3rd Talk at Café Weimar

Drug In

Stefan Kubicek, Joanna Loizou,
Georg Winter, Giulio Superti-Furga

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Uncover the mechanism of action of known and new chemical entities to modulate cellular function, identity, differentiation and growth to ameliorate disease, increase precision and avoid resistance to therapy

What are the chemical entities that you are using? Where do they come from? Do you design them?

Kubicek: We have a CeMM library which has around 90,000 compounds, for 2,500 of which we have an idea of what their molecular target is. These are approved drugs, compounds that are currently being tested in the clinic, but these are also so-called chemical probes for certain classes of enzymes that we have, a CeMM-wide interest, including kinases and chromatin-modifying enzymes. Then, we have a large set of compounds that we selected for their chemical diversity, and so these are structures we know very little about, except for their performance in the screens that we have running at CeMM. They are selected to cover as broad a chemical space as possible.

Loizou: Do you have in your collection any FDA-approved drugs and are there any examples of repurposing these from your studies?

Kubicek: Yes, we have. We have purposely tried to cover the FDA-approved and generally approved drugs as widely as possible. In this context, we made a specific collection that we named the *CeMM Library of Unique Drugs or CLOUD*, in which we attempted to keep all the diversity there is in these approved drugs but fitting it to the format that we like to use in drug screens which is a 384-well plate. What we do a bit differently than all the other screening approaches, is that for all these drugs we have annotated them very well with the plasma concentration that is achievable in the clinic.

Superti-Furga: In 2018, what we have started using more intensively is this library of metabolic drugs. They have been assembled by my laboratory together with PLACEBO in the Kubicek laboratory, as a collection that covers enzymes and transporters that affect intermediate metabolism. We use them to probe the effect that interfering with metabolism creates on some of the interesting assays we have. Typically, we also look at the expression of transporters in response to that. The other group of chemical agents we are using we could call "tool compounds" that affect cell biological properties, typically affecting proteostasis and ER homeostasis. Lastly, in 2018, we started to design some new kinds of PROTACs together with Georg Winter and we are looking forward to testing them.

Winter: This is one of the things we are most interested in. Typically, we get these compounds either from collaborative efforts with laboratories in Boston with which I worked during my postdoc years. We also design them ourselves and the gist of these small molecules is really that, as opposed to most other molecules that bind and inhibit a particular protein, these bind and degrade them. So, we can eliminate entire proteins out of the cellular environment. We are particularly interested in applying this technology to probe and understand gene control to be able to potentially drug proteins so far considered undruggable.

Is it difficult to plan or to predict drug action?

Loizou: Technologies such as machine learning have been instrumental within the last 2 to 3 years in helping us predict and design more efficient ways of utilizing drugs. I think this is going to be a tool and a way to push forward by which we can plan and predict drug action in a cheaper, more efficient, faster and more precise manner.

Superti-Furga: So, when comparing profiles, using artificial intelligence makes sense …

Loizou: To design, to predict and to speed up the drug discovery purpose.

Winter: I think, based on the protein class, it's sometimes straightforward to predict or design them to achieve a certain on-target effect. So, we know that they inhibit a particular enzyme. I think what is always interesting to investigate is what else these molecules might do. Traditionally, we have been thinking about small molecules as something that competes off endogenous ligands or metabolites, but more and more we realize that small molecules can do much more. They can change the properties of proteins, they can change stability, they can change interaction partners within cells by just contributing a certain type of binding energy and, all of a sudden, one protein is able to talk to another protein inside a cell. I think this is something that we'll see much more of in the years to come and I believe this will be what is hardest to predict as opposed to target effects inside the same protein family.

Kubicek: I also feel that the proteins and domains for which we have structural resolution, particularly for the structurally guided large-scale efforts to predict targets, still only represent a very limited fraction of all the possible proteins and protein interaction sites that are out there. So, finding targets from compounds that are active in phenotypic screens – and a large proportion of the screens that we do are cell-based and phenotypic – is still a major challenge. Certainly, some technologies that have also been developed at CeMM allow us, in a global picture, to ask the question, "What are all the proteins that are potential interaction partners for certain drugs?", in a cellular context. They are very valuable, both for finding a relevant target but then, of course, also for cases where a drug is designed to have a certain action and mechanism, and is optimized for that, and then for figuring out what else it is doing in terms of off-targets.

Superti-Furga: I think this is probably the area where CeMM is most highly competitive at the international level. I think collectively, the different groups have a good set of approaches and capabilities to figure out the mechanism of action of chemical agents, but I think it is still very, very difficult. I think this is for several reasons. It starts with the identity of cell lines, that often needs to be determined first, the purity and the conformation of the individual chemical agents is sometimes unclear. Many agents may have cell-type- or even cell-state-specific effects and often they are composite effects or compound effects of hitting multiple targets. Sometimes they are of the same class and sometimes of different classes. So, true chemical probes that hit a few very well-characterized targets are not many.

Loizou: And how has CeMM contributed to experimental approaches aiming at nailing down a specific target that a drug may have?

Superti-Furga: Traditionally, the ability to look at proteins engaged by drugs through proteomics has been a very powerful approach, despite all its limitations that have to do with concentration of the target and the affinity. But also, in recent times, I think that has been very nicely complemented by genetics, where we have an increasingly versatile tool box to interrogate by genetic modifiers of drug action what is going on. Then, as you mentioned, the sophistication in the phenotypic characterization of cellular effects leads to the ability to compare and parse and cluster effects so that you can also try to do it that way. All those things together, you know, the molecular and the phenotypic profiling, are becoming more and more powerful.

Is it not presumptuous to do in academia what pharmaceutical companies can clearly do much better?

Superti-Furga: For sure, yet we consider our activities complementary to the activities of pharmaceutical companies. We can do crazier things, for example we have developed this technology that looks at mononucleated cells of the blood for changes in viability and interaction, something that perhaps would not have been done in pharma. We don't believe we can be drug developers. In terms of drug discovery, we are limited by the diversity of the compounds that we have, but we have the freedom of engaging in crazier kinds of assays and doing things that most industry scientists could never do, but that we do. And by so doing, we identify interesting effects that are worth following up.

Loizou: I would also agree that I think drug development is very complementary between what academic research institutes do and big pharma does. As Giulio said, research institutes can be more exploratory, whereas pharmaceutical companies tend to go for a low risk and short-term gain in terms of time invested in developing products. And indeed, we have seen that a collaboration between industry and academia is very fruitful and there are several examples of very efficient pharmaceuticals, including chemotherapeutics, which have been developed by a close collaboration between academic exploratory work and big pharma developing the drugs.

Kubicek: I would also agree, right, that what pharma is particularly good at is developing a candidate or a target to further an approved drug. But what academia can do much better and much more freely is to really understand targets and come up with ideas. That's what it is also doing to bring good rationale related to why it is important to target or not to target a particular pathway, and on the other hand, to really bring novel, overwhelming concepts that would really turn the whole concept of drug ability upside down by finding new, entirely new mechanisms, by which proteins can be targeted, degraded and so on.

Winter: I also think that some of these fundamentally new aspects of how drugs can be made, they really need to stem from a deep understanding of underlying biology. Particularly interesting cases are when drugs or drug ideas emerge from laboratories that have been devoting years of research to really understanding one particular pathway, one particular protein. I think this is also something that academia can contribute and is already contributing to the entire drug development and drug discovery process, simply because this is time- and curiosity-driven research that pharma can't afford, where they would typically take something that seems like a sure theme and then spend a lot of efforts in hit and lead generation, come up with a metabolically stable molecule that is tested in every type of stability assay to then be able to really test it first in animals and then in patients eventually.

Kubicek: I dug out a recent PNAS paper that tried to analyze what is actually academia's contribution to new drug approvals by analyzing how many novel chemical entities were approved from 2010 to 2016, and then, for each of them, trying to analyze what are the old publications on the target of that drug – if it was a drug for a new target – or on the drug itself. They came up with a number of more than 800 million in public spending for each novel one.

Are there any breakthroughs or new trends we can associate with 2018?

Loizou: For me, what was striking about 2018 was that it was a record year for the number of FDA-approved drugs. In fact, in 2018, there were 61 drugs approved by the USFDA, which is the US Food and Drug Administration. But the striking thing is actually what type of drugs were approved to treat which diseases, because 51% of those drugs were actually released in order to treat rare or orphan diseases. I thought that was really quite surprising and interesting because this is normally a group of diseases for which, in each individual disease, there are very few patients, but when taken as a whole, of course, they affect a large majority of the population. These patients tend to have very few treatment options. So, moving towards a more beneficial therapeutic avenue for them is, I think, really important.

Kubicek: I think also, when you look at the structures of these newly approved drugs, you can really see that they cover a wide spectrum, from RNA-based therapeutics to very small, rather small molecules, larger small molecules. This really highlights that there is a broad spectrum of agents that are approved in the clinic and certainly, yes, also shows clear contributions of academic research, which has highlighted that all of these were druggable pathways.

Winter: I think from a chemical biology perspective or chemistry perspective, it's pleasing to see that the majority of all newly approved drugs are still small molecule therapeutics as opposed to cellular therapies or biologicals like antibodies. So, I think there is still reason to be excited about chemistry and biology for drug discovery.

Superti-Furga: I think what really has changed is the appreciation that drugs may have these additional mechanisms of action where they modulate and create interactions. So, it's really like a gain-of-function kind of situation. Contributions that we probably would not have anticipated. It is not clear how generally this is applicable and how often it occurs, but conceptually, we can say in 2018, we all became more aware of that very, very important, additional perspective, which is that drugs may just manage the interaction among the existing molecules more often than we imagine.

Kubicek: Maybe also very fragment-based discovery. It's a trend that has been around for some years, but now really, the concept that using not only crystallography and NMR-based methods on an isolated target but really that also very, very small molecules, so to put fragments in a cellular context, can be used to elucidate targets and potentially new targeting mechanisms.

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Is there anything regarding this area that was achieved in your laboratory or at CeMM that you think is worth mentioning?

Winter: One thing in this field that we now have learned, if we stick with PROTACs for a minute, is that the idea that everything that these molecules would bind, they would also degrade, is not correct. So, you could make a multi-functional binder that would bind to many different kinases and a PROTAC-derived form that degrades different, but not all, kinases. You need particular steric requirements to be able to recruit the protein to the E3 ligase. These steric requirements really dictate the selectivity space of degradation. It turns out that you can take an inhibitor that binds to multiple proteins and by, for instance, systematically altering the linker region, tune these ligands in a way that they can degrade particularly interesting subsets of the kinases. This is an incredibly powerful tool to engineer selectivity in a molecular scaffold, or a chemical scaffold, that is otherwise unspecific.

Superti-Furga: Let me ask you, is it just steric properties, or are there also properties of the targets themselves that make them more or less accessible to degradation?

Winter: I think this hasn't been addressed in sufficient detail. Limitations could for instance be the particular cellular compartment a target resides in. I think what has been clear, what has been surprising, is that just the affinity per se is not that relevant, so you can degrade proteins even though the kinase only binds in the single-digit, two-digit micromolar range.

Superti-Furga: What was interesting is to see that by studying the genetics of drug resistance in vitro, which is in a petri dish with cell culture, you may not only learn something about the drug in question, but you may also discover entirely new, regulatory properties of the target itself. This was the case for the protein called LZTR1 that clearly regulates a pathway that is centered around RAS.

Loizou: Well, in my lab we are interested in understanding and studying diseases that occur due to defects in DNA repair genes. One of the diseases we were focusing on is Fanconi anemia that consists of an inability to remove cross links from DNA. The way that we were asking this question was to basically question how we can rewire cells which are deficient in DNA repair to now become DNA-repair-proficient. Through genetic approaches, we identified a novel deubiquity-lating enzyme called USP48. What we identified is that USP48 has the ability to restore DNA repair in the cell lines defective for Fanconi anemia in an error-free manner. We are exploring ways of how to inhibit USP48 as a potential therapeutic avenue.

Kubicek: We are excited about the fraction of small molecules in the nucleus of a cell and their contribution to gene expression and control of transcription. This is based on a project where we were interested in regulators of BRD4-mediated transcriptions and where we found a folate metabolic enzyme, MTHFD1, to be physically recruited to certain regions in the genome where BRD4 acts as an enhancer of transcription, and it has just been accepted, so we are happy! We are excited about that and it's hopefully also what we are going to solve in the next five years. In my ERC project, we really want to ask the question: What are all the endogenous metabolites contributing to the regulation of gene expression in the nucleus? And we develop methods that would specifically target these metabolic enzymes in the nucleus while leaving them intact and functional outside in the mitochondria and in the cytoplasm.

Do you have the necessary tools? Is there anything that needs to be developed further?

Kubicek: Understanding small molecules and where in a cell they travel, where exactly which metabolite is, and so on, is an immensely challenging question, because diffusion is very fast, on the millisecond scale. We don't have a single published nuclear metabolome, so a description of all the metabolites in a cell's nucleus versus the cytoplasm.

Superti-Furga: Are you able to do that?

Kubicek: I mean we have it in the paper that is now accepted. We use a fast protocol of nuclear isolation where it takes us just three minutes to kind of get the nuclei out and then compare to cytoplasm. The metabolite composition is very, very different, but then still, on a free-flowing time scale, it would take milliseconds for a metabolite to cross the cells… so three minutes is an enormous time scale. We are also thinking of, say, finding methods to somehow block the nuclei, a pause that would give us a longer time scale. We've done some very preliminary experiments where we would just incubate nuclei at buffers for different times.

Winter: What we want to implement next year maybe, is to also integrate synthetic DNA approaches, so basically to be able to synthesize drug-resistant alleles at scale. Or synthesize parts of proteins that might determine whether a particular protein is degraded by a certain drug or not. And therefore, have a more rapid method to assess different drug candidates, how easy it might be to degrade a protein, what are general rules that make a protein degradable, and so on.

Superti-Furga: I think it is my obligation as Scientific Director to be constantly mindful of what kind of technologies are limiting our approaches. I think the difficulty everyone has, is to have enough access to medicinal chemistry or generally, organic synthesis, to the point that we can experiment with things more and play around with them. Just as Georg mentioned, the ability of doing this at the protein level from the target is fantastically powerful. The enlargement of our faculty, to include two new faculty members who are chemists, will represent – certainly a cultural enrichment – and also a practical enrichment of the tool box. But in the medium-long run, we need to further build the internal capacity. Clearly, these are some of the things we are planning to do in 2019: proceed with the plans around chemical biology and create a center that is competent in identifying new chemical entities that have not been seen yet.

Loizou: From my perspective, it is important to remember that useful cellular assays are very important tools also. So, with my lab being interested in understanding DNA repair and how DNA repair pathways are rewired, we traditionally do that by assessing how DNA damage will impact on cell survival, but of course that's not a very accurate measure of how DNA damage is dealt with. So now we are setting up more relevant tools which will directly allow us to measure effects of drugs, compounds or chemotherapeutic agents on DNA repair rather than on cell survival.

Kubicek: We've been doing most of our chemistry so far, following up on a hit from a screen in collaboration and with chemists outside of CeMM or at contract research organizations, where we designed the molecules, but we have them synthesized elsewhere. That is working well, but clearly there is a benefit of doing it in-house. We are very happy that now we have a chemist in the lab, Marton Siklos, who has already – in the last month – made 20 new molecules.

Superti-Furga: Wow.

By when do you think will we see impact of this research in medical practice?

Winter: In the targeted protein community, 2019 will be a very interesting year because this is the year where these specifically designed heterobifunctional molecules will be first tested in clinical trials. So, these are the first programs that will likely be targeting the androgen receptor specifically in a therapy-resistant subpopulation of patients. To the best of my knowledge, these clinical trials are to start at the beginning of 2019. Interestingly, there are already drugs in the clinic that act as degraders, most notably the so-called immunomodulatory agents, or IMiDs, such as the drug Revlidmid, which is a blockbuster drug for the treatment of multiple myeloma and 5q- myelodysplastic syndromes. It took the scientific community sixty years or more to understand that IMiDs follow this unique mechanism of action of changing the function of the ubiquitin E3 ligase CRBN, but there will be plenty of opportunities to try to copy this particular drug's mechanism of action. One example similar to the IMiDs are the sulfonamides that work via a mechanism that is very similar. They lead to degradation of the particular splicing factor which otherwise would also be undruggable and those molecules were tested in phase II clinical trials, but again, at this point, with a wrong understanding of their actual mechanism of action and thus likely following a suboptimal trial design that lacked relevant and informative biomarkers.

Superti-Furga: I just would like to say that, from my perspective, I think this is really a wonderful challenge for CeMM. It may take years, but I think the entire organization is now reaching a certain level of maturity that may set – as future goals – the ambition to try to see some of the, let's say, chemical biological or, in a bold sense, drug discovery projects, coming from a lab at CeMM eventually entering a clinical program. I think it's a reasonable goal. It will take, I assume, let's say, five years, typically, to come to that point, but that should not let us shy away from that goal. That is sort of my perspective.

Kubicek: Yes, certainly. Following up a bit on what Georg said, I was recently trying to see, whether anyone had done global proteomics of approved drugs at early time points. We also concur with Giulio that, yes, our ambition should be to move some of the findings forward to the clinic. For the folic pathway, of course, it is also a pathway that is already in the clinic, so we hope that understanding the contributions of the pathway in the cytoplasm, in the mitochondria and in the nucleus, will also have a clinical impact through better patient stratification and better drugs.

Loizou: Well, the development of inhibitors for DNA repair enzymes has received a lot of attention in recent years, not only for the treatment of cancer but also for other diseases associated with DNA repair defects. Indeed, it was a synthetic lethal interaction between BRCA and PARP-1 which led to the development of PARP inhibitors in the clinic, which was the first example of a specific synthetic lethality in the treatment of cancer. So, I think, considering the interest not only from academic labs but also from pharmaceutical companies in developing such inhibitors as a way to treat diseases, this will become a reality in the coming few years.

Superti-Furga: Joanna, did pharmaceutical or biotech companies become interested in your research based on your chemical, biological findings and identification of new effects of known compounds and so on and so forth?

Loizou: We've certainly been contacted by both academic groups and also pharma companies for several of our projects. More recently, on the project we just published this year, so in 2019, on understanding how kinases, which are enzymes that phosphorylate substrates, are involved in the DNA damage response and DNA repair. Because kinases are enzymes, firstly, but also because they are frequently deregulated in many cancers, they are a group of proteins that received a large amount of attention from pharma-ceutical companies.

Superti-Furga: And druggable …

Loizou: Yes, they are druggable as enzymes. Recently we were contacted by a pharmaceutical company that wanted us to advise them on developing a program they have on targeting an enzyme with inhibitors as a novel therapeutic, chemotherapeutic approach.

Superti-Furga: I think this is something that, maybe to wrap up, the organization is learning to do better and will need to do yet more efficiently, namely to partner with dedicated companies that are professional in the ability of taking some of these projects along, particularly in those situations, where we still would be involved somehow, by contributing some of the understanding on the target or the mechanism of action of the drug. That is something we have already seen, we have multiplied our relationships, and we are trying to do that, eye to eye, without losing, let's say, control of the projects, but at the same time being adults and partners and acknowledging contributions fairly.