Five hundred years ago, Leonardo da Vinci drew this sketch of incendiary bombs. In case someone else is sketching a bomb today, in particular a dirty bomb or other radioactive weapon, a team at Pitt is developing experimental protective therapy.
It’s as alarming as it is plausible to Joel Greenberger. He believes that at some point in the next decade or so, someone will detonate a fission bomb, a dirty bomb (in which radioactive material is dispersed by conventional explosives), or release radioactive material into the wind.

“When I go around to meetings, I carry around a briefcase with a 25-pound barbell plate in it,” Greenberger says. Twenty-five pounds of fissionable material is all that’s needed to build a Hiroshima-type bomb. “I put it in the back of the room, and I have someone open it. Then I explain that, essentially, if it went off in this building, it would be enough to wipe out Oakland, and there would be a good million people who’d experience some degree of fallout.”

Obviously, some people would be beyond help in such a grim scenario, but Greenberger is looking for a way to reduce or eliminate the deleterious effects of fallout for those who’d survive. With others, he’s testing new therapies that seem to offer protection from radiation exposure.

Greenberger, an MD who is chair and professor of radiation oncology in the University of Pittsburgh School of Medicine, has about $13 million in federal grant money set aside for the job.

In 2005, the National Institute of Allergy and Infectious Diseases bestowed $10 million, with which Pitt established a Center for Medical Countermeasures Against Radiation. Greenberger, the grant’s principal investigator, was charged with developing and testing small molecules that can be used to mitigate the effects of ionizing radiation.

The Biomedical Advanced Research and Development Authority of the U.S. Department of Health and Human Services awarded a second grant of $2.7 million in 2008. This grant can be renewed for up to three years and $9.8 million.

That money is intended to help Greenberger and colleagues develop and deliver drugs that combat radiation exposure. His collaborators include Valerian Kagan, a PhD and Dsc professor in Pitt’s Graduate School of Public Health; Peter Wipf, a PhD University Professor in Pitt’s Department of Chemistry; and John Lazo of the School of Medicine, a PhD who is the Allegheny Foundation Professor in the Department of Pharmacology and Chemical Biology and codirector with Wipf of Pitt’s Drug Discovery Institute.

This journey began, as many things do in science, with a different intention. In 1993, Greenberger was investigating ways to improve cancer care by selectively protecting normal tissue from radiation therapy.

Ionizing radiation harms cells by creating superoxide, which causes DNA damage and leads to cell death. Greenberger found that overexpressing manganese superoxide dismutase (MnSOD), an enzyme that all cells possess, converts superoxide into hydrogen peroxide. Other molecules then come along and turn the hydrogen peroxide into water. Removing superoxide prevents DNA damage.

Wipf, the chemist, has engineered a drug called JP4-039 that functions like MnSOD. Both target the mitochondria—the cell’s power plant. When MnSOD is delivered directly to the mitochondria, Greenberger says, it disrupts the chemical signals caused by radiation that lead to DNA damage and cell death.

Greenberger and colleagues are now in the process of testing JP4-039’s safety and efficacy. They’ve begun animal testing. Shortly after the results are in, Greenberger hopes to begin a phase I trial in nonirradiated people to show JP4-039 is safe for humans.

In another avenue of study, a Pitt research team led by Greenberger found in 2004 that resveratrol, an antioxidant in red wine and many plants, also offers effective protection against radiation exposure. Researchers are investigating whether it can be used clinically.

Greenberger says a drug like JP4-039 could be distributed at an earlier point in the testing cycle if the situation requires it. “Let’s say we’re in the middle of a phase I trial, and we’ve given the drug, and people aren’t getting sick [from it]. A bomb goes off someplace; the FDA could fast-track production of what’s in the pipeline.”

Work is progressing on delivering JP4-039 through a transdermal patch, similar to how some birth control medications are administered. Safety is especially vital in this case, Greenberger says, because there won’t be time to determine who has been exposed to radiation in the wake of a nuclear incident. The drug would be given to everyone who seeks treatment. “A lot of people who come in to emergency rooms 24 or 48 hours after a bomb won’t need anything, but they won’t know they don’t need anything, and they’re going to be scared,” he says.
Herpes is Greek for “crawl” or “creep,” referring to the way its lesions spread across the body. Known to science since the Greeks, it’s creepy in another way. It hangs around the body in a latent state, often going years without causing any symptoms. At certain times and under certain conditions, the virus reacts, causing infections and collateral damage the immune system exacts on the body’s own tissues. Why the virus reacts, or remains latent, has been a major source of interest to immunologists and virologists for decades.

The University of Pittsburgh’s Robert Hendricks has been fixated on this question for almost 20 years. Hendricks, a PhD, and his team recently found out how the body keeps these virus sleeper cells at bay, through what amounts to an immunological commando raid. Hendricks is the Joseph F. Novak Professor and vice chair for research in ophthalmology; he’s also a professor of immunology, as well as microbiology and molecular genetics. His lab studies herpes simplex virus type 1. HSV1, as it’s called, is the lesser-known, “above the belt” cousin of HSV2, or genital herpes.

HSV1 typically hides out in the trigeminal ganglion, a bundle of nerves just underneath the brain at the top of the brain stem. These nerves innervate the cornea and parts of the mouth and face, offering a convenient portal through which the virus spreads. The viral DNA migrate into the cellular nuclei of neurons in the ganglion, where the virus goes into a latent state. When reactivated, it travels down the axons from the ganglion to the outer tissues, vexing its host with cold sores or “fever blisters” on the mouth and lesions on the cornea, which can lead to blindness.

Until about 10 years ago, scientists assumed the immune system had nothing to do with keeping the virus in a latent state because the latent virus didn’t emit any telltale proteins. But in the 1990s, Hendricks and colleagues at Pitt were among the first to document the immune system’s role in keeping the virus dormant. The researchers found a class of lymphocytes called CD8T cells conspicuously clustered around latently infected neurons, like guards around a “high-value” prisoner.

But what is it about these CD8T cells that prevents the virus from reactivating? These cells can work in one of two ways—by scrambling the viral replication sequence or by killing the host cell. This latter function is performed by cytotoxic molecules called lytic granules. The granules are more or less immunological grenades. This is a common way CD8T cells kill viruses. Mice deficient in lytic granules, for instance, are inordinately susceptible to Ebola and HIV infection. The method works relatively well in muscle and skin cells, but neurons are another matter: They don’t grow back, at least not as easily.

So the investigators assumed the CD8T cells were scrambling the viral DNA to prevent replication. Jared Knickelbein, an MD/PhD student in the Medical Scientist Training Program and a member of Hendricks’ lab, tested this theory. He gave one group of latently infected neuron cultures CD8T cells with faulty lytic granules, the other normal lytic granules. Knickelbein and Hendricks expected the groups to have the same incidence of reactivation. They didn’t. Neuronal cells were more likely to reactivate if their T cells were deficient in lytic granules. “Jared went ahead and showed us we were wrong,” Hendricks says. They looked closely at the neurons. The lytic granules weren’t killing the host cell.

The Hendricks team had found something new—lytic granules that attacked the virus but kept the host cell alive. Kill the soldiers, save the building.

But how?

The group focused on a common and potent lytic granule component, granzyme B, which is a protease—an enzyme that cleaves proteins.

“We thought, ‘What if this protease was cleaving a viral protein essential for the virus to replicate?’” Knickelbein recalls. With help from bioinformatics software, Knickelbein and Hendricks examined the amino acid sequence of potential target proteins—cleavage sites, in scientific argot—and found one sequence within an important protein in viral replication, ICP4 (infected cellular protein 4). This seemed to solve the puzzle—granzyme B cleaved ICP4 early on in the viral replication process, stopping the process inside the neuron, with minimum damage to the host cell.

This discovery, published in Science in 2008, could pave the way toward a herpes vaccine.
When MD/PhD student Jenny (pronounced Yenny) Linnoila began her PhD studies at the University of Pittsburgh in 2003, her adviser, Zuo-Zhong “Z.Z.” Wang, then an associate professor of neurobiology, invited her to investigate one of the great unknowns of the neuromuscular junction—the place where, as the name implies, nerves and muscles meet.

But first he warned her that it wouldn’t be a walk in the park. “Do you want to do something that the world hasn’t figured out yet?” she recalled him saying. “It’s really exciting, but it isn’t easy.”

After decades of research, neurobiologists learned that as a fetus develops, the motor neuron secretes a protein called agrin, which binds to a receptor on the muscle surface called muscle-specific kinase, or MuSK. If anything keeps agrin and MuSK from getting together—for example, an autoimmune disease that can damage MuSK—the lines of communication between nerve and muscle become disrupted, and the junction cannot function.

Normally, the brain’s messages to get moving travel down the spinal cord and through the motor neuron. When a message reaches the neuromuscular junction, the nerve releases onto the muscle a neurotransmitter called acetylcholine. In a healthy muscle, clusters of acetylcholine receptors (AChRs) are already formed along the neuromuscular junction, right where they’re needed. Because of this “clustering,” the AChRs can work together to relay the message to the muscle, causing it to contract. When these clusters can’t form, the junction loses function, and the muscle weakens.

“Say you have a nerve injury,” says Linnoila. “When the nerve degenerates, all these receptor clusters spread out with no direction and just go anywhere on the muscle-cell surface.”

Wang posed to Linnoila a fundamental question about what puts bodies in motion: What causes clusters to form during development and to hold their ground throughout our lives? MuSK seemed a likely party to it. For one thing, MuSK is a kinase, a class of proteins known to be great communicators. For another, MuSK isn’t found anywhere else in the body besides the neuromuscular junction. So what does MuSK talk to?

At Wang’s suggestion, Linnoila did a biological assay to hunt for proteins that bind to MuSK; if any proteins did, he reasoned, they were likely to be involved in clustering.

“Lo and behold,” says Linnoila. “In particular, one protein called Tid1 bound very strongly to MuSK. Basically, you put those two together, and they’re married.”

Linnoila believed strongly in the hypothesis and spent the next four years building the case. Studying nerves and muscles in rats from embryo through adulthood, Linnoila found MuSK and Tid1 in wedded bliss. She showed that if you knock out Tid1 in a model of neuromuscular development, clusters never form. And if you knock out Tid1 at a later stage in the life cycle, the clusters fall apart, the AChRs scatter, and the muscle weakens.

Wang accepted a faculty position at the University of Southern California in 2005, so Linnoila moved to Los Angeles to continue her work. (She continued to meet regularly with her Pitt PhD committee.) Wang shepherded her through the exhaustive process of getting a paper on her studies accepted in *Neuron*. It was published in November 2008.

Then, last summer, Wang died in a hiking accident. “It was horrible,” says Linnoila. Like any scientific father and child, they’d grown close. She spoke at Wang’s funeral and dedicated the paper to him. She marvels at the skills he demonstrated as a scientist and as a mentor.

Now Linnoila is about to begin her residency in neurology at Harvard University. Once she finishes, the young physician-scientist looks forward to resuming her research, excited by the patient populations that might eventually benefit from it, among them people with myasthenia gravis and muscular dystrophy.

And when she talks about these possibilities, she looks up and smiles.

“It’s like Z.Z. said, ‘It isn’t easy.’ But it’s worth it.”