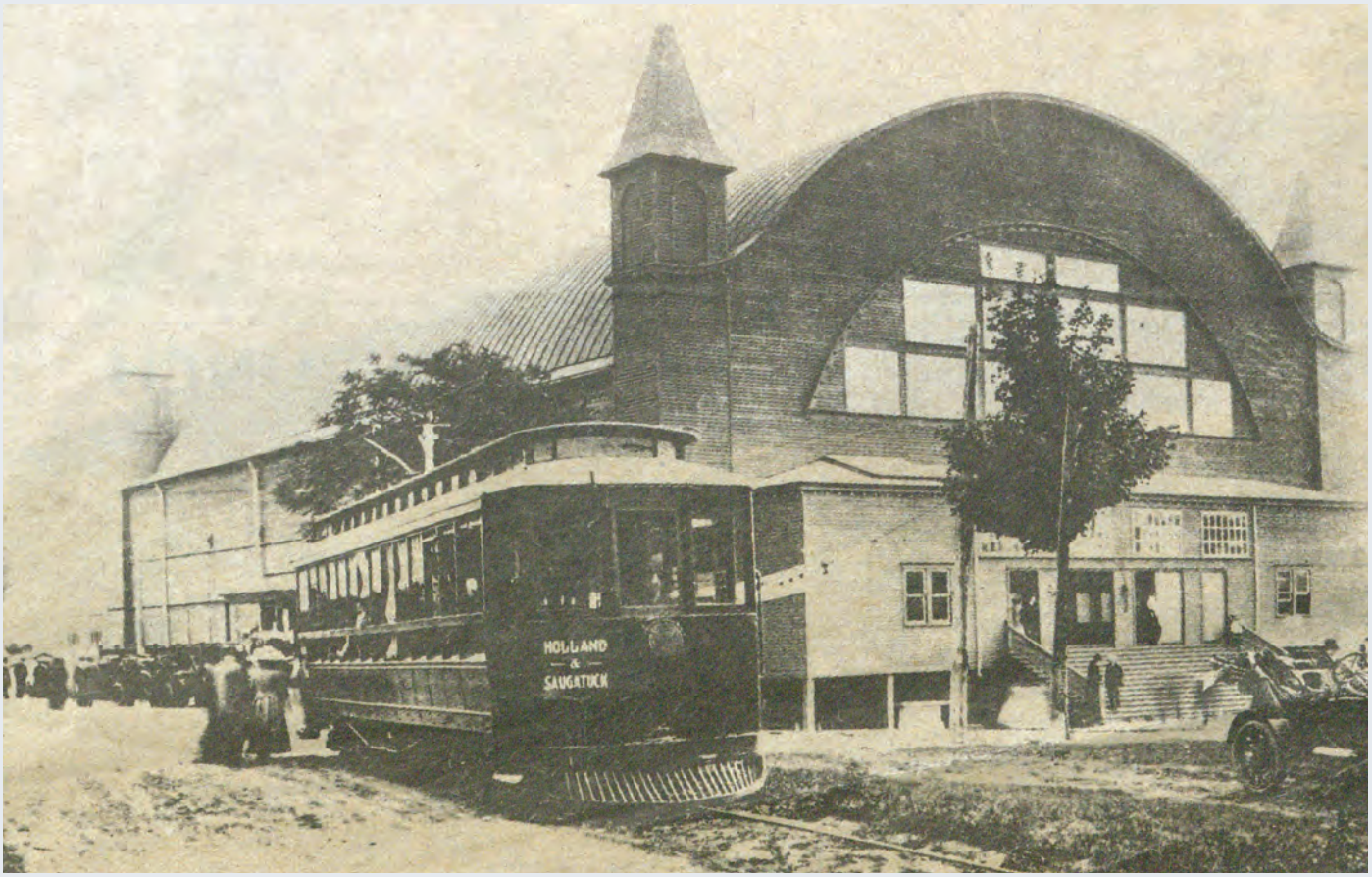


You probably know Ludwig Cancer Research is named after a billionaire. Many of you might even recall that the billionaire in question, Daniel Keith Ludwig, made his fortune in shipping and, perhaps, that he is credited with inventing the modern supertanker. But we suspect very few, even in the extended Ludwig Cancer Research community, know much more than that about the man, such as where he came from, what he was said to be like or how he built the sprawling conglomerate that made him one of the richest men in the world. This is not your fault. Ludwig cherished his privacy and worked hard to protect it—even from the prying gaze of posterity.

If you're curious to learn more about the man, read on. The article that follows is carved out of a book on the history of Ludwig Cancer Research prepared by the Communications department. That history owes its creation to chairman-emeritus of the Ludwig Institute's Board of Directors John Notter, who suggested that somebody write the story of the Institute, which he helped conceptualize and establish and whose founding Board he chaired until 1980. He returned to the Board in 2009 and took the chair once again the following year. After 14 years at the post, John was replaced in July by the Institute's retiring CEO and President Ed McDermott, though he remains on the Board (see previous page).

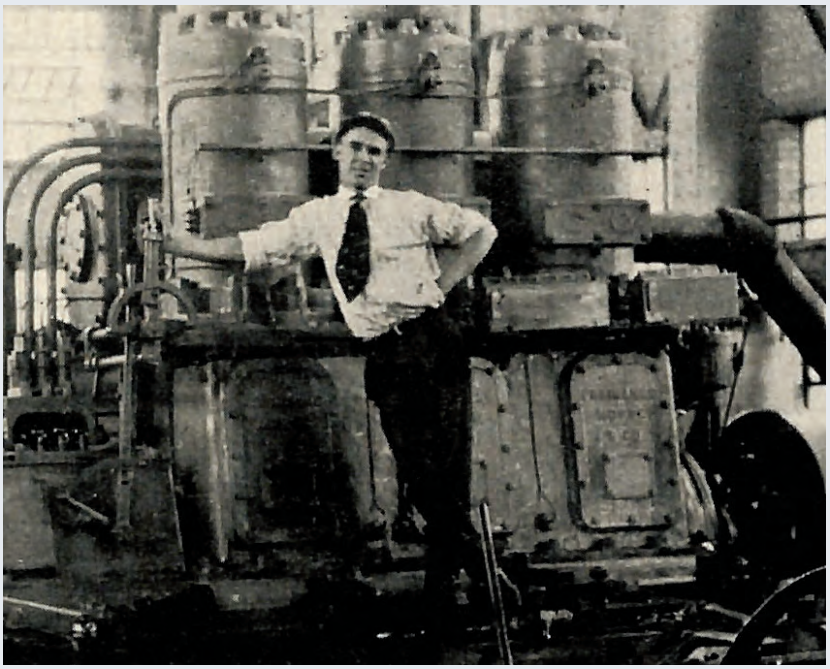
We thank them both for their support and contributions to the Ludwig Institute history, from which we excerpted the following biography.

The making of a magnate



Above: The pavilion at Kalamazoo Lake in Michigan where Ludwig shined shoes and sold popcorn as a young boy.

Right: Ludwig at about 17 years old, working on marine engines for Fairbanks, Morse and Co.



Daniel Keith Ludwig was born on June 24, 1897, in South Haven, a small port town on the Lake Michigan shore, where a pier built by his grandfather bore the family name.

Shipping was in his blood. As, clearly, was business. He told a *Fortune* magazine reporter in 1957 in an exceedingly rare, sanctioned profile that when he was just nine years old, he pulled together $75 to buy a sunken, 26-foot boat and toiled through the winter to fix her up. He then hired a crew and chartered her out the next summer for twice as much as he'd paid for her, all while earning extra on the side shining shoes and selling popcorn.

The *Zulia*, a large and highly effective side-casting boom and hopper dredge, was conceived, designed and built by Ludwig. Created to deepen the entrance to Lake Maracaibo in Venezuela to open access to massive oil tankers, it removed more "spoil" in 10 days than small hopper dedges had removed in two years.

When the young Ludwig's parents divorced six years later, he dropped out of school and followed his father, a real estate agent, to Port Arthur, Texas, where he endured a singularly lonely childhood. Ludwig eventually found work selling supplies to ships anchored at the local port while attending night school to pick up the math he needed for marine engineering. He then moved back to Michigan and completed his training working at 20 cents an hour for the manufacturer Fairbanks, Morse and Co., which subsequently hired him and sent him off to the Pacific Northwest and Alaska to install ship engines.

Ever the entrepreneur, Ludwig freelanced his services in his spare time and soon decided he preferred being his own boss. With $5,000 borrowed on his father's signature, the 19-year-old Ludwig bought an aged side-wheel excursion steamer named *Idlewylde*, paid back the loan by selling off its machinery and boilers, and converted the ship into a barge. The conversion, which entailed extensive welding of bulkheads in the cargo spaces using a simple but effective method, left a lasting impression on Ludwig and later influenced his pioneering construction of supertankers. Buying some wooden boats to assemble a ramshackle fleet, Ludwig began hauling liquid molasses up the Hudson River to distilleries in Canada during World War I.

That business was, however, short-lived. Ludwig sold his barges to his erstwhile client and stayed barely a step ahead of bankruptcy using his decrepit tugs for general hauling during an ensuing downturn in the shipping business. He noticed around this time, however, that transporting oil was about four times as profitable as hauling molasses. So he chartered out a small, nearly finished tanker from the United States Shipping Board, sold his tugs to complete its construction and began oil deliveries for a Massachusetts refinery.

In 1923, he bought an antique, partly sail-driven tanker, the *Wico*, for $25,000 from a scrap metal dealer named Boston Metals Co., claiming outright ownership of an ocean-going vessel for the first time and starting a lasting business relationship with the dealer. But a partner he enlisted in that business soon elbowed him out. Undeterred, Ludwig established a company named American Tankers Corp. with new partners a couple of years later, this time buying a tanker named the *Phoenix* from the United States Shipping Board.

The making of a magnate

Seeking to expand his business, Ludwig next returned to New York and bought a coal-hauling vessel named the *Ulysses*, which he converted into a 14,000-dead-weight ton (dwt) tanker—enormous by the standards of the day (dwt refers to the total weight a ship can carry, including cargo, fuel, ballast, passengers and everything else onboard). That move nearly bankrupted Ludwig when delays in the collier's conversion led to the loss of its charter. But the failure would ultimately spark a rally in Ludwig's fortunes when he managed, in 1937, to offload his white elephant to a whaling concern for four times its value as a tanker.

The proceeds pulled him out of debt and financed the hiring of his first full staff. Around the same time, Ludwig also obtained from New York's Chemical Bank a loan he considered the most consequential of his career, using it to buy several government cargo vessels, which he converted into tankers. By 1942, Ludwig had his own shipyard for building and converting ships into tankers—Welding Shipyards, the first of two he'd operate in Virginia.

He was innovating on the financial front as well. In 1938 Ludwig pioneered a mechanism for financing his growing fleet that would later become standard in the industry. He would charter a tanker to an oil company for a certain number of years and borrow from a bank for the same term to finance the construction of new vessels or support other investments. The oil firm would then pay the monthly charter fees to the bank, which would take its cut and transfer the rest to Ludwig. Comprehensive insurance coverage of the ship would protect the bank. Ludwig, for his part, could borrow on existing vessels, sure that the loan would be repaid; and the new vessels, which he owned entirely, could serve as additional collateral.

By the late 1940s, under the skeptical gaze of his competitors, Ludwig was building larger and larger tankers on the calculation that they'd be more profitable because operating costs do not rise in direct proportion to ship size. His hunch proved correct and, encouraged by the results, he signed a deal with the Japanese government in 1951 to

The tanker *Universe Burmah* at Ludwig's Kure shipyard in Japan.

lease the Kure shipyard, where the graving docks and other factors greatly eased the application of his many innovations in supertanker construction. His tankers grew from the neighborhood of 23,000 dwt, considered gargantuan when introduced in the 1940s, to a staggering 326,000 dwt a couple of decades later. By the late 1960s Ludwig had six such "Bantry Bay Class" Goliaths plying the oceans, part of a fleet that grew to number more than 60 ocean-going ships at the height of his career.

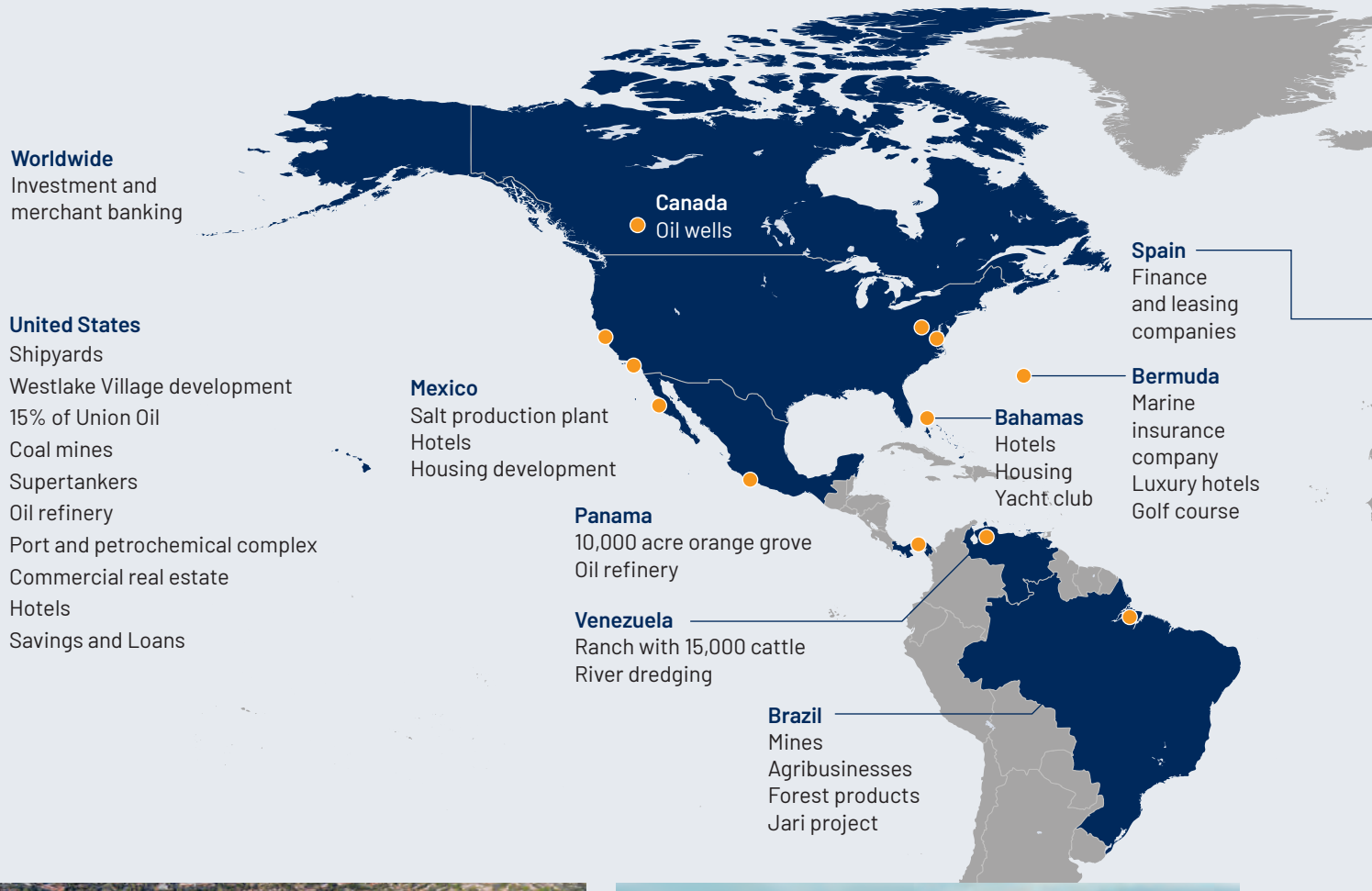
Ludwig's engineering chops were a core asset of his businesses. Having developed pioneering welding techniques at his Virginia shipyards, he continued innovating at Kure, where his use of prefabrication and sectional pre-assembly streamlined the production of his supertankers. The components and designs of his ships were largely interchangeable, ensuring further efficiency in not only their construction and maintenance but their operation as well: crews could be moved around as needed from ship to ship and feel at home wherever they were dropped.

Like the man himself, the tankers were notably frugal. They lacked basic comforts such as air conditioning, let alone frills like swimming pools, luxurious captain's quarters or "owner's cabins." Yet, as characteristically, Ludwig was happy to pour large sums into structural and mechanical components that would improve their profitability. He similarly spared no expense in hiring only the best officers to run them. As the American Society of Mechanical Engineers noted in posthumously awarding Ludwig the Elmer A. Sperry Award for Advancing the Art of Transportation in 1992, "His ships are known the world over as lean and austere in appearance, but they are recognized as exceptionally durable and reliable in machinery, equipment and basic structure." His design innovations in shipbuilding, it additionally noted, extended well beyond the supertanker to encompass dredges and bulk carriers and even a floating power plant that could be hauled across oceans and dropped off at remote locations.

On the foundations of this fleet, Ludwig

The Ludwig empire

Daniel Ludwig's conglomerate spanned the globe, employing tens of thousands in enterprises as varied as they were ambitious. Here's a sampling of the businesses he owned at various points over the course of his career.

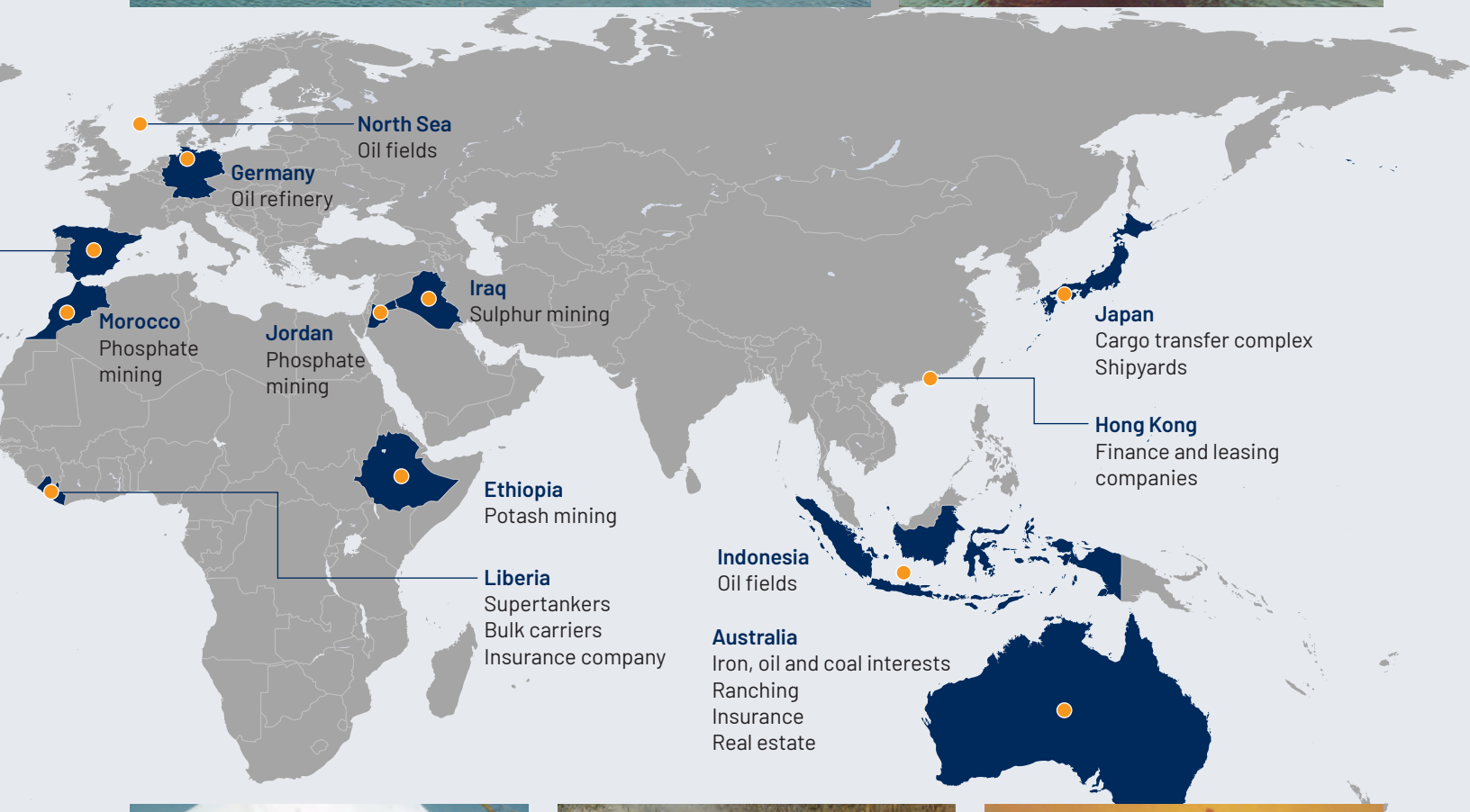


Westlake Village in California

The Acapulco Princess Hotel

The Princess Hotel Bermuda

A Bantry Bay class supertanker



Ludwig, center, at his salt production facility in Mexico

Kaolin mine in Brazil

Mining operation in Australia

The making of a magnate

Gertrude Virginia Higgins was married to Daniel Ludwig from 1937 until his death in 1992.

built a commercial empire. His investments certainly overlapped at times: If his ships needed coverage, he established an insurance company; if the shipyards needed steel, his cargo ships, chartered out to U.S. Steel, hauled iron ore from a Venezuelan mine to smelters in the U.S. Inventive as ever, Ludwig personally designed gigantic bulk carriers for that purpose and had them ferry the ore over a channel deepened by a dredge of his invention.

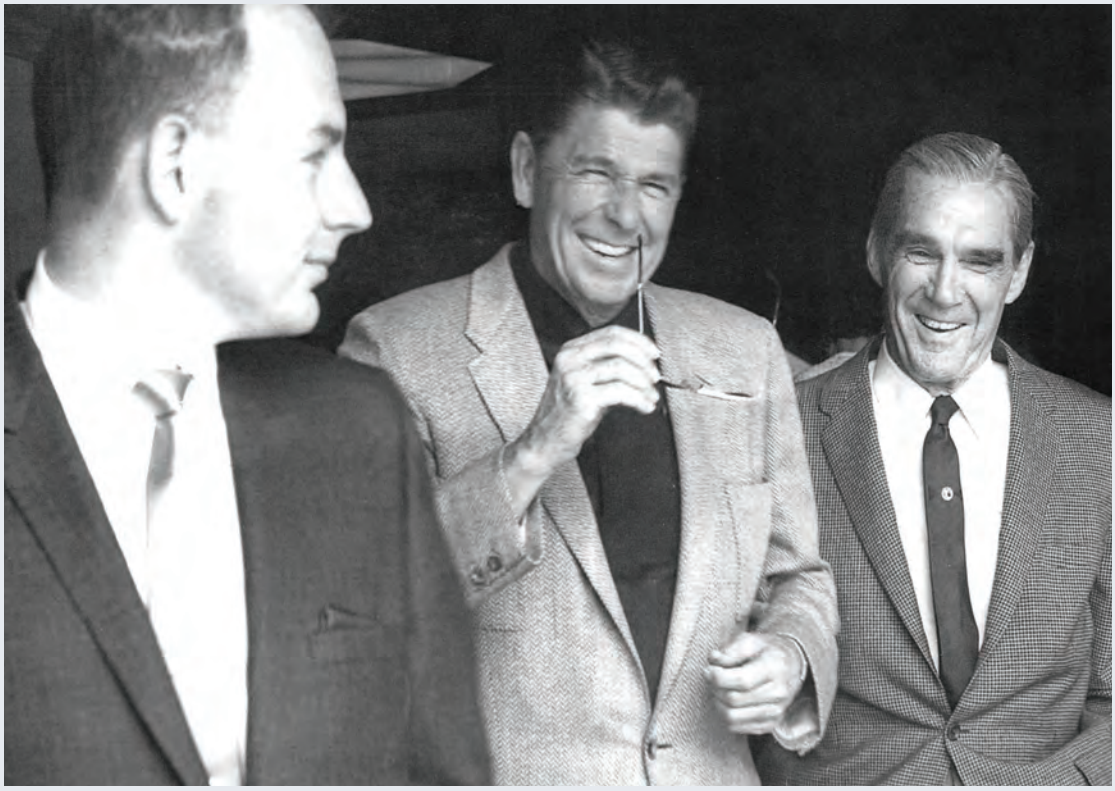
By 1963, his stack of holding companies—of which he was sole owner—had interests in an oil refinery and an orange grove in Panama; a potash mine in Ethiopia; iron, coal and oil interests in Australia; an international chain of luxury hotels; an oil refinery in Germany; the Kure shipyard and a cargo transfer complex in Japan; a 650,000 acre cattle ranch in Venezuela; interests in oil companies in Canada and California, a state where he also controlled a clutch of savings and loan companies; the world's largest manufacturer of salt by solar evaporation—Exportadora de Sal—in Mexico, whose salt harvesting and other machinery he developed and built, and that he serviced with self-discharging carriers of his design that docked at a deep-sea port he constructed at Cedros Island; and, of course, a fleet of 22 bulk carriers and 28 supertankers that was expanding at a steady clip.

Ludwig left his mark in residential real estate as well—most notably Westlake Village, which he built on a storied 11,780-acre ranch he bought in 1963 for $32 million (the equivalent of $328 million today) just 40 miles outside of Los Angeles. What Ludwig saw at the time, and others did not, was that it was only a matter of time before a highway to the nearby city ran past the ranch. With that, the natural beauty of the land—hundreds of Hollywood movies had been filmed there—and its proximity to L.A., any development at the location was likely to be successful, if done correctly. To ensure it was, Ludwig established a subsidiary of his American-Hawaiian Steamship Co. (AHS), named American-Hawaiian Land Co., to manage its development. Notter, who was chairman of the AHS board and now chief of its subsidiary, retained a civil engineering firm to design not just a housing development but an extensively planned city.

The effort involved the integrated contributions of hundreds of experts in dozens of specialties—from schools to healthcare to hydrology to cemeteries to land use—working in concert to create a master plan for a city of tens of thousands, complete with homes, parks, schools, greenbelts, lakes and marinas, shops and industrial zones. The project involved the construction of a $3.5 million lake, stocked with catfish and bass, boasting eight elegantly designed miles of shoreline. Westlake Village was a spectacular success and is still considered among the best planned cities in the country.

By the early 1970s, Ludwig's net worth was estimated to be in the billions. He had added oilfields in Indonesia, real estate in Australia, skyscrapers and other properties in the U.S., iron and kaolin mines in Brazil and a whole lot more to his skein of enterprises.

Yet Ludwig's confidence in his own vision could be blinding and would lead him, in the late stages of his career, into a mire of his own making. Anticipating, correctly, an impending fiber shortage, Ludwig bought a tract of land more than twice the size of Delaware in the Brazilian Amazon for $3 million in 1967. His plan, named the Jari project, was to raze most of the rainforest on his property and replace it with the fast-growing Burmese gmelina tree, supplementing that fiber-making enterprise with mining and ranching operations. The project was highly controversial and became something of a political lightning rod in and even outside Brazil. Despite ample warning to drop the project, Ludwig would persist until 1982 and leave only after the political



Daniel Ludwig, right, with future President Ronald Reagan, center, and John Notter.

situation in Brazil became untenable. By some estimates, he lost nearly $1 billion, in 1981 dollars, in the enterprise.

Still, even with the press generated by the Jari project, hardly anyone outside the shipping industry knew who Ludwig was. This was entirely by design: laconic and intensely private, Ludwig detested publicity of all kinds. If frequently blunt, cantankerous and openly bored by small talk, he was also very loyal to the few friends he had, who were mainly business partners and lawyers he'd known for ages. His other friends included the actor Clark Gable, who Ludwig revered, and Richard Nixon, who was a guest at his home before he was elected president. A conservative in the old sense of the word, Ludwig held Ronald Reagan in high regard, prominently displaying a picture of himself and the future president in his Manhattan penthouse. He was said to be devoted to his wife, Gertrude Virginia Ludwig, whom he had married in 1937, just a couple of months after divorcing his first wife, from whom he seems to have been estranged soon after their marriage began in 1928.

In public, and especially in his old age, the titan kept a low profile—though it would be an exaggeration to say he was a recluse. He flew economy, used public transportation, walked to work and otherwise played the part of an ordinary if somewhat enigmatic old man with determined fidelity. He went to great lengths to keep his name out of the press, even taking his executives to task when it cropped up in print unexpectedly. And though, being one of the richest men in the world, he could have done almost anything he wanted, he confessed he had no hobbies or even interests beyond business.

Except, evidently, an abiding fascination with the conquest of cancer.

Ash Alizadeh

Risk of Second Tumors and T-Cell Lymphoma after CAR T-Cell Therapy | *The New England Journal of Medicine*, 2024 June 12

The risk of secondary cancers caused by CAR-T cell engineering found to be relatively low

As chimeric antigen receptor (CAR) T-cell therapies have been deployed in the clinic, researchers have become increasingly concerned about the risk of secondary tumors arising after treatment, especially T cell cancers related to viral vector integration into the genome during the engineering of the therapeutic T cells. A study co-led by Ludwig Stanford's Ash Alizadeh explored the actual risk of such secondary cancers in patients who had received CAR-T therapies. He and his colleagues, including several at the Stanford Center, examined outcomes for nearly 800 CAR-T treatments in 724 patients treated at Stanford since 2016 and found the risk of such cancers to be relatively low: only 25 were detected, suggesting a 6.5% risk in the three years after completion of therapy. They also examined in depth a single lethal case of T-cell lymphoma following CAR-T therapy for diffuse large B cell lymphoma, deeply profiling the cells of both lymphomas. The two lymphomas were molecularly distinct, though both were positive for Epstein-Barr virus, which can promote cancer. The researchers found no evidence of oncogenic retroviral integration as a cause for the second cancer after exploring the possibility using multiple techniques. Aside from addressing that issue, their study provides a framework for monitoring viral vectors used in T cell engineering and improving risk assessments for such therapies.

Melita Irving

Combining SiRPα-decoy coengineered T cells and antibodies augments macrophage-mediated phagocytosis of tumor cells | *The Journal of Clinical Investigation*, 2024 June 3

A new two-in-one strategy for cancer immunotherapy

Ludwig Lausanne's Melita Irving, recent PhD graduate Evangelos Stefanidis and colleagues reported in a June paper in *The Journal of Clinical Investigation* a two-pronged strategy to drive a simultaneous T cell and macrophage attack on tumors. Their approach modifies T cells equipped with affinity optimized cancer antigen-targeting receptors to additionally secrete CV1—a high-affinity version of SiRPα, which effectively silences a "don't eat me" signal cancer cells transmit to avoid being gobbled up by macrophages. SiRPα normally interacts with CD47 on the cell surface to issue that signal. Irving's team had previously engineered therapeutic T cells to produce a CV1-Fc decoy to engage a combined macrophage and T cell attack on tumors. But the first run at that strategy failed because the antibody tail fragment (Fc) they'd appended to the CV1 decoy provoked a macrophage attack on the T cells, which were coated with the construct. Expressing CV1 in T cells without the Fc tail, however, circumvented attack by human macrophages. Moreover, combining the T cell therapy with anti-PD-L1 and anti-EGFR antibodies—both used for cancer therapy—further enhanced the dual attack in preclinical studies by giving the macrophages new antibody tails to target. Aside from presenting a new therapeutic strategy, the study suggests efforts to target the SiRPα-CD47 axis have been clinically unsuccessful in part because they stimulate the clearance of tumor-targeting T cells by macrophages.

Metabolomics study suggests a strategy for improving cancer immunotherapy

Researchers led by Ludwig Princeton's Kellen Olszewski and Director Joshua Rabinowitz reported in a May publication in *Cell Chemical Biology* that tumor-infiltrating T lymphocytes (TILs), which can target cancer cells, favor the use of the de novo purine biosynthetic pathway, which requires copious 1-carbon units generated by folate metabolism. This contrasts with T cells in lymph nodes and in the spleen, which mainly rely on nucleoside salvage for purines, which are key DNA and RNA components. Like TILs, cancer cells too synthesize purines de novo. This sets up a competition for 1-carbon units between cancer cells and T cells in tumors. 1-carbon units can circulate in the body as formate. Supplementing 1-carbon units by infusing mice with formate—or orally administering low doses of methanol, which the researchers show boosts formate levels—drives purine biosynthesis in TILs. Further, methanol augments anti-PD-1 checkpoint blockade in a mouse model of colorectal cancer, tripling the incidence of durable tumor regressions. These findings align with a study reported last year, led by Ludwig Harvard's Marcia Haigis and Arlene Sharpe, showing formate supplementation boosts T cell fitness and enhances PD-1 blockade in a mouse model of melanoma. All this suggests the availability of 1-carbon units is a limiting factor in T cell-mediated anti-tumor immunity. It further offers a readily testable strategy for improving responses to immunotherapy.

One-carbon unit supplementation fuels purine synthesis in tumor-infiltrating T cells and augments checkpoint blockade | *Cell Chemical Biology*, 2024 May 16

Kellen Olszewski

Joshua Rabinowitz

Andrew Lane

Qingyu Luo

CRISPR screen identifies a testable treatment for a subset of AMLs

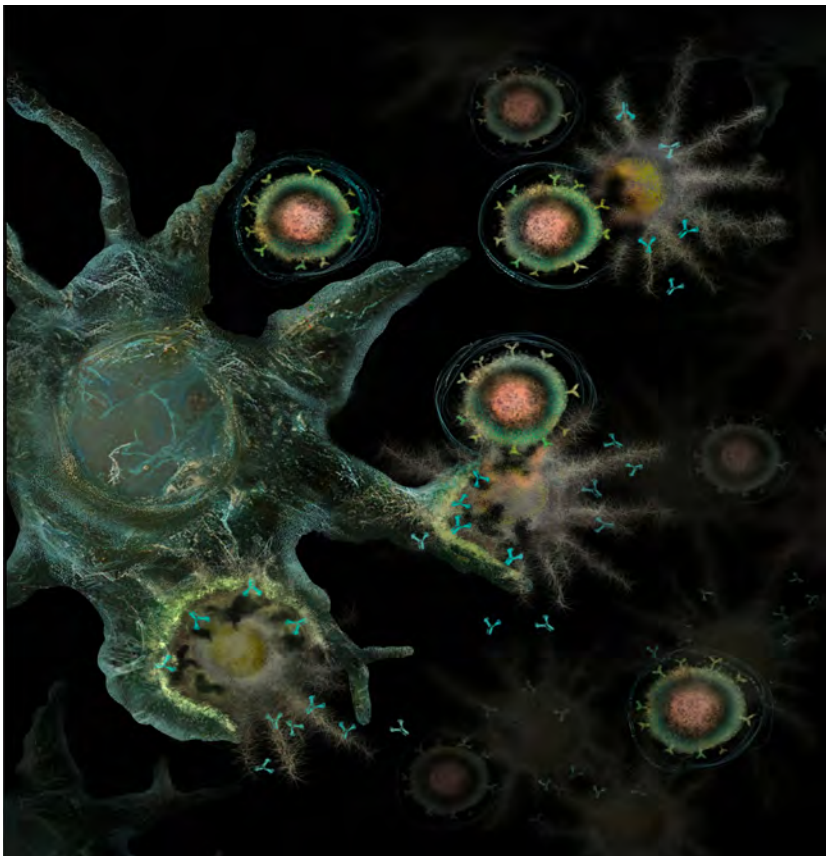
Researchers led by Ludwig Harvard investigators Andrew Lane and Qingyu Luo reported in a May publication in *Nature* that the cells of a subset of myeloid, and some lymphoid, leukemias rely on a molecular complex called PI3Kγ for survival. They also showed in preclinical studies that eganelisib—a PI3Kγ inhbitor—may prove effective in treating such cases alone or in combination with cytarabine, a drug often used to treat acute myeloid leukemia (AML). Using genome-wide CRISPR interference to identify genes that AML cells depend on for growth, Qingyu, Andrew and their colleagues discovered that the gene for PIK3R5, a component of the PI3Kγ complex, is essential to the survival of a subset of leukemia cells. Animal models of patient-derived leukemias predicted to be dependent on the complex survived longer with eganelisib treatment. Combined treatment with eganelisib and cytarabine extended survival more than cytarabine alone, regardless of the leukemia's sensitivity to PI3Kγ inhibition, suggesting the two medicines have synergistic effects. The researchers found that PI3Kγ inhibition suppresses a metabolic process called oxidative phosphorylation that susceptible leukemia cells are especially dependent on for energy generation. Cells that survive cytarabine treatment—and might seed relapses—are similarly vulnerable to eganelisib. The findings offer a scientific rationale for testing the drug combination in AML patients whose cancers express biomarkers of susceptibility to the therapy.

Targetable leukaemia dependency on noncanonical PI3Kγ signalling | *Nature*, 2024 May 8

Making peace between two promising strategies for immunotherapy

Administering CAR-T cells or T cells with engineered antigen receptors together with antibodies that block the CD47–SiRPα axis—which transmits a "don't eat me" signal to macrophages—should be a promising therapeutic strategy. But Ludwig Stanford's Crystal Mackall and her colleagues reported in a *Nature* paper in May that the combination leads in animal models to rapid clearance of both types of therapeutic T cells by macrophages, which appear to help regulate T cell persistence in tumors. The researchers noted that clearance of CAR-T cells bound by anti-CD47 antibody was in fact so swift and thorough that it could serve as a safety switch to shut down CAR-T cells if necessary. To fix the problem, the researchers engineered therapeutic T cells to express a CD47 mutant ($CD47_E$) that is still bound and activated by SiRPα but is not recognized by anti-CD47 antibodies. This protected the therapeutic T cells and CAR-T cells from macrophages by their continuing transmission of the "don't eat me" signal while leaving tumor cells susceptible to macrophage targeting after treatment with anti-CD47 antibodies. The researchers showed that the combination therapy induces significant and sustained recruitment of macrophages to the tumor, and even though many of these macrophages had pro-tumorigenic properties, the combined treatment proved sufficiently synergistic to enhance anti-tumor effects in mouse models.

Engineered CD47 protects T cells for enhanced antitumour immunity | *Nature*, 2024 May 15



Gerardo Sotillo

Artistic representation of macrophages and CAR-T cells cooperatively killing tumor cells after CD47 blockade. T cells engineered to be protected from anti-CD47 binding synergize with activated macrophages to enhance tumor killing.

Crystal Mackall

AI model predicts the best T cell receptors for personalized immunotherapy

Researchers led by Ludwig Lausanne's Alexandre Harari and Rémy Pétremand reported in a May paper in *Nature Biotechnology* a powerful computational tool for identifying the most potent tumor-infiltrating T lymphocytes (TILs) for use in personalized cancer immunotherapies. Cellular immunotherapies of this kind involve extracting immune cells from a patient's tumor, optionally engineering them to better combat cancer and reintroducing them to the body after they've been expanded in culture. But only some TILs actually kill cancer cells, and even fewer do so effectively. The tool developed by Alexandre, Rémy and colleagues to identify the best T cell receptors (TCRs) for the job, MixTRTpred, combines an AI-driven predictive model called TRTpred that can rank TCRs based on their tumor reactivity with two other algorithms developed by Ludwig Lausanne's Vincent Zoete. One predicts which of these TILs' TCRs bind most strongly to tumor antigens and the other helps maximize the diversity of targeted antigens. To validate their tool, the researchers cultivated human tumors in mice, extracted TCRs from their TILs and applied MixTRTpred to optimize their selection of TCRs. They then engineered T cells from the mice to express those TCRs and showed that these cells could eliminate tumors when transferred back into the mice.

Identification of clinically relevant T cell receptors for personalized T cell therapy using combinatorial algorithms | *Nature Biotechnology*, 2024 May 7

Alexandre Harari

Rémy Pétremand

Vincent Zoete

Liron Bar-Peled

Mapping cysteines in cellular proteins to target for cancer therapy

Roughly 400 protein drivers of carcinogenesis have so far been identified in cells, but only about 10% of these have been effectively targeted, and most of them are protein kinases—which add phosphate groups to proteins—or other enzymes. Drugs that target oncogenic drivers in different ways include a set whose members covalently and irreversibly bind the amino acid cysteine on proteins. These drugs have had some success in the clinic. Researchers co-led by Ludwig Harvard's Liron Bar-Peled applied proteomics methods to identify new targetable cysteines in key cellular proteins. They reported in *Cell* in April a quantitative portrait of such "cysteine-ligandability" across more than 400 cancer cell lines, establishing an initial framework for a cysteine ligandability map. Liron and his colleagues found that the variability in responses to cysteine-targeting stems in part from differences in the redox environment of cancer cells and mutations near the targeted cysteines. They applied discoveries made using their dataset, named "DrugMap," to develop covalent ligands that disrupted the oncogenic activity of transcription factors NF-κB1 and SOX10. DrugMap, they suggest, could help systematically uncover the rules for cysteine ligandability and aid drug development.

DrugMap: A quantitative pan-cancer analysis of cysteine ligandability | *Cell*, 2024 April 22

How radiotherapy gives a boost to metastasis—and how that might be countered

Radiotherapy (RT) can in some circumstances stimulate anti-tumor immune responses but just as effectively suppress them in others. Ludwig Chicago researchers led by Hua Laura Liang and Director Ralph Weichselbaum reported in a *Clinical Cancer Research* paper in May that immunosuppression induced by RT is systemic and can promote metastasis. The researchers implanted on each side of a mouse a tumor from a distinct source—so they'd induce distinct immune responses—and hit only one of them with radiation. This allowed them to specifically identify the immune effects of RT without having to worry about the confounding effects of shared anti-tumor immune responses. These studies showed that local irradiation elevates systemic levels of myeloid-derived suppressor cells, which inhibit immune responses, while simultaneously inducing the systemic expression of the immune checkpoint molecule PD-L1 on dendritic cells and other myeloid cells that are critical to anti-tumor immunity. These systemic effects aided metastasis from the untreated tumor to the lung. The phenomenon was accompanied by increased PD-L1 expression in the lung, which was dependent on elevated serum levels and signaling of a chemotaxis factor, CXCL10, that was produced predominantly by myeloid-derived suppressor cells. Targeting this axis, they showed, slowed tumor growth and metastasis in mice treated with RT, suggesting a therapeutic strategy for further evaluation.

Radiotherapy Enhances Metastasis Through Immune Suppression by Inducing PD-L1 and MDSC in Distal Sites | *Clinical Cancer Research*, 2024 May 01

Hua Laura Liang

Ralph Weichselbaum

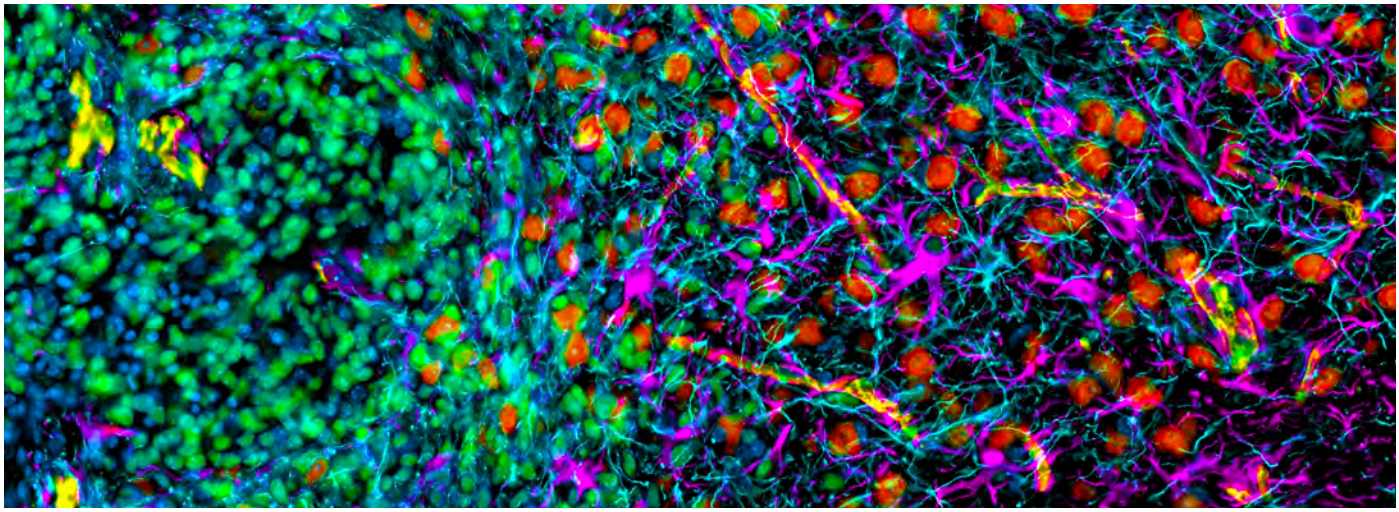
Pierpaolo Ginefra

Nicola Vannini

A potential dietary intervention to improve T cell surveillance for cancer

Researchers led by Ludwig Lausanne's Pierpaolo Ginefra and Nicola Vannini reported in an April publication in *Cancer Research Communications* that the urolithin-A (UroA)—which is produced by the metabolism of ellagic acid by certain types of gut bacteria and has been shown by Nicola's lab and others to promote mitochondrial health—can also, through an entirely different mechanism, improve T cell surveillance of cancer in mice. Its benefits for mitochondrial health derive from the metabolite's promotion of mitophagy, or the recycling of damaged and aged mitochondria, a process that slows down with aging. Pierpaolo, Nicola and their colleagues found that UroA activates the transcription factor FOXO1 in CD8+ T cells by reducing its phosphorylation and promoting its localization to the nucleus. Sustained FOXO1 activation boosts the expression of the cell adhesion protein L-selectin, which in turn drives the expansion of naïve T cells in the lymph nodes and spleen of mouse models—and this delays cancer onset. T cells grown in the presence of UroA were also better suppressors of melanoma tumors when transferred into mice. The findings suggest UroA may be a useful nutritional booster of cancer immunosurveillance, though further studies would be required to confirm the relevance of these findings to humans.

Urolithin-A promotes CD8+ T cell-mediated cancer immunosurveillance via FOXO1 activation | *Cancer Research Communications*, 2024 April 16



HIFI, a complete analytical workflow based on open-source tools, can capture cellular organization patterns across large image areas. Because it is nondestructive, HIFI could be applied to examine the cell types and structures in very fragile glioblastoma samples. Glioma cells in the image above are labeled green, vessels are yellow, astrocytes are magenta, neurons are red, neuronal axons are cyan and all other nuclei are labeled blue.

A more affordable and accessible approach to high-dimensional imaging

The tumor microenvironment (TME) undergoes profound changes in its cellular and molecular characteristics and spatial organization in response to therapy. Capturing the immense complexity of these spatial rearrangements has, however, proved difficult due to technical challenges and the cellular heterogeneity across large sections of whole tumors. Researchers led by Ludwig Lausanne's Spencer Watson and Johanna Joyce have addressed these issues with a new and complete analytical workflow, which they term Hyperplexed Immunofluorescence Imaging (HIFI), reported in an April paper in *Nature Communications*. HIFI employs open-source tools to enable whole-slide, high-magnification, high-dimensional imaging—resulting in the identification of complex cellular organization patterns across large image areas. To also help lower barriers to such analyses and increase accessibility for researchers, HIFI was devised to require no special reagents or expensive equipment. The researchers integrated HIFI—which permits the simultaneous analysis of more than 45 markers at high magnification—with machine learning and other analytical methods to examine differences in the effects of radiotherapy on the TME of glioblastoma and breast-to-brain metastases in mouse models. They discovered that despite equal benefit from radiotherapy, glioblastomas undergo extensive reorganization of structural architecture and immune cell populations in response to treatment, while brain metastases do not. This innovative approach not only provides deeper insights into tumor biology but also democratizes access to advanced imaging techniques for research and discovery.

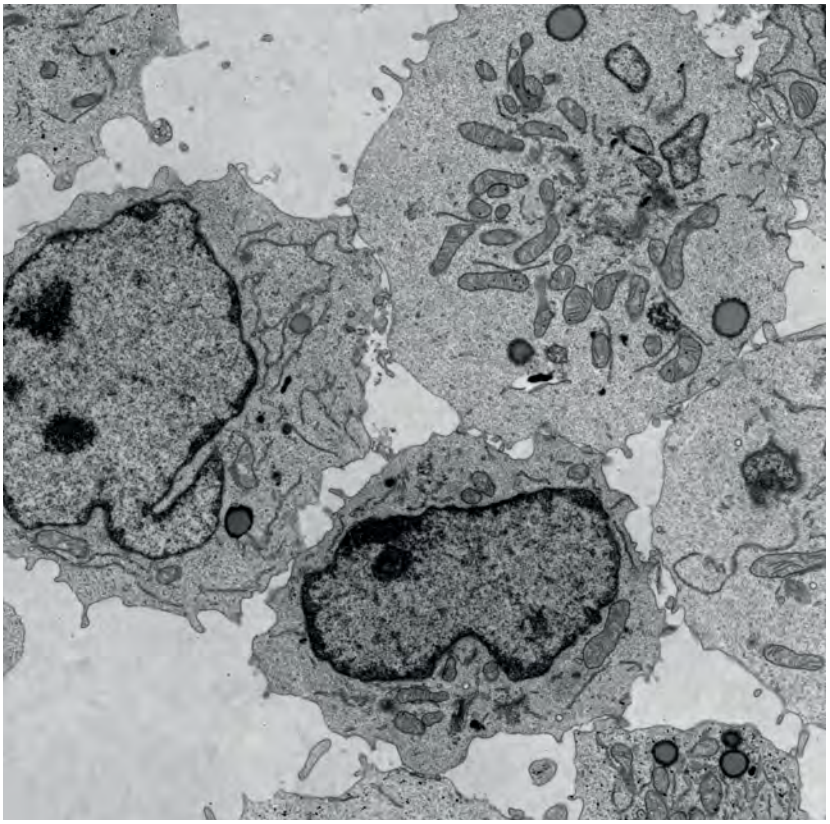
Spencer Watson

Johanna Joyce

Microenvironmental reorganization in brain tumors following radiotherapy and recurrence revealed by hyperplexed immunofluorescence imaging | *Nature Communications*, 2024 April 15

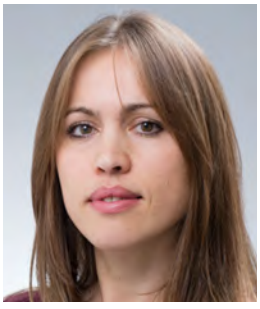
How PGE_2 saps the vitality of tumor-infiltrating T cells essential to immunotherapy

Researchers led by Ludwig Lausanne's Matteo Morotti, Alizee Grimm, Denarda Dangaj Laniti and Director George Coukos reported in an April publication in *Nature* their discovery of a mechanism by which the lipid prostaglandin E2 (PGE_2) poisons the metabolism of tumor-targeting CD8+ T cells to serve as a checkpoint against their activity. Their study relied in part on the analysis of data and samples from clinical trials conducted in Lausanne of an immunotherapy known as adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TIL), or TIL-ACT. Those studies showed that TILs bearing markers of response to interleukin-2 (IL-2)—a factor essential to the functional vitality of T cells—were the ones that expanded best in culture, a finding that was verified in cell culture experiments. The researchers discovered that PGE_2 disrupts the ability of T cells to respond to IL-2 by monkeywrenching the assembly of the IL-2 receptor's component proteins. The resulting loss of IL-2 stimulation induces metabolic dysfunction, causing a functional lethargy in the TILs known as "anergy" and ultimately triggering ferroptosis, a type of programmed cell death. Disrupting that mechanism during the cell culture processes employed in TIL-ACT dramatically improved the therapeutic potential of transferred TILs in mouse models of cancer. Their findings were confirmed by a companion study in the same issue of *Nature*.



Matteo Morotti

Alizee Grimm



Denarda Dangaj Laniti

George Coukos

G.Weber / CHUV

Above: Electron microscopy of T cells treated with PGE_2, causing lipid droplet accumulation.

PGE_2 inhibits TIL expansion by disrupting IL-2 signalling and mitochondrial function | *Nature*, 2024 April 24

The value of invalid outputs

Generative models based on deep neural networks have emerged as a powerful framework for structure-based drug design by enabling more efficient exploration of chemical space, a universe estimated to contain some 10^{60} possible molecules. The most productive of those are trained on SMILES (for Simplified Molecular-Input Line-Entry System)—chemical language models that represent molecules as strings of text. Some fraction of SMILES strings generated by these models are not viable chemical structures, and researchers have strenuously sought to limit their generation or to correct for them. Ludwig Princeton's Michael Skinnider showed in a *Nature Machine Intelligence* study solely authored by him that those efforts are unnecessary, and even detrimental: the ability to produce invalid outputs provides a self-corrective mechanism that filters low-likelihood samples from the language model output, improving the model's performance. Further, enforcing valid outputs hampers learning and limits the exploration of chemical space. Invalid SMILES, he argues, are a feature of such models, not a bug. Beyond enabling more efficient drug design, optimized models were able to anticipate the existence of so far undiscovered dietary small molecules, which could provide a technological platform to identify diet-derived small molecules that modulate cancer progression or therapeutic efficacy.

Michael Skinnider

Invalid SMILES are beneficial rather than detrimental to chemical language models | *Nature Machine Intelligence*, 2024 March 29

A faster, more accurate way to analyze the effects of thousands of mutations at once

Mutations of all sorts—deletions, amplifications, single nucleotide changes—initiate cancer and drive its evolution. But it has not been possible to swiftly and comprehensively assess the different biological effects of the thousands of distinct mutations found in cancer genomes. Researchers led by Ludwig MIT's Francisco J. Sánchez-Rivera reported in a March publication in *Nature Biotechnology* their adaptation of a type of CRISPR genome-editing system known as prime editing (PE) to do just that. PE, which employs a PE guide RNA (pegRNA) to target and directly edit the genome at specific sites, can be used to generate any type of small mutation without interfering with surrounding sequences. Francisco and his colleagues devised a method to measure the efficiency and accuracy of each pegRNA used for editing and integrated it with experimental and computational methods to interrogate the cellular effects of thousands of mutations at once. They applied their system, which could be a useful tool for precision medicine, to examine the effects of more than 1,000 mutations in the tumor suppressor p53 that are frequently observed in cancer genomes. Aside from validating their methods, Francisco and his colleagues showed that some p53 mutations are far more pro-cancerous than previous studies relying on gene overexpression had suggested—demonstrating the value of examining mutations in the more physiological context permitted by PE.

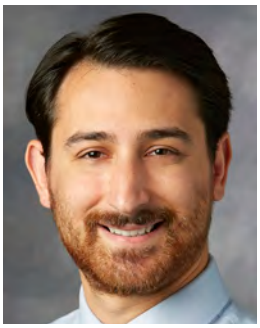
Francisco J. Sánchez-Rivera

High-throughput evaluation of genetic variants with prime editing sensor libraries *Nature Biotechnology*, 2024 March 12

A possible strategy for immune system rejuvenation

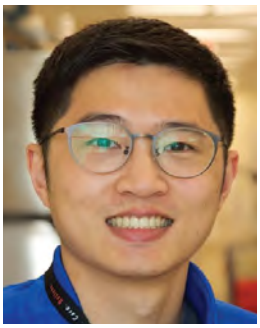
The aging of the immune system is frequently characterized by a decline in the production of lymphocytes—B and T cells, which execute adaptive immune responses—and an increase in inflammation and other pathologies associated with myeloid immune cells. Chronic inflammation, meanwhile, is associated with an elevated risk for multiple ailments, including cancer. Age-related changes in self-renewing hematopoietic stem cells (HSCs), which make all the cells of the blood, are thought to underlie these phenomena: HSCs with a balanced output of lymphoid and myeloid cells (bal-HSCs) predominate over HSCs with myeloid-biased output (my-HSCs) during youth, while aging brings with it increased proportions of my-HSCs. Transferring bal-HSCs into mice whose HSCs have been cleared has been shown to restore a healthy balance of lymphoid and myeloid cells; the opposite occurs with the transfer of my-HSCs. Researchers led partly by Ludwig Stanford's Jason Ross and Director (emeritus) Irving Weissman explored whether depleting my-HSCs might similarly restore an appropriate immune balance. They reported in a *Nature* paper in March that antibody-mediated depletion of my-HSCs in aged mice restored characteristic features of a more youthful immune system and improved primary and secondary adaptive immune responses to viral infection. Their findings may have relevance to understanding, preventing and treating diseases exacerbated or caused by my-HSC dominance of the blood generation system.

Depleting myeloid-biased haematopoietic stem cells rejuvenates aged immunity | *Nature*, 2024 March 27



Jason Ross

Irving Weissman



Hai-Tsang Huang

William Sellers

Identifying the targets of E3 ubiquitin ligases

Ubiquitylation, a biochemical modification made to proteins, is involved in multiple cellular processes, including protein degradation, and its dysregulation plays a role in several cancers. The enzymes that add ubiquitin groups to proteins, known as ubiquitin ligases, are an emerging class of drug targets. Even though 600 E3 ubiquitin ligases have been identified in humans, their substrates remain largely unknown and existing methods for identifying these targets have limitations. Researchers led by Ludwig Harvard's Hai-Tsang Huang and William Sellers reported in a March issue of *Nature Chemical Biology* their development of a method, named E3-substrate tagging by ubiquitin biotinylation (E-STUB), that broadly and accurately identifies targets of E3 ubiquitin ligases. Their method specifically labels ubiquitylated substrates in proximity to an E3 ligase of interest with biotin to enable their subsequent identification. The researchers applied E-STUB to characterize the actions of protein degraders (i.e., PROTACs and molecular glues), revealing direct as well as collateral targets—including ubiquitylation events that do not lead to substrate degradation. These results suggest E-STUB could aid new strategies for drug design, like inducing ubiquitylation of proteins central to disease processes that aren't amenable to binding by inhibitory drugs. Beyond that, E-STUB has great potential as a tool for the functional study of E3 ligases, advancing our understanding of their roles in human health and disease.

Ubiquitin-specific proximity labeling for the identification of E3 ligase substrates | *Nature Chemical Biology*, 2024 March 21

Tushar Nichakawade

Jiaxin Ge

Bert Vogelstein

Suman Paul

A new way to treat T cell malignancies

Patients with T cell leukemias and T cell lymphomas, collectively called T cell cancers, have poor prognoses. Innovative new immunotherapies that have improved survival in B cell leukemias and lymphomas have the potential to make a major impact on the treatment of T cell cancers if they could be developed. A team consisting largely of Ludwig Johns Hopkins researchers and led by Tushar Nichakawade, Jiaxin Ge, Director Bert Vogelstein and Suman Paul devised an approach using antibody-drug conjugates (ADCs) that targeted T cell cancers while sparing half of normal T cells. The rationale for this approach was that every T cell cancer in a patient expresses only one type of T cell receptor, whereas half of the normal T cells in an individual express one type, and the remaining half express the other. The remaining half of the normal T cell population maintains a functional immune system necessary for human survival. The Ludwig Johns Hopkins team's ADCs, which recognized the T cell receptor β-chain constant region 1 (TRBC1), selectively delivered a potent cytotoxin to the T cell cancers, leading to long-term cancer regressions and cures in pre-clinical models. The investigators hope that the ADCs can be tested in human clinical trials in the future.

TRBC1-targeting antibody-drug conjugates for the treatment of T cell cancers | *Nature*, 2024 March 27

Tracing how early oncogenic events influence tumor evolution

Defining how early molecular events in carcinogenesis—like the activation of an oncogene or the loss of a tumor suppressor—shape the cellular responses and later characteristics of tumors has proved challenging. Researchers led by Ludwig Oxford's Samvid Kurlekar, Joanna Lima and Peter Ratcliffe reported in a March *Cancer Research* paper their development of an experimental platform to address this challenge. They applied their method to examine the longitudinal changes induced by the loss of the tumor suppressor, VHL, which drives the kidney cancer clear cell renal cell carcinoma. The loss of VHL expression in their system was linked to the expression of a genetic reporter, tdTomato, permitting the researchers to isolate and analyze cells of interest over time via microscopic and single-cell RNA analysis. Their analysis uncovered distinct responses to loss of the Vhl protein in different cell types. The study also defined a proximal tubular cell class with oncogenic potential and revealed long-term adaptive changes in areas of the renal epithelium caused by the disruption of Vhl activity. Aside from its presentation of a new system for tracking how early oncogenic events alter cancer initiation and progression, the study offered specific insights into how Vhl suppresses tumorigenesis and how its loss drives the evolution of this kidney cancer.

Oncogenic cell tagging and single-cell transcriptomics reveal cell type-specific and time-resolved responses to Vhl inactivation in the kidney *Cancer Research*, 2024 March 19

Samvid Kurlekar

Joanna Lima

Peter Ratcliffe

Joshua Rabinowitz

Challenging an assumption about a peculiarity of cancer and T cell metabolism

When oxygen is in short supply, cells employ glycolysis rather than respiration to burn sugar for energy. Otherwise, most cells respire. This makes sense, as respiration produces 15 times as much ATP—the main currency of cellular energy—as does glycolysis. But cancer cells and activated T cells resort to glycolysis regardless of how much oxygen is available, a phenomenon known in cancer as the Warburg effect. Scientists have long believed that this is mainly because glycolysis is faster in terms of generating ATP per unit mass of protein. Researchers co-led by Ludwig Princeton's Joshua Rabinowitz interrogated this assumption and reported in a March issue of *Nature Chemical Biology* that aerobic glycolysis does not in fact generate ATP faster than respiration. Their studies employing proteomic and metabolic flux analysis found this to be true across different yeast species, T cells, cancer cells and tissues and tumors. Aerobic glycolysis instead correlates with higher levels of glycolytic protein expression, which can support the proliferation of cells if hypoxia occurs. Josh and his colleagues show that yeast strains that perform aerobic glycolysis do not grow better in aerobic conditions but do outgrow their respiratory counterparts in anaerobic conditions. Based on their findings, the researchers propose that aerobic glycolysis emerges in cells that need to maintain the capacity for growth in both low and normal oxygen conditions.

Mitochondrial ATP generation is more proteome efficient than glycolysis | *Nature Chemical Biology*, 2024 March 6

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