DEEP DIVE, **FEATURES**

Building a better drug

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Photography by Pier Constantini

Laura Cancedda didn't set out to develop a drug for autism traits. In fact, **Cancedda**, senior group leader of the Brain Development and Disease Laboratory at the Istituto Italiano di Tecnologia in Genoa, Italy, had never studied autism at all when she realized her research on how developing neurons maintain inhibitory signaling might point toward new medicines. But it wasn't until she met researcher **Marco De Vivo** in 2015 that the idea came into focus. Together, they decided to test whether dialing down inhibitory brain activity by modulating chloride ion transport can treat the **repetitive behaviors** and social challenges that are associated with autism.

In November 2021, they co-founded a startup, Iama Therapeutics, and a month later raised 8 million euros ($8.9 million) from two Italian venture capital firms. The company plans to start clinical trials in the second half of next year to test the drug's safety in neurotypical people, and it aims to begin testing the drug in children with idiopathic autism by the end of 2024.

Yet the drug, called IAMA-6, treads familiar territory: It targets the same mechanism as an already approved blood pressure drug called bumetanide. Since 2010, at least two independent groups have run trials testing bumetanide for conditions associated with autism, and the outcomes have been highly variable. Last year, the **two most advanced clinical trials** to date testing whether bumetanide could ease autism traits in children and adolescents were halted because they did not appear to be effective.

For some autism researchers, this history is difficult to overlook. "I don't view the collective data on bumetanide as suggestive that it is helpful in autism spectrum disorder," says **Jeremy Veenstra-VanderWeele**, professor of developmental neuropsychiatry at Columbia University. "I think this program of research has been adequately explored — and is negative." But Cancedda and De Vivo are not bothered. They are working with a new class of molecule with a better safety profile. "If we maintain the efficacy but get rid of the side effect," says De Vivo, "then you can test efficacy more clearly."

The brain needs both excitatory and inhibitory signaling to function, but the balance between the two changes throughout development. Early in life, most signaling is excitatory, building new connections between neurons. Then, in childhood and adolescence, inhibitory signaling comes online, dampening connections between neurons. Inhibitory signaling is regulated by the neurotransmitter gamma-aminobutyric acid (GABA).

Chloride ions are crucial for tuning GABA signaling, and their levels are regulated by a push and pull between two transporter molecules — NKCC1, which brings chloride ions into neurons, and KCC2, which carries them out. Bumetanide blocks NKCC1, thereby dialing down chloride levels in neurons and increasing inhibitory signaling — though it's unclear how much of a given dose gets to the brain.

According to the **signaling imbalance theory** of autism, this shift from excitatory to inhibitory signaling does not fully occur in autistic people — thus the interest in using bumetanide to ease some behavioral traits associated with autism. But the drug also affects a version of NKCC1 found in the kidneys (called NKCC2) and causes a strong side effect — diuresis — which limits the dose people can take. Plus, participants in clinical trials needed frequent blood tests in the first couple of weeks to check potassium levels, and that can be intrusive, "especially for children with sensory issues," says **Hilgo Bruining**, professor of child psychiatry at Amsterdam University Medical Center in the Netherlands, who has run several clinical studies of bumetanide in autism.

> "I had this working hypothesis that was initially very wrong." Laura Cancedda

Cancedda first began exploring the therapeutic potential of chloride transporters through a series of failed studies. She was working with a Down syndrome mouse model, which faithfully recapitulates the triplication of part or all of human chromosome 21. Other studies had hinted that the issues with memory and cognition observed in these mice were caused by having too many neurons that rely on GABA, and therefore too much inhibition in the brain. But when she and her colleagues used a KCC2 inhibitor to decrease GABA activity, the animals' performance on cognitive tests plummeted even further. "I had this working hypothesis that was initially very wrong," Cancedda says.

The result was so disheartening that she halted the studies altogether. But she soon began to wonder how and why the KCC2 inhibitor was making things worse. She came across early clinical studies by Yehezkel Ben-Ari, president and co-founder of the French biotech company **Neurochlore**, which owns the patent for bumetanide as an autism treatment. At the time, Ben-Ari was also director of the Mediterranean Institute of Neurobiology in Marseille, France. He had been studying the role of GABA signaling in development and had published studies showing a

link between chloride ion transporters and GABA signaling in **epilepsy** and autism. In one, his team published a small trial showing that **bumetanide slightly improved social behaviors** in autistic children aged 3 to 11. About a year later, he reported that the drug **normalized electrical activity in the brain**, as well as social behavior, in a mouse model of fragile X syndrome and a rat model of idiopathic autism.

Some researchers were divided about the work — particularly the small clinical trial. But Cancedda saw these and other emerging studies as support for the therapeutic effect of adjusting chloride levels in the brain — not just for autism, but for other conditions, too, such as epilepsy and schizophrenia. “It became very clear that the possibility to modulate the chloride transporters — both NKCC1 and KCC2 — would be something very interesting,” she says.

In 2015, Cancedda and her colleagues showed that NKCC1 was present at higher than normal levels in the brains of Down syndrome mice, and that treating the animals with bumetanide fully **fixed their cognitive symptoms**. The result surprised her. “In Down syndrome, we have more than 300 genes that are dysregulated, so I was not expecting a full rescue,” she says. That result spurred the team on, but because of bumetanide’s troubling side effects, Cancedda thought, “OK, we need to find a new drug.”

Shortly before the paper was published, De Vivo, a medicinal chemist with a lab at the institute, attended a departmental seminar where Cancedda presented her work on Down syndrome. He approached her afterward, and a collaboration was born. They decided to try to build on work by Ben-Ari and others who were by then testing bumetanide as a therapy for autism. “The idea was pretty simple,” De Vivo says. “Can we [make] a molecule that acts the same way as bumetanide does, but without touching the protein in the kidney, which is responsible for the diuresis?”

To create the molecule, De Vivo and his team first used computational methods to virtually screen thousands of compounds in search of those that block NKCC1 but not its cousin in the kidney, NKCC2. Then, using computer modeling, they superimposed these blockers on the bumetanide molecule and fine-tuned each of the components of bumetanide to identify several new molecules that are optimized to selectively and potently target NKCC1. Then they designed, synthesized and tested the most promising ones, eventually homing in on a **new chemical class of molecule**, the most potent of which was IAMA-6. “And that is where Laura and I thought, ‘Well, maybe at this point we can start our own startup,’” De Vivo says.

This year, De Vivo and Cancedda collaborated with U.S. biologists to **publish the structure** of

NKCC1, which will allow them to more easily create other NKCC1 blockers for future studies.

Meanwhile, Iama's scientists say IAMA-6 avoids bumetanide's issues by making a beeline for the brain, dodging the kidneys completely. "Our compound so far is much more selective against NKCC1 in the central nervous system," says **Andrea Malizia**, Iama's chief executive officer. "It is much more brain penetrant and at least 50 times more selective than bumetanide — and has no toxicity at all."

Cancedda and De Vivo tested the new drug in a widely used mouse model of idiopathic autism in which the animals receive a prenatal injection of a seizure medication called valproate. Valproate-treated animals tend to groom excessively, a behavior thought to share biological roots with repetitive behaviors in people. The mice also tend to spend less time than typical mice sniffing a novel animal of the opposite sex placed in their cage, and to avoid other mice in a classic **three-chambered test**, behaviors researchers often interpret as reflecting difficulties with social interaction.

> "Our compound so far is much more selective against NKCC1 in the central nervous system." Andrea Malizia

Treating the animals with IAMA-6 **reversed these symptoms completely** — as with the Down syndrome mice — a surprising result because of autism's complexity, Cancedda says, though she does not expect to see such a dramatic effect in clinical trials. Iama is now collaborating with the contract research organization PsychoGenics to test IAMA-6 in an animal model of epilepsy. The company is also testing a different NKCC1 inhibitor in mice carrying genetic mutations that are found in autism-related conditions, such as **fragile X syndrome**, **Rett syndrome** and **tuberous sclerosis complex**.

The autism mouse data align with other animal studies suggesting that manipulating NKCC1 may change social behavior, and they provide support for testing the hypothesis in people, Veenstra-VanderWeele says.

But mouse models, and especially behavioral tests in mice, often do a poor job of predicting a drug's effect in people, says **John Jay Gargus**, director of the Center for Autism Research and Translation at the University of California, Irvine. He agrees that the company's results so far lay the groundwork for a clinical trial.

Iama is still planning its clinical trials strategy, and two members of its scientific advisory board will be helping: **Antonio Hardan**, professor of psychiatry and behavioral sciences at Stanford University in California, and **Luigi Mazzone** at the Tor Vergata University of Rome in Italy. Both conduct clinical research on autism.

The company plans to test the drug in two age groups of autistic children: 7 to 14 years and 14 to 18 years. Malizia, meanwhile, is building connections with advocacy groups and within the broader autistic community. He's well aware that clinical trials are notoriously challenging for autism drugs, which often fail in late-stage trials. "Bumetanide is proof of this," he says.

Another issue before Iama is how to best select participants for trials. Although manipulating NKCC1 to boost GABA activity makes sense for epilepsy, where hyperexcitability is a core part of the disease mechanism, says Veenstra-VanderWeele, in autism that mechanism will likely only be relevant for a subset of autistic people.

Bruining agrees. "Drugs that are developed from a specific mechanistic hypothesis will not have broad applicability in a very heterogenous spectrum," he says. That means researchers will have to use clear and specific inclusion criteria.

Last year, Bruining and his colleagues **reported** that they could use electroencephalography to determine whether children had atypical brain signaling — and they could successfully predict which children would respond to bumetanide. Also, he says, it might be possible to identify specific behavioral or sensory profiles that are likely to be present in responders.

It will also be important to pin down methods that gauge whether participants are responding, Hardan says. "There are probably compounds that we've studied over the years that failed to show positive findings — not because they aren't effective, but because the scales we are using are not sensitive enough to pick up a difference between baseline and the end of the trial."

It's not clear how changes in chloride levels would lead to the traits seen in autism — or to the other neurodevelopmental conditions in which they have been observed. And some researchers say that it's still unclear which molecule to target. The biotech firm Ovid Therapeutics is testing whether a drug that activates KCC2 can treat refractory epilepsy.

Jeremy Levin, Ovid's chairman and chief executive officer, notes that KCC2 expression increases during development, parallel to the rise in inhibitory signaling, unlike NKCC1, which is normally present at constant levels throughout life. KCC2 is also present exclusively in the central nervous system, unlike NKCC1, which is expressed in multiple cell types throughout the body, so the thought of using NKCC1 as the chloride ion transporter target gives Levin pause.

He also notes that the behavioral assessments that are used in clinical trials for autism drugs “are very difficult to define objectively.” That makes autism a risky place to start, compared with refractory epilepsy, where success or failure is much clearer to see, he says.

But even if NKCC1 regulates chloride ion levels only indirectly, the biological effect of blocking it and activating KCC2 should be the same, says Claudio Rivera, research director in neuroscience at the University of Helsinki in Finland and professor of neuroscience at Aix-Marseille University in Marseille, France. “It’s a very complicated mechanism which people actually still don’t really understand,” he says, adding that a drug such as IAMA-6 that hits NKCC1 with specificity could be enormously valuable. It would enable researchers to cleanly test the hypothesis that adjusting the level of inhibitory activity in the brain can improve autism traits. To try it, he says, “makes a lot of sense.”

The hard part of demonstrating IAMA-6’s benefits for easing autism traits in the clinic is still to come, but the company’s team is confident that the drug will make it to the finish line. Either way, De Vivo says, Iama’s strength as a company lies in its ability to create novel compounds that regulate chloride ion levels without the side effects of prior drugs — “and that is very promising.”