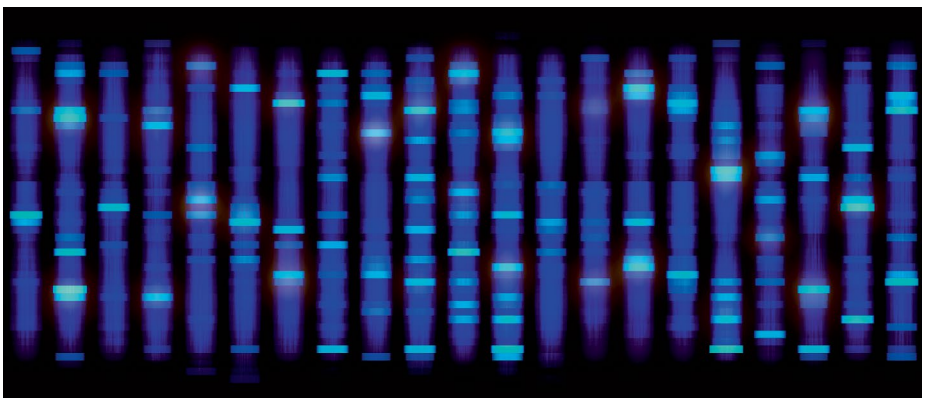
Startups probe hidden viruses in the 'dark genome' to treat disease

Drug hunters are finding that ancient virus-like artifacts in the human genome could offer new avenues to treat neurodegeneration, cancer, autoimmunity and even aging with antibodies, vaccines and antiretroviral agents.



LINE-1 and other retrotransposons have helped drive genomic evolution.

By Michael Eisenstein

A new wave of therapies is taking aim at virus-like elements spread throughout the human genome. These genomic parasites, which have accumulated over the course of human evolution, are embedded in the vast expanse of DNA sequences that dwell in the spaces between our genes — what scientists call the 'dark genome'. Although they were largely ignored in the past, growing scientific evidence indicates that these normally dormant elements — including retroviruses, transposons and other repetitive sequences — can reactivate, triggering inflammation, cancer and other disease-related cellular damage.

Several companies have recognized this reactivation as an untapped clinical opportunity and are developing therapeutics to stifle these ancient interlopers. For example, Transposon Therapeutics' lead drug candidate, TPN-101, was originally devised as an antiretroviral drug to block proliferation of HIV-1, but also appears to be a potent inhibitor of a dark genome-dwelling transposon known as LINE-1 (long interspersed nuclear element-1). This February, Transposon Therapeutics announced promising results from a phase 2 trial testing TPN-101 as a treatment for the neurodegenerative disorder progressive supranuclear palsy (PSP). Venture investors and pharma are paying attention. In September 2023, dark genome startup Rome Therapeutics reported $77 million in series B funding from major players including Sanofi, Bristol Myers Squibb and Johnson & Johnson — bringing their total take for this round to $149 million.

Many startups initially faced an uphill battle persuading investors of the clinical opportunities in the dark genome. "They're tainted with the assumption of non-functionality and non-importance," says Joseph Dukes, CSO at Oxford, UK-based Enara Bio, a company engaged in scanning the dark genome for antigens that may offer fruitful targets for cancer immunotherapy. Rosana Kapeller, CEO and co-founder of Rome Therapeutics, recalls being greeted with skepticism the first time she presented the company's strategy at the J.P. Morgan Healthcare Conference in 2020. "People looked at me and said, 'You're nuts'," she recalls.

The skepticism was understandable given that, until relatively recently, drug discovery has nearly exclusively focused on the exome: the 2% of the genome that codes for protein. But upwards of half the human genome consists of repetitive dark genome elements that have accumulated throughout evolution. For example, LINE-1 has a history dating back over 100 million years, and Kapeller estimates that this element composes roughly 20% of the genome. Most of these sequences are defunct fragments, but the roughly 150 intact LINE-1 sequences can potentially proliferate via a copy-and-paste mechanism driven in part by the LINE-1-encoded reverse transcriptase (RT) enzyme.

Human endogenous retroviruses (HERVs) — which retain similar protein-coding genes to those seen in retroviruses like HIV, but lack the ability to produce replicating particles — are another important target. Both LINE-1 and HERV sequences are normally maintained in a quiescent state via DNA methylation, but events that lead to demethylation can cause these stowaways to 'wake up', potentially triggering a powerful immune response or other pathological outcomes.

In certain cancers, for instance, there are many studies showing that certain HERVs have become reactivated. "It's basically just a reflection of tumor-dysregulated gene expression," says Birgitte Rønø, CSO at Evaxion Biotech in Hørsholm, Denmark. "Once cells undergo malignant transformation, they start expressing sequences that are normally silenced." The proteins encoded by these reawakened HERVs — particularly the envelope protein, Env — can be presented at the surface of tumor cells and contribute to the anticancer immune response. For example, George Kassiotis of the Francis Crick Institute in London and colleagues showed in 2023 that people with lung cancer who had a robust antibody response against tumor-expressed HERV proteins had better clinical outcomes and were more likely to benefit from immunotherapy.

This newly uncovered understanding that protein-coding genes are not always sufficient to identify potent antigens led Evaxion and Copenhagen-based Hervolution Therapeutics to exploit antigens expressed by reactivated HERVs to develop anticancer immunotherapies. Evaxion uses artificial intelligence (AI)-powered computational methods to identify abnormal 'neoantigen' peptides that are exclusively expressed in tumor cells as a result of cancer genome mutation and dysregulation. Their platform identifies neoantigens likely to elicit a robust T cell response,

News in brief

FDA approves first MASH drug

The first drug to treat fatty liver disease due to metabolic dysfunction-associated steatohepatitis (MASH) has been given a green light by the US Food and Drug Administration. Madrigal Pharmaceuticals' Rezdiffra (resmetirom) received an accelerated approval to treat the disease, previously known as non-alcoholic steatohepatitis (NASH). In this progressive liver condition fat buildup triggers inflammation and cell damage, leading to fibrosis and eventually cirrhosis. The complex pathophysiology of the condition and need for long-term safety have until now hindered drug discovery efforts. Rezdiffra is an oral thyroid hormone receptor-β (THR-β) agonist that improves lipid metabolism and mitochondrial activity in liver cells.

The FDA's go-ahead was based in part on a phase 3 trial published in the *New England Journal of Medicine*. Biopsy samples from over 900 people showed that fat buildup and inflammation cleared without worsening fibrosis in 25% of patients at 12 months, compared with approximately 10% on placebo. In addition, fibrosis improved without worsening fatty liver symptoms in around 25% of patients, compared with 14% of patients in the placebo group. The drug also improved low-density lipoprotein cholesterol levels.

Other clinical trials in MASH are testing candidates with diverse mechanisms of action, including Boehringer Ingelheim and Zealand Pharma's peptide agent survodutide, a dual glucagon and glucagon-like peptide 1 receptor agonist. Recent phase 2 results showed that the drug cleared liver disease in 83% of patients versus 18% in the placebo group, signaling that MASH drugs with improved efficacy might be on the horizon.

and the best candidates are incorporated into therapeutic vaccines. Evaxion added a HERV-focused algorithm called OBSERV to their workflow early this year. This approach is already bearing fruit: HERV activation seems to improve the immune system's response in some cancers. "We did a lot of bioinformatic analysis and saw that, in patients with low mutational burden, there was a benefit of having a high HERV burden in terms of survival," says Rønø. Evaxion's preclinical work has confirmed that HERV antigens can elicit an antitumor response, and Rønø says they are continuing to explore how such antigens might enhance the efficacy of both personalized and off-the-shelf therapeutic vaccines.

At Hervolution, the aim is to provoke a two-pronged immune response by eliciting both anti-viral antibodies and CD8 killer T cells against reactivated HERVs. The company is targeting solid tumors with a vaccine design that incorporates the Env protein from the retroviral element HERV-K, with a modification that makes this protein extra-immunogenic. The Hervolution team is exploring both adenoviral-vectored and mRNA-based vaccine formats, and the company has observed robust antitumor activity in animal models when their vaccine approach is combined with checkpoint inhibitors. Founder and CSO Peter Holst says Hervolution is now pursuing Investigational New Drug application-enabling studies, with an eye toward beginning clinical trials in 2025.

Cancer is not the only condition in which suppressed repetitive elements can reawaken, notes Holst. Aging, infection, environmental conditions and other factors induce cellular stress pathways, which he describes as "key inducers of demethylation." This demethylation reactivates HERVs and retrotransposons, and these elements are copied into RNA, which is then enzymatically reverse-transcribed into DNA in the cytosol, setting off alarm bells in affected cells. "Cytosolic DNA is very toxic — it's like having a virus," says Kapeller. "And then your body responds and it's like having a horrible viral infection."

An important demonstration of this pathological mechanism came in 2018 from researchers led by Yanick Crow at the University in Edinburgh. They were working with children born with Aicardi–Goutières syndrome, an ultra-rare disease in which the immune system attacks and destroys white matter in the central nervous system. They focused specifically on the role of reactivated transposable elements. Eckard Weber, co-founder and chief innovation officer at Transposon, says, "There was very compelling preclinical evidence that the interferon activation in this disease is caused by excessively elevated LINE-1." Crow and colleagues demonstrated that a cocktail of HIV RT inhibitors greatly reduces production of the pro-inflammatory interferon-α, demonstrating that they could mitigate this immune disorder by interfering with LINE-1 replication.

Transposon and Rome are both working with nucleoside analog drugs — similar to those used to inhibit HIV RT — to contain the damage in other immunological and neurological disorders where similar mechanisms are thought to be at play. However, most HIV drugs are a poor fit for LINE-1, and a 2023 *Nature* paper that included Rome researchers demonstrated why. The study produced a detailed structural and functional analysis of LINE-1 RT that revealed striking differences from its HIV counterpart. Transposon's TPN-101, licensed from Japanese biotech Oncolys BioPharma, is one of the few cross-reactive HIV RT inhibitors, whereas Rome has generated an entirely novel nucleoside analog for LINE-1 RT.

Many autoimmune conditions may involve LINE-1 reactivation, and this is the initial focus of Rome's drug development programs. The company presented promising results from a preclinical investigation of their LINE-1 RT inhibitor, RPT-A, at the American College of Rheumatology Convergence meeting in Boston this past fall. They demonstrated sharp reduction in LINE-1-associated organ inflammation in a mouse model of Aicardi–Goutières syndrome, and that abnormal LINE-1 activation is also a feature of skin biopsies from people with lupus. Kapeller says Rome is on track to launch a lupus trial this year, and is enthusiastic about the opportunities ahead. "If this is correct, this has the potential to be the first non-immunosuppressive drug for autoimmune diseases," she says, noting that other interferon-fueled autoimmune conditions could respond as well.

Transposon's Weber says that LINE-1 reactivation is a common hallmark of neurodegenerative disorders that involve tau protein aggregates in the brain, including Alzheimer's and PSP. Evidence suggests that tau accumulation induces the opening of the heterochromatin that normally suppresses LINE-1 elements. "[This] exposes these elements and gives them the opportunity to make RNA copies of themselves," he says, adding that these changes induce a damaging inflammatory response. In the company's recently completed PSP trial, they found that disease progression seemingly halted for patients on the highest

dose of TPN-101 after six months of treatment, alongside clear reductions in neuroinflammatory biomarkers. The study was small, with 42 participants, but Weber says, "There's more than enough evidence to now proceed to a pivotal trial."

Amyotrophic lateral sclerosis (ALS) is another disorder with dark genome ties. Transposon has released interim results from a phase 2 trial in ALS, and Weber says they have seen promising evidence that LINE-1 inhibition may preserve patient respiratory function — an important predictor of survival. HERVs may also offer a fruitful target here. A 2021 trial led by Avindra Nath at the National Institute of Neurological Disorders and Stroke showed that inhibition of HERV-K with antiretroviral drugs helped preserve neurological and respiratory function in patients with ALS, and a large phase 3 trial is now underway. HERV-K is a major focus of Geneva, Switzerland-based GeNeuro's research, and the company is now collaborating with Nath on a therapeutic monoclonal antibody that targets the HERV-K Env protein, which has been linked to neurological damage in ALS.

GeNeuro has employed a similar approach in other indications, with mixed results. A phase 2 clinical trial of temelimab, an antibody against the Env protein from another retroviral element, HERV-W, proved ineffective at preventing new lesions in multiple sclerosis (MS) despite considerable evidence linking HERV-W reactivation to this disease. But the company is pursuing other opportunities for temelimab, including an ongoing phase 2 trial to assess whether the antibody mitigates systemic inflammation associated with long COVID. "We see this HERV-W protein in one-third to 40% of patients," says co-founder and CSO Hervé Perron. In October 2023, Alphabet subsidiary Verily entered a collaboration with GeNeuro to further explore this connection, and Perron proposes that this reactivation could be a feature of other postinfectious syndromes as well.

The broader systemic decline associated with aging also has a dark genome component. A 2023 paper from Guang-Hui Liu of the Chinese Academy of Sciences and colleagues presented evidence that HERV-K reactivation contributes to senescence and age-related inflammation. Notably, this team included researchers from Altos Labs, the secretive anti-aging venture backed by Jeff Bezos and Yuri Milner, and the company recently filed a patent describing the use of antisense oligonucleotides to inactivate LINE-1 in age-related diseases. "I'm not surprised that they are latching onto this," says Weber. "I think a lot of people are starting to think along those lines now."

At this year's J.P. Morgan event, Kapeller described a warmer reception than the first time. "Everybody knew about the dark genome," she says. "People were informed and people were excited." But the field is still small — primarily the domain of startups, rather than big pharma — and unusually collegial. In 2022, Rome hosted the first Dark Genome Symposium, which has now become an annual opportunity for startups, clinicians, and researchers to exchange insights. "Our mantra here is: collaborate where we can, compete if we have to," says Dukes, whose company, Enara, hosted last year's meeting at the Crick Institute in London.

This collaboration is important because many challenges and mysteries remain. For example, when the causative factor is unique to humans, it becomes difficult to develop disease models. "Mouse and human are actually completely different in the dark genome space," says Marie Classon, who until recently conducted dark genome-related cancer research at the now-shuttered Pfizer Center for Therapeutic Innovation in South San Francisco. "There are some regulatory mechanisms and whatever that are the same, but the actual 'repeatome' is not the same," referring specifically to the highly repetitive elements that comprise most of the dark genome. She also notes that informatic pipelines for genome analysis have historically elided repetitive genome regions, and many 'omic procedures may need retuning to account for these blank spots on the map.

Add to that the many unknowns about dark genome structure and function, and this field can appear intimidating. But that also brings opportunity, and Kapeller is confident that it is worth the effort. "If we had given up every time that we hit the wall, we would not be here today," she says. "You have to be persistent in this business."

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News in brief

World's priciest drug treats MLD

The US Food and Drug Administration has approved the first therapy for metachromatic leukodystrophy (MLD), a rare fatal genetic disorder. The lysosomal storage disease affects about 40 children each year in the USA. It is caused by a mutation in the gene encoding the arylsulfatase enzyme that leads to progressive demyelination and progressive loss of motor and cognitive functions. There were previously no treatments. The new gene therapy, Orchard Therapeutics' Lenmeldy (atidarsagene autotemcel), has a price tag of $4.25 million, making it the world's most expensive drug. It inserts functional copies of the arylsulfatase A (*ARSA*) gene into the patient's own hematopoietic stem ex vivo with a lentiviral vector. The repaired stem cells are re-infused, correcting the enzyme deficiency and preventing the harmful buildup of sulfatide fats that cause nerve cell demyelination.

The approval is based on results from 37 pediatric patients showing that Lenmeldy improved motor impairment and survival compared with the natural history of MLD. All presymptomatic patients treated with Lenmeldy who had the late infantile form of MLD were alive at age 6 years, compared with just over half of the natural history group. Treated patients could walk and had normal language and cognitive skills. In patients with pre- or early symptomatic juvenile forms of MLD, the gene therapy also slowed motor and cognitive decline.

Lenmeldy was developed by Italy's San Raffaele Telethon Institute for Gene Therapy in partnership with GlaxoSmithKline, which sold the asset to Orchard, now part of Japanese pharma group Kyowa Kirin. The gene therapy received European Commission approval as Libmeldy in 2020. Lenmeldy carries warnings for the risk of infections, blood clots and brain swelling, among others.