



It's All About the Client

The Development of Hsp90 Inhibitors as Anti-Cancer Agents

Approximately one to two percent of all proteins found in a cell are the evolutionarily conserved heat shock 90 (Hsp90) proteins. These proteins, so ubiquitous and functionally complex, are a challenge to study in mammalian cells. For a long time after the identification of Hsp90, the most anyone knew was its size (90 kilodaltons), that it was activated as part of the cellular response to stress (e.g., heat), and that it performed “housekeeping functions” in the cell. In the 1990s, Len Neckers, Ph.D., Senior Investigator in CCR’s Urologic Oncology Branch, was among the first to recognize that Hsp90 inhibitors could be powerful drugs in the fight against cancer. Today, 19 inhibitors of Hsp90 have been approved for clinical trial as targeted anti-cancer agents, and the Neckers laboratory is combining yeast genetics with work in mammalian cell lines and mouse models to define the next generation of Hsp90 inhibitors.

The Trials of Hsp90

Hsp90 works hard, serving over 200 “client” proteins in the cell, helping them to fold correctly as they take up their rightful positions in the cell. For reasons that are still not well understood, Hsp90 has a special fondness for oncoproteins whose structures shift according to functional state. Among Hsp90’s clients, a surprising number are well recognized targets in oncology, including HER2, a member of the epidermal growth factor receptor (EGFR) family, the fusion protein kinases BCR-ABL and EML4-ALK, the receptor tyrosine kinases KIT and MET, and the steroid

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hormone receptors for androgen and estrogen. As a result, Hsp90 is predicted to have activity against a variety of cancers.

“Theoretically, heat shock proteins are a very interesting class of drug targets because they are involved in oncogene protein

folding in many different tumor types, so their inhibitors should cause downregulation of multiple oncogenes. So, they have the potential to treat different tumor types,” said Giuseppe Giaccone, M.D., Ph.D., Chief of the Medical Oncology Branch at CCR. “And

Hsp90 inhibitors may be particularly effective in combination with other therapies.

the preclinical data are really incredible—these drugs work at nanomolar concentrations *in vitro*.”

Because Hsp90 affects multiple oncogenic pathways, Hsp90 inhibitors may be particularly effective in combination with other therapies to lessen development of resistance. “One of the major reasons for drug resistance is the cancer’s resourcefulness: if you shut down one pathway, then a parallel pathway will take over. Hsp90 inhibition could prevent activation of the parallel

pathway. So there is a clear role for Hsp90 inhibition in resistant tumors, either upfront to prevent the development of resistance, or later to reverse the resistance once it starts,” said Giaccone. Several clinical trials are under way to test Hsp90 inhibitors in drug-resistant settings.

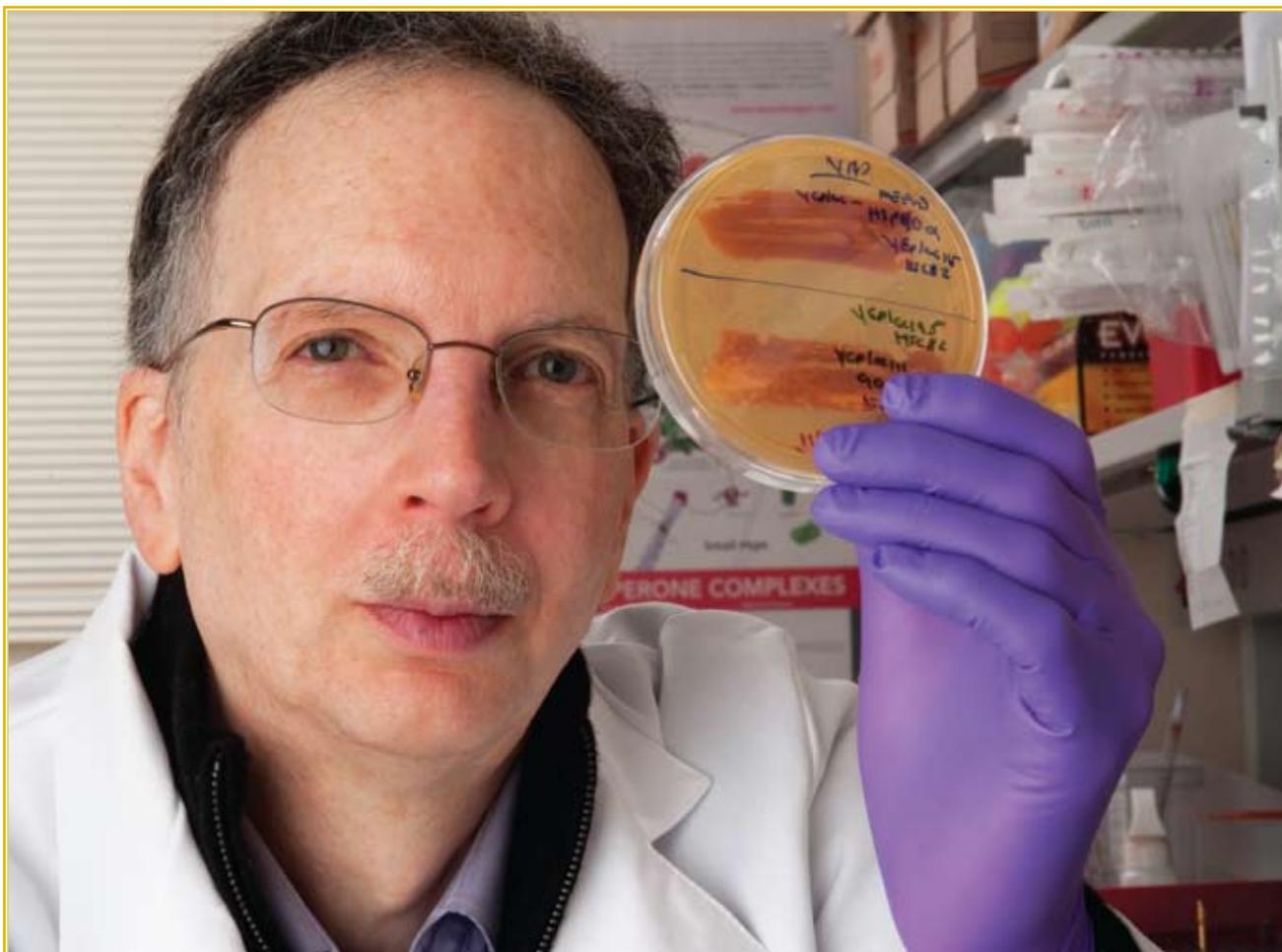
Neckers agrees that Hsp90 inhibitors could be highly effective in combination with agents targeted against particular oncoproteins. But his research also points to a way forward that utilizes alternative strategies to

interfere with Hsp90 in cancer cells. This is important because the first generation of inhibitors have not yet achieved the expected success. “These should be the perfect cancer drugs,” said Neckers, “but so far, the activity in patients is less than you would predict.”

In the Beginning

The Neckers laboratory did not always study Hsp90. “My lab in the late 1980s was working on something completely different: antisense technologies,” said Neckers. However, he was increasingly concerned about off-target effects, that is, interactions that could not be predicted by the sequence of the antisense probe. Neckers had just completed a successful review of his laboratory by the Board of Scientific Counselors, so he felt he had an opportunity to explore new research directions.

(Photo: R. Bauer)



Len Neckers, Ph.D.

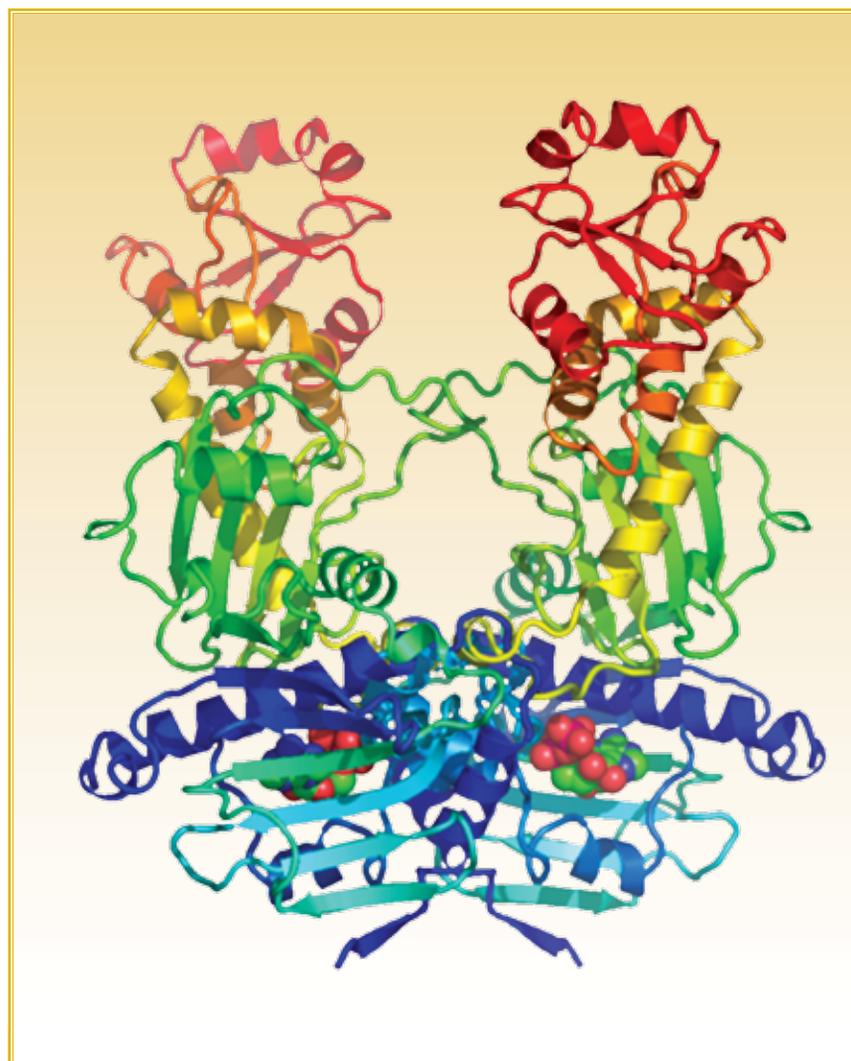
Luke Whitesell, M.D., Ph.D., now at the Whitehead Institute for Biomedical Research at M.I.T., was working as a Research Fellow in the Neckers laboratory and had decided to search for novel inhibitors of protein kinases to study their effects on tumor cell morphology. Learning of the antibiotic geldanamycin, isolated from *Streptomyces hygroscopicus*, that was reported to inhibit the viral oncoprotein v-SRC, Whitesell acquired it and a set of structurally similar compounds called benzoquinone ansamycins from the Natural Products Repository of NCI's Developmental Therapeutics Program (DTP).

Although the compound did block the ability of v-SRC to transform cells in culture, Whitesell and Neckers soon found that this was not because the compound was directly interfering with the activity of the v-SRC protein. Instead, geldanamycin caused the v-SRC protein to degrade. When they did an experiment to pull out cellular proteins that bound directly to geldanamycin, only one came up—a protein 90 kilodaltons in size, which subsequent experiments confirmed was Hsp90.

"Initially, we thought this was so depressing," said Neckers. The team was disappointed to pull down a huge amount of what seemed to be a boring cellular housekeeping protein, and nothing else. Fortunately for cancer research, that disappointment didn't last for long.

"A light bulb went off, and we realized this may be more interesting than we thought. What if this heat shock protein was associating with v-SRC and what if the loss of v-SRC had something to do with the binding of geldanamycin to Hsp90?" asked Neckers.

A series of experiments revealed that geldanamycin bound to an ATP-binding site in the N-domain of Hsp90 and inhibited its ATPase function, that is, the ability of Hsp90 to use energy from ATP to operate



Molecular model of heat shock protein 90 (Hsp90).

(Image: L. Neckers, CCR)

as a protein chaperone. Once free of Hsp90, v-SRC became vulnerable to protein degradation machinery in the cell. "That showed us that Hsp90 binds to a client protein and protects its stability," said Neckers.

Although geldanamycin is itself too toxic to be used in humans, Neckers worked with NCI clinical translation programs—DTP and the Cancer Therapy Evaluation Program (CTEP)—to develop a discontinued compound from Pfizer, 17-AAG, a close chemical relative of geldanamycin, which had been identified in a HER2 inhibitor screen. They tested 17-AAG in preclinical models, verified its Hsp90 inhibitory activity, and began a phase I clinical trial in the late 1990s. Neckers noted that his former NCI

colleague, Edward Sausville, M.D., Ph.D., now at the Greenbaum Cancer Center at the University of Maryland, was instrumental in initiating these clinical trials.

"We found that the less frequently the drug was administered, the higher the doses were that could be tolerated before toxicity became a problem." At that point, drug companies began to get interested in Hsp90 as a target, considering the ATP-binding site as "druggable."

Turning to Yeast

Mehdi Mollapour, Ph.D., came to the Neckers laboratory as a Visiting Fellow with a very different background in Hsp90 research. Several years after the Neckers lab

Neither dose was effective in preventing tumor growth in mice, but together, they were highly effective.

identified geldanamycin's target and mechanism of action, Mollapour was working on his doctoral degree at University College London where investigators first crystallized Hsp90 from the yeast, *Saccharomyces cerevisiae*, and visualized the ATP binding site in its N-domain.

"Up to that point, it was very controversial as to whether Hsp90 bound and hydrolyzed ATP. By crystallizing the protein in the presence of ATP, we could see the ATP binding site. That really changed the whole field," said Mollapour.

Mollapour was motivated to join the Neckers laboratory because of the finding that geldanamycin bound to the same site on Hsp90 as ATP. "I wanted to learn more about cancer, but also continue to build on my expertise," said Mollapour.

Both Mollapour and Neckers had become interested in how Hsp90 itself is regulated. Neckers and other investigators had observed that all the Hsp90 inhibitors that have been tested to date in animals uniformly concentrate in tumors, whereas they are cleared relatively quickly from the blood and from normal tissues. Neckers had also seen that Hsp90 in tumors seemed to be more sensitive to drugs like 17-AAG and felt that if there were differences in Hsp90 in different cell types, they were most likely a result of modifications made to the Hsp90 protein after it was translated from mRNA.

"Before I joined Len's lab, I had a series of questions about Hsp90,

and I felt that the next big question was how this protein gets modified post-translationally," said Mollapour. "When I emailed him about coming to the lab, I asked Len about post-translational modifications and remarkably, he was interested in addressing the same question."

Up until Mollapour joined the laboratory, Neckers and his colleagues had focused on Hsp90 in mammalian cells. But because there is so much Hsp90 in these cells, it is difficult to study the protein by genetic manipulation. Yeast, on the other hand, provides a perfect model system for using genetic tools to study how modifications to Hsp90 affect its function.

Who Is Regulating Whom?

"We published a paper in *Molecular Cell* last year that is a perfect example of integrating insights from yeast into translational research," said Neckers.

Mollapour and Neckers demonstrated in yeast that Hsp90 was modified—phosphorylated—by a protein kinase that is itself a client of Hsp90. They identified the site of phosphorylation in Hsp90 in yeast and confirmed this by mutational analysis. They further demonstrated that if they prevented Hsp90 phosphorylation by silencing or inhibiting the protein kinase, Hsp90 inhibitors were more effective.

"We went on to show in prostate cancer cells that preventing Hsp90 phosphorylation made these cells

dramatically more sensitive to our Hsp90 inhibitors," said Neckers. Most recently, they have shown a synergistic effect *in vivo* between very low doses of both the kinase inhibitor and the Hsp90 inhibitor. Neither dose alone was effective in preventing tumor growth in mice, but together, they were highly effective.

"We started something in simple eukaryotic cells—in other words, yeast—and made that huge jump to mammalian systems; first to cancer cell lines and then to mouse cancer models," said Mollapour. Along the way, we benefited enormously from the diverse expertise of our collaborators, which helped us to bring all the pieces together expeditiously. "My next goal is to see whether this principle of a client protein modifying Hsp90 is true for other protein kinases," said Mollapour.

"The kinase inhibitor we used is in phase 1 and 2 clinical trials for different cancer types," said Neckers. "So we hope that soon it will be feasible to combine this kinase inhibitor with an Hsp90 inhibitor in a combinatorial clinical trial."

Improving Efficacy

"We are continuing to collaborate with companies that have Hsp90 inhibitors in the clinic," said Neckers. He is particularly interested in the efficacy of Hsp90 inhibitors in cancers that are driven by known client proteins like HER2 and mutated EGFR. "HER2 remains among the most sensitive



(Photo: R. Baer)

Len Neckers, Ph.D., and Mehdi Mollapour, Ph.D.

client proteins of Hsp90 that have so far been identified in tumors.”

The most famous example of HER2-positive tumors is in a subset of breast cancers, for which the targeted drug Herceptin is prescribed. “Herceptin frequently stops working after some time, but in initial trials with the Hsp90 inhibitor 17-AAG added in, there was additional clinical benefit,” said Neckers. HER2 is also overexpressed in a certain percentage of patients with bladder cancer. “The program in the Urologic Oncology Branch for bladder cancer is expanding, so we are hoping to test Hsp90 inhibitors on these cancers as well.”

Interestingly, normal EGFR is not highly dependent on Hsp90, but

mutations in EGFR that confer drug resistance to the targeted agent, Tarceva, also confer dependence on Hsp90. Accordingly, clinical benefits of 17-AAG and other Hsp90 inhibitors in drug-resistant non-small cell lung cancer have also been observed.

“A lot of what we do now in our laboratory is to focus on identifying more modifications of Hsp90 and understanding how they regulate function and specificity,” said Neckers.

Neckers and his team have identified several modifications—phosphorylation and acetylation sites—on Hsp90, not all of which have the same impact on function. They have found sites that inhibit the binding of client proteins,

and increase or decrease Hsp90’s sensitivity to inhibitors. Neckers is hopeful that within all this information, there is going to be a way to explain and exploit the difference between Hsp90 in tumors and healthy tissues.

To learn more about Dr. Neckers’ research, please visit his CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=neckers>.