That observation 10 years ago sent Lyden’s career in a new and controversial direction. The physician-scientist at Weill Cornell Medicine in New York City has since become convinced that a primary tumor—the first to grow in a person’s body—spews protein- and RNA-packed vesicles known as exosomes into the blood that have a powerful impact in distant tissues. Far from being passive trash bags, as some have thought, the exosomes help cells from the primary tumor take root in other organs, or metastasize, the process that ultimately kills 90% of cancer patients.

In a series of provocative papers, he and his colleagues have injected exosomes from cultured tumor cells into mice and observed striking effects on metastasis. They conclude that exosomes “prime” certain organs by making them more hospitable to primary tumor cells and “educate” other, nontumor cells to help the new cancer flourish.

Lyden’s ideas run counter to the traditional view of metastasis, which is that cells shed from the primary tumor simply take root in distant organs and grow. “Most cancer biologists think the tumor cell dictates every part of the metastatic cascade,” Lyden notes. But he believes that exosomes prepare metastatic sites by changing tissue so it can nourish tumor growth long before cancer cells arrive.

“Tumor cells are pretty much an innocent bystander. They go to organs where pre-
metastatic niches are already established by tumor-secreted factors including exosomes,” he maintains.

If Lyden is correct, tests for blood-borne tumor exosomes might provide a warning that a cancer is about to metastasize. His work also raises the prospect that targeting these vesicles with drugs could thwart the growth of metastatic tumors.

Lyden’s ideas are “very interesting,” says cancer biologist Raghu Kalluri of MD Anderson Cancer Center in Houston, Texas, who also studies the vesicles. But in his view, “It’s an open book still. Nothing is completely proven about exosomes and metastasis”—much less the relevance to human disease and treatments. Indeed, some biologists say Lyden has stretched his conclusions beyond what his data support. They question his work on several grounds—for example, that injecting mice with a large number of exosomes from cultured cancer cells may not entirely reflect what happens in a living animal.

One Lyden collaborator says skepticism is a natural reaction. His proposal “is mind-boggling,” says cancer biologist Mina Bissell of Lawrence Berkeley National Laboratory in Berkeley, California. “When someone is brave enough to make observations that are turning the field on its head, that’s where the uncomfortableness comes in,” she says. “People start bashing it.”

EXTRACELLULAR VESICLES, membrane-enclosed packets released by many types of cells, were first noticed more than 30 years ago emerging from red blood cells. But interest picked up in the mid-1990s when immunologists realized these vesicles did more than help cells discard unneeded proteins—they could also help immune cells communicate (Science, 24 June 2005, p. 1862). Many cancer biologists have homed in on the smallest of these vesicles: exosomes, vesicles less than 100 nanometers in diameter, which are produced in abundance by tumor cells.

In the last few years, researchers have found that these tumor-derived exosomes can help a primary tumor invade neighboring tissue by transferring their cargo to adjacent, normal cells and influencing their behavior. Some studies have found that exosomes and larger vesicles can pass on oncogenic proteins that make the cells malignant in the first place.

Lyden, however, thinks exosomes also help tumors leapfrog to distant sites. Primary tumors metastasize by shedding cells into the bloodstream, but only a tiny fraction of those cells become established and grow in other organs. The successful colonists have help, Lyden showed about a decade ago. Building on evidence that bone marrow cells somehow nurture new tumors, Lyden's group gave mice transplants of green fluorescent–tagged bone marrow cells, then injected red-tagged melanoma or lung cancer cells under the animals' skin. The cells formed tumors and later metastasized to the lung.

The surprise was that green bone marrow cells appeared in the lungs several days before the metastatic tumor cells, and at exactly the same sites. Lyden concluded that the primary tumors somehow sent a message to the bone marrow cells to home in on future metastatic sites and produce proteins that would help blood-borne tumor cells take hold and grow, a phenomenon he dubbed the “pre-metastatic niche” in a widely cited 2005 paper in Nature. Even the liquid from cultured tumor cells seemed to recruit bone marrow cells, Lyden's group reported, suggesting factors within the liquid were conveying the messages.

Those factors, Lyden's lab eventually concluded, were carried by exosomes from primary tumor cells. In an attention-grabbing study published in 2012 in Nature Medicine, Lyden's team injected mice with one of three different treatments: exosomes from cultured, highly metastatic melanoma cells; exosomes from low-metastatic melanoma cells; or control particles. They then implanted highly metastatic melanoma cells under the animals' skin. Those given the exosomes from highly metastatic cells developed much more metastatic tissue.

Lyden concluded that the tiny vesicles were creating his premetastatic niche, transporting an oncogenic protein called MET that prepared organs for tumor cells to take root and “educated” bone marrow cells to assist. Consistent with that scenario, Lyden reported in the same study that exosomes from the blood of cancer patients with advanced melanoma contained much higher than normal levels of MET.

Lyden and collaborators followed up with a similar paper last May in Nature Cell Biology, this time on pancreatic cancer, which tends to metastasize to the liver. Again they looked at how exosomes from cultured cells affected the spread of cancer in mice. They concluded that exosomes from pancreatic cancer cells educate certain liver cells to secrete molecules that create the fibrous environment conducive to the growth of tumor cells—this time via a protein called MIF.

Then last November in Nature, Lyden's team published an even more radical study, arguing that exosomes actually dictate which tissues a cancer will spread to. Exosomes carry integrins, the sticky proteins that cells use to interact. Lyden's group reported that exosomes from different types of cancer cells carry different sets of integrins. These direct the exosomes—and subsequent metastasis—to specific organs.
Two takes on metastasis

According to the traditional view, cells from a primary tumor enter the bloodstream, then a few lodge in distant organs such as the lungs. These cells then grow into new tumors. An alternative view is that primary tumors send out tiny, protein- and RNA-packed vesicles called exosomes that prepare distant sites for tumor cells to take root and recruit bone marrow cells to assist. This “pre-metastatic niche” nourishes the tumor cells that arrive later.

Exosomes from cancer cells that metastasize to the lungs, for example, were rich in two specific integrins. Giving these exosomes to mice with a type of breast cancer that normally spreads to the bones caused the cancer to spread to the lungs instead. These integrin “zip codes,” Lyden’s team suggested, could be used to predict where a patient’s cancer would metastasize. The team also showed in mice that blocking certain integrins curbed metastasis, pointing to a possible antimitastasis treatment.

CRITICS have several concerns. Lyden’s reliance on injections of cultured exosomes is one source of the skepticism. Some also question his evidence that certain proteins help exosomes prepare the ground for metastasis. For example, Lyden’s group used a short “hairpin” strand of RNA (shRNA) to block a particular gene in tumor cells to curb their output of exosomes and, in other papers, limit production of MIF and integrins. But anonymous commenters on PubPeer, a website for discussing papers after publication, have complained that the researchers didn’t rule out the possibility that these shRNAs have “off target” effects, outside the exosomes, which could have skewed the results.

Kalluri and others also question his claim that the integrins on exosomes serve as zip codes for metastasis. They note that often exosomes from cell lines that metastasize to different organs have the same integrins, so it’s not clear how the proteins could target exosomes to specific organs.

Lyden responds his group has done “multiple tests” to back up their experiments with shRNA. For instance, when they used peptides to block the binding of integrins, they saw the same effects. As for the role of integrins, he says critics have missed a key point: that it’s not only the presence of the integrin, but the amount that matters.

Recent work by other groups supports some parts of Lyden’s proposal. A study published last May in Cell for the first time followed exosomes produced by primary tumors in living mice. Cancer biologist and imaging expert Jacco van Rheenen of the Hubrecht Institute in Utrecht, the Netherlands, and colleagues found that exosomes from highly metastatic tumors could make less malignant cells in distant organs more aggressive. “It is very much in line with the work of Lyden,” van Rheenen says. But he notes that his experiments don’t say whether the exosomes exert their effects even before primary tumor cells reach the sites of metastasis, as Lyden’s work suggests they do.

More validation—or more questions—could come soon as part of the Reproducibility Project: Cancer Biology, an effort run by a nonprofit group to reproduce key experiments from 50 high-impact cancer papers. Lyden’s 2012 Nature Medicine paper is among them, and the replication results could be out later this year.

Even if Lyden’s claims hold up in animals, some are not convinced that the premetastatic niche—and the role of exosomes in shaping it—has much relevance for patients. “There is a disconnect between the animal studies and what is seen in the clinic,” says Joan Massagué, a metastasis researcher at Memorial Sloan Kettering Cancer Center in New York City. He explains that by the time most patients’ cancers are discovered and the primary tumor removed, a few metastatic cells have usually spread and lodged in other organs, where they can lie dormant for years, then awaken and grow. In that case, Massagué argues, it’s not clear what role exosomes from a primary tumor that is no longer present would have.

But Lyden insists that exosomes—which he says metastatic tumors produce, too—are important to the spread of cancer even at that point. “If you could inhibit the role of exosomes, cancer progression could be thwarted and patients could live with cancer for a long time.” If that promise pays off, those little red dots he saw a decade ago could signal hope as well as a warning.