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VA Research Currents

RESEARCH NEWS FROM THE U.S. DEPT. OF VETERANS AFFAIRS

For a new generation of cancer drugs, scientists look to targeted nanomedicine

Chemotherapy for cancer has saved many lives. But at the same time, the treatment has a serious drawback: It can be toxic to healthy cells along with cancerous ones. That can cause short-term side effects that make treatment difficult to tolerate, such as hair loss, mouth sores, and anemia. And even when chemotherapy succeeds in curing cancer, it can leave in its wake permanent "collateral damage" such as sterility and a weakened immune system.

For years, scientists have been in hot pursuit of what they see as a way to overcome the challenge: "smart" chemotherapy that zaps bad cells without harming good ones.

A team at the Minneapolis VA Health Care System and the University of Minnesota is developing a form of smart chemotherapy they think holds special promise. "[Our] concept is completely different than what's out there in the literature and what's being pursued in many labs," says lead investigator Khalil Ahmed, PhD.

The approach relies on a capsule so small that 40,000 of them could fit on the head of a pin. Both the capsule and the drug inside it are designed to home in only on cancer cells.

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Thinking 'small'—A team at the Minneapolis VA Health Care System and the University of Minnesota, including (from left) Drs. Khalil Ahmed, Gretchen Unger, and Janeen Trembley, is working on a targeted nanomedicine approach to infiltrate and kill cancer cells without harming normal tissue.

Photo by April Eilers

Study links gene to PTSD risk

Based on DNA analysis on hundreds of trauma-exposed Veterans and other volunteers, researchers with VA and Boston University have pinpointed a gene variant they say substantially increases the risk of posttraumatic stress disorder. The discovery could eventually help guide PTSD care. The findings, now online in *Molecular Psychiatry,* are the first published results from a genome-wide association study on PTSD.

Senior author Mark Miller, PhD, says the new finding is preliminary but very promising. "I'm optimistic that we're onto something real." He says the results may lead to a better understanding of exactly what goes awry in the brain in PTSD, and aid in the development of new drugs and diagnostic or preventive measures.

Miller is a clinical and research psychologist with the Behavioral Sciences Division of VA's National Center for PTSD, and an associate professor at Boston University School of Medicine.

In a genome-wide association study, or GWAS, researchers scan the entire genetic material of a person—all his or her DNA. They use chips that contain probes for millions of possible gene variants. The goal is to pinpoint variants that are more common in people with a certain disorder than in those without it.

The Boston study, which included more than 500 Veterans—many of them with

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combat trauma—homed in on the retinoid-related orphan receptor alpha (RORA) gene and one of its variants, rs8042149. The variant is a single nucleotide polymorphism, or SNP. In simpler terms, it is a change in one of the chemical bases that make up the gene. One gene can potentially have up to thousands of such variants.

Searching the entire genome for a risk variant

Most published studies to date on the genetics of PTSD have been "candidate gene studies." In these studies, researchers first sift though biomedical evidence to identify genes that appear to be involved in a certain health condition. They then examine those specific genes in patients' DNA and look for variants that may be associated with the disease. The focus, says Miller, is only on "the usual suspects"—genes known to play a role in that area of health. Such research often follows "knock-out" studies in mice, in which scientists genetically engineer the lab animals to lack a certain gene and then observe the effects on health and behavior.

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Shedding light—In the first published results from a genome-wide association study on PTSD, a team with VA and Boston University describe a gene variant that may increase risk for the condition. Research on U.S. troops who have served in Iraq and Afghanistan suggests 10 to 18 percent are likely to have PTSD after they return home.

'Our theory is that for people [with this gene variant], their neurons are less able to mount a defense against the damaging effects of stress on the brain.'

In a GWAS, in contrast, researchers start with no set idea of which gene will emerge as a marker of the condition. The human genome contains some 23,000 protein-coding genes in all.

"You just look at the data," says Miller. "It's more of a discovery approach. You can come up with a finding anywhere in the genome." He adds that since most of the genome is still an unknown—uncharted territory—and scientists know little about the role of various genes and other pieces of DNA, "chances are you're going to find an association in an area of DNA whose function you have no idea about."

The psychologist says his team was "particularly excited" when they realized that the RORA gene pinpointed in their study has been the focus of other recent research on mental health conditions. It's been implicated in attention deficit hyperactivity disorder, bipolar disorder, autism, and depression, which frequently accompanies PTSD. The gene is known to affect brain development, neuroprotection (protection of brain cells from injury or degeneration), and hormone production.

On a basic level, says Miller, the gene "detects changes in the biochemical cellular environment and responds to those changes." He and his coauthors believe changes in RORA may lead, in essence, to a meltdown on the part of brain cells when the stress level rises. In their article, the researchers wrote that the particular gene variant their study homed in on, rs8042149, may "reduce the capacity of neurons to respond to the biochemical stressors induced by traumatic stress," such as elevated hormone levels, inflammation, and free-radical damage.

"Our theory is that for people who have the RORA risk variant, their neurons are less able to mount a defense against the damaging effects of stress on the brain," Miller explains.

Findings support anatomical evidence from brain scans

He cites further evidence bolstering a possible link between PTSD and RORA. For starters, the gene codes for proteins found in

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NANOMEDICINE *(from page 1)*

The ultra-tiny capsule, or nanocapsule, is less than 50 nanometers in diameter (a nanometer is a billionth of a meter). It's the product of a collaborating lab, that of Gretchen Unger, PhD. A consultant to VA, Unger is also the founder and chief scientific officer of a Minnesota company called GeneSegues, which is now making the capsules for research. The technology is being used by scientists studying cancer, infectious disease, and other conditions.

Unlike capsules you buy at the drug store or vitamin shop, this one is invisible to the naked eye. Rather than being taken orally, the microscopic capsules would be suspended in a solution and given to patients intravenously.

Nanocapsule made from natural protein

Unger's company formulates the capsules in test tubes from a natural protein, or biopolymer. In lab experiments, the polymer breaks down within a few hours of entering a cell membrane. The cargo inside—a drug—is then freed to do its job in the cell nucleus.

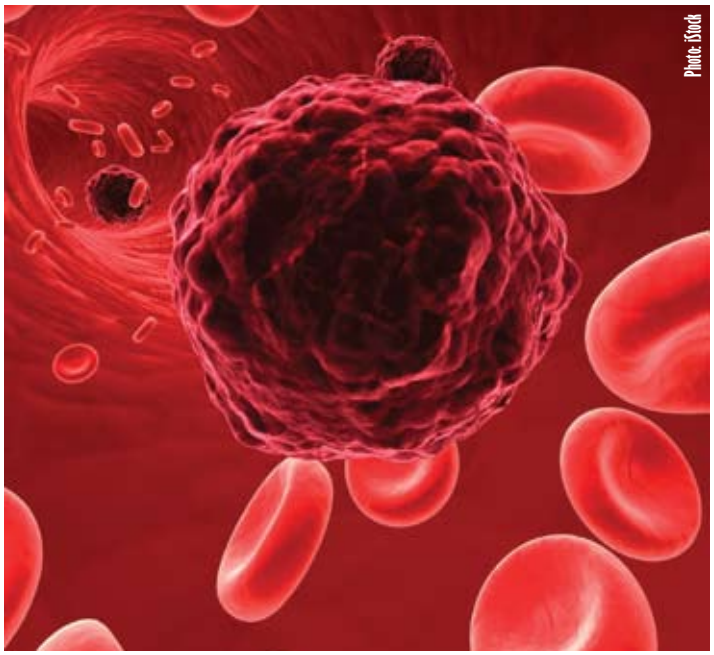
The capsules enter cells through the "lipid raft" pathway. Lipid rafts are cholesterol-rich structures in cell membranes. They manage the traffic of proteins and other molecules into and out of the cell.

The nanocapsules act like commandos that slink past guards at a tightly controlled checkpoint, headed to their target. Because of their ultra-tiny size, the capsules slip through cell membranes without setting off immune responses that could degrade the capsule or its payload on the way to the nucleus.

There's another plus to the capsule: The protein from which it's made can be changed based on the target. Ahmed's studies use a capsule made from tenfibgen, derived from a larger protein called tenascin. Importantly, tenascin receptors are abundant in the membranes of cancer cells. The capsules are drawn to them like heat-seeking missiles.

"The concept is to target a receptor [a protein on the surface of cells that acts like a molecular docking site] that's elevated in cancer cells but that is not present in normal cells," says Ahmed.

Another plus of the nanocapsule is that it is able to penetrate tumor cells within organs, such as the prostate, as well as in areas of the body to which cancer has spread, such as lymph nodes or bone. That's an important factor, says Ahmed. "A particularly serious problem in cancer therapy is dealing with metastases, as they are difficult to target."



Targeting tumors—Cancer cells, such as the one seen in this medical illustration, are the target of an experimental nanonmedicine treatment being developed by VA researchers and colleagues in Minneapolis.

Lab tests suggest the tenfibgen-based nanocapsule would be safe in humans. Partly because the capsules are made from a natural protein, says Ahmed, "They don't generate immunotoxicity, and they don't affect blood chemistry."

Double dose of targeting

Ahmed's team has tried a couple of different "cargos" inside the nanocapsule—all designed to target a substance found in higher amounts in cancer cells. This boosts the chance that only cancer cells will be affected by the therapy.

The drugs inside the nanocapsule in Ahmed's experiments target a protein called CK2. More than a decade ago, Ahmed's lab became the first to discover this molecule as a marker of cancer cells. "It's elevated in virtually every cancer we've looked at," says the scientist.

Currently, the lab is focused on targeting CK2 with two therapeutic agents, both members of the nucleic acid family that includes DNA and RNA. The two agents are known as CK2 antisense and siRNA.

CK2 appears to be crucial for cell survival. So knocking it out in cancer cells ensures the death of those cells. While some other

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Study finds 'observation' as effective as surgery in early prostate cancer

A major federal study led by VA researchers found no difference in survival between men with early-stage prostate cancer who had their prostate surgically removed and those who were simply watched by their doctors, with treatment only as needed to address symptoms if they occurred. The findings appeared July 19 in the *New England Journal of Medicine.*

"Our data show that observation provides equivalent length of life, with no difference in death from prostate cancer, and avoids the harms of early surgical treatment," said lead author Dr. Timothy Wilt. He called observation "a wise and good choice for many men." Wilt is a physician-researcher with the Center for Chronic Disease Outcomes Research at the Minneapolis VA Healthcare System and a professor at the University of Minnesota.

Photo: iStock

Tough decisions—A VA-led clinical trial has shed new light on the treatment options available to men with early-stage prostate cancer. There are some 240,000 new cases of the disease each year in the U.S.; about one in six men will get prostate cancer during his lifetime.

Study involved more than 730 men at 52 sites

The randomized trial involved 731 men and took place at 44 VA sites and eight academic medical centers. Known as the Prostate Cancer Intervention Versus Observation Trial, or PIVOT, the study was conducted and funded by VA's Cooperative Studies Program, with additional funding from the National Cancer Institute and the Agency for Healthcare Research and Quality.

One group of men in the study had a radical prostatectomy—surgical removal of the walnut-sized prostate. Surgery is generally performed in the belief it can lower the risk of prostate cancer spreading and causing death. Evidence has been lacking, though, as to whether the treatment is worth the risks, especially for men whose cancer was initially detected only on the basis of a blood test—the prostate specific antigen (PSA) test. In most cases, these tumors are not large enough to be felt during a doctor's exam and do not cause any symptoms.

The other men in the trial were in the "observation" group. With this approach, physicians generally do not provide immediate surgical or radiation therapy. Rather, they carefully follow men and provide treatments aimed at relieving symptoms, such as painful or difficult urination, if and when the cancer progresses and causes bothersome health problems.

The trial followed patients between 8 and 15 years. When Wilt's team analyzed the results, they found no difference in death rates between the two groups, either from any cause or specifically from prostate cancer.

In terms of quality of life, the surgery group experienced nearly double the rate of erectile dysfunction—81 percent versus 44 percent—and roughly three times the rate of urinary incontinence—17 percent versus 6 percent. Bowel dysfunction was similar between the groups, 12 percent versus 11 percent.

Wilt said that while the results show observation is a good choice for many men, the equation may be different for those with higher-risk tumors. PIVOT found no difference in outcomes between the two groups for men with PSA values of 10 or less. But Wilt said there may be a survival benefit to surgery for men with PSA scores above 10, or other clinical results indicating more aggressive tumors.

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It's not a stretch—Recent research at the San Diego VA Medical Center confirms the benefits of yoga for women with low back pain. *See item below.*

Vitamin D and prostate cancer—Investigators at the Charleston VA Medical Center and the Medical University of South Carolina have seen encouraging, if somewhat mixed, results so far from research on the effects of vitamin D supplementation on men with low-risk prostate cancer. About 45 patients took part in the trial. All were being treated with "active surveillance"—repeated tests of their prostate-specific antigen (PSA) levels, and periodic biopsies to check for cancer progression. The men all took daily soft gels containing 4000 IUs of vitamin D. No significant changes were noted in the men's PSA levels, on average, but 55 percent showed improvements in biopsy results after one year on the vitamin D supplements. In comparison, 11 percent showed no change on this measure, and 34 percent showed some worsening. The researchers are now conducting a follow-up study to further test the theory that vitamin D may help slow prostate cancer progression. (*Journal of Clinical Endocrinology and Metabolism,* July 2012)

Women with back pain may benefit from yoga—A study at the VA San Diego Healthcare System suggests that women Veterans with chronic low back pain can see improvements in pain and energy levels and overall mental health status from participating in yoga. The study assessed the women before and after they took part in 10 weeks of yoga practice in classes and at home. According to the researchers, the women in the study showed greater improvements than did men who had completed a similar program. A more rigorous trial is now being designed to further study the benefits of yoga for low back pain in both male and female Veterans. Yoga programs are now in place at numerous VA medical centers, and VA researchers at several sites are studying yoga's potential benefits for a wide range of physical and mental health conditions. (*Journal of Complementary and Alternative Medicine,* online July 19, 2012)

Hormone boosts resistance to obesity—In a recent review article, researchers with VA and the University of Minnesota explained what they have learned so far about an obesity-fighting hormone called orexin. The hormone helps regulate what the scientists call "spontaneous physical activity," or SPA. The work on orexin and SPA is part of ongoing research by the team on brain mechanisms that trigger weight gain and weight loss. Unlike higher-intensity formal exercise, SPA is the simple moving around and unconscious fidgeting that people do throughout the day. The researchers have found that orexin increases SPA and in turn can reduce the likelihood of weight gain. In lab experiments, for example, rats fed a high-fat diet were less prone to gain weight when they received brain injections of orexin. The scientists say learning how to safely alter orexin levels—or otherwise affect the brain's SPA pathways—is "an attractive target for obesity prevention and therapy." (*Annals of the New York Academy of Science,* July 17, 2012)

Can expressive writing ease Veterans' readjustment?

"I was a fighter jet mechanic in the military. I could handle 20 some-odd jets on an hour turn time with people screaming at that they needed their stuff done yesterday. I used to be able to struggle with a part for hours, in the freezing cold, again with people screaming at me some more, and not really feel the stress. Yes, there was stress. But possibly it was the 'good' stress that people talk about. Now, something goes a little wrong and I flip out and get angry, and anger leads to not thinking straight, and not thinking straight leads to rage! It just isn't a good thing for anyone involved. ..."

The former Air Force staff sergeant who wrote the words above, part of a blog post on the Web site *notalone.com,* is one of thousands of Iraq and Afghanistan Veterans who are expressing their feelings and struggles in writing—some publicly on the Web, in writing groups, or in published books; and others in the privacy of their own journals.

Now, VA researchers are studying the power of expressive writing to help Veterans readjust. More than 1,000 Veterans nationwide have taken part in a study based at the Minneapolis VA Healthcare System. The study is the largest yet on expressive writing. The researchers, led by Nina Sayer, PhD, are assessing the impact of writing on posttraumatic stress, social support, physical health, life satisfaction, and other outcomes.

The Iraq and Afghanistan Veterans in the study, recruited through VA and Department of Defense sources, are randomized to either expressive writing or one of two "control" activities. One control group is asked to write factually about the information needs of new Veterans. The other is not given any writing assignments.

The writing participants visit a Web site where they receive instructions and write

for at least 20 minutes, on up to four separate days within a 10-day window. For the expressive writing group, the topic is their reintegration challenges, the transition from soldier to civilian. They are asked to "write about their deepest thoughts and feelings around the topic," says Sayer, and to not worry about grammar and spelling.

Past studies on expressive writing have shown its therapeutic value for people with various health conditions and adjustment problems. But this is the first time the approach has been studied specifically as a way to help Veterans cope with reintegration.

To make the therapy as accessible as possible, the researchers created a Web site where the Veterans could do their writing.

"You don't need to be clinically diagnosed," notes Sayer. "You don't need a clinician to refer you to a specialist. You don't need to wait for an appointment with that specialist. And you don't need to leave work or travel to a clinic."

Expressive writing can stand alone as a therapy, or potentially work as an adjunct to other treatment, such as psychotherapy, says Sayer. She says some Veterans may think about starting psychotherapy as a result of their writing, and some may bring issues from their writing into their therapy sessions.

"It could be that for some people, the experience of writing about their reintegration issues may lead them to think about trying therapy, when they hadn't thought about doing so before," notes Sayer.

To help create a therapeutic writing experience for participants, the writing samples, all done online, are not shared with anyone. Sayer's team, however, does review them for safety concerns, such as violent or suicidal thoughts. When appropriate, the team reaches out directly to Veterans to try to connect them with the help they need, or refers the writing samples to a clinician outside the research team for possible follow-up with the Veteran. The writing Web site itself also offers referral information.

"We have over 2,500 writing samples at this point," says Sayer, "and there were only about a dozen we flagged and followed up on in one way or another." She says they have not had to do a "warm transfer" to VA's suicide hotline team, or summon the police to a Veteran's home, though they are able to take such actions in extreme cases.

While the researchers don't anticipate dramatic impacts from the writing intervention, they do expect positive results. The key, they point out, is that expressive writing is a low-tech, low-cost activity that Veterans from all backgrounds can easily access. "It has the potential to help a large number of people, even if the effect is small to medium," says Sayer. "Because of this reach to a wide population of Veterans, it could make an important difference."

Couples coping with PTSD—A clinical trial conducted by the Boston VA Healthcare System and a university research center in Toronto found benefits from a 15-session therapy program for couples in which one partner had posttraumatic stress disorder. Eight in ten of the PTSD-affected partners who received the treatment no longer met the diagnostic criteria for PTSD, versus only two in ten among those on the wait list. The gains were largely sustained three months after therapy ended. (*Journal of the American Medical Association,* Aug. 15, 2012)

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drugs might deliver a crippling blow to cancer cells, anti-CK2 drugs inflict a fatal wound—at least in cell culture and animal studies to date.

Some researchers have expressed concerns because CK2 is also found in normal cells, and the protein is essential for those cells to stay alive as well. So delivering an anti-CK2 therapy that is somewhat sloppy, that is not perfectly targeted to cancer cells, could be highly toxic and have dire side effects. Normal cells, however, show some resistance to undergoing down-regulation of CK2, as compared with cancer cells. Moreover, it appears that cancer cells are strongly "addicted" to CK2, such that blocking production of the molecule rapidly kills off these abnormal cells.

Packaging CK2 inhibitors in a nanocapsule, says Ahmed, is further insurance that only cancer cells would be zapped. In an article earlier this year in *Cancer Letters,* he and his coauthors wrote: "[Nanoencapsulation] of a CK2 small molecule inhibitor or siRNA would hold an even greater potential for elimination of tumor in a targeted manner and with minimal potential of host toxicity."

He hopes the pairing up of the anti-CK2 drugs with the nanocapsule will be the perfect marriage: a happy occasion for medical research, and one that spells doom for cancer.

Millennium Cohort Study finds sparse evidence of lung damage from burn pits—Analyzing the military and health records of nearly 23,000 Iraq and Afghanistan Veterans, researchers with VA and the Department of Defense found little evidence of a higher risk of respiratory problems among those who served near burn pits. The military had used open-air burn pits to incinerate a wide range of trash items, such as chemicals, paints, medical and human waste, plastics, wood, and food. The new analysis was part of the large, ongoing Millennium Cohort Study (MCS). The investigators looked for links between service within two, three, or five miles of known burn pits and new cases of bronchitis, emphysema, asthma, and other breathing problems. The Service members and Veterans in the study had served with the Army or Air Force and were surveyed during 2004 to 2006 and again during 2007 to 2008. Increased symptoms were seen among those who served within two miles of Joint Base Balad in Iraq, but the finding was statistically weak and no trend was apparent. Last year, the Institute of Medicine found inconclusive evidence of a link between burn pits and long-term respiratory problems but recommended further study. VA is continuing to look at the issue and is hosting a scientific conference, along with Defense, on Aug. 21–23 for government and academic experts to review scientific and medical evidence and consider approaches to diagnosis, exposure assessment, care, surveillance, and research. The meeting is part of a broader VA-DoD action plan on the issue. For more information, visit *www.publichealth.va.gov/exposures/burnpits/index/asp.* (*Journal of Occupational and Environmental Medicine,* June 2012)

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Only about one in five men in PIVOT had tumors classified as high-risk. Wilt said this proportion is representative of U.S. men with an early-stage prostate cancer diagnosis based on PSA testing and follow-up biopsy. Prostate cancer is usually slow-growing, and most men with PSA-detected prostate cancer do not die from the disease or develop health problems related to it, even if it is not treated with surgery or radiation. Nonetheless, many men choose more aggressive treatment nonetheless. Says Wilt, "Concerns about cancer spread and death often fuel the desire on the part of both patients and providers for some type of early intervention."

Wilt's view was echoed by Durado Brooks, MD, MPH, director of prostate and colorectal cancers at the American Cancer Society. He said that when faced with a diagnosis of prostate cancer, most men choose to take some action. "This is partly because we haven't had any good long-term studies, but it's difficult to get past the fear of the word 'cancer,' he told *Bloomberg News*. "The [PIVOT] study shows the value in taking a step back and looking harder at watchful waiting or active surveillance."

Cutting-edge research— A genome-wide association study that included more than 500 Veterans—many of them with combat trauma—identified a particular gene variant that may boost the risk of PTSD.

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brain regions that help with emotional regulation and behavioral control, such as the hippocampus, anterior cingulate cortex, and prefrontal cortex. And at least one study has linked RORA expression levels to the thickness of the brain's white and gray matter. Interestingly, brain scans of those with PTSD have also found reduced volume in certain brain areas, including the hippocampus. One theory is that the volume shrinks in response to trauma and the chronic stress that follows. Another is that people who inherently have less volume in those areas may be more susceptible to PTSD in the wake of trauma.

Either way, Miller's team is eager to further explore the RORA-PTSD link. "We hope to connect with colleagues doing structural imaging," he says. The team plans to work with colleagues at VA's Boston-based Translational Research Center for TBI and Stress Disorders. One of the questions to be answered: Will Veterans with PTSD who have the "rs8042149" variant show signature patterns of structural brain changes?

The group will also look at other RORA variants that may play a role. The probe-containing chip they used in their GWAS tests for only 600 or so RORA variants. "There are actually thousands of potential variants of the gene that could be tied to PTSD," Miller points out. The researchers have their work cut out for them, but having a focus on a particular gene may pave the way for future progress.