

ties: Impacts of lifelong parenting. *American Journal on Intellectual and Developmental Disabilities* 116(6), 479-499.

Sibling Support Project (2015). Sibshops. Retrieved July 22, 2015 from <https://www.siblingsupport.org/about-sibshops>.

U.S. Census Bureau (2013). FM-3: Average number of own children under 18, for families with children under 18. Current Population Survey. Retrieved December 6, 2014 from <https://www.census.gov/hhes/families/data/families.html>.

Hsp90 Inhibitors: What's Taking So Long?

CLAIRE JARAMISHIAN



WRITER'S COMMENT: When I received the literature review assignment in my UWP 104F class, I was excited to write about a project that I worked on with Dr. Ken Kaplan in the Molecular and Cellular Biology Department at UC Davis. This review synthesizes the conclusions we made based on the literature published about a class of chemotherapeutic drugs called Hsp90 inhibitors. These drugs have been in the clinical testing phase for decades, yet none of them have been approved as a standard treatment for cancer. I hope you enjoy reading this piece as much as I loved writing it. Thank you Dr. Amy Clarke and Dr. Ken Kaplan for all of your guidance and expertise throughout this process.

INSTRUCTOR'S COMMENT: In Writing in the Health Professions, students spend the quarter focused on a narrow topic of their own choosing. They research new findings in the peer-reviewed literature and write a series of pieces, culminating in that most difficult of undergraduate assignments: the literature review. When she came in to discuss topic selection, Claire described a project she had worked on previously – tracing the path of drug development of several cancer therapies. She had already read and collated some 25 experimental reports on the topic, but she felt that there was something missing, some room to more definitively answer the research question. But she was hesitant to revisit a topic on which she had already spent so much time. I often tell students to build on their strengths and to follow their instincts. I also tell them that they will get more from less – a narrower approach will allow for deeper analysis. Whether she took my advice or just knew what to do, Claire chose to revisit the topic but to narrow the focus. The genius of the resulting literature review, a model of economy and precision which focuses on a single, long-delayed but potentially groundbreaking drug, is all Claire's doing.

– Amy Clarke, University Writing Program

Abstract

Heat Shock Protein 90 (Hsp90) is a chaperone protein that stabilizes a wide array of client proteins. Hsp90 clients are over-expressed in cancer cells, causing uncontrolled cell proliferation. Although multiple drug formulations of Hsp90 inhibitors have undergone clinical testing, none has been approved as a standard treatment for cancer. This review follows the clinical development of Hsp90 inhibitors as a novel class of anticancer drugs. It considers the drug mechanism in treating cancer and the use of biomarkers as an indication of drug efficacy; it also analyzes published clinical trial results of different formulations evaluated as single agents or in combination with other therapies. We conclude that the clinical paths investigational drugs take appear to affect their development time-line.

Background

Discovery and drug mechanism.

Heat Shock Protein 90 (Hsp90) is a molecular chaperone protein that stabilizes a wide array of client proteins. In cancer cells, many Hsp90 clients are over-expressed and contribute to the canonical cancer phenotypes (e.g., increases in cell proliferation, growth, and survival). The first Hsp90 inhibitor was discovered 38 years ago. Since then, the compound has undergone multiple formulation changes to make the drug more effective and less toxic to the human body (1,8). According to the Drug Discovery and Development timeline, this process should take on average 10 years, but Hsp90 inhibitors are an exception to this estimate (2).

Hsp90 inhibitors have been shown to reverse cancer cell growth in the laboratory, yet no inhibitor has been approved as a standard cancer treatment. Factors that have impeded the clinical success of Hsp90 inhibitors are unclear but might be associated with both the biology of current inhibitors and the economics of drug development. To identify the issues impeding Hsp90 inhibitor clinical success, a review of clinical trial literature that involves investigational Hsp90 inhibitor compounds is herein undertaken.

Hsp90 is part of the heat shock protein pathway. When a cell is under stress, Hsp90 is activated. Cellular stress is caused by many factors, one being the presence of misfolded proteins (3). Hsp90 sequesters these proteins, allowing them to fold inside the matrix of Hsp90. Some of the

misfolded proteins are oncogenic. When Hsp90 rescues these proteins, they are allowed to remain in the cell and proliferate (4). Pre-clinical studies show that chemical inhibitors of Hsp90 cause degradation of clients in cancer cells.

Hsp90 inhibitors are compounds that bind to the active site of Hsp90, preventing it from hydrolyzing ATP at the gamma phosphate. This prevents the release of bond energy, resulting in a protein that is unable to chaperone oncoproteins. Ubiquitin is a protein that makes post-translational changes to other proteins, tagging them for destruction. The proteins, in this case Hsp90, are then degraded through the proteasome (4,5) (Fig. 1). The drug mechanism behind Hsp90 inhibitors is not specific to a form of cancer; in theory this drug treats every type of cancer.

Hsp90 Inhibitor	Pharmaceutical Company	Year Drug Entered Clinical Testing	Development Phase Reached	Reason for Termination
Geldanamycin	Upjohn	N/A	N/A	Toxicity
Tanespimycin	Bristol-Myers Squibb	2005	Phase III	Nonclinical, patent expired
Retaspimycin	Infinity	2011	Phase III	Lack of clinical efficacy
Ganetespib	Synta	2013	Phase III	Currently undergoing Phase II and III clinical trials

Issues with Hsp90 Inhibitor Development

Evaluation of Drug Efficacy.

Biomarkers are used to evaluate a patient's response to therapy. They are grouped into three categories: stratification, pathway, and substrate. Stratification biomarkers use gene expression to separate patients into different cohorts in clinical studies. Pathway biomarkers involve the target protein pathway of the experimental drug; for Hsp90 inhibitors, it is the Heat Shock Protein pathway. Substrate biomarkers are the putative

clients of Hsp90.

The use of biomarkers is variable in the Hsp90 inhibitor studies (Fig. 3a). 20% of the clinical trials did not evaluate any biomarkers. Pacey et al. investigated Tanespimycin as a single agent in patients with metastatic melanoma, concluding that the pathway biomarker Hsp72 was reached. However, the results were not consistently detected. True drug efficacy is difficult to determine without monitoring the cancerous regions of a patient's body. Extracting malignant tissue from patients is very invasive, and thus limited. This explains a lack of consistency among many Hsp90 clinical trials.

Investigators lack a homogeneous way to measure drug efficacy in patients, meaning the success or failure of Hsp90 inhibitors cannot confidently be determined on a biological level. Drug efficacy is a crucial factor that determines whether or not an experimental drug can advance in clinical development or is terminated. Therefore variable data among the biomarker data prolongs clinical testing.

Biomarker Assay Quality

	Tanespimycin	Ganetespib	Retaspimycin	Percentage of clinical trials	Herceptin Percentage of clinical trials
No Biomarkers Evaluated	6	1	1	20%	N/A
Pathway Biomarker	21	2	2	63%	100%*
Stratification Biomarkers	3	3	1	18%	100%
Substrate Biomarker	19	1	0	50%	100%
# of Clinical Trials Reviewed	31	5	4	Total: 40	

Fig. (3) Table summarizing the different biomarkers evaluated in 3 different drug compounds published in 40 clinical trials.

A phase II trial investigating Tanespimycin as a single agent measured client protein Hsp72 levels in the Peripheral Blood Mononuclear Cells (PBMC) of patients. The investigators presented this data in a non-quantitative Western blot, but there was a problem with the assay because no band spanned the pre-load lane. Even if the level of Hsp72 were minimally expressed in patients, there would be a faint band across the entire cell (6).

Also, the data provided was from only 2 patients. However, investigators have access to blood samples from all the patients included in the study; the absence of tissue samples already makes it difficult to draw conclusions from clinical trials. Such a small sample size and lack of quantification does not allow investigators to confidently determine that the protein expression of Hsp72 changed between pre and post dose administration. There is no clear-cut evidence that client protein expression changed in response to the drug instead of other confounding variables. Similar to inconsistent use of biomarkers, variability in percent of evaluable patient blood samples in clinical trials extends the development time of investigational drugs.

Single Agents vs. Combination Therapies

The Overall Response Rate (ORR) of a drug measures the positive response patients achieve at the conclusion of a study. It is the number of Complete Responses (CR) and Partial Responses (PR), divided by the total number of evaluable patients. CR indicates remission: all symptoms and lesions of cancer have dissipated. PR is achieved when 30% or more of the symptoms and cancerous lesions have resolved.

All known phase I and II clinical trials of Tanespimycin and Ganetespib yielded a 24% and 4% ORR, respectively, in patients that received the Hsp90 inhibitor as a monotherapy or in combination with a standard chemotherapeutic drug or radiation. Retaspimycin did not have any CR or PR in clinical studies; trials administering Tanespimycin were the only ones to investigate combinatorial therapies. The most notable combination therapy study, Modi et al., used Tanespimycin and Herceptin, resulting in a 22% ORR. The patients who previously progressed on Herceptin responded to this combination therapy. The study concluded that Hsp90 inhibitors have the ability to sensitize cancer cells by either decreasing or overcoming their resistance to standard chemotherapeutic drugs (7).

Among all the combinatorial trials, there was a 48-fold increase in the ORR when compared to monotherapies. This discrepancy between the different therapies is significant; there is a lack of combinatorial trials among Hsp90 inhibitor literature despite the evidence that combination trials yield a higher ORR in patients. Absence of such literature could have caused the early termination of some Hsp90 inhibitors like Retaspimycin, which was terminated on the basis of a lack of clinical efficacy. Based on the recent findings of increased efficacy in combinatorial trials, this decision could have been premature. However, Ramalingam et al. are currently investigating Ganetespib in combination with docetaxel in the phase III GALAXY-2 trials (9). Results of the trials will be available later this year.

The ORR is a critical indication of drug efficacy. Examining either progressive or reductive cancerous lesions is the most powerful indicator of whether or not an investigational drug is successful in a clinical trial.

Conclusion

Based on the analysis of drug mechanism, indications of efficacy through biomarkers, assays, and different clinical trials, it is clear that these factors have impeded the clinical success of Hsp90 inhibitors. Without a consistent extraction of tumor samples and blood specimens, investigators do not have a complete baseline comparison of all the patients who have participated in Hsp90 inhibitor clinical trials. Variability in biomarker evaluation, along with the assays produced from that data, make it difficult to conclude whether or not the clinical trials generate statistically significant data.

The results of the newest Hsp90 formulation, Ganetespib, in combination with docetaxel, will help investigators and pharmaceutical companies gain a better understanding of whether or not this class of drug should be further developed (9). Another promising combinatorial trial with a discontinued Hsp90 inhibitor, Tanespimycin and the protease inhibitor Bortezomib, yielded a 15% ORR (10). There is an ongoing clinical trial combining Ganetespib and Bortezomib, and based on previous combinatorial trials involving Bortezomib, the results that will be published later this year should help determine the clinical path of Hsp90 inhibitors (11).

Although drug development is financially risky, correct determination of success or failure is needed; neglecting to do this impedes the

developmental timeline of Hsp90 inhibitors.

References

- National Cancer Institute. Geldanamycin (NSC 122750)17-AAG (NSC 330507)17-DMAG (NSC 707545) [Internet]. National Cancer Institute. Available from: https://dtp.cancer.gov/timeline/flash/success_stories/S3_geldanamycin.htm
- Pharmaceutical Research and Manufacturers of America . 2007 Feb. Drug Discovery and Development [Internet]. Washington (DC):Pharmaceutical Research and Manufacturers of America; [cited 2015 June] . Available from: http://www.phrma.org/sites/default/files/pdf/rd_brochure_022307.pdf2.
- K.B. Kaplan, R. Li. A prescription for 'stress' – the role of Hsp90 in genome stability and cellular adaptation. *Trends Cell Biol.*, 22 (2012), pp. 576–583.
- Li J, Soroka J, Buchner J (2012) The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. *Biochim Biophys Acta* 1823: 624–635.
- Soga S, Akinaga S, Shiotsu Y. Hsp90 inhibitors as anti-cancer agents, from basic discoveries to clinical development. *Curr Pharm Des* 19: 366–376, 2013.
- Pacey S, Gore M, Chao D, Banerji U, Larkin J, et al. (2012) A Phase II trial of 17-allylamino, 17- demethoxygeldanamycin (17-AAG, tanespimycin) in patients with metastatic melanoma. *Invest New Drugs* 30: 341–349.
- Modi S, Stopeck AT, Gordon MS, Mendelson D, Solit DB, Bagatell R et al (2007) Combination of trastuzumab and tanespimycin (17-AAG, KOS-953) is safe and active in trastuzumab- refractory HER-2 overexpressing breast cancer: a phase I dose- escalation study. *J Clin Oncol* 25(34):5410–5417.
- Williams, Robert R. 24. Discontinued in 2013; oncology. Expert opinion on investigational drugs [cited 2015 March 3] 24(1):95-110. *J Clin Oncol* 32:5s, 2014 (suppl; abstr TPS8118^).
- Richardson PG, Chanan-Khan AA, Lonial S, Krishnan AY, Carroll MP, Alsina M, et al. Tanespimycin and bortezomib combination treatment in

patients with relapsed or relapsed and refractory multiple myeloma: results of a phase 1/2 study. *Br J Haematol* (2011) 153:729–40.10.1111/j.1365-2141.2011.08664.x

“A Phase I Study of Ganetespib +/- Bortezomib in Patients With Relapsed And/or Refractory Multiple Myeloma.” A Phase I Study of Ganetespib +/- Bortezomib in Patients With Relapsed And/or Refractory Multiple Myeloma. N.p., n.d. Web. 25 June 2015

Tissue Engineering: a Potential Therapy for Anterior Cruciate Ligament Reconstruction



REBECCA MILLER

WRITER'S COMMENT: After my horse tore a ligament and had to retire from competition, I became interested in soft tissue injuries and their capacity for healing, or lack there-of. Since then, I have been lucky enough to perform research in Dr. Keith Baar's laboratory, which has exposed me to the field of tissue engineering. So when Dr. Amy Clarke gave us the seemingly daunting task of writing a literature review on a health topic of our choice, I immediately knew my subject. ACL ruptures and their subsequent surgeries are common; however, the emerging science of bioengineered replacement ligaments remains largely unnoticed. Through my work in the lab, I have become acutely aware of many of the major pitfalls of the current surgical reconstruction options for ACL tears. As an aspiring orthopedic surgeon, I recognize the importance of finding a surgical option that will provide the patient with the best outcome. For this reason, I was compelled to write a review comparing the current surgical options with the potential use of engineered ligaments. I hope this review excites people about the research that is out there: Ligaments are being grown in the lab, and it's happening right here on our campus.

INSTRUCTOR'S COMMENT: My abiding image of Rebecca is this: on the day of oral presentations, as she taught a mini-seminar on the topic of her literature review, she pulled a small glass vial seemingly out of thin air. In it was a bioengineered anterior cruciate ligament, its size disguising the enormity of the achievement it represents. Rebecca works in the lab that created this prototype of what could become a standard of ACL reconstruction. As a future orthopedic surgeon, she might even use a version of this manufactured ligament to help those hobbled by ACL tears regain full function. It was certainly one of the best visual aids I'd ever seen a student use to underpin a presentation. But it is so like Rebecca that this little marvel of science took a backseat to her calm, absolutely clear discussion of the problems of ACL reconstruction and the role of bioengineering in addressing them. Her splendid literature review reflects her deep understanding of these problems and, more than that, her ability to collate and condense the relevant research so elegantly.