Researchers synthesize compound to flush HIV out of hiding

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Any hunter will tell you that when your quarry goes into hiding, you have to flush it out to get a good shot at it. Such is the case with HIV, the virus that causes AIDS.

Though antiretroviral "cocktails" can target an active infection, they cannot get at the virus when it retreats inside the host's T cells, where it may lie dormant for decades, waiting for an opportunity to burst forth in a fresh round of infection. What HIV hunters need is a good bird dog.

Now, Stanford chemist Paul Wender and his coworkers have found a way to synthesize better bird dogs, agents that can be tailored to flush HIV out into the open where the immune system and antiretroviral therapies can destroy it. Wender is senior author of a paper about the research in the May 2 issue of *Science*.

"We're not sure how far this will go, but certainly, from a theoretical point of view, it has promise of taking therapy to the next level-that is, addressing issues related to eradication of the disease, of the virus, at least," said Wender, the Francis W. Bergstrom Professor.

Wender and his co-workers Jung-Min Kee and Jeff Warrington have developed a way to synthesize prostratin and DPP, two compounds that occur naturally in plants, in the laboratory. Prostratin, found in the Mamala plant (Homalanthus nutans) that grows in the Samoan rainforest, has shown promise in previous studies as an activator of dormant HIV. DPP, a molecular relative of prostratin found in resin spurge (Euphorbia

resinifera), which grows in arid regions, also has shown potential.

Research has been hampered, though, because the compounds are difficult to obtain, particularly in the quantities needed for practical lab work on their mode of action and therapeutic potential. The yield from both plants is low and highly variable; the availability of the plants is limited; and isolating the compound is difficult. Heavy harvesting of the wild plants, especially in Samoa, also could cause ecological damage.

But synthetic prostratin and DPP, which now can be readily made in the lab, changes that equation.

"We have now minimized, if not eliminated, the issue of availability of prostratin and DPP," Wender said. "But equally, if not more importantly, we have opened access to other compounds that might be similar in structure but superior in function."

Previous work done in mice by researchers at the University of California-Los Angeles indicates that prostratin, used in combination with interleukin-7, an immune system stimulator made in bone marrow, managed to flush out and eliminate approximately 80 percent of the dormant virus. But with HIV, 80-percent efficiency is not enough. Anything less than 100 percent means the virus is still lurking in the T-cells and will spring back to action as soon as an opportunity presents itself.

"Nature has produced these compounds for various reasons in the plants from which they're derived, but certainly not to treat human maladies," Wender said. "They're not optimized for human therapy."

But with synthetic prostratin and DPP available, researchers can take the basic compounds and tinker with the structure and related function. "We could find out how to improve them by reverse engineering: figuring out

what is important and what isn't important," Wender said. "We could begin to design and synthesize molecules that would never be found in nature but might actually be therapeutically more beneficial than what has been found thus far."

In the *Science* paper, Wender and his team detail how both compounds can be synthesized, but also show the initial phase of designing and making new derivative compounds.

Although prostratin has long been used by traditional Samoan healers without their patients experiencing acute side effects, it is possible that undesirable effects could show up in an immune-impaired patient taking prostratin or DPP. But Wender noted that engineering the compounds in a lab would permit scientists to circumvent these problems. "Usually these kinds of side effects are a result of a drug hitting multiple targets. So it hits one target, which is beneficial, but then it hits some other target, too," he said. "But by modifying the structures, you could select for the beneficial activity over the non-beneficial activity."

"It's a little bit like draw poker," Wender said. "The important point is that we're not forced to use the hand we get. We'll get a hand and we'll return a few cards if we don't like it, because we can keep on tuning this until we get it right, so that a royal flush, hopefully, can be realized."

Wender's team developed their method of synthesizing prostratin and DPP by using a renewable resource, croton oil, made from the seeds of a small tree (Croton tiglium) cultivated in Asia. They derived phorbol from the croton oil and then converted it into the structure of prostratin.

The conversion process required some engineering finesse; they had to overcome a hurdle when, by removing an oxygen atom, they triggered a series of anticipated but seemingly undesired changes.

"To the credit of my coworkers, Jung-Min Kee and Jeff Warrington, they employed a strategy that sometimes is missed," Wender said. "Rather than fighting the flow, they went with it." They found a way to redirect the chemical complications into a solution to the problem that proved even better than the route they had initially sought to follow.

"Eventually they produced a shorter, more economical way of connecting our starting material, phorbol, to our target, prostratin," Wender said. The process Kee and Warrington came up with requires only five steps, which is of tremendous importance in making it economically feasible. As Wender pointed out, "steps cost money and human time."

Wender emphasized that the work of his team is the most recent chapter in efforts of a truly global community, starting with the Samoan healers, who willingly shared their knowledge with Paul Cox, an ethnobotanist who saw them prescribing a tea made from Mamala bark for patients with hepatitis-like symptoms. Cox, in turn, sent samples to the National Institutes of Health, in hopes that the bark might have antiviral properties useful in fighting some cancers. Researchers at NIH then analyzed the bark and isolated prostratin.

Prostratin belongs to a class of compounds called tiglianes, many of which promote tumor growth, so it had no initially perceived use in fighting cancer. But NIH researchers found that prostratin was not a tumor promoter and checked to see if perhaps it could help combat HIV, which is when its remarkable ability to flush out the dormant virus was discovered. Significantly, prostratin has also been found to block uptake of the purged virus, offering yet another potentially therapeutic benefit.

"The whole effort is a testimonial to a global community working to deal with what I think is a global, and top priority, problem," Wender said.

Source: Stanford University