Breakthrough Medicines

Q&A

WITH TOM TUCKER

ON THE DISCOVERY OF MK-0616 (ENLICITIDE), A NOVEL ORAL MACROCYCLIC PEPTIDE PCSK9 INHIBITOR

I have recently retired from Merck & Co., Inc., Rahway, NJ, USA (hereinafter "MSD") and my comments are solely my own opinions and perceptions. I've tried to be frank and honest in my comments but also have purposefully and deliberately avoided anything proprietary that hasn't been previously published or presented at various external meetings. - Tom Tucker

For this issue of the PDHC Chronicle, we had the opportunity to speak with Mr. Tom Tucker, corresponding author on two landmark Journal of Medicinal Chemistry papers describing MK-0616. This investigational oral macrocyclic peptide represents a breakthrough in targeting PCSK9, a key regulator of LDL cholesterol. Remarkably, MK-0616 has now advanced to Phase 3 clinical development, underscoring both the scientific and translational impact of the program. In this conversation, Mr. Tucker reflects on the journey from concept to clinic, the challenges faced, and what this program means for the future of peptide drug discovery.

Why MK-0616 Matters

- **First-in-class oral macrocyclic peptide targeting PCSK9, a key regulator of LDL cholesterol.**
- **Now in Phase 3 clinical development, with potential to become the first oral alternative to PCSK9 antibodies and siRNA therapies.**
- **Demonstrates that oral peptide therapeutics can achieve drug-like PK/PD profiles, challenging the notion that peptides are limited to injectables.**
- **Built on a multidisciplinary platform approach combining mRNA display, medicinal chemistry, structural biology, and innovative assays.**
- **Could impact patient accessibility by enabling convenient daily oral dosing in cholesterol management.**
- **Serves as a proof-of-concept for expanding peptide drug hunting into other "undruggable" targets.**

Q **The development of MK-0616 was a tour de force effort involving a large multidisciplinary team and platform technologies. How did you each get involved in the project, and what role did you each play on the team?**

I became involved in the project from day one. At that time, MSD was just reentering the peptide space and beginning to rebuild its peptide capabilities. A member of my team at West Point (Chengwei Wu) who is an experienced peptide synthesis expert taught most of the chemists on the project at that time (who were historically small molecule chemists), how to do peptide synthesis, and the effort expanded from there. MSD has since hired many dedicated peptide synthesis specialists as well as continued to give small molecule chemists the opportunity to cross train and learn to do peptide synthesis. I think this blend of unique perspectives and experiences provides the opportunity for very novel solutions to difficult design and development problems.

Q **Looking back, what was the spark that convinced you and the team that PCSK9 could be drugged with an oral peptide, despite the conventional wisdom that this was nearly impossible?**

Oral bioavailability in this space was deemed to be a necessity from day one. I think early on we had some doubters as we began to make peptides and realized what the properties of these early hits were like – the molecules were poorly permeable, unstable, and had poor PK properties. But we continued to work hard, and I have to give a lot of credit to a scientist in our DMPK team by the name of Ken Koeplinger (who has since retired from MSD) who I worked very closely with. Ken was convinced based on what he had seen in the literature as well as in conversations with academic consultants that enhanced formulation based oral delivery of peptides was possible and practical. Ken convinced our chemistry team as well as our management that this could be done successfully and safely, and we worked together to demonstrate the feasibility in early animal experiments. We as Med Chemists realized that stability in the gut would be a key and necessary component of oral delivery, and we worked collaboratively with our DMPK colleagues to establish the assays needed to fully interrogate and solve these issues.

Q **What was the most difficult medicinal chemistry challenge on this program? How did you decide which scaffolds to prioritize, and what were the most informative assays?**

The biggest Med Chem challenge was solving the gut stability issue. We had four key amide bonds in our early leads that were all critical for interaction with PCSK9 that were being metabolized, and fixing these issues necessitated that we design some very complex scaffolds that required a lot of out of the box, non-conventional thinking. Interestingly, coming out of the early mRNA Display screening collaboration with Ra Pharma, we had found two distinct chemical lead series. The one that most of the team was following up showed some very good oral bioavailability when dosed with our enabled formulations on the front end, but after initially working on this we chose to pursue the other lead class. We just had a good feeling about these other hits and felt that they might be more optimizable than the prior series. Ultimately this second series led to the clinical compound. We also made a decision early on to take a valuable external collaboration with our IRBM colleagues for peptide design and synthesis. They focused on replacing the original dibenzyl xylene (dbx) linker in the molecules and it's two thioether moieties with alternative amino acid-based linkers. Interestingly, there were no metabolic nor other issues associated with the dbx linker, but ultimately when we found a chemical stability issue in the presence of specific permeation enhancers. We averted disaster by having a plug and play solution ready to go. In terms of assays, the key assay for us was our Target Engagement assay which allowed us to accurately relate PK and PD in plasma and help us to accurately predict our human doses. We worked closely with our DMPK colleagues to develop this assay using a biotinylated version of one of our inhibitors. The procedure is described in our publications. Amazing cross-disciplinary collaboration was a key part of this effort from end to end, and I can't say enough about the ability of this team to collectively solve each complex challenge that came along during this work.

MK-0616 in the Spotlight

- ***Featured as a "Molecule of the Month" by Drug Hunter, a leading resource for medicinal chemistry insights.***
- ***Voted 2023 Molecule of the Year by the Drug Hunter community, reflecting its impact and excitement across the global drug discovery field.***
- ***Celebrated not only for its therapeutic potential in lowering LDL cholesterol but also for demonstrating the feasibility of oral macrocyclic peptide medicines.***

Q **Can you comment on the research operating plan and share how the workflow was structured to keep the team moving forward?**

We had a well-defined ROP and we stuck to it methodically throughout the project. Fret assay on the front end to determine potency, followed by IV PK in rats and assessment of stability and metabolic ID. We also used ID /oral gavage dosing in rats to guide our progression of compounds forward to our target engagement assay and more advanced PK/PD studies in primates.

Q **If you had the chance to go back and change any decision that the team made, what would it be?**

I don't think I would change a thing. That's not to say we were perfect and didn't make mistakes because we made many. But the entire project worked out so well and found such exceptional chemical matter that in the end I

wouldn't change anything. I would readily admit that several very serendipitous events throughout the process fell the right way for the team, but as any experienced medicinal chemist knows serendipity is always a key and welcome component of any successful drug development program!

Q **Peptides often face hurdles in permeability and stability. What specific strategies proved most effective in overcoming these liabilities for MK-0616?**

In our case, since our molecules were targeting a circulating target in plasma, having poor intracellular permeability was an advantage and really helps to prevent our molecules from engaging other potential targets inside cells. In terms of gut permeability, we were able to show early on in our leads that gut permeability was low even with fully stabilized molecules. We knew early on that if we were going to get an orally bioavailable molecule, we would likely need to use an enabled formulation-based approach that focused on allowing our molecules to be absorbed via paracellular absorption through transient opening of the tight junctions in the gut. This enabled early commitment to and focus on this approach.

In terms of stability, this is critical. If you want to be orally bioavailable with a peptide therapeutic, stability in the gut is everything! We were able to assess stability to the key gut enzymes on our key molecule and were also able to take advantage of having access to metabolic ID studies early on. We were able to systematically identify the vulnerable spots on our peptides and develop specific synthetic strategies to block these metabolic sites and stabilize the molecules. We identified four major vulnerable amide bonds and guided by this data and structure- based design, we were able to design some complex cross linking strategies to modify the molecules that not only fully stabilized them to metabolism but also greatly increased the potency of the molecules, down to the picomolar level!

Q **What were the largest bottlenecks in the program, and are there any lessons learned that could help the broader field accelerate peptide medicines to the clinic?**

The largest bottlenecks throughout this project were related to the complex synthesis of our key molecules throughout the progress of the program. As I said in order to solve the metabolic issues as well as some off target issues, the amount of complexity that had to be built into the molecules was quite amazing. I still look at the structures in awe and amazement that we were able to turn these complex peptide molecules into drug candidates. Early on we used SPPS to build these complex structures and were able to adapt a lot of chemistry to both on and off-resin synthesis. Later, it became necessary to move to solution-based approaches to allow for more efficient scale up. The work done by our process chemistry group to improve the scaleup and synthesis of MK-0616 is world class and equally as impressive as the discovery effort. The development of biocatalytic approaches to most of the steps in this synthesis really enabled the clinical development. Our process team recently published this work in JACS, and I would encourage everyone to read this manuscript.

Q **Were there any unexpected PK/PD findings when moving from preclinical to clinical evaluation?**

Not really – our Target Engagement model was so robust that it was able to help us clearly build solid and predictable PK/PD correlations that carried over into the clinic. We were pleasantly surprised in the clinic as we were able to get sufficient exposures to reach the Target Engagement levels with the desired efficacy at doses that were even lower than our early human dose projections.

Q **MSD has a history of exploring macrocyclic peptides. How did MK-0616 build on prior internal knowledge, and how does it inform MSD's broader peptide platform strategy?**

In many ways, we were able to blend MSD's strong background in small molecule drug discovery with our growing expertise in peptide therapeutics to create complex structures for challenging biological targets. The demonstrated success with MK-0616 has allowed the cyclic peptide platform to become a key focus of novel therapeutic discovery efforts at MSD and has helped to solidify our "Modality Agnostic" approach to novel therapeutic discovery.

Q **Do you see PCSK9 inhibition via peptides as a unique case, or do you believe this program could catalyze a wave of peptide design across other undruggable targets?**

This has already happened at MSD and is happening across the pharmaceutical industry. Our internal peptide discovery efforts have clearly been bolstered, and cyclic peptides have become a key modality for ongoing and future drug discovery efforts at MSD. The rapid explosion of interest in this space across the industry catalyzed by the success of MK-0616 and other similar molecules from other pharma companies has been remarkable and I believe this revolution in peptide therapeutics will continue and will accelerate.

Q **The PDHC emphasizes the "Life of a Peptide"—from de novo design through translation into the clinic. Which stage of MK-00616's journey did each of you feel was most transformative, and why?**

Several stages were transformative. First of all, the early design of the peptides and the unique solutions discovered for the numerous issues we encountered were revolutionary to the field. From the design of multiple cross links to rigidify the molecules and block metabolism, to the use of the quaternary amine sidechain to help physical properties and also help eliminate several off target issues,to the development of our Target engagement assay, the early discovery and lead optimization parts of this program required novel, outside the box thinking and approaches. The other transformative stage was already mentioned – the incredible process chemistry work and the extensive use of biocatalysis to make the scaleup feasible and cost efficient.

Q **How important were external collaborations, or cross-disciplinary knowledge-sharing in making MK-0616 possible?**

As I mentioned earlier, we entered into a collaboration with Ra Pharma to apply their mRNA display screening capability to identify cyclic peptide starting points for our hit validation efforts. We also partnered with IRBM on the medicinal chemistry efforts across lead identification, lead optimization and finally candidate selection. Importantly, we were able to work closely with them to solve a key issue we had with the dbx crosslinker which came from the mRNA display post-translational macrocyclization chemistry. We did this work at risk and under pressure before we even knew this would be a problem. It was one of those experience/intuition things; there were no observed issues early on, but something told us there would be at some point and this was done in parallel, in anticipation of potential downstream issues. I'm a big believer in the intuition of experienced medicinal chemists, and I trust that immensely. In this case it paid off handsomely! Both Ra Pharma and IRBM proved to be an incredible collaborators, and they have demonstrated expertise and a proven track record of making important contributions to clinically advanced peptides.

Q **In your opinion, what does the peptide field need most right now—new synthetic methods, better predictive models, translational biomarkers, or something else?**

It's all of the above and more! Small Molecule drug discovery and antibody therapeutic discovery have been given huge investments by pharma in the past twenty years, however the "space in between" occupied by peptides and especially cyclic peptides has been largely ignored until more recently. In many ways, we are having to learn how to most efficiently design, develop, and market these unique molecules. I also think that in the past, peptide chemists had very limited views of how to make a peptide therapeutic based on their backgrounds and experiences. Technologies like mRNA display are revolutionizing peptide lead finding, and this has in turn fueled a revolution in the development of novel chemistry with the design and synthesis of non-natural amino acids. This revolution has just begun and I hope we will see it continue to amplify and expand. I also think that molecules like MK-0616 start to somewhat blur the line between peptides and small molecules, and I think that as more historically small molecule focused medicinal chemists begin to appreciate and work in the peptide therapeutic space, they will bring even more synthetic creativity and novel design concepts that I think have been on hold in the peptide space for a while. This has not been the fault of peptide chemists, but more the deemphasis of the peptide therapeutics space throughout the 1990s and the early 2000s by the pharma industry. New ways of thinking about molecular design and synthesis are expanding the pallet of tools available for novel peptide design and synthesis.

Solving issues related to the oral delivery of peptides designed to reach intracellular targets remains a huge problem, and I believe a lot of focused work will need to go into solving this complex problem. How can we design

chameleonic properties into molecules from day one while also simultaneously retaining potent and specific biological activity and drug-like properties remains a massive challenge that will be difficult to solve and not something that can be easily adapted at this point to discovery technologies like mRNA Display. The use of AI/ML in the future may be able to provide insights and creative, outside the box solutions here, especially as computing capacity and capabilities continue to grow exponentially.

Q **If you imagine peptide therapeutics in 2030, what do you think will be the most striking differences compared to today?**

I really believe that there will be a continuing and expanding revolution in novel peptide design, synthesis, and development over the next five years. I believe more and more companies will jump back into the peptide therapeutics space and continue to fuel this revolution. I firmly believe that we will see a huge increase in the use of synthesized non-natural amino acids as a common route to solving multiple issues in peptide design and development. Structures like MK-0616 will become commonplace as companies become more willing to take on complex drug discovery problems that they would never have thought about taking on in the past. I also believe we will see a new revolution in peptide delivery and formulation that will make oral and other routes of delivery much more routine for peptidic molecules. Also, will the anticipated contributions of AI/ML come to fruition in the drug discovery space – this is a key question for the future?

Q **What advice would each of you give to young scientists and drug hunters who want to contribute to the next generation of peptide-based breakthrough medicines?**

First of all, learn about the peptide space and educate yourself through talking to those working in the field and immerse yourself in the peptide literature. Familiarize yourself with the key issues for drug discovery – potency, specificity, stability, PK, PD, formulation, and how these issues relate to design and development of peptides. Learn the fundamentals of peptide synthesis. But most of all, don't be afraid to jump into the field and contribute!

Q **Finally, do you view MK-0616 as primarily a proof-of-concept for oral peptides broadly, or as a case study in what it takes to industrialize such a modality?**

I view MK-0616 In both ways. First, it shows that the peptide modality is alive and well, and the once thought to be impossible goal of oral delivery of peptides is well within our grasp. Secondly, I think this work also clearly demonstrates the complexities associated with modern oral peptide therapeutics design and development and does indeed serve as a case study for future oral peptide therapeutics.

This project has been a crowning achievement for my career and as I just retired from MSD after 36+ years, I feel like I'm going out on a real positive, and I'm proud to have been a contributor to what I view as a game changer to peptide therapeutics. It was an amazing team effort and truly the highlight of my career at MSD.

Closing Note

Mr. Tucker's reflections highlight not only the scientific ingenuity behind MK-0616, but also the collaborative spirit and persistence required to push the boundaries of peptide drug discovery. With the program now in Phase 3 clinical trials, MK-0616 stands, and how these properties relate are both a milestone and a springboard for future innovation in the peptide therapeutics field.