SYNTHETIC PEPTIDES COME OF AGE

New generation of imaging drugs receives increased attention—potential for detecting tumors, artherosclerosis, thrombus, inflammation, and infection

SINCE THEIR INTRODUCTION to biomedical research in the late 1980's, radiolabeled peptides have inspired hopes in nuclear medicine for new imaging methodology, but after presentations at the Society of Nuclear Medicine's annual meeting last June, the technology has sparked even more interest within the community. The spectrum of response ranges from skeptical optimism to complete enthusiasm for the technology's possibilities. "The field [of peptide tracer imaging] is interesting, promising," said an investigator who has worked for years on monoclonal antibodies as imaging agents. "I think the future of small receptor molecules is enormous," said an investigator conducting clinical trials on a radiolabeled peptide. And another clinician remarked, "The area of peptides as radiotracers is intellectually exciting and full of creative potential."

Though only time will tell the real impact of radiolabeled peptides in imaging and therapy, results from the initial tide of research may hint how high or low the marks may reach. Understandably, nuclear medicine practitioners dream of a magic bullet that targets only certain tissues, and peptides that specifically bind with certain receptors might make good magic bullets.

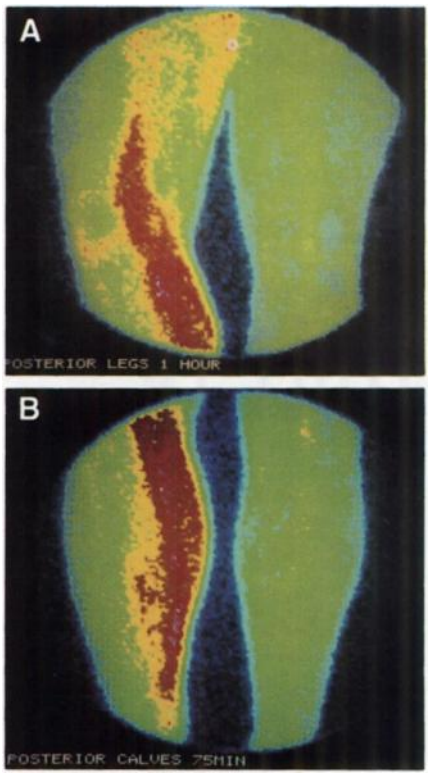
The Peptide Concept

The history of nuclear medicine essentially began in 1938 with the idea of using a radioactive isotope of iodine, to trace function of a specific tissue type, namely thyroid. This idea evolved into the idea of identifying receptor-ligand pairs and labeling the ligand messengers with radionuclide markers. Biochemists also began uncovering several kinds of biochemicals—antibodies, somatostatin, lipoproteins—that had affinities for particular binding sites in the body and discovering what parts of those biochemicals were responsible for the binding—and the potential for new tracers for nuclear medicine grew.

Stanley J. Goldsmith, MD, clinical director of nuclear medicine at Memorial Sloan-Kettering Cancer Center (New York, NY), who has worked with various peptide markers for many years, described one of the concepts underlying the use of peptides as tracers, that is "to identify and duplicate the key part of a protein and to synthetically improve the rest of the molecule to amplify its pharmacological properties: binding affinity, mode of distribution, and mode of excretion. At the same time," he cautioned, "the wisdom—several million years of human evolution—which has resulted in the biology and structure of antibodies shouldn't be dismissed lightly, without careful analysis and comparison of their features."

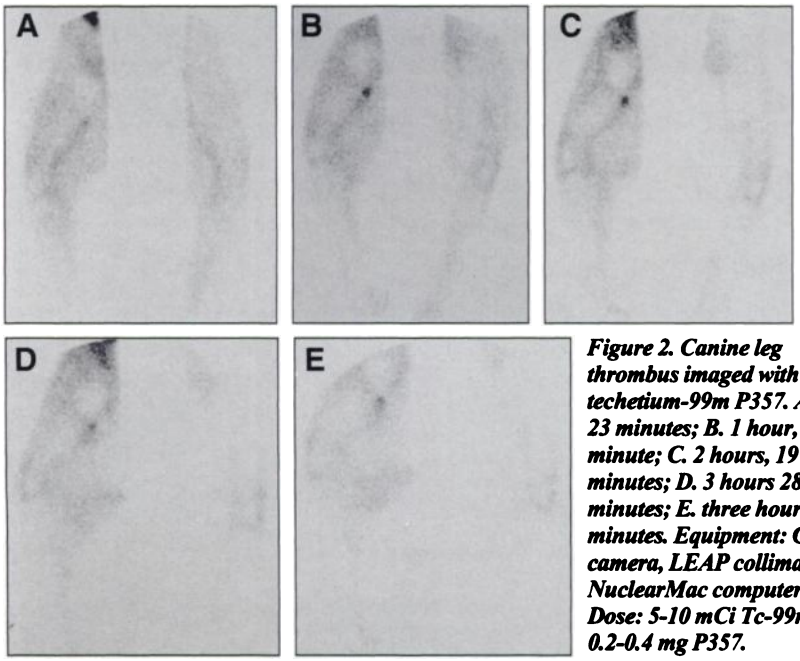
The binding sites of monoclonal antibodies have provided one model for peptides as radiotracers. "I have seen the whole area of monoclonal antibodies develop from using whole antibodies, then fragments, then subfragments," said David M. Goldenberg, ScD, MD, president of the Center for Molecular Medicine and Immunology (Newark, NJ) and chairman of immunomedics (Morris Plains, NJ). "Now we're just going even smaller to synthetic peptides." Nature makes monoclonals to bind uniquely to an antigen. By breaking down the antibody into fragments and then subfragments, some biochemists have attempted to isolate that area of the molecule that binds with the antigen, then connect that portion to a radionuclide to make an imaging agent without the rest of the antibody molecule. Taking this logic one step further, some chemists attempted to mimic that binding site by constructing the smallest molecule possible that will bind with the antigen. But other investigators began modifying messengers like somatostatin and adding a radionuclide while optimizing binding affinity.

One such peptide is Octreoscan from Mallinckrodt (Petten, The Netherlands), the trade name for ^{111}In-pentetreotide, a modified somatostatin molecule with a DTPA-linker added for labeling with ^{111}In. Since somatostatin receptors are particularly increased in neuroendocrine tumors, and in gangliomas, medullary thyroid car-



Photos: Carol S. Marcus, Harbor-UCLA Medical Center

Figure 1. Thrombus in the left leg of a 37 year old male, imaged with technetium-99m P280. A. thigh and knee, 1 hour; B. calf, 1 hour, 15 minutes. Equipment: Elscint 415 with high resolution collimator, 128x128 matrix, Sopha computer. Dose: 20 mCi in 3 ml, approx. 250 μg P280.



Photos: Linda C. Knight and John Lister-Jumes, Temple University Hospital

Figure 2. Canine leg thrombus imaged with techetium-99m P357. A. 23 minutes; B. 1 hour, 1 minute; C. 2 hours, 19 minutes; D. 3 hours 28 minutes; E. three hours 42 minutes. Equipment: G.E. camera, LEAP collimator, NuclearMac computer. Dose: 5-10 mCi Tc-99m; 0.2-0.4 mg P357.

cinoma, gastrointestinal tumors, and lymphomas, Octreoscan is useful in results reported from several European centers for imaging these tumors.

The clinical use of somatostatin and its derivatives dates back to the work of Roger Guillemin and Andrew Schally, two of three recepients of the 1977 Nobel Prize in Medicine and Physiology, awarded for their work on hypothalamic peptides. (Rosalyn S. Yalow, PhD, senior investigator emeritus at Veterans Administration Hospital (Bronx, NY) shared the other half of the prize for developing radioimmunoassay, which made Guillemin and Schally's work possible.) One peptide that Guillemin identified was somatostatin, which inhibited the growth hormone somatotrophin but later proved to have other endocrologic functions, including a general antigrowth effect on normal and tumor cells. Initial attempts at modifying the peptide to make a diagnostic imaging agent employed a tyrosine substitution for phenylalanine in an eight amino acid sequence and an ^{123}I label, which proved to have a too short a half-life for the biologic half-life of the peptide, required a difficult labeling process, and was scarce in the world market. Investigators further modified the molecule's amino acid sequence, deriving an eight amino acid chain, pentetreotide, a variation on the earlier octreotide, with somatostatin's key binding amino acid sequence—phenylalanine-D-tryptophan-lysine-threonine—linked into a ring by a disulphide bond between two cysteines; an added DTPA linker enabled ^{111}In-labeling.

Since somatostatin receptors are highly expressed in some tumors, its analogs have been reported to be useful in shrinking these tumors, and radiolabeled analogs, including Octreoscan, have undergone extensive clinical trials in Europe to image somatostatin positive tumors in the brain and pituatary adenomas, endocrine gastrointestinal and gastropancreatic tumors, paragangliomas, and medullary thyroid carcinoma. Randy McBeath, senior product manager (for Octreoscan) at Mallinckrodt, said that the company has applied for FDA approval of the drug.

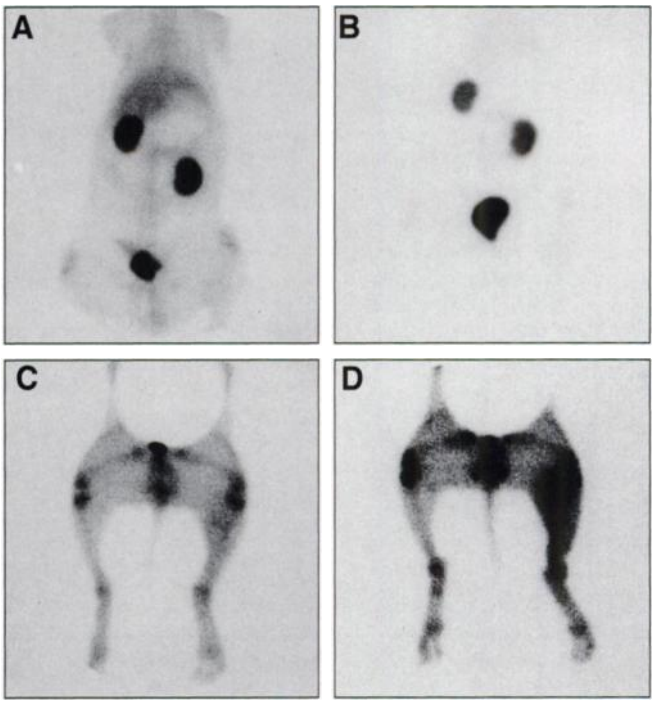
The concept of using peptides as carriers of radiolabels expanded even further when Robert Lees, MD, at the Harvard/MIT Artherosclerosis Center identified the amino acid sequence of apoprotein of lipoprotein. Because lipoproteins are taken up by atherosclerotic plaques, a peptide based on the key amino acid sequence promised to make a good tracer of these plaques. Dr. Lees synthesized the peptide sequence and labeled it with ^{123}I to image artherosclerotic lesions. This work led to the founding of Diatech (Londonderery, NH), a start-up biotecnology firm which has since extended the work on apolipoprotein-derived peptides. This group developed techniques to label with ^{99m}Tc, which is more suitable for the shorter half life of the apoprotein-derived peptides. Jeffrey Borer, MD, professor of radiology at New York Hospital Cornell Medical Center (New York, NY) carried out preclinical trials with these peptides in rabbits, working with a series of peptides of about 2 kilodalton in size, which come from the binding region of the Apo B protein of the low density lipoprotein (LDL) molecule. The Watanabe rabbits—which are genetically disposed to hyperlipidemia—developed artherosclerotic lesions in the aortic wall. When the team injected the rabbits with ^{99m}Tc-labeled peptides, the peptides localized on lesions in the aorta and provided images of artherosclerosis. Similar work was carried out at Mt. Sinai Medical Center (New York, NY) by Shankar Vallabhajosula, PhD, and Dr. Goldsmith, using normal rabbits fed high cholesterol diets. Dr. Borer is now proceeding to clinical trials aimed at imaging carotid arteries in patients with artherosclerotic lesions, with groups at Mt. Sinai Medical Center and Massacusetts General Hospital (Boston, MA). They will compare the peptide-tracer images with subsequent surgical findings.

Diatech has proceeded to develop peptides to image other targets also, including thrombi (Fig. 1). The company synthesized a small cyclic peptide that binds to receptors on activated platelets, which are associated with thrombotic clots. Preclinical trials on canine thrombus, under Linda C. Knight, PhD, and Alan H. Maurer, MD, at Temple University (Philadelphia, PA) using a dimer of a cyclic peptide, with a tripeptide sequence chelating ^{99m}Tc, revealed thrombus in the animals' legs

(Fig. 2). A pilot clinical study in Naples, Italy, in June showed that thrombus-binding peptide produced clear images of thrombotic lesions in nine patients. As the peptide binds ten times more strongly to human platelets than to canine, it promised to be an effective diagnostic tool. Carol S. Marcus, PhD, MD, director of the Nuclear Outpatient Clinic at Harbor UCLA Medical Center (Torrance, CA) is beginning physician-sponsored IND on infectious imaging agents and Phase II trials on the thrombus agent, and has obtained some strong images in a human leg (Fig. 1). Hirsh Handmaker, MD, vice chairman of the Department of Nulcear Medicine at the Children's Hospital of San Francisco, is also conducting Phase II clinical trials of the thrombus imaging agents.

Researchers at Cytogen (Princeton, NJ) are developing a peptide that binds gastrin-releasing (or bombesin-like) peptide receptor, said Vernon L. Alvarez, PhD, senior director of discovery research at Cytogen. When activated, this receptor stimulates the release of gastrin and growth factor for cells, and it is greatly overexpressed in small-cell lung cancer. With a linker and chelator added to the peptide, the investigators add either ^{111}In or ^{99m}Tc to make an agent for diagnostic imaging, or ^{90}Y or ^{186}Re to make an agent for therapy. Cytogen has conducted preclinical animal trials, but they will not be testing on patients until next year.

Research is also underway into radiolabeling chemotactic peptides for imaging inflammation and infection. Naturally occurring chemotactic peptides bind to receptors on white blood cell membranes and cause the cell to move along the chemoattractant gradient toward a site of inflammation in the body. Alan J. Fischman, MD, PhD, has led research at the Massachusetts General Hospital (Boston, MA) in chemically modifying chemotactic peptides to increase receptor binding, and adding ^{99m}Tc labeling to image sites of inflammation. Diatech has also been developing chemotactic peptides to image infection. A team under Dr. Vallabhajosula at Mt. Sinai Medical Center has used analogues of peptides that are chemotactic for neutrophils and modified them to contain moieties chelating ^{99m}Tc. After injecting bacteria into rabbit muscle in vivo then injecting the imaging agent, the investigators obtained images of infection between 30 minutes and four hours (Fig. 3).



Photos: Shankar Vallabhajosula and Brian M. Mayer, Mt. Sinai Medical Center

Figure 3. P322 biodistribution in the rabbit 24-hour E. coli infection model; A. upper torso, 30 minutes; B. upper torso, 4 hours; C. lower legs (infection in left leg), 30 minutes; D. lower legs, 4 hours. Equipment: Siemens camera (LFOV), high resolution collimator, 256x256 matrix. Dose: approx. 3mCi in 1 ml, approx. 75 ug P322.

Resolution (Missassauga, ON), a joint venture between Nordion International and Allelix International, is investigating the commercial use of chemotactic peptides for diagnostic imaging. The company is also investigating peptides that will bind to drug-resistant cancer cells. Another U.S. company, Rho-Med (Albuquerque, NM) has broken down a membrane protein, laminin, which has small chains of amino acids that bind to specific molecules. Working under Cooperative Research and Development Agreements with Los Alamos and Brookhaven National Laboratories, the company's researchers have sythesized peptides based on these binding amino acid sequences and are adding technetium label to image lung tumor and inflammation.

Peptides' Promise as Imaging Agents

Though clinical results on peptides are just coming in, many researchers working with them already anticipate several perceived advantages to the drugs. Though peptides may have a particular set of tasks they can accomplish in nuclear medicine, and monoclonal antibodies another set, many peptide researchers, with due respect for monoclonals, speak of peptides' potential as imaging agents by comparing them with the earlier technology. "The hope is that peptides will be superior to monoclonal antibodies in terms of cost, in terms of technetium-labeling, and in that peptides are biologically inactive, increasing patient safety," said Dr. Handmaker. Since peptides are small and can be made synthetically without the lengthy, costly processes to derive antibodies from animals, he said, they should be much cheaper to manufacture. Also, there has been difficulty achieving in vivo stability of ^{99m}Tc labeling with antibodies, as much of the reduced metal binds to the molecule's nonspecific binding sites; peptides, though, offer essentially an unlimited number of ways to chelate

the metal into stable complexes with the molecule. But the question of antibodies' biological activity and its effects on patients—a source of a flurry of research trying to curtail such activity—is not a cut-and-dried question of peptides' being safe and antibodies unsafe, as anti-antibody response has yet to harm anyone.

"HAMA is not a health/safety problem," Dr. Alvarez said, referring to the human anti-mouse antibody response. Rather than affecting patient health, he said, it is more of practical problem, because the immune systems of the 50% of patients who show HAMA destroys the antibody molecule upon subsequent injections. "The other 50% can be reinjected," he said, and as for the affected 50%, "you can wait three months and most are ready again" for another injection because the response has subsided. "Fragments are even less immunogenic; only about five percent of patients show HAMA." A recent solution to HAMA has been to "humanize" all parts of the monoclonal antibody molecule but the immununotype; though these can still cause a reaction in some patients, the percentage is very small.

Dr. Marcus sees more than a mere practical problem with all the extra mouse-derived material on the antibody molecule. "The big molecules lumber along [in the body] and are metabolized in the liver, so you get big liver doses," she said. "You can get big enough doses to knock out the liver, despite the specificity for the tumor. But peptides can be made to go out the kidney so the liver doesn't get a big radiation dose."

Other qualities that some researchers see in peptides include lower regulatory hurdles, greater control of sterility in production, smaller molecular weight and thus higher penetration, and greater control over linker attachment and chemical activity. Paul Abrams, MD, JD, president and CEO of NeoRx (Seattle, WA), a maker of monoclonal antibodies, pointed out that peptides will be regulated as drugs rather than as biologicals (like monoclonal antibodies), and that means a simpler development process. "With biologicals, you are expected to have the same techniques [and facilities] operating that will produce the product" for Phase III trials, he said. "Thus you must know your market in advance." There is an historical reason for this, he said, that goes back to when the Bureau of Biologics was constituted under the Public Health Service and regulated the production of blood products like human sera, for which a different process gave a different product. "I expect the requirements will become less stringent over time," he said—but in the meantime, companies still have to deal with this regulatory code for biologicals and have the commercial facility running before beginning Phase III.

One peptide researcher pointed out that, in antibody production, one must always check cell lines for viruses and for viral DNA that may contaminate the antibodies and pose a problem in purifying; this is not a question in peptide production. At the microscopic level, a peptide's small molecular weight, and thus small Stokes radius, allows the molecule to penetrate the cellular space much more rapidly. J.D. Bernardy, JD, director of clinical and regulatory affairs at Diatech, sees this smallness as one of the major attractions of the genre. "Since they clear rapidly, there is no long-term blood issue to deal with; therefore you obtain visualization of the pathology more rapidly and the target-to-background ratio is presented more rapidly," he said. Furthermore, since they are smaller, "you can do designer chemistry needed for receptor localization on a small level. Now you can target for specific 'business' sites."

Complete control over the chemistry of peptides may be the characteristic that most appeals to their supporters—but it is both a bane and blessing. Complete control over the chemistry means a peptide's success depends upon a mixture of random amino acid combinations and human creativity and insight into what those combinations may accomplish. With such control, a chemist may determine where to attach a linker or alter the chemical activity to improve binding to the target. Yet there are technological limits to control over peptide construction: "At 40 to 50 amino acids, now you've reached the limits of the current technology," Mr. Bernardy said. "Once you get bigger than that, the molecule's conformation starts to become an important item." The tertiary structure is not important with small peptides, though it can be with larger peptides like interleukin, which has about 200 amino acids. These limits serve as reminders that control means forfeiting the billions of years of evolutionary wisdom behind the antibody molecule.

Drawbacks to Peptides

Dr. Goldenberg basically questions whether peptides can perform as well as antibodies. "There's no real evidence of the rapidity of imaging, of the same tumor-to-background ratio, and of targeting after many hours: thus there's not enough clinical evidence" that peptides can perform any better. He grants that labeled somatostatin has been shown to be clinically useful, but with other peptides, "We may be looking at a vascular effect not related to receptor binding," he said. "There need to be proper controls to show that the proper binding is happening," and researchers need to demonstrate "that the synthetic peptides are actually showing the same targeting as the specific peptide sequence: there's no evidence in any of the peptide studies that any of this is done." He pointed out how, with hypervascular tumors, a lot of nonspecific agents can target either tumor or infection, "so you don't know what you're seeing. Therefore you need controls to show whether this is a specific targeting or an irrelevant effect due to hypervascularization." As he summed up, "Getting small is important from a manufacturing perspective, but are you trading off for specificity?"

Even peptide supporters concede the difficulty in getting their method to do the job that nature does naturally: it takes a lot of work to get a good peptide. After all, you can inject an antigen into a mouse, get an antigenic response, then screen all cells producing monoclonals for strong

binding to antigen: the animal is doing your work for you. But peptide enthusiasts have answers to the problems. As for the question of peptides' rapidity of imaging, Dr. Marcus offered anecdotally, "In clinical trials, I was getting pictures in five minutes. I've done six patients and the images have been rapid. The problem was it went there very quickly then faded. You start imaging immediately, whereas antibodies take hours and hours (though the IgM I was using gave me images early, too)."

Dr. Alvarez found that a rapidity of clearance would have a certain advantage. "Ninety-five percent is out of the body in half an hour, only five percent goes on to localize, which offers a nice clearance for nontarget organs and the blood," he said. "Antibodies have a slow clearance—you may wait several days but you deliver much more to the tumor—say, five percent of the dose, whereas only 0.05% of the dose of peptides" may reach the tumor. "So peptides will be more difficult for therapy." Mr. Bernardy contended that rapidity of imaging was a function of the biology. "Some peptides weakly held to their target site," he said, "but that problem may be as simple as modifying amino acids on site to alter conformational fit, which is hard to do with antibodies." The criticism against many peptides' slowness, he said, "is as good as saying, 'Some peptides are not as good as others.'"

Peptide supporters often defend peptides' shortcomings by saying the problem is not inherent but only due to the current progress of chemistry. Answering the objection to peptides' tumor-to-background ratio, Dr. Marcus said, "If there's a problem, it's not with the generic group but with the particular drug. You just have to be smart enough to design for the receptor itself." She offered an anecdote, "I saw Octreotide images last week in D.C. and they lit up beautifully." But to defend shortcomings by saying the problem only lies in making the chemistry good enough begs the question of whether the chemistry can ever be made good enough, which is what the critics are saying.

Dr. Marcus granted the criticism that there is no targeting of peptides after many hours: "Of the six patients I did, that's true. A lot of the peptide had been urinated out; it leaves the targeted region early. Again, we just need to understand the receptor itself and build a molecule well so it doesn't fall off early, that fools enzymes that may gobble it up, and so on--granted it's going to take a lot of creative [work] to design one." As to the problem of hypervascularization, she conceded, "There are peptides whose distribution is due to high blood flow in an area and are nonspecific. You could get the same distribution with labeled albumin, say, and so Dr. Goldenberg is right." As he suggested, researchers have to choose a peptide that doesn't have a specificity and use that as a control. "But bad science—without controls—isn't limited to peptides. If the distribution is due to blood flow, just design something better."

The Need for Data

The truth behind the exact power of peptides in imaging may never be settled until interested parties get together and agree on exactly what needs to be shown, then present their data clearly and analyze whether the data fulfill the requirements. But in the meantime, pharmaceutical companies are homing their researchers on what, in just a few years, has mushroomed into a vast garden of apparent potential. Besides the research going on at Cytogen, makers of the monoclonal antibody imaging agent OncoScint, another monoclonal maker NeoRx (Seattle, WA) is looking into peptides, despite its skepticism (NeoRx is awaiting FDA approval of its OncoTrac, a monoclonal antibody imaging agent). "I think ultimately peptides ought to be cheaper to produce," said NeoRx's Dr. Abrams. "But I don't see evidence they perform better than monoclonal antibodies—in fact they perform worse. Affinity is the question." His company has contracted with an outside research group to form random peptides until they find one with the same affinity as a monoclonal antibody. But he adds the caveat, "In small-cell lung cancer, Oncotrek is the single most accurate test to determine whether the patient has the disease. It would take a major undertaking to find a molecule as good."

Still, not surprisingly, a genre of drugs that has some researchers resorting to rhapsodic metaphor would have many potential profiteers clambering onto the bandwagon, if only to see if it will hold. "This is a candy store, and we're just entering it," cooed one researcher. "Peptides are like an envelope with an addres, and what's inside can vary infinitely—technetium or gadolinium or chemotherapy." Dr. Borer spoke of using imaging agents to prognosticate cardiopathology more efficiently by looking directly at a biological effect and not just a statistical effect. Exercise electrocardiography and coronary arteriography allow statistical correlations to a risk of death from an associated cardiological problem, he said, "but we don't look at the lesion itself. With peptides we can do this—we can look at fatty plaques or fibrous plaques." As fatty plaques are more unstable and thus more likely to "pop" first, he said, and as peptides hold the potential of targeting one type of plaque or the other, more accurate prognostications would be possible.

Mr. Bernardy described the peptide phenomenon as a way of starting with the basic building blocks and constructing from the ground floor up—the opposite of antibodies, which are the complete edifice that researchers are taking apart. "We're headed toward the middle ground," he said. Dr. Alvarez said, "I think both monoclonals and peptides have their place. To get to that middle ground, peptide enthusiasts may have to heed Dr. Abrams's advice: "With any pharmaceutical agent, it's the dirty details that count: what does the product do, how is it most cost-effective, how is it most beneficial for the patient? You need details of what the product really is doing—what do the data say?" From the hard data so far, the critics do not find that peptides are doing their job.

Lantz Miller