Newsletter:
Summer 2014

Drug Discovery Toxicology Specialty Section (DDTSS)

President's Message

DDTSS President, Andrew Olaharski, shaking hands with Muhammet Ay at the 2014 DDTSS reception

I am pleased to note that as incoming president, 2014 marks the 10th year of the Drug Discovery Specialty Section. Completion of a single decade (or four or five of them for some of us), is a special event often accompanied by significant reflection and thoughts about the future. I feel very fortunate to be the one to state that the DDTSS is currently on solid footing, with a membership just shy of 400 and an Emil A. Pfitzer endowment fund that enables us to continue our generous awards for both students and postdocs who have impactful abstracts and posters at each annual meeting. I would be remiss not to mention that this is a direct result of those dedicated individuals who have preceded me (a list of the past presidents is on page 3).

The DDTSS officers are very excited about what the future holds and are currently planning activities throughout the rest of the year. Most relevant to the here and now is the special guest contribution by Dolo Diaz of Genentech who provided a thoughtful and thorough overview of how a discovery/investigative toxicologist identifies whether an observed toxicity is due to an on-target pharmacology. The guest contribution will be a new feature to the newsletter moving forward and we hope to highlight various aspects of drug discovery toxicology in each of the future newsletters. Please reach out to us if you have an idea that you would like to share with the DDTSS membership. In addition, a 2 hour webinar tentatively titled "You are what your microbiome eats: Recognition of how the microbiome is changing toxicology" is being scheduled for this fall and will contain pieces directly relevant to drug discovery. This webinar will also be recorded, allowing individuals that cannot attend the live event the opportunity to revisit the presentation at a time that better suits their schedule. A number of events are also currently being planned for the 2015 annual meeting to be held in San Diego, including a mentoring luncheon where 20 students & postdocs will have the opportunity to meet with 5-6 experts representing different facets of industry (lunches will be provided) as well as an expert panel discussion at the DDTSS reception (topic TBD). In addition, we will be providing a $500 student travel award to the 2015 SOT meeting in San Diego. Please keep an eye out for additional communications that will provide more specific information on each of these activities.

The webinar, student travel award and mentoring lunch are all being generously sponsored by Charles River Laboratories.

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DDTSS 2014 Reception

Yvonne Will with two of the post-doc poster competition award winners Hua Shen and Rachel Goldsmith (Rachel Church absent).

Yvonne Will standing with the graduate student poster competition award winners Roshni Rao, Kazuhisa Miyakawa and Muhammet Ay.

The 2014 SOT in Phoenix was filled with terrific science and we in the DDTSS were very excited to have had some of that highlighted within our graduate student and post-doctoral poster competitions. It was a very challenging job for those judging the competition but after some significant debate the following winners were announced…

The 2014 graduate student poster competition award winners are:

1) Kazuhisa Miyakawa from Michigan State University ($1000); abstract entitled: CONTRIBUTION OF PAR-4 AND THROMBIN TO ACETAMINOPHEN HEPATOTOXICITY IN MICE
2) Roshni Rao from the University of South Carolina ($400); abstract entitled: TETRAHYDROCANNABINOL PREVENTS MICE FROM STAPHYLOCOCCAL ENTEROTOXIN B-INDUCED TOXIC DEATH BY THE MODULATION OF THE MIR-17-92 CLUSTER AND DE NOVO INDUCTION OF T-REGULATORY CELLS
3) Muhammet Ay from Iowa State University ($150); abstract entitled: QUERCETIN TREATMENT PROTECTS PROGRESSIVE NIGRAL DOPMINERGIC NEURONAL DEGENERATION IN CELL CULTURE AND MITOPAK ANIMAL MODELS OF PARKINSON'S DISEASE BY ACTIVATING PKD1 SIGNALING .

The 2014 post-doctoral poster competition award winners are:

1) Rachel Goldsmith from The National Institutes of Environmental Health Sciences ($1000); abstract entitled: ANALYSIS OF HIGH-THROUGHPUT, HIGH-CONTENT DATA IN A *C. ELEGANS* BASED TOXICITY ASSAY
2) Rachel Church from The Hamner Institute ($400); abstract entitled: DOXORUBICIN-INDUCED GLOMERULAR INJURY IS ASSOCIATED WITH URINARY MICRORNA ALTERATIONS IN THE RAT
3) Hua Shen of the University of California, Berkeley ($150); FUNCTIONAL GENETIC SCREEN IN HUMAN HAPLOID CELLS TO IDENTIFY GENES INVOLVED IN SUSCEPTIBILITY TO CHEMICAL EXPOSURE

Yvonne Will with our two departing DDTSS officers. A large thank you to Eric Harstad (left; outgoing councilor) and John Wisler (right; outgoing past president) for their dedication over the last several years to the specialty section.

List of Past Presidents

Drew Badger: 2005-2008
Kyle Kolaja: 2008-2009
John Davis: 2009-2010
Cindy Ashfari: 2010-2011
Craig Thomas: 2011-2012
John Wisler: 2012-2013
Yvonne Will: 2013-2014

Post-doctoral representative vacancy

The DDTSS is currently searching for a post-doctoral representative to serve as an officer through May of 2015. The elected individual will act as a liaison for the post-doctoral membership, representing their interests and needs and helping to set agenda topics for future newsletters, webinars and meetings. Interested individuals should reach out directly to Andrew Olaharski (Andrew.olaharski@agios.com) and Dan Kemp (daniel.c.kemp@gsk.com).

Special Guest Contribution

Weight of Evidence Criteria to Differentiate On-Target from Off-Target Toxicity for Small Molecules

Dolo Diaz PhD, DABT; Senior Scientist; Discovery Toxicology Group Leader, Genentech

Oftentimes it becomes critical to differentiate on-target from off-target toxicities in the drug discovery space. On-target (mechanistic, pathway-related, class-effects) toxicities are driven by the intended target and therefore they are tightly linked to the intended pharmacological effect, such that safety margins or therapeutic indexes cannot be increased. Off-target (chemically-driven, structurally-driven) toxicities are driven by one or more known or unknown targets other than the intended target, or directly related to physicochemical or structural properties. Awareness of what on-target toxicities are expected may help in the decision to pursue or abandon a certain target, whereas off-target toxicities are typically considered solvable with chemical modifications, ranging from minor changes within a chemical series to more profound changes with alternative chemical series. The following ten criteria and associated caveats should be helpful in building a weight of evidence towards determining on-target toxicity.

1) One of the most obvious signs of on-target toxicity is toxicity that is consistent with the biology of the target. Due to the complexity and interconnectedness of molecular pathways and the vast amounts of available literature, it is often not difficult to find links between a particular toxicity and target biology, but caution should be taken since these links might not always be relevant. On the other hand, because of the unknowns in target biology, a link might still exist in the absence of data suggestive of it, and in this sense, ruling out biological plausibility can be difficult (akin to proving a negative).
2) Expression of the target in the tissue that manifests the toxicity is also an important indicator of on-target toxicity. Tissue expression is not always clear and oftentimes different data sources offer conflicting information. Furthermore, mRNA expression does not always imply expression of the protein, and sometimes unspecific antibody signals can be misleading in their indication of tissue expression. On the other hand, lack of expression in a tissue can be hard to prove since available methods and reagents might not offer sufficient sensitivity. Finally, consideration must also be given to potential differences in expression across species and to the possibility that the target tissue might be different from the tissue that manifests the toxicity. For resources on tissue expression see http://www.proteinatlas.org/, http://biogps.org/, http://www.genecards.org/ and http://www.ebi.ac.uk/.
3) Effects that are consistent with the toxicity caused by inhibitors of other targets within the pathway is another sign of potential on-target toxicity. This criterion is more informative for linear molecular pathways versus pathways that are more complex, for which modulation of different nodes of the pathway can lead to different toxicities.

4) One of the most useful tools in determining if a toxicity is on-target is whether the toxicity is similar to the phenotype of target null (knock-out) animals (for target inhibitors). The most relevant knock-out models are the ones that most closely approximate the effect of the molecule, i.e. typically inducible models that reproduce the effects in adulthood (as opposed to embryonic knock-outs), and models where the target protein is still present but lacks function to ensure that scaffolding effects are retained, i.e. kinase binding domain-dead, ligand-binding-domain dead knock-outs [1]. In addition to overestimating the toxicity of a molecule due to potential developmental roles of the target that are not relevant in adulthood, embryonic knock-outs can also underestimate the toxicity if compensatory mechanisms have been triggered during the course of development. An additional consideration is that knock-out phenotypes can vary across species, and since the rat is typically the rodent species of choice for toxicology studies, it is worthwhile considering the generation of knock-out rats early in the drug discovery process, since they can prove very useful in interpreting toxicity findings [2]. Finally, human mutations in the target, i.e. loss or gain of function phenotypes, can be very informative to identify potential undesired, on-target effects. For resources on knock-out models see http://www.informatics.jax.org/; for resources on human mutations see http://www.ncbi.nlm.nih.gov/omim.
5) Knock-out mice and rats can also be used as experimental models to test molecules of interest with the understanding that if the toxicities under evaluation are on-target, they should be absent when testing the molecule in a target knock-out animal. Due to the potential for different strains of wild-type animals and knock-out backgrounds, care must be taken to reproduce the toxicity in the wile-type littermates to ensure that absence of the toxicity in the treated knock-out is not due to lack of sensitivity of the particular strain [3].
6) Some of the of the most direct evidence of a toxicity being on-target is presence of the toxicity with two or more chemically-distinct target modulators, ideally from two different chemical series [4, 5]. Caution must be taken since even very chemically-diverse molecules can share common off-targets that could be responsible for the toxicity.
7) Demonstrating absence of the toxicity with an inactive analog is also good evidence that the toxicity is target-driven. For this approach to be useful, analogs should be as structurally-similar, i.e. inactive enantiomers, as possible, since very minor structural changes can sometimes dramatically alter the off-target profile of molecules [6].
8) A strong relationship between toxicity and target potency is also strongly suggestive of on-target toxicity. This relationship is more straightforward to demonstrate using in vitro models (if the toxicity can be recapitulated in vitro), since exposure/potency can be challenging to control in vivo. A dose-response effect should be present over a wide-range of target potencies (i.e. 10-100-fold) to be able to establish a clear relationship.

9) Good in vitro selectivity as determined by a "clean" in vitro off-target profile, i.e. no strong hits in secondary pharmacology screens, good kinase selectivity, etc., can also be supportive evidence of on-target toxicity. However, these screens only cover limited pharmacological space and therefore a limited number of off-targets, clean profiles cannot rule out the possibility of off-target hits driving toxicity. On the other hand, if a molecule is very promiscuous and non-selective, it is more likely to suggest that the observed toxicity might be off-target.
10) Finally, if the toxicity is consistent across multiple species, i.e. same toxicity present in rodents and non-rodents, on-target toxicity is more likely to be suspected, especially if the target is well-conserved across species (although the toxicity could also be driven by an off-target hit that is also conserved across species).

Clear proof that a toxicity is on-target is hard to obtain, but a thorough consideration of the criteria described above can provide a weight-of-evidence that is adequate to reasonably conclude whether a toxicity is on-target, and therefore enable decision-making.

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