



PODCAST

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WENDY CORNELL, Director, Chemistry Modeling and Informatics at Merck, moderates an expert panel discussion on targets in drug discovery. The panelists are: **JAMIE BAUMGARTNER, PH.D.**, Senior Director of in vitro Pharmacology at MDS Pharma Services, **BRIAN LIGHTBODY**, Vice President of Business Development of Small Molecule Drug Discovery for MicroCal Products Group, GE Healthcare, and **PATRICK ZARRINKAR**, Vice President of Technology Development at Ambit BioSciences

WENDY CORNELL What are the implications of target/drug molecular recognition in the drug discovery process, and how can these interactions be better understood?

BRIAN LIGHTBODY The understanding of the molecular recognition of a target in a potential pharmacophore is a very important part of the drug discovery process, since it's ultimately this process that will be the basis for some form of pharmacological intervention. Establishing a basic understanding of the binding mechanism of a small molecule to its target, which then leads to a control of the biological response, can have a significant impact in a downstream success of a drug discovery program. So this knowledge is really critical to the design of molecules, with the optimum structural features leading to better potency selectivity into reducing the likelihood of clinical failures due to factors like off-target effects.

PATRICK ZARRINKAR The foundation of any successful drug is a compound that has appropriate potency and selectivity and the more you understand about the recognition process between the compound or a series of compounds and the target, the better chance you have of finding compounds that have appropriate potencies and selectivity. That's only the first part.

The tricky part is to maintain that appropriate potency and selectivity while you're optimizing the other properties of the compound that are important in having an actual drug from pharmacokinetics to solubility, formulability and so on. The more you understand about the molecular recognition event, the more you know about where you can modify the compound and how you can modify the compound and still maintain that appropriate potency and selectivity. I think an in-depth understanding of the molecular recognition event often can come from not only from understanding the interaction with the target of interest, but also from understanding how the compound interacts with other related targets and the effect of changes in the molecule across the pattern of interactions with targets. This actually tells you even more about the importance and the role of any particular interaction between the molecule and the particular target of interest. So I think the more you know, the better your chance is of getting an appropriate molecule with the properties you want.

WC I agree with both of you. I think selectivity is becoming more of an issue for everyone — the more tools that we have to get at that early, the better. **Given that not all biological targets can be reasonably accessed, what role should chemistry play in target choice?**

PZ Traditionally, the way drug discovery is done is biology-driven. Decisions about which targets to pursue are more or less based on the biology of those targets, assuming that you are working in a protein class that is considered drugable. But, of course, even within a drugable class, not all targets are equally drugable, and this target-centric approach really makes that assumption, which isn't really true. Any given organization only has access to a small fraction of chemistry space, either due to the physical materials that are available and/or due to the intellectual property space that it is free to work in. With the chemistry available to any one organization, certainly not all interesting targets are equally accessible. So it seems that there's more efficiency to be had by having chemistry feed into the decision about which targets to pursue along with biology. What I mean by that is to look at a number of targets that are biologically interesting, and then ask which one of these is most easily accessed by compounds that we actually have at our disposal. The way we put this idea into practice at Ambit is by screening compound libraries, not just against individual targets that we decide are interesting, but against the whole panel of kinases that includes most of the ones, if not all of the ones, that are biologically interesting either today, or will become biologically interesting. Then we can pick and choose and say, "Here are eight targets that we would be happy to pursue from a biological perspective, and three of these we can actually access really well with compounds we have on hand." So that's where we should put our efforts. So the chemistry plays an almost equal role in deciding which targets to pursue along with the biology of those targets, and that makes the process more efficient, and gives you a chance to focus your discovery resources on those projects where you have the best starting points that are most likely to succeed in the shortest period of time.

JAMIE BAUMGARTNER There are two things that we are actually seeing. One is fragment-based discovery, where chemistry is not taking the final molecule, but taking fragments that may have biological activity and starting to class those. The second thing is, instead of looking at individual targets in isolation, one of the trends that we are seeing is taking integrated cell-based disease models and doing "chemical genetics": It's where the biologists are meeting the chemist halfway, in terms of looking at more targeted libraries in more disease context and instead of looking at an individual's targets, looking at a cell-based readout that would be a mimic of that disease. So you can take a look at immune response, for example, and peripheral blood mononuclear cells and looking at a specific cytokine profile, which are compounds that have a desired profile



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for what you think a therapeutic would look like, screen a focus set of compounds and then start choosing your hits out of there, and then work backwards using a platform like Ambit's to find out where that compound actually interacts. In that particular situation, you're screening against the whole kinome or the whole genome in that cell-based context instead of looking at individual targets. One of the trends that we are actually seeing is more of a retreat from the traditional high throughput screening. So instead of looking at a million compounds or 10 million compounds against one particular target, we're seeing more focused sets and trying to get more biological context early with those particular hits.

BL We're seeing an increasing interest in trying to screen things in the most biologically relevant way possible, as well as trying to be rigorous about determining the mechanism of action for some kind of pharmacological intervention using things like more endogenous reagents, enzymes, substrates and so on. The purpose is just to make sure that the model system represents the most biologically relevant system possible, so that the knowledge and understanding gained is relevant, and that's one big area we're seeing, especially in the label-free world.

WC There definitely seems to be more of a movement to go back to biological relevance and not have the system be so isolated that it becomes simple to analyze, but is not as relevant as it should be.

BL It gives you more confidence in the data.

WC What advice can you give on how to achieve success once a hit is identified?

JB We're actually seeing two different modalities in terms of characterizing hits. One is a classical approach to where we're seeing iterations to where companies are trying to optimize potency initially against a particular target to try to drive down an nanomolar or picomolar type of potencies of a compound against a particular target, and then what we start to see is exploration of the selectivity of that particular compound. So that's a more traditional approach. Some of the more successful projects that we've interfaced with have taken a different approach of doing a lot more parallelism having small iterative pharmacological tests to test for selectivity among subclasses early on. That actually leads to better decisions in terms of how that compound or how that lead series is actually characterized, and also puts in place some of the ADME assays earlier on, so by the time you start looking for more potency, you have a better idea of whether that is going to be druggable molecule, and you also will be able to take a look at what you want to have downstream in your development pathway that would actually address some the adverse events or toxicities that may hang up or kill that particular compound or project later on in the development phase. More parallelism

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and adding more context to that particular molecule or that series is very important, and actually leads to better decisions and downstream success. You do get earlier triage or earlier failures with those compounds, but the end result is more robust. We're seeing more success with our clients and their ability to achieve IND filings.

PZ The broader you look, the earlier and the better you understand the hits that you have and how to move forward with those hits, the better the chance you have of actually ending up with a drug. Again, going back to the theme of chemistry helping to decide which programs to work on, one approach is to start with hits against several different targets and to do some exploratory chemistry to see where you get the best traction, and then focus your resources on that. So you don't assume that you have a 100 percent success rate on the programs you start; you start three programs and then narrow that to one, based on which one looks the most promising, focusing your resources on the programs that are most likely to succeed in the shortest period of time.

WC I would agree with both of you, and I think having as many well-characterized hits to choose from at the start is really important. Certainly once chemistry begins — and a lot of SAR is developed around a particular lead series — then there can be reluctance to switch to another series and possibly lose a lot of that information. I think the choices that we make at the beginning are very important. **How do you see the role of biophysical testing relative to evaluating small molecule inhibitors or activators of drugable targets?**

BL Biophysical methods like microcalorimetry and SPR really play an important role in the selection and design of small molecule pharmacophores for two important reasons. First, these methods are label-free, allowing for interaction analysis of native molecules that more accurately represent a true picture of the biological system. Secondly, in relation to the overall binding affinity, the information that these technologies can provide, such as the thermodynamics of binding and the kinetics of binding, yield more important insights into the binding mechanism and residence time of a small molecule to its target partner. Knowledge of the binding mechanism for example, is crucial in selecting molecules with the best drug-like features for optimum potency and selectivity. This, of course, results in more confident decisions in the drug discovery process. One trend that we are seeing relative to the previous question is that people are more hesitant these days to make their decisions about which hits to move forward based on binding affinity alone getting more rigorous about trying to peel back the underlining factors in the binding affinity. In some cases, may even choose molecules with a lower binding affinity, because they really have the desirable structural features for a potential drug candidate.

WC I think when Pfizer came out with the rule of five, however many years ago that was, it really "codified" the way a lot of people were thinking anyway without realizing it. I think it's clear we need to go well beyond just counting molecular weight and individual donors and acceptors, and so on, and really characterize these molecules.

PZ I would definitely agree with Brian that a greater understanding of how compounds interact with the targets, in addition to how potently they interact really helps one understand the compounds and helps guide which hits to actually focus on. One thing that we've done at Ambit along those lines is to build assays that are specific for a particular activation state of a kinase. So we have separate assays for an activated and an unactivated kinase, and we can understand whether the compounds prefer one state or another, or

whether they don't care. For example, there are two classes of kinase inhibitors that are broadly known as Type I and Type II. Type II inhibitors prefer an unactive-like confirmation of the kinase, and it manifests itself with much higher affinity to the unactivated state relative to the activated state, and we can see that in those binding assays. So actually starting to build assays where you screen compounds against a panel of those assays, you start to understand, not just which targets compounds hit, which is usually what you're interested in when you're profiling a compound against kinases but how each compound might interact with those kinases — is it a Type I inhibitor or Type II inhibitor? What's the binding mode? That allows you to start to ask structural questions in biochemical assays without necessarily having to do a lot of structural work up front.

JB One of the things that we actually come up against is: How do you interpret that early testing data in a biological context? What we're concerned about is how those assays or those early determinations, or binding mechanisms are influenced by protein. All the drugs that we are talking about for small molecules have to reach target with respect to binding up to human serum, for instance, and how does that effect these early interpretations? It's something that we're continuing to have vigorous debate around.

WC What must be considered when adding more biological context to compounds early?

JB In essence, I think the protein binding pieces is one. For anybody offering pharmacological testing whether its kinase testing or broad pharmacological places, the question is: How can you take a look at an early test and make a prediction downstream about what that drug would do if it was administered in man?

It's about adding more context earlier and using that to compare to marketed drugs or drugs in the clinic, as well as pre-clinical models and trying to squeeze out or trying to get more information from those in vitro tests to predict in vivo activities.

BL It's obviously very difficult to predict downstream effects of potential pharmacophore. What we're seeing with biophysical techniques is increasing interest in factors like the hydrophobicity of a drug or drug candidate and how that relates to factors like non-specificity. So that bit of information is something that can be gleaned from something like a calorimeter where you can relate the entropy to the hydrophobicity and the enthalpy is factors like relating the degree of hydrogen bonding and much more ionic and more specific binding events, which is additional information that can be provided by biophysical techniques to more specific binding to places like a catalytic domain or it may be an allosteric site. It's interesting with things like kinases — the fact that you can do some really interesting multi-factorial experiments with things like non-activated and activated kinases in the kinases cascade in addition to the binding mechanism — the mechanism of action can definitely give you more confidence in what's going on and what factors underlay the binding mechanisms, and they can be related downstream to potential secondary effects like off-target effects. For example, if a potential drug candidate has a high degree of hydrophobic interaction underlying its total binding affinity, there's a good chance that it's going to be a very non-specific binder because hydrophobicity is caused by the repulsion of the molecule from the cell and the environment that it's in and not so much the attraction to any given specifics on a target molecule. There's been several retrospectives looking at this, such as the HIV 1 protease inhibitors and the statins to equate the history of the evolution of different drug candidates and their subsequent increase in hydrogen bonding and decrease in hydrophobic interactives to more downstream specificity,

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The Chemistry Modeling and Informatics Group supports the drug discovery efforts at the Rahway, NJ site. In addition, the group is active in methodology development in areas such as virtual screening, kinase similarity prediction, ADME modeling, structural bioinformatics, and target assessment. She is a member of Merck's New Technologies Research Licensing Committee and is active in the Computers in Chemistry (COMP) Division of the American Chemical Society (ACS) where she is a past Program Chair and currently holds the position of Immediate Past Division Chair.



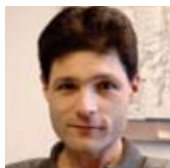
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Mr. James (Jamie) Baumgartner is the Senior Director at MDS Pharma Services, a provider of innovative early stage drug development solutions from discovery through Phase IIa. Previously Dr. Baumgartner was a Research Scientist at Amgen and prior to that he worked as a Research Scientist at ZymoGenetics.



BRIAN LIGHTBODY
VICE PRESIDENT
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Mr. Lightbody is responsible for developing and orchestrating programs that will facilitate the adoption of microcalorimetry into small molecule drug discovery programs. He has over 25 years of experience in developing and commercializing products for biotech and pharmaceutical research.



PATRICK ZARRINKAR, PH.D.
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Dr. Zarrinkar has been at Ambit Biosciences since 2000, and currently is Vice President of Technology Development. He has led the development of Ambit's KinomeScan profiling platform, and was the biology project team leader for the discovery AC220, a FLT3 inhibitor currently in phase II clinical trials. Prior to joining Ambit Dr. Zarrinkar was a Staff Scientist at the Genomics Institute of the Novartis Research Foundation (GNF) (1999-2000).

and lack of drug polymorphism in viral molecules, and how that would effect the drug potency. I think that is interesting work.

PZ It's very helpful and important to add biological context early-on to understand your compounds and that one has to think carefully in how to interpret those results for biological assays and interpret them in light of what one knows about the properties of the compound. I think it boils down to knowing your assays and knowing your compounds and how an assay is run. The details of an assay can affect the outcome and one has to keep that in mind when looking at the results. For example, if it's a cellular assay with cell stimulated, what was the concentration of the stimulant? How long was the stimulant present? Was the compound added before, during or after the stimulant was added? What's the serum concentration that goes to the protein binding issue that Jamie mentioned? All these factors can affect the outcome of an assay. They have to understand the details and the compound.

In the oncology space, a lot of biological assays are cellular proliferation assays — they're neither in sub cultures or in xenografts, which are just cell proliferation experiments in animals — and you might be working with an inhibitor of say, kinase X and you're working a cell system that is believed to be dependant on kinase X for growth, and you see good activity, but unbeknownst to you, your compound may be hitting other kinases that are also important. For example, we know that a fraction of kinase inhibitors have activity against aurora kinase, which is an important kinase with a cell cycle. So if you inhibit an aurora kinase, you are going to inhibit proliferation of most cell types. So if you get a result with your supposedly kinase X inhibitor and a proliferation assay, you might ascribe that to inhibition of kinase X, where as you might have also been inhibiting aurora kinase, and that's what you're seeing and the effect of doing that. Of course, aurora is only one of many kinases that are involved in the cell cycle. So to interpret the biological assay, you really have to understand the properties of your compound, because you think it might be an inhibitor of a particular target, but your compound doesn't know that that's what you expect it to do, and it's going to do whatever it is going to do. I think if you understand your assays and understand your compounds in some detail and think hard about how to interpret the results of the assay in light of that knowledge, that's really where you are going to get some real information about what you have.

JB We talked about adding biological context. Certainly the assays themselves we're seeing a different type of trend of adding multiplex readouts, so in the oncology field for example, the aurora kinase example is fantastic in the sense that if you take a look at only cellular proliferation, you miss the boat. You don't know what your global mechanism is, whether you're hitting specifically on one kinase or whether you're hitting on a node in terms a signaling pathway, or whether you're hitting a multiple kinase where you can do multiple mechanisms of action. For example, in the cancer field, what we're actually seeing this measure cellular proliferation, which has been the gold standard to add reads that would look at apoptosis for phospho sonatri that would measure that cell cycle block. It may be a specific modal measure, like a phosphor-specific substrate inside them, like ATT1 for example. Adding more context just from the assay itself and getting more reads out of that compound can actually address many of the questions that Patrick is talking about in terms of adding more context or why that compound is having that effect on that particular cell line. When we have that type of information, you make much better decisions about which compound, which hit, which lead you actually want to put forth, and again increase your probability of success in terms of development and clinical candidate. **FP**