Newsletter: Fall 2023

Drug Discovery Toxicology Specialty Section (DDTSS)

INSIDE THIS ISSUE

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See you in Salt Lake City in 2024!

See you in Salt Lake City in 2024!

President's Message

As I procrastinated writing something thoughtful and witty that might be of interest to discovery toxicologists, I decided to multitask this newsletter and farm out some of the brainstorming to ChatGPT on relevant topics so I could concentrate on finding cheap tickets to the U2 concert. Then I thought, well, let's just have ChatGPT write a song about toxicologists in the style of U2! Work smarter, not harder, right? *Side Note: I really like toxicologists are the heroes in the hive mind of the Great Computer Cloud in the Sky.*

Discovery toxicologists are typically on the forefront and some of the first line users of these technologies. In my deep, meaningful conversation with the AI prompt, it says that toxicology in pharma will change dramatically in the coming decade. Will the paradigms of drug development change in the dawn of this era? Will the reductions in *in vivo* studies come to fruition with the advent of *in vitro* and *in silico* models? Sadly, it did **not** predict that AI advances would increase my vacation time. Clearly AI needs to learn to be a team-player.

My former PI used to repeat often that a toxicologist must have the intangible quality of good judgment and constantly highlighted the concepts of risk assessment. Echoes of this hit me in the "safety's grace" line—that a well-trained toxicologist with good judgement will always be invaluable to risk assessment and the health of their respective institution. Ultimately toxicologists play a valuable role in the community and lives of patients. We should be proud of our unique role.

On the issue of new technologies, we are appreciative to the authors/experts and honored they took the time to write "new modality" reviews in this issue. It's something the officers thought you might enjoy—highly recommend.

Finally, I hope to see you all in Salt Lake City for the Annual Meeting! By that point, I hope to hear Bono crooning about toxicologists as drug heroes.

DDTSS President
Jon Maher

Toxicologists: Drug Heroes

Oh, toxicology, it's in our veins,
A deadly dance of deadly fate,
The hidden dangers that we face,
It's us who will decide our fate.

The damage done, we can't deny,
With every breath, we take it in,
A lesson to be learned,
In toxicology.

In the search for safety's grace,
They [sr. management] put the risks in their right place,
With knowledge, wisdom,
and steady pace, in the toxicologist's embrace.

ChatGPT (in the Style of U2)

Opportunities and Considerations for *Cell and Gene Therapy* Safety Assessment

Amy Pointon
Safety Sciences, BioPharmaceuticals R&D
AstraZeneca, Cambridge, UK

Cell and gene therapies offer the potential to transform disease treatment with the ability to have curative benefit across a range of disease indications. Gene therapy involves the modification or manipulation (i.e. repair, knock-in or knock-out) of a gene either *in vivo* via viral or non-viral delivery approaches or *ex vivo* cell manipulation. While cell therapies involve the transfer of living cells with a desired function to patients, resulting in tissue regeneration or the removal of disease causing or dysfunctional cells using immune cells e.g. T-cells, that can be manipulated via gene engineering prior to administration. Each cell or gene therapy can ultimately be composed of multiple components that make up the final drug product, each component can influence the overall safety profile adding additional complexity to preclinical toxicology evaluation. How the benefit risk profile is evaluated for these complex biological therapies is evolving and the approach required needs to be tailored for different therapies. For example, a virally delivered DNA such as an AAV gene therapy requires a different approach to identify potential risks to therapeutic gene editors delivered by both viral and non-viral technology. Similar differences are also present within cell therapy, for example, between direct organ administration of regenerative cells to the administration of genetically engineered T-cells. This diversity of therapies presents a number of challenges and opportunities to toxicologists as we develop risk identification and mitigation strategies. The overall aim of the preclinical safety evaluations is to establish understanding of the safety profile of the product manufactured and administered using the same process and route of administration as the intended clinical material prior to first time in human. To aid in the design of these evaluations 'guidance to industry' has been published providing a framework for development of appropriate preclinical strategies.

When designing preclinical safety strategies several challengers need to be taken into account. For example, selection of appropriate model systems either *in vitro* or *in vivo* and whether these are diseased or healthy, study length and need for temporal assessments, route of administration, selection of evaluated tissues and or cell types and what test article to evaluate, the human material or comparable analogous cells. When facing these questions, often a robust scientific justification, taking into account specific test article considerations is made to support the proposed approach.

Preclinical approaches for cell and gene therapies need to be able to identify potential safety consequences over various biological resolutions, including genomic, chromosomal, cellular and whole body. This requirement is driven by the potential risks of these therapies which predominantly focus on off-target toxicity targeting 'healthy' cells, off-target gene editing, on-target rearrangements, tumorigenicity and unfavourable immune responses.

These considerations are enabling novel models and methods to be developed enabling humanised evaluation in a robust manner, for example, the evolution of genomic sequencing technology i.e. long read sequencing instead of image-based karyotyping methods and humanised *in vitro* and *in vivo* models i.e. human cell primary screening and humanised mouse studies. As technology develops further, the depth and translational relevance of these technologies is likely to increase and to enable not only detection of potential safety liabilities but also quantitative risk benefit decision making.

Perspectives on Novel Modalities: *Personalized Cancer Vaccines* and *TCR T Cell Therapies* for Cancer Immunotherapy

Gautham K. Rao
Safety Assessment, Genentech
South San Francisco, CA

With the rapid progress in genomic sequencing technologies and human leukocyte antigen (HLA)-epitope binding prediction algorithms, it has become possible to identify, predict and prioritize neoantigens within human tumors. This has resulted in the development of therapeutic personalized cancer vaccines (PCVs) targeting patient-specific neoantigens, which aim to induce T cell responses against the patient's tumor. At present, PCVs representing a variety of platforms–RNA, DNA, and peptide–are in early and late-stage clinical development, and the field has been bolstered by recent demonstration of preliminary clinical benefit. A major challenge to the manufacture of PCVs is vaccine generation and delivery on a patient-by-patient basis within a reasonable timeframe since the mutational landscape of tumors can change over time. From the preclinical side, PCVs also face multiple development challenges. A major challenge is the lack of a pre-defined clinical candidate that can be tested in nonclinical species since PCVs target patient-specific neoantigens that are not defined prior to patient screening nor expressed in animals. As a result, *in vivo* toxicity studies can only test the safety of the clinical vaccine platform. Key considerations during the discovery stage for PCVs are to choose a platform that can be assessed for safety and efficacy in at least one preclinical species, and to ensure that pharmacology and toxicology studies are conducted with a molecule that is adequately representative of the clinical platform. PCV platforms generally leverage elements of the innate immune system to stimulate and enhance the magnitude and durability of immune responses. Since there may be differences in the innate immune responses between nonclinical species and humans, it is also important to consider biology differences that may influence vaccine tolerability and toxicity. Finally, depending on the platform, assessments of vector biodistribution and persistence may be relevant as they also inform the overall safety assessment. To date, PCVs have demonstrated proof-of-principle and an acceptable safety profile in clinical studies. PCVs hold the promise of expanding clinical benefit to patients, especially in the combination immunotherapy setting, and continue to be on the forefront of individualized medicine for the treatment of cancer.

T cell receptor-engineered T cell (TCR T cell) therapies are a new generation of cellular immunotherapies. Multiple TCR T cell therapies

are under development in preclinical and clinical stages. With the clinical success of chimeric antigen receptor (CAR) T cell therapies in hematological malignancies, the focus has increasingly shifted to TCR T cells as efforts to treat solid tumors with adoptive cell therapies have intensified. Unlike CAR T cells, which recognize cell surface antigens, TCR T cells recognize peptide antigens presented in the context of HLA restriction and, therefore, are capable of recognizing antigens from intracellular compartments. TCR T cells are generated by *ex vivo* gene editing of T cells to knockout the endogenous TCR and express a tumor antigen-specific TCR in its place. The first generation of TCR T cell therapies use T cells sourced from the same patient (autologous); however, there are growing efforts to enable an off-the-shelf approach using donor- or iPSC-derived T cell (allogeneic) sources to reduce turnaround times associated with patient screening and manufacture. Tumor antigens targeted by TCR T cells may be shared across patients or patient-specific neoantigens. While TCR T cells offer promise as a novel treatment for intractable cancers, they also present unique development challenges. Human TCRs recognize antigens in the context of human HLA molecules; therefore, the main challenge for preclinical safety assessment is the lack of relevant *in vivo* models. Early TCR T cell therapies were marred with severe adverse events in patients due to on-target/off-tumor or off-target reactivity. Subsequently, sophisticated preclinical safety assessment paradigms have been developed to inform clinical safety, which rely on new approach methods (NAMs) utilizing a variety of *in silico* and *in vitro* assays. During preclinical stages, key safety considerations are on-target/off-tumor and off-target reactivity potential. Assessments of on-target/off-tumor reactivity use a variety of approaches including evaluation of expression and level of expression of target antigen in healthy tissues, and testing for reactivity to human cells expressing both the target antigen and cognate HLA. De-risking of off-target reactivity potential include assessments of TCR to recognize variants of target peptide that may exist in the human proteome, testing for reactivity to normal human tissues expressing the cognate HLA but not the antigen, and ability of the TCR to

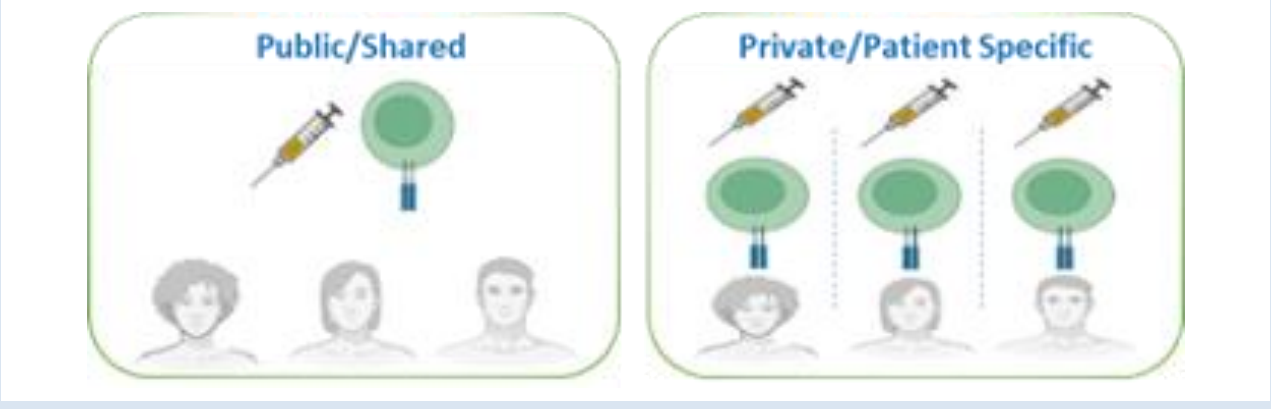

Figure 1. *Vaccines and TCR T cell therapies targeting tumors may be placed in two broad categories – those that target tumor antigens shared across patients (public antigens such as tumor-associated antigens) and those that target tumor antigens specific to individual patients (private antigens such as neoantigens). Approved therapeutic cancer vaccines such as Provenge® fall in the former category, while personalized cancer vaccines fall in the latter. TCR T cell therapies also straddle both categories with current efforts mainly focused on targeting shared antigens.*

recognize peptides in the context of other HLA molecules (alloreactivity). Finally, since TCR T cells are manufactured by gene editing, thorough assessments of off-target gene editing must also be conducted. Autologous TCR T cell therapies have demonstrated proof-of-concept in early clinical trials, and the field is slated to grow significantly as efforts for off-the-shelf approaches using allogeneic platforms intensify.

Perspectives on Novel Modalities: *Heterobifunctional Targeted Protein Degraders*—An Exciting New Modality

Katie Stamp
Nonclinical Safety, Bristol Myers Squibb
New Brunswick, NJ

Harnessing the ubiquitin proteasome system to degrade proteins of interest (POI) with small molecules to treat diseases is not new–molecular glues (MGs), such as thalidomide have been around for many decades, and there are many molecular glues used clinically. MGs are typically low molecular weight molecules and have drug-like physical properties, but they have some known safety risks such as potential to degrade unwanted targets, that can result in extremely severe toxicities, eg teratogenicity. A rapidly expanding, more novel small molecule approach to harnessing the proteasome comes in the form of heterobifunctional targeted protein degraders or TPDs (eg PROTACs® - proteolysis targeting chimera). TPDs contain a target-binding domain and an E3 ligase binding domain; the target binding domain can bind to any part of the POI to bring it into proximity of the cellular degradation machinery to be degraded and does not have to modulate target activity. This modality offers tremendous potential to target previously undruggable targets and to overcome resistance mechanisms. In addition, these molecules are catalytic in nature, able to repeatedly cycle through POI binding, degradation and release. This stoichiometry results in potentially lower efficacious concentrations compared to a conventional inhibitory molecule. As with any therapeutic modality, there are potential challenges and risks associated with heterobifunctional degraders. Due to their two-part nature, they tend to be high molecular weight small molecules, and often violate 'Lipinksi's Rule of 5', resulting in challenging physical properties, such as high lipophilicity and poor solubility. Additionally, some theoretical safety concerns to address include interference with homeostasis of the cellular degradation machinery and potential accumulation of natural substrates; degradation of unwanted proteins; and risks associated with the hook effect, where degradation is less efficient at higher concentrations due to formation of binary complexes (POI-drug or drug-ligase) rather than active ternary complexes (POI-drug-ligase). Another critical consideration for nonclinical safety assessment is the relevance of the toxicology species to predict effects that may be observed in humans due to either on- or off-target effects. This may be of particular significance for cereblon modulators since there are known cereblon sequence differences across species that have been shown to render some species insensitive to the effects of these molecular glues. With targeted protein degraders, both target and ligase expression and homology across species must be considered. Conceptually, the safety assessment of targeted protein degraders is similar to typical small molecules; however, the techniques used, assay conditions/study endpoints, and timing of assessments may need to be modified to address differences in mode of action of the class, such as inclusion of

proteomics to assess degradation of unwanted targets, *in vitro* assay modifications to accommodate lipophilicity and low solubility and extended assessment periods to account for degradation and resynthesis of the POI. Heterobifunctional degraders are a very exciting class of drugs with much potential and much to be learned.

DDTSS SOT Activities and Competitions (2023)

Previous DDTSS Officers:

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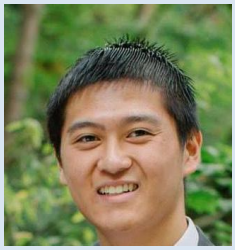
Brandon Jeffy

Councilor
Saurabh Vispute

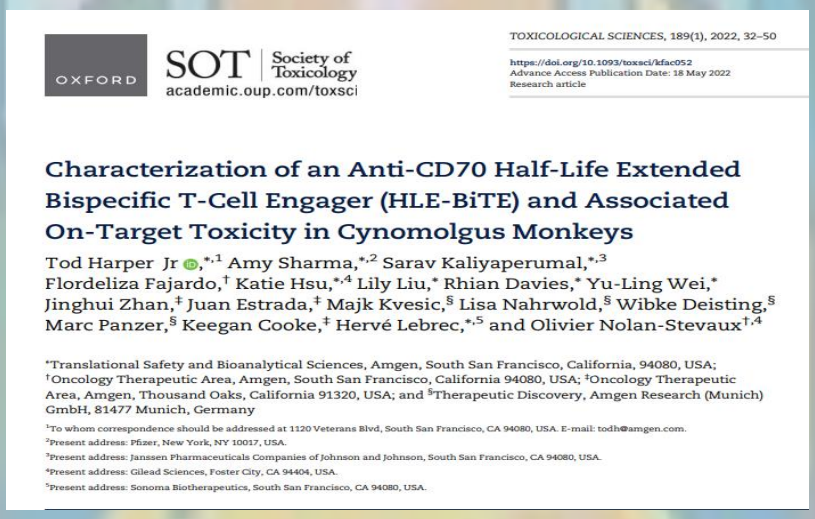
Postdoctoral Rep
Souvarish Sarkar



Graduate Student Rep
Ray Hau

2023 Drug Discovery Toxicology Paper of the Year Award

Harper T, Sharma A, Kaliyaperumal S, Fajardo F, Hsu K, Liu L, Davies R, Wei YL, Zhan J, Estrada J, Kvesic M, Nahrwold L, Deisting W, Panzer M, Cooke K, Lebrec H, Nolan-Stevaux O. Characterization of an Anti-CD70 Half-Life Extended Bispecific T-Cell Engager (HLE-BiTE) and Associated On-Target Toxicity in Cynomolgus Monkeys. Toxicol Sci. 2022 Aug 25;189(1):32-50.



OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 189(1), 2022, 32–50
https://doi.org/10.1093/toxsci/kfac052
Advance Access Publication Date: 18 May 2022
Research article

Characterization of an Anti-CD70 Half-Life Extended Bispecific T-Cell Engager (HLE-BiTE) and Associated On-Target Toxicity in Cynomolgus Monkeys

Tod Harper Jr,[*,1] Amy Sharma,[*,2] Sarav Kaliyaperumal,[*,3] Flordeliza Fajardo,[†] Katie Hsu,[*,4] Lily Liu,[*] Rhian Davies,[*] Yu-Ling Wei,[*] Jinghui Zhan,[‡] Juan Estrada,[‡] Majk Kvesic,[§] Lisa Nahrwold,[§] Wibke Deisting,[§] Marc Panzer,[§] Keegan Cooke,[‡] Hervé Lebrec,[*,5] and Olivier Nolan-Stevaux[†,4]

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[4]Present address: Gilead Sciences, Foster City, CA 94404, USA.
[5]Present address: Sonoma Biotherapeutics, South San Francisco, CA 94080, USA.

Dr. Tod Harper Jr. presented this work in our DDTSS-sponsored webinar on Sept 14th, 2023, and accepted this award at the 2023 DDTSS reception at the SOT Annual Meeting in Nashville, TN. See page 9 for some great photos of our reception!

DDTSS SOT Annual Meeting Poster Awards (2023)

Graduate Student Winners

1st Place: Danielle Kozlosky, Department of Pharmacology & Toxicology, Rutgers University, New Brunswick, NJ, USA

Poster title "Enhanced Fetoplacental Cadmium Toxicity in Mice Lacking the Placental Bcrp Transporter"

2nd Place: Piyush Padhi, Department of Pharmacology & Toxicology, University of Georgia - Athens

Poster title *"Harnessing a Programmable, Microbiome-Based Genome Engineered Live-Biotherapeutic for Sustained Levodopa Delivery in Parkinson's Disease*

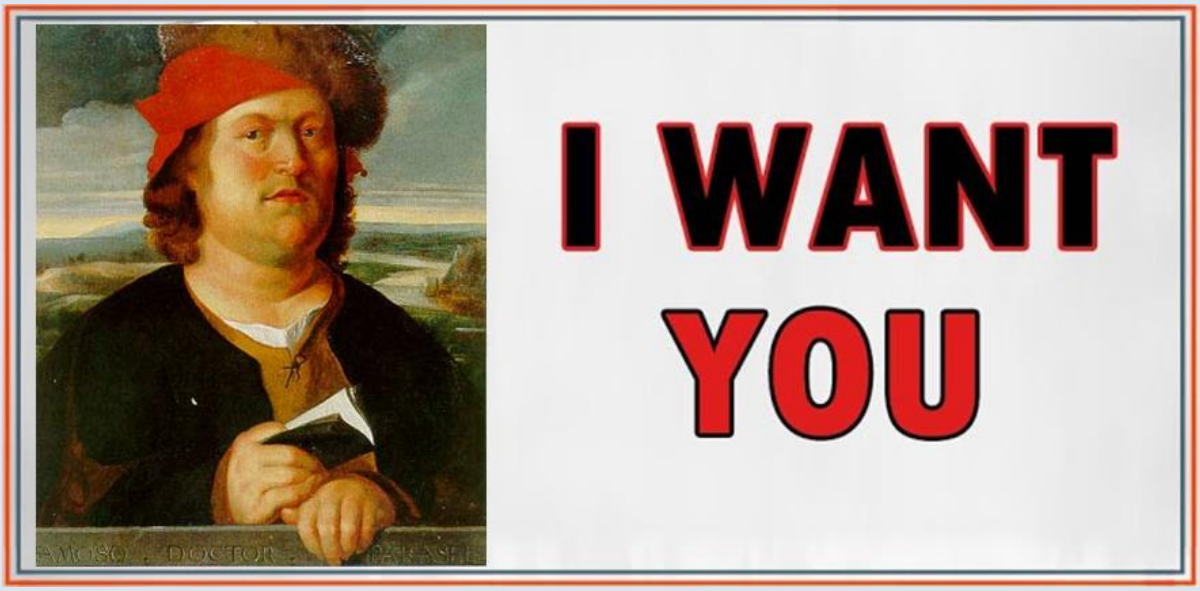
***3rd* Place: Zakiyah Henry, Department of Pharmacology & Toxicology, Rutgers University, New Brunswick, NJ, USA**

Poster title *"The Role of Tissue-Restricted FXR Deletion in NASH Development in Mice"*

Unfortunately, there were no successful Postdoctoral applicants last year…… BUT …… we hope to change that this year with your help! We encourage all postdocs with abstracts relevant to Drug Discovery Toxicology to apply for our upcoming poster awards!
See page 10 for more info!

Award Ceremony at DDTSS SOT Annual Meeting Reception (2023)

Thank you for attending the CE course (Advanced Discovery Toxicology) and DDTSS reception at the 2023 SOT meeting!



…..to submit your ground-breaking drug discovery toxicology publications for…

Our Annual Science Competition! – Drug Discovery Toxicology Paper of the Year Award!

We are pleased to announce that for the seventh year running we will be awarding a prize for the drug discovery toxicology "Paper of the Year." The winner will receive a plaque of recognition and a financial award at the 2024 SOT Drug Discovery Reception. There will also be an opportunity for this work to presented at the Reception. The application is open to all DDTSS members. You must be senior or first author, and the paper must have been accepted or published in 2023. Papers for consideration can be submitted at any time before the **15th December 2023 deadline** to SOT Apply. Please feel free to encourage students and/or postdocs and to reach out to colleagues and make them aware of this exciting opportunity to share their work!

…and to submit your outstanding research for…

The 2024 DDTSS Student and Postdoctoral Fellow Poster Competition and Emil A. Pfitzer Travel Award!

Abstracts should describe original research with high relevance for the field of drug discovery and investigative toxicology. All abstracts will be evaluated for scientific merit and relevance. First, second, and third place winners will be announced at the DDTSS reception and cash prizes will be awarded from the Emil A. Pfitzer Endowment Fund for winning entries. Learn about the awards on the DDTSS website and use SOT Apply to submit your application by **29th December 2023**.

SOT SALT LAKE CITY MARCH 10–14

DDTSS Activities at the 2024 SOT Meeting

CE Course

Bridge over Adverse Waters: Integrating Pathology Findings into the Interpretation of Toxicology Studies (March 10th, 2024)

Session Chairs: Jonathan Maher, Pliant Therapeutics; and Marie Lemper, UCB

Primary Endorser: Drug Discovery Toxicology Specialty Section

Other Endorsers: Biotechnology Specialty Section; Comparative Toxicology, Pathology, and Veterinary Specialty Section

- **The Interconnectivity between Pathology and Toxicology.** Satoko Kakiuchi-Kiyota, Genentech, South San Francisco, CA.
- **Understanding the Aversity to Adversity: Pathologists' Perspectives on Adversity in Nonclinical Toxicity Studies.** Helen Booler, Novartis, Basel, Switzerland.
- **Integrating Clinical Pathology into the Broader Pathology Readout.** Paula Katavolos, BMS, New Brunswick, NJ.
- **Pathology Evaluation in Adversity and Weight of Evidence Decisions for Neurotoxic Findings in Nonclinical Studies.** Brad Bolon, GEMpath Inc., Longmont, CO.
- **Alternative Approaches to Routine Histopathology in Drug Development: How Can Alternative Technologies Inform on Overall Risk Profile?** Mark Hoenerhoff, Inotiv, Kalamazoo, MI.
- **Interactive Session: How to Efficiently Anchor Pathology Discussions to Toxicology.** Marie Lemper, UCB, Cambridge, MA.

Mentoring Luncheon

We will be holding a ***Mentoring Luncheon for Students and Postdocs*** from 12 Noon–1 PM ***on Tuesday, March 12*** at the 2024 SOT meeting in Salt Lake City. Members of the DDTSS leadership committee will be available to discuss careers in pharmaceutical drug discovery toxicology and to answer any questions. More info to come. Anyone wishing to attend can already contact Satoko Kiyota (kiyota.satoko@gene.com). Detailed information will be announced prior to the meeting.

DDTSS Reception

Please join us for our ***DDTSS reception*** at the Hyatt Regency ***on Wednesday, March 13*** at the 2024 SOT meeting in Salt Lake City. Stay tuned for a time and location! We hope to see you there!

Upcoming

Advancing Toxicology in Drug Discovery using Generative Adversarial Networks

Speaker 1: **Dr. Weida Tong, PhD, US FDA**

Speaker 2: **Dr. Zhichao Liu, PhD, Boehringer Ingelheim**

Thursday, February 8, 2024, 2:00 PM–3:00 PM EST

Register for free on the DDTSS website!

Past

"Characterization of an Anti-CD70 Half-Life Extended Bispecific T-Cell Engager (HLE-BiTE) and Associated On-Target Toxicity in Cynomolgus Monkeys"

Organized by Satoko Kakiuchi-Kiyota, Genentech; and Lauren Lewis, BMS

Speaker: **Dr. Tod Harper, PhD, DABT, Amgen**

Thursday, September 14th, 2023

Recording

Stay Tuned — More to Come in the Spring!!!

1. **Webinar Geared Toward Undergraduates**
2. **Webinar Geared Toward Graduate Students**

Engage Undergraduates in the Pursuit of Toxicology Careers!

Access ToxScholar Outreach: https://www.toxicology.org/awards/gf/toxscholar.asp

List of Past Presidents

Brandon Jeffy	**2022**
Marie Lemper	**2021**
Zoe Zhong	**2020**
Dinah Misner	**2019**
Howard Mellor	**2018**
Peter Newham	**2017**
Ray Kemper	**2016**
Dan Kemp	**2015**
Andrew Olaharski	**2014**
Yvonne Will	**2013**
John Wisler	**2012**
Craig Thomas	**2011**
Cindy Ashfari	**2010**
John Davis	**2009**
Kyle Kolaja	**2008**
Drew Badger	**2004–2007**

Open DDTSS leadership positions in this coming year's election:

- Vice President-Elect (4-year commitment)
- Secretary/Treasurer (2-year commitment)
- Councilor (2-year commitment)

If you're interested in becoming part of the leadership team, please submit your Biosketch **ASAP** to kiyota.satoko@gene.com. Self-nominations are welcome. Deadline to receive nominations is **November 17th, 2023.**

To-do list:

1. **Submit your:**
   - Paper of the Year
   - Poster (Grad Student or Postdoc)
2. **Make sure to attend:**
   - **CE course:** *Bridge over Adverse Waters: Integrating Pathology Findings into the Interpretation of Toxicology Studies* ***(Sunday, March 10th)***
   - **DDTSS Reception:** More info to come!
   - **DDTSS Mentoring luncheon:** More info to come!

See you in Salt Lake City in 2024!