TACONIC
Models For Life.

Meeting Report

Virtual Workshop - IBD Mouse Modeling: Best Practices for Drug Discovery, Emerging Models, and Preclinical Research

Overview

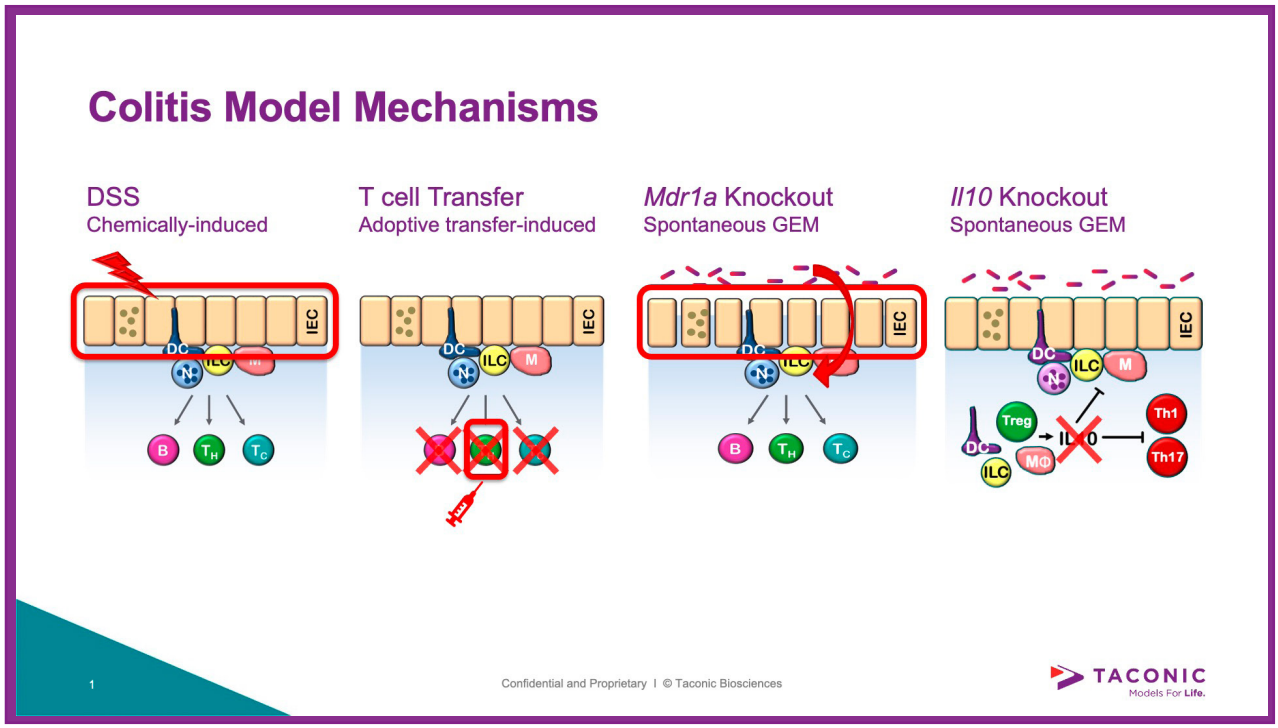
A four-hour virtual workshop titled *IBD Mouse Modeling - Best Practices for Drug Discovery and Emerging Models* was held on March 31st, 2021 and organized by Taconic Biosciences. The workshop consisted of a symposium of invited speakers from academia, industry, and regulatory agencies with the goal of providing a forum for experts in the field of inflammatory bowel disease (IBD) to come together and highlight critical new science, share strategies, and discuss the path forward in colitis research. The symposium included talks from Dr. Philip Dubé of Taconic, Dr. Benoit Chassaing of Inserm, Dr. Mark Sundrud of Scripps Research Institute, Dr. Jeremy Goettel of Vanderbilt University, Robert O'Connell and Dr. Julie White of Bolder BioPATH, Dr. Philip Smith of Roche, and Dr. Odile Engel of the FDA. The full event recording, agenda, and speaker bios are available **online**.

Dr. Philip Dubé
Modeling IBD: Challenges and Opportunities

The workshop began with Dr. Dubé introducing some of the challenges and opportunities of modeling IBD in mice. He first highlighted the many challenges with colitis models, including selecting the appropriate model based on the mechanisms of the model and drug targets, the characterization and translational relevance or validity of the models, and the importance of the microbiome in reproducibility, pathway compatibility, and drug targeting and discovery. Some of the factors critical to colitis mouse models include genetic and microbiome contributions to colitis phenotypes. He did an excellent job of defining the differences in model validity and gave a great overview of the mechanisms of the commonly used models (Figure 1). His presentation includes a discussion of non-scientific factors which can impact IBD research including licensing barriers, timelines involved in animal research, and expenses incurred from colitis studies. He also touched on Taconic's TruBIOME® program which offers a solution to maintain gnotobiotic models with custom microbiome profiles. His introductory talk set the stage for the other speakers' presentations and ensuing discussion throughout the symposium.

Figure 1

Colitis Model Mechanisms



DSS is a popular model used due to its acute disease onset (1-2 weeks). This model is chemically induced and based on injuring the gut epithelium and assessing the regenerative and inflammatory properties after induction and treatment. The T cell transfer (TCT) model is also very popular due to its focus on T cells in the gut. While this model is great at showing the role of T cells in the model, it focuses on one T cell population. The final two models listed are the Mdr1a and Il10 knockouts. These are spontaneous colitis models. Mdr1a knockout mice have a barrier defect that leads to spontaneous colitis in response to the enteric microbiota. Il10 knockout mice develop spontaneous colitis and inflammation in response to microflora. Both models have longer disease onsets (1-6 months) (Dr. Philip Dubé, Taconic Biosciences).

Dr. Benoit Chassaing

Mouse Models of IBD - a Microbiome Perspective

Dr. Chassaing gave an overview of the role that the microbiota plays in health and disease. He touched on dysbiosis and the alterations in microbiota function that typically lead to colitis or IBD. His research uses dietary emulsifiers to drive alterations of the microbiota composition and promote chronic inflammation in a Black 6 Il10 knockout model[1]. One of the highlights of Dr. Chassaing's talk was the use of gnotobiotic models to determine correlation vs. causation of the microbiome's role in colitis. He utilized fecal microbiota transplantation (FMT) into germ-free (GF) mice to demonstrate that the microbiota is required and sufficient for driving intestinal inflammation[2]. More importantly, he showed that minimally complex microbiota is "resistant" to colitis in these models. Specific types of bacteria and more complex flora tend to lead to the development of colitis in Crohn's patients[3]. While his approach cannot be applied to all IBD models, it emphasizes the critical importance of the role of the microbiota in IBD.

Dr. Mark Sundrud

Lymphocyte Sub-specialization in the Small Intestine: Mechanisms, Implications for IBD Therapy, and Utility of Preclinical IBD Animal Models

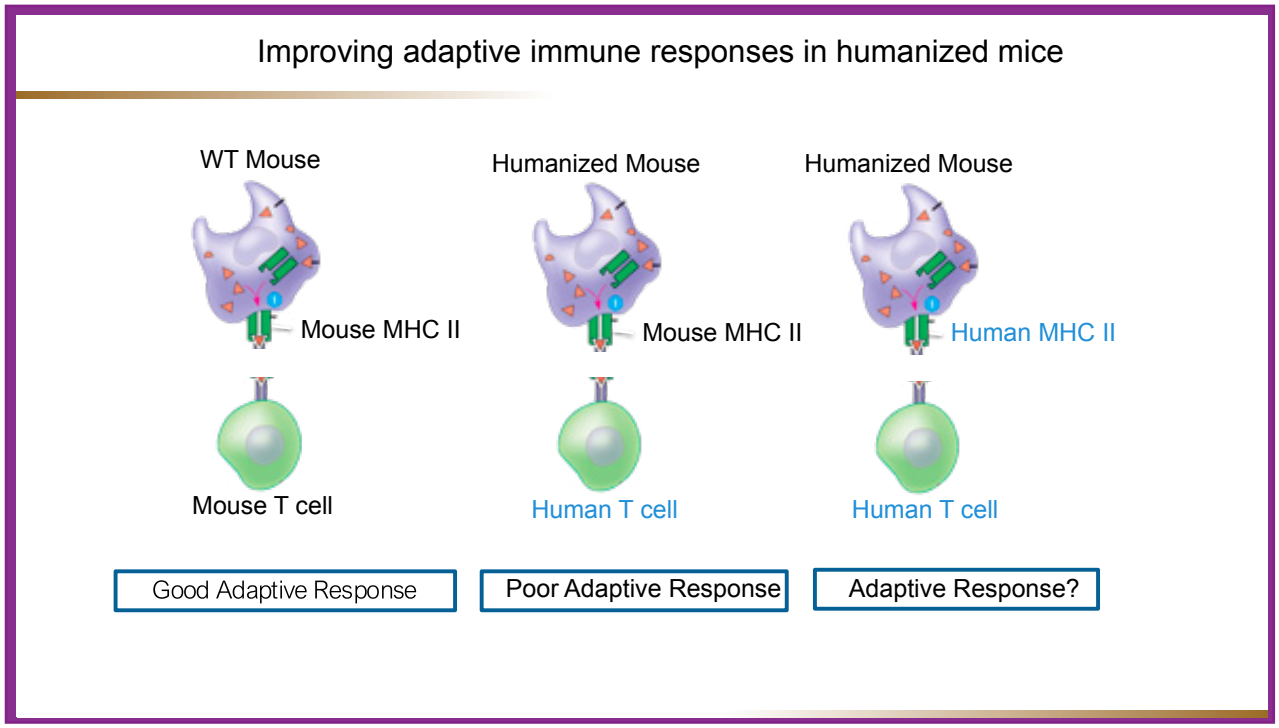
Dr. Sundrud gave a very interesting talk based on the disease area and mechanisms involved. One of the more refreshing parts of his presentation was his distinction between Crohn's disease (CD), specifically small bowel Crohn's disease, and ulcerative colitis (UC). While there are similarities in immune pathways between the two diseases, there are differences in how these pathways impact the diseases[4]. These differences also extend into the clinical presentation and location of the inflammation of both diseases. Most CD models largely focus on the colon and are affected by the microbiota. However, there is evidence of ileitis (and a few colitis) models not being affected by dysbiosis but displaying the presence of distinct immune cell subsets seen in IBD[5]. Dr. Sundrud's research has shown that T cells in the distal ileal gut have acquired a different mechanism to halt the development of ileitis; the presence and upregulation of the Mdr1a transporter, which serves to protect the intestine (along with other receptors) from bile acid-induced oxidative stress and colitis[6,7]. Overall, IBD models should not only consider the microbiota but also other important factors such as inflammation and specifically T cell function and regulation in the intestine and IBD. His research took a slightly different approach with a classic model (TCT, Mdr1a model) to allow the mouse to inform the IBD polygenic disease landscape through human immune cells and pathways.

Dr. Jeremy Goettel
Humanized Mice for IBD Research

Dr. Goettel discussed the use of humanized immune system mouse models in IBD. One of the limitations of murine models is that they do not always reflect what is in the patient and fail to predict the outcome of human therapeutics. Humanized models present a viable translational platform to study human immunobiology *in vivo*. After giving a thorough background and definition of humanized immune system mice, he went in-depth about the major caveat of these models, the lack of the adaptive immune response. Dr. Goettel's approach to overcoming this challenge is the development of a humanized mouse that expresses human HLA-DR1, which allows for proper education of T cells on a human MHC molecule (Figure 2). These mice present with a strong and robust immune response driven by T cells and mature B cells which display class switch recombination[8]. He combined this novel humanized model with two different methods to induce colitis: TNBS chemical induction and humanization with hematopoietic stem cells (HSCs) from patients suffering from immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX). This showcased the versatility of this model by engrafting these mice with HSCs from an autoimmune patient to recapitulate disease
(See next page)

Figure 2

Adaptive Immune Response in Humanized Mice



Adaptive immune responses in models can vary based on how T cells are selected for in the mouse. In wild type (WT) mice, selection of T cells and development is done in a mouse thymus on a mouse MHC class I or II molecule resulting in a good or normal adaptive response. However, in many humanized models, after human HSC (CD34+) engraftment and human immune cell development, human T cells are selected on mouse MHC molecules and develop in a mouse thymus resulting in a poor adaptive response. Due to this caveat, many humanized models fail to have a strong adaptive response because human peripheral T cells have been educated on a mouse MHC and fail to recognize human antigen presenting cells for activation. The research strategy to overcome this (seen above) was the development of a humanized mouse that expressed human MHC or HLA for human T cell selection (Dr. Jeremy Goettel, Vanderbilt University).

Humanized Mice for IBD Research
(CONTINUED)

development. He also used the model to show efficacy for therapeutic testing in IBD. He found that HLA restriction is not required for certain treatment efficacy with his humanized TNBS model[9,10]. Further, he was also able to use this same model to show that infusing ex vivo induced or activated human T regulatory cells prevents colitis[11]. The highlight of his presentation was his ability to show the power to develop more evolved and advanced IBD humanized models to assess human target engagement. The potential to continue this trend in research is within reach.

Robert O'Connell and Dr. Julie White

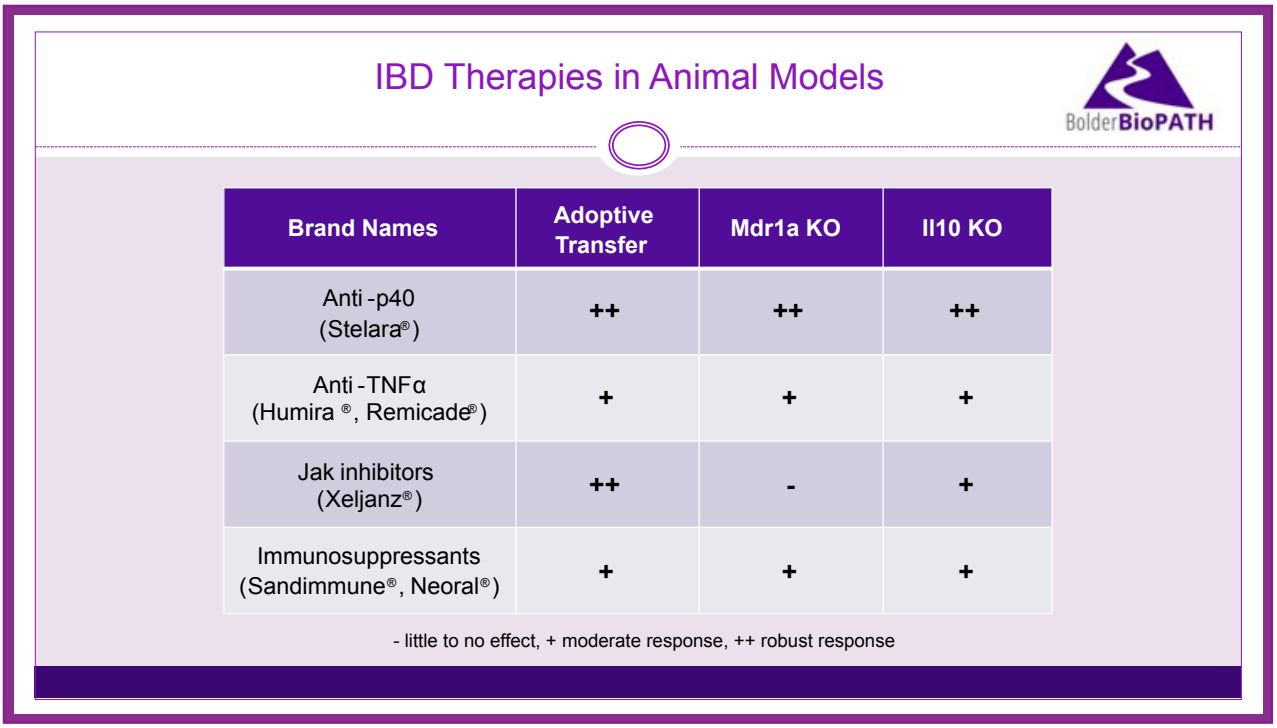
Comparing *in vivo* and Histopathological Endpoints in the T-cell Transfer and Spontaneous Mouse Models of IBD

Mr. O'Connell and Dr. White gave an overview and a CRO perspective of the IBD models used at Bolder BioPATH. Bolder BioPATH has collaborated with Taconic over the years on numerous colitis models, including the TCT, Mdr1a, and Il10 knockout mice, and they have experience with more acute models like DSS and TNBS[12]. They discussed the histopathological presentations in several different mouse IBD models. Overall, their presentation covered important practical considerations for successful IBD studies. Some of the highlights of their talk included discussion of the use of endoscopy and scoring, histopathological assessment and scoring, differences in colitis location and phenotype with each model, and the correlation of the models to human UC and CD. Figure 3 summarizes their findings from treatment studies in TCT, Il10, and Mdr1a knockout mice. Most of the pathways targeted are similar, and each model has its strength and weaknesses. Based on Bolder BioPATH's presentation, observations, and opinions, these three models have the most relevant pathways and tend to be the strongest predictors of clinical success of therapeutics.

(CONTINUED)

Figure 3

Chart of IBD Therapeutic Use in Animal Models and Response or Outcome



Brand Names	Adoptive Transfer	Mdr1a KO	Il10 KO
Anti-p40 (Stelara®)	++	++	++
Anti-TNFα (Humira ®, Remicade®)	+	+	+
Jak inhibitors (Xeljanz®)	++	-	+
Immunosuppressants (Sandimmune®, Neoral®)	+	+	+

- little to no effect, + moderate response, ++ robust response

TCT, Mdr1a, and Il10 knockout models are strong predictors of clinical therapeutic success. All three models respond well to anti-p40 (Il12/23) treatment. The opposite occurs for anti-TNF and Jak inhibitor treatments. These results hold true for clinical outcomes in humans (Robert O'Connell and Dr. Julie White, Bolder BioPATH).

Dr. Philip Smith
The Use of Mouse IBD Models in Drug Discovery

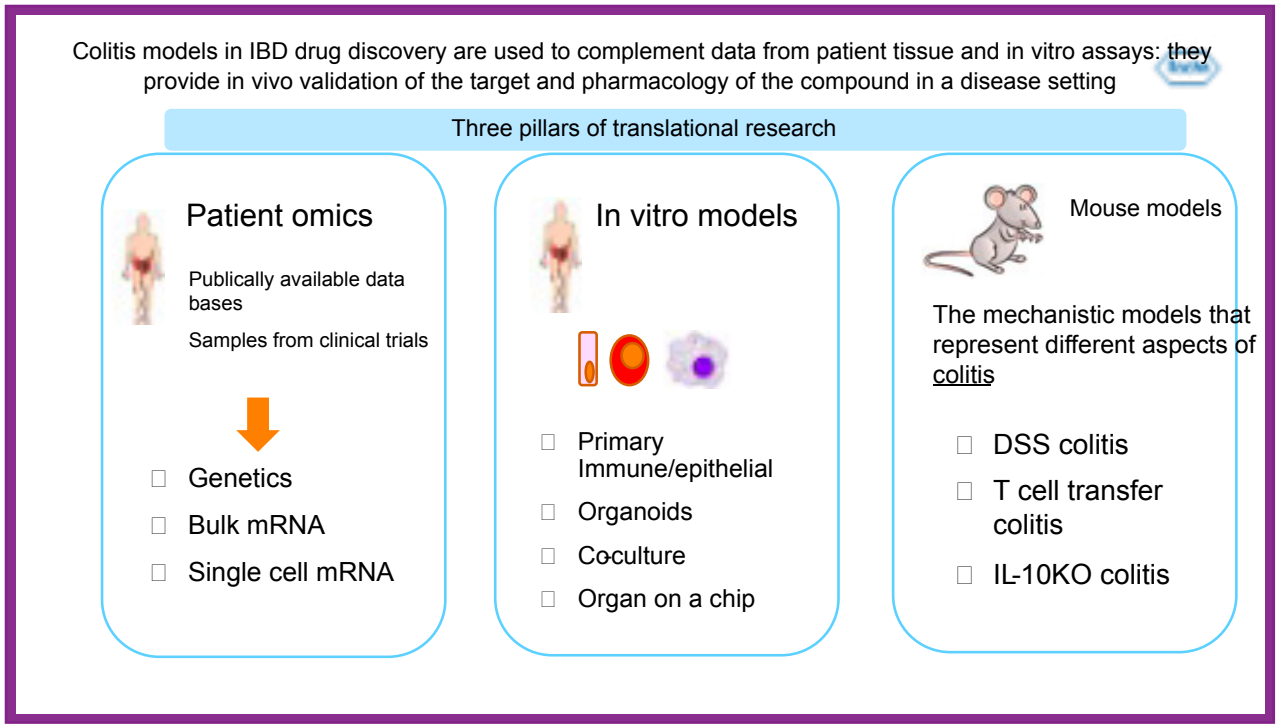
Dr. Smith brought an industry perspective on IBD drug discovery and IBD mouse models to this workshop. He eloquently explained that the strengths of the colitis models are that they model different aspects of the disease. DSS induction models epithelial damage as well as permeability seen in colitis. The compounds that target the epithelial barrier (GLP-2, EGF, etc.) prove to be effective in this model based on the mechanism. This holds true for other models, including the TCT which replicates T cell migration, and the Il10 knockout which promotes TLR-mediated cytokine release. It is important to match the therapeutic under evaluation with the model which demonstrates the mechanism of action targeted by the drug. Dr. Smith also highlighted one of his more masterful studies which determined that the Fc receptor on the anti-TNFα antibody is required for efficacy. He also pointed out that the typical laboratory mouse has a relatively immature immune system compared to a normal human immune system and noted how difficult it is to compare mice to humans without similar immune education. The use of colitis models for drug discovery follows a reductionist path, with most models being used for target discovery, exploratory biology, and biomarker research. Once researchers near early clinical trials, the need for these models decreases. The key concept from his presentation was that there are no IBD models, just numerous colitis and ileitis models that represent certain pathogenic processes of human IBD disease.

(See next page)

(CONTINUED)

Figure 4

Colitis Models Generate Data to Complement Patient-omics and *in vitro* Assays



There has been a new approach to our understanding and use of mouse models for drug discovery research. Mouse colitis models are now being used to complement data generated from transcriptomics of single cell RNA seq of patient tissue and *in vitro* primary cell and co-culture assays. These experiments provide *in vivo* validation of the target and pharmacology of the compound in the disease setting (Dr. Philip Smith, Roche AG).

Dr. Odile Engel

The Role of Gut Microbiota in Autoimmune Diseases: Focus on Inflammatory Bowel Disease (IBD)

Dr. Engel concluded the presentations with an overview of her microbiome research and use of the FMT model. Her talk took a different perspective focusing on IBD as a systemic disease driven by TNFα. TNFα plays a critical role in IBD leading to "leaky gut" symptoms which include impaired barrier function, increased immune cell infiltration, and gut tissue inflammation and remodeling, to name a few. Dysbiosis of the microbiome, which was mentioned throughout the presentations and discussed in more detail during Dr. Chassaing's talk, is a major marker for IBD and other human disease. Dr. Engel was able to show that these alterations in the composition of the microbiome and resulting disease phenotypes can be transmitted. Using $TNF^{\Delta ARE}$ mice, which overproduce TNFα and display severe systemic and IBD symptoms similar to CD. She transferred their microbiota through FMT to germ-free

(See next page)

The Role of Gut Microbiota in Autoimmune Diseases: Focus on Inflammatory Bowel Disease (IBD)
(CONTINUED)

and control conventional B6 mice. Her research showed that transferring the microbiome from "sick donors" ($TNF^{\Delta ARE}$ mice) transmits colitis disease (in the form of gut inflammation through increased Il17 and TNFα levels and altered gut microbiome, and specific bacterial strain changes, similar to those seen in $TNF\Delta^{\Delta ARE}$ mice) in germ-free animals[13]. Interestingly, conventionally colonized or "healthy" mice were resistant in principle to these changes or alterations. Dr. Engel did an excellent job of not only exploring the potential for the microbiome to transmit the disease experimentally in preclinical models but also shed light on its promising ability to treat IBD in humans[14]. Her research also emphasizes the need for careful donor selection for human therapeutic FMT to avoid unintended consequences.

Conclusion

Overall, the presentations did a thorough job addressing the challenges and opportunities of IBD research. Prior to the event, many attendees expressed their inability to choose the best IBD or colitis model. What we learned is that there are no perfect IBD models. You must choose the appropriate model based on your understanding of the: 1) mechanisms of actions in the disease you are investigating, 2) pathways you are targeting and validating for drug or therapeutic treatment, and 3) translational relevance to human IBD disease. Any one of the models discussed during the workshop (and in the field) can be used for colitis and IBD research. The more intuitive question is, *What is the best approach to choosing the most appropriate model?* The speakers did a great job of answering this by allowing the mechanisms and science to guide and lead their research decisions. One of the highlights of the meeting was discussing the opportunities in IBD modeling and research. There is significant potential in designing novel IBD humanized immune system models. There are also interesting translational opportunities to address the microbiome through FMT in germ-free, conventional, and/or antibiotic-treated mice. More importantly, the most prominent take-home themes from the speakers on IBD modeling is to consider the importance of the microbiome and human immune system in studying IBD and colitis disease. After the formal presentations, the event concluded with an interactive panel session that also touched on more specific challenges and opportunities in the field and a networking segment that gave attendees a chance to interact with the speakers. The end of this report includes the question-and-answer transcript from the event.

Acknowledgements

The Virtual Workshop: *IBD Mouse Modeling - Best Practices for Drug Discovery and Emerging Models* was produced by a consortium of contributors at Taconic Biosciences (listed below). We would like to acknowledge those individuals and thank them for their hard work, assistance, and perseverance in achieving a great event.

Contributors

SPEAKERS

Dr. Philip Dubé – Associate Director of Global Applications Sciences and Senior Scientist, Taconic Biosciences

Dr. Benoit Chassaing – Assistant Professor, Inserm

Dr. Mark Sundrud – Associate Professor of Immunology and Microbiology, Scripps Research Institute

Dr. Jeremy Goettel – Assistant Professor of Medicine and Assistant Professor of Microbiology and Immunology, Vanderbilt University Medical Center

Mr. Robert O'Connell – Vice President of Research, Inflammatory Bowel Disease, Bolder BioPATH Inc.

Dr. Julie White – Comparative Pathologist, Bolder BioPATH Inc.

Dr. Philip Smith – Discovery Leader for Gastro-Immunology Research, Roche Holding AG

Dr. Odile Engel – Senior Staff Fellow, US Food and Drug Administration (FDA)

MODERATOR

Dr. Courtney Ferrebee – Field Applications Scientist, Taconic Biosciences

ADDITIONAL SUPPORT

Dr. Megan MacBride, Dr. Fred Beasley, Dr. Moriah Jacobson, Dr. Terina Martinez, Dr. Ivan Gladwyn-Ng, Dr. Caroline Horizny, Stacy Wennstrom, Kelly Grover, Jason Barnes, Justin Grosskurth, Kristofer Wildermuth

References

1 Chassaing, B. et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature 519, 92–96 (2015).
2 Viennois, E., Gewirtz, A. T. & Chassaing, B. Chronic inflammatory diseases: Are we ready for microbiota-based dietary intervention? Cell Mol Gastroenterology Hepatology 8, 61–71 (2019).
3 Viennois, E. et al. Dietary Emulsifiers Directly Impact Adherent-Invasive E. coli Gene Expression to Drive Chronic Intestinal Inflammation. Cell Reports 33, 108229 (2020).
4 Chen, M. L. & Sundrud, M. S. Cytokine Networks and T-Cell Subsets in Inflammatory Bowel Diseases. Inflamm Bowel Dis 22, 1157–1167 (2016).
5 Pizarro, T. T. et al. SAMP1/YitFc mouse strain: A spontaneous model of Crohn's disease-like ileitis. Inflamm Bowel Dis 17, 2566–2584 (2011).
6 Cao, W. et al. The Xenobiotic Transporter Mdr1 Enforces T Cell Homeostasis in the Presence of Intestinal Bile Acids. Immunity 47, 1182-1196.e10 (2017).
7 Chen, M. L. et al. CAR directs T cell adaptation to bile acids in the small intestine. Nature 1–5 (2021) doi:10.1038/s41586-021-03421-6.
8 Goettel, J. A. et al. Fatal autoimmunity in mice reconstituted with human hematopoietic stem cells encoding defective FOXP3. Blood 125, 3886–3895 (2015).
9 Tyagi, R. K. et al. HLA-Restriction of Human Treg Cells Is Not Required for Therapeutic Efficacy of Low-Dose IL-2 in Humanized Mice. Front Immunol 12, 630204 (2021).
10 Goettel, J. A. et al. Low-Dose Interleukin-2 Ameliorates Colitis in a Preclinical Humanized Mouse Model. Cell Mol Gastroenterology Hepatology 8, 193–195 (2019).
11 Goettel, J. A. et al. AHR Activation Is Protective against Colitis Driven by T Cells in Humanized Mice. Cell Reports 17, 1318–1329 (2016).
12 Fitzpatrick, L. R. et al. VPR-254: an inhibitor of ROR-gamma T with potential utility for the treatment of inflammatory bowel disease. Inflammopharmacology 28, 499–511 (2020).
13 Gabay, O. et al. Exposure to TNF Antagonist Therapies Induces Variations of the Gut Microbiota in an in vivo Model Using Healthy Mice. Joint Bone Spine 87, 175–178 (2019).
14 Levy, A. N. & Allegretti, J. R. Insights into the role of fecal microbiota transplantation for the treatment of inflammatory bowel disease. Ther Adv Gastroenter 12, 1756284819836893 (2019).

Selected Q&A Transcript from the Event

There was robust discussion during the presentations via the chat function, and answers were submitted by the speakers as well as other attendees.

Questions for Dr. Philip Dubé, Modeling IBD: Challenges and Opportunities

Are there any sex differences with the colitis phenotype? Are females more susceptible?

Dubé: No. Not in our (Taconic's) hands. We have not noticed or identified any sex differences in the Taconic colitis models.

Are Taconic SPF mice associated with a defined consortium?

Dubé: All Taconic mouse strains begin associated with ASF (Altered Schaedler Flora) but, depending on the husbandry conditions, will accumulate additional microorganisms. Our standard SPF, known as "Murine Pathogen Free™" (MPF™), is not gnotobiotic. We do offer some strains as either GF or with a defined flora, and we are able to generate these as custom projects. More details regarding Taconic's production health standards and custom microbiome services are available online.

What is the role of the microbiome in the T cell transfer model (immune-compromised)?

Ferrebee: There has been an extensive amount of data collected in immunocompromised mice (SCID, Rag1 or Rag2 KO, etc.) on the adoptive TCT model showing the importance of the microbiome in T cell-induced colitis. Some studies have been able to show that in GF TCT recipients, histopathological scores are low (indicating no colitis detected) when compared to TCT recipient mice colonized by specific bacterial strains. Research groups have demonstrated that these microbial strains and communities (mono- and multi-associated) can determine disease induction, susceptibility, and severity (with the presence of segmented filamentous bacteria (SFB) and SPF conditions being one of the leading combinations to induce colitis). The microbiome is critical for colitis in the TCT model.

Can Taconic supply SCID mice colonized with SFB for the T cell transfer model?

Dubé: Taconic supplies Rag2 KO mice that are from SFB+ colonies. These work very well for TCT model.

Questions for Dr. Benoit Chassaing, Mouse Models of IBD - A Microbiome Perspective

What are the limits of the gnotobiotic approaches?

Chassaing: There are a lot of limits but many values. This approach is not an easy model to use. You need to have specific equipment and maintain or keep a specific gnotobiotic status. You need a specific isolator or housing system to maintain this status. From a technical approach, it is definitely challenging. This approach can be quite expensive to work with. But if you have all the tools needed, there can be a lot learned and gained from this model.

What about the emulsifier effect in WT mice?

Chassaing: As we reported in 2015 (https://www.nature.com/articles/nature14232), in WT mice, low grade chronic intestinal inflammation is observed, which leads to altered metabolism (insulin resistance, increased body weight, etc.). This is actually a very important finding. Not all individuals with genetical susceptibility seem to be impacted by these commonly used dietary emulsifiers.

Is AIEC LF82 alone + emulsifier sufficient for colitis?

Chassaing: Yes, it is. Il10 KO mice mono-colonized with AIEC LF82 bacteria + emulsifier develop chronic intestinal inflammation.

Is it better to treat mice post-T cell transfer as conventional (handling outside of biosafety cabinet) or handle as immunodeficient?

Dubé: I think this would depend upon your animal facility, the additional opportunistic agents that might be present, and the additional biosecurity measures that you have in place. Rag2 KO or SCID mice are still immunodeficient post-T cell transfer, so if there is a risk of being exposed to opportunistic agents, I would recommend handling them in a biosafety cabinet.

Questions for Dr. Mark Sundrud, Lymphocyte Sub-specialization in the Small Intestine: Mechanisms, Implications for IBD Therapy, and Utility of Preclinicial IBD Animal Models

Is Mdr1 also upregulated in the epithelial cells?

Sundrud: Yes, Mdr1 is high in the intestinal epithelial cells. Philip (Dubé) touched on this. If you look at both data from mice and humans, from what I have seen, it does appear that epithelial Mdr1 expression is also highest in the ileum. Though it is clearly also high in the epithelium of the colon. Obviously, the really interesting question is why Mdr1a KO mice develop colitis and not ileitis. The "short" answer is we don't know. But potentially the "longer" answer is that there is also evidence of hepatic dysfunction in the liver of Mdr1a KO mice. The way that we think about bile acid-induced ileitis is that the liver is essentially the "gun" that is firing the bile acids ("bullets") that then get reabsorbed in the ileum and Mdr1 is the shield that protects against those "bullets". If you take away or reduce the "bullets" then you need less of a shield. So, we think that may explain why these KO mice don't develop ileitis. But there are other important considerations. One is that Philip (Dubé) mentioned that the Mdr1a KO model and colitis involves just Mdr1a. It lacks Mdr1a not Mdr1b. We and others have shown that Mdr1b is upregulated in a compensatory manner by T cells and in many other tissues where Mdr1a is normally high. What we don't know potentially is whether Mdr1b gets upregulated in T cells in those mice (Mdr1a KOs) in the ileum and if that is sufficient to protect against ileitis. Whereas potentially in colonic epithelial cells for whatever reason, it's possible that the compensation by Mdr1b either doesn't happen or is not as necessary.

Is Mdr1 expression restricted to ileal CD4 T cells, but not colonic CD4 T cells? What subset of immune cells are CD4 T cells, Th1, Th17, and/or αβ TCR restricted?

Sundrud: That is a great question. I tried to touch on this. I apologize if I wasn't clear. We published some of this data. What I would tell you is any cell in the ileum has a very high expression of Mdr1. And I also want to make it clear that it's not as if T cells outside of the small intestine just don't express Mdr1. Ist's just that they express Mdr1 at much lower levels. It's really clear to determine the expression either by dye efflux assay or by using the Mdr1 reporter mouse. But we have looked at a number of T cell subsets and it appears that the pattern (as I mentioned before) shows any lineage pro or anti-inflammatory in the ileum markedly upregulates Mdr1 expression. And that also extends to intraepithelial lymphocytes including gamma delta cells. So, we think that this is not a lineage-specific pathway. Essentially, any cell that's in the ileum, especially the intraepithelial cells, are going to be exposed to very high concentrations of local bile acids and you need Mdr1 to prevent oxidative stress.

Do ileal CD patients have increased bile acids in the lumen/feces compared to UC or CD colitis?

Sundrud: This is a great question. You might imagine that our understanding of bile acid dysmetabolism in inflammatory bowel disease is not as advanced as our understanding of the microbiome and dysbiosis. And to be fair, I don't follow the clinical literature as much. I have not seen any clear sets of studies that develop a consensus that there is a certain pattern of bile acids that are seen in ileal Crohn's vs. "UC" (ulcerative colitis) vs. healthy adults. The one point that I would say is that chronic inflammation of the ileum has been shown in some cases to decrease expression of the ASBT transporter. Chronic inflammation of the ileum or ileal Crohn's patients undergoing a surgical resection has been known to impair bile acid reabsorption in the ileum. The result of this is an increased concentration of bile acids in the colon and that induces this phenomenon called bile acid diarrhea. Post-operatively (following surgical resection), a lot of ileal Crohn's patients present with bile acid diarrhea and are placed on cholestyramine and/or other bile acid sequestrants for treatment. More recent data suggests that inflammation in ileal Crohn's disease by itself is not sufficient to predict bile acid malabsorption. There's so much more information that we need to understand about how bile acids are regulated and how they potentially become dysregulated in both ulcerative colitis and Crohn's disease.

Alterations in bile acid composition have been described in multiple forms of intestinal inflammation, including lower GI inflammation (inflammation in pouchitis or from C. difficile infection). Is bile acid biology very different in colon vs. ileum in human disease?

Sundrud: The biggest difference in bile acid physiology between the small and large intestines (abbreviated SI or LI) is concentration. The concentration of bile acids in the SI is ~ 1-10 millimolar, which approaches critical micelle concentrations (CMCs) for most species and can cause stress and inflammatory responses in local/resident cells. By contrast, and because of ileal reabsorption, bile acid concentrations in the LI/colon are low micromolar (~ 10-50μM). At these concentrations, bile acids appear to have more hormone-like signaling effects through cell surface and nuclear receptors. In addition, the secondary bile acids produced via microbial metabolism in the colon have different preferences for host receptors, compared with primary (and aminoacylated) bile acids in the SI. So, whereas our works suggest that high bile acid concentrations in the SI have predominantly pro-inflammatory effects on mucosal T cells (https://pubmed.ncbi.nlm.nih.gov/29262351/; https://pubmed.ncbi.nlm.nih.gov/33828301/), other recent studies indicate that low-level bile acid signaling through alternative nuclear receptors in LI/colonic immune cells may generally promote tolerance and suppress inflammation (https://pubmed.ncbi.nlm.nih.gov/31875848/).

Questions for Dr. Mark Sundrud, Lymphocyte Sub-specialization in the Small Intestine: Mechanisms, Implications for IBD Therapy, and Utility of Preclinicial IBD Animal Models (CONTINUED)

Is there any impact of bile acid sequestration on secretory lineages like tuft cells in SI?

Sundrud: The absorptive enterocytes in the SI are tightly regulated by bile acid-driven FXR signaling. But I am not aware of studies that have looked at bile acid effects on secretory/tuft cells.

Questions for Dr. Jeremy Goettel, Humanized Mice for IBD Research

Does the humanized mouse model of TNBS-induced colitis work with PBMC reconstitution rather than CD34+ cells? If so, how well does this compare to CD34+ reconstituted mice?

Goettel: That is a great question. The tricky part about the PBMC infusion is that the animals have a very short lifespan. The reason we chose to move towards the fully immune humanized animals is because while you get circulating PBMC in the blood, very few of them actually migrate to the colon and lamina propria. You do find them there but just in smaller numbers. For the complicating reasons of GvHD (or reactivity), as well as fewer cells in the gut, we moved away from doing that. But I understand that it's much easier to take PBMCs and infuse them. But just be aware that those animals will get a strong xenoreactivity.

Someone from Taconic may want to comment but based on my understanding, the PBMC version would not work for any model that takes longer than 24-48 hours because the human cells will be cleared within the first couple days of the experiment. With the CD34+ mice, the human cells will be present for the duration of the experiment.

Goettel: As far as PBMC model, after a few days only T cells will persist along with some B cells, but most other types are cleared or die.

Dubé: In super-immunodeficient mice (e.g., NOG), various human immune cells will survive and expand for extended periods of time following PBMC engraftment. T cells, in particular, will expand over the course of 3-4 weeks and these cells will drive the development of xenogeneic GvHD. NK cells and most myeloid cells are very short-lived following PBMC engraftment in standard NOG.

Does the induction of Tregs by agonist 2-(10 H-indole-30 -carbonyl)-thiazole-4- carboxylic acid methyl ester (ITE) occur locally in the gut or Induced and then recruited from spleen?

Goettel: We have not infused ITE into the animals and then assessed it. The model that I introduced was actually taking the regulatory T cells, inducing them *ex vivo*, and infusing them into the animals. The induction was in the culture dish and then (the cells were) infused back (into the mouse).

Do you generate B cell responses in the HLA-DR1 Tg mice?

Goettel: We are able to see OVA-specific IgG in the HLA-DR1 mice.

Questions for Mr. Robert O'Connell and Dr. Julie White, Comparing *in vivo* and Histopathological Endpoints in the T-cell Transfer and Spontaneous Mouse Models of IBD

In the Mdr1a model, do you have problems with mice fighting? What about sex differences with regard to disease severity and progression?

O'Connell: Early on we looked at females vs. males and we didn't necessarily see a difference in disease severity or progression. With a lot of models we run in our facilities, we try to work with females over males because of aggression and fighting. Fighting induces a stress which can cause disease differences. We run that Mdr1a model in females.

A general question, are all colon weight/length data representing colon including feces?

O'Connell: The colon weight/lengths are collected without feces. Once we remove the colon from the animal, we will measure the length, remove any fecal content, and then weigh.

Questions for Mr. Robert O'Connell and Dr. Julie White, Comparing *in vivo* and Histopathological Endpoints in the T-cell Transfer and Spontaneous Mouse Models of IBD (CONTINUED)

What would the recommended group allocation be for this and other IBD models if you don't have access to endoscopy?

O'Connell: I think sample sizes or numbers of 10 or 12 are a good place to start. Endoscopy is primarily performed to gauge severity of disease. If you're looking for more subtle treatment effects that you necessarily won't pick up with, say a disease activity index, it is useful for that. Sometimes we can run models and a particular batch of mice may come in and seem to progress a little bit faster. So, with that situation, endoscopy is useful if we need to terminate a study a few days or a week early. The opposite may be true as well. If we are seeing nice effects, we could extend the study out based on those endoscopic scores to see a larger (longer) treatment effect or difference. If you don't have an endoscope, it's not necessary to run the model. It's just nice to track disease. But if you started with numbers of 10 or 12, I think that's a good place.

Do you observe fibrosis in the Mdr1a KO mouse or any mouse models? What is the best way to assess fibrosis in any of these models?

O'Connell and White: That is the one thing that we are currently looking into. With the Mdr1a model in particular, this model has been reported for fibrosis. Early on we did some (fibrotic) staining at day 35 and did not see a difference. So, we are currently running those studies again. We are planning to run the study out a bit farther and then perform the staining and see if that's viable in our facility.

The stain we usually recommend for intestinal fibrosis is the Picrosirius Red (PSR) stain for two reasons. One is that you already have quite a bit of collagen in the intestine and if you use something like the trichrome stain, it tends to be a little harder to read through that background staining of the collagen. Two, the PSR has the additional ability to be visualized under polarized light so you can see the newly laid collagen vs. the pre-existing collagen. PSR is usually what we recommend for histological assessment of intestinal fibrosis.

Is endoscopy too invasive if performed once a week?

Dubé: In my experience, endoscopies are well tolerated if performed properly by an experienced endoscopist. The exception being DSS where the gut is very fragile. I will let Bolder comment on their experience.

O'Connell: We have performed weekly endoscopy in all of the chronic models without any evidence of damage caused by the procedure.

Do you know if there is a very different microbiome in immune compromised models (like TCT) vs. other models in immune competent mice?

O'Connell: We know there are differences between vendors, and we imagine there are also differences between the strains. However, we do not have any data to directly support this.

Ferrebee: It has been reported that the microbiota or microbiome composition in the IBD models is actually very different (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3937555/). I think Dr. Chassaing and Dr. Engel did a great job of discussing the differences in their gnotobiotic FMT models which showcase this. Those differences in microbiota/microbiome compositions hold true for all the IBD models across the board.

What is the maximum number of endoscopies that can be conducted per animal in a study, particularly in chronic models?

O'Connell: Once a week is fine, and I imagine 2 or 3 times weekly would be ok as well. However, I don't think you gain much information performing the procedure more than once weekly, and the anesthesia component also needs to be considered for chronic studies.

Questions for Dr. Phil Smith, The Use of Mouse IBD Models in Drug Discovery

One of the challenges is that a pathway that is demonstrably altered in some patients may not be altered in a given model. Should target activity be assessed in various models before choosing which one(s) to use to evaluate a given intervention? Second, thoughts on why neutralizing soluble TNFα is ineffective in IBD?

Smith: For question one, I think this goes to the last point in my talk. In order to choose the correct model to assess the efficacy of compound "X" targeting pathway "Y", we need to have a deep understanding of the models down to the single cell level. I think once we have this understanding of

Questions for Dr. Phil Smith, The Use of Mouse IBD Models in Drug Discovery
(CONTINUED)

Smith: the pathways involved in the different models that we've talked about today, we can say this model represents this pathway very strongly. For example, we can say we see this pathway upregulated in this patient population from the single-cell data of patients. Therefore, we can bring together this model and this patient population. With some biomarker research, we could even identify these patients in the clinic and treat the patients with specific medication. Models and target patient populations should be paired. Biomarker pairing can inform appropriate medication strategies. For question two, there are various hypotheses on the role of the Fc receptor on TNFα antibodies. One is that the antibodies with a functional Fc receptor induce apoptosis of T cells expressing membrane-bound TNFα. The other is that the antibodies form immune complexes with TNFα which then down modulates macrophage activity through FCγRIIa.

Sundrud: For question two, the poly-arthritis in TNFα mice is T/B-independent, whereas the ileitis is T cell-dependent (as determined by crossing with Rag1-/- animals): https://pubmed.ncbi.nlm.nih.gov/10204494/

Questions for Dr. Odile Engel, The Role of Gut Microbiota in Autoimmune Diseases: Focus on Inflammatory Bowel Disease (IBD)

Did your FMT study analyze mucosal inflammation and cytokine production in the colon, ileum, or both tissues?

Engel: What we do when we collect the colon or gut is collect the middle of the gut. That means we always select samples from the same area which is midway between the stomach and the colon. For microbiome studies, we extract genomic DNA from the colon.

Did you measure the TNFα level in FMT before the transfer?

Engel: Yes. As I have shown, you can see the TNFα levels by histology in the donors before the transfer. We did some Luminex before the transfers and we see very high levels of TNFα in the gut of the donor mice.

Has transmission of any sort of autoimmune disease phenotype been identified in humans treated with FMT (i.e., for C. difficile infection)?

Engel: That is a very good question. We (at the FDA) regulate biotechnology and TNFα antagonists that are going to be used to treat IBD. We also regulate fecal transplants, and they are now being regulated through the FDA more and more. We screen those fecal transplants submitted to the FDA to be sure that we do not give a fecal transplant to a patient with very severe IBD. In which case, this transplant would be deleterious to the patient. However, the screening process is still in its infancy. We are still not largely regulating this process as much as we can. I think this is the reason why the FDA is very interested in looking at the microbiome and the transmission of disease through transplantation. You do not want to transmit another disease while treating IBD. So, this is a very crucial issue.

Have you tried to treat WT mice with antibiotics before you did the $TNF^{\Delta ARE}$ FMT and did you get a disease phenotype?

Engel: We thought about doing it, but finally decided to use GF mice because it was straightforward for us to perform those studies. Both methods (antiobiotic use and GF mice) hold just as much impact in the FMT research world. But yes, it will distinguish if the microbiome itself is transmitting the phenotype, or a more immunologic cross-talk mechanism is occurring.

Is there a role for combination therapy in IBD? For example, a microbiome manipulation/therapeutic combined with a biologic?

Engel: Definitely yes. Therapy combinations are the future of treating diseases. I am not aware of studies showing a treatment with FMT associated with biologics, but it is certainly a way to go, with all the precautions we take.

Interactive Panel

What does it mean to have a humanized IBD mouse model? What are the challenges and opportunities surrounding humanized IBD mice?

Goettel: I think what would really usher in a new era is if we could get to a place where we could do a humanized model like the adoptive transfer type colitis models. The difficulty in the humanized mouse models of IBD is that you don't have control over the genetics as much. And I've always thought the "holy grail" will come when we can culture HSCs indefinitely so that we can target certain genes much like we do for mice and then generate immune humanized mice that have certain genetic defects. While it's possible to look at this for the monogenetic disorders (for example I mentioned the FoxP3 deficiency), for other things that might be more polygenetic IBD, it is hard to tease that apart because the adults themselves are a single individual or "one". Usually, you don't have many donors and you don't identify what their genetic polymorphisms may be before you proceed to perform engraftment and *in vivo* research in mice. I feel like those are some of the limitations and I think on the future horizon if we can somehow manipulate the genetics of the stem cells prior to performing engraftment in mice, that would really start to allow us to find genes that function in IBD and determine how those genes play out in human immune cells.

Smith: Absolutely. So, one thing that we really are short of and would allow us to bring forward new therapies, particularly large molecules or biologics, are targets. Many of the targets we are going after do not cross-react with human targets. So, we are left with developing very potent antibodies to an exploratory receptor or target. But really, we don't have any models that enable us to generate the *in vivo* "proof of concept." This would give us confidence that it would have an effect in the clinic. A lot of the projects either don't make it to the clinic because the confidence is not there and we don't understand the biology in the disease setting OR they go straight into the clinic and because we don't understand the biology generally, they fail. So having a mouse model of intestinal inflammation that will enable us to target a human receptor on a T cell (IL23 for example) would allow us to generate an antibody to block it. One of the downfalls is that we have no way to test it. (We would need to develop the surrogate antibody which again is very cumbersome.) For example, to have a model such as the TNBS model that Jeremy (Goettel) shared where (it doesn't have to be translational and) it is sensitive to ustekinumab (anti-IL12/23p40) is really valuable. We could use this model to examine the IL23 pathway in human cells *in vivo*.

Sundrud: I guess maybe one point that I would ask Dr. Goettel for clarification on would be the potential application of CRISPR-based gene editing in the human progenitor cells prior to putting them in to a mouse model. I understand that you are not going to be able to target dozens of individual loci. But you probably will target at least a couple. Is that being explored as far as you are aware of (in the context of humanized mouse models)?

Goettel: So, not that I am aware of. We had proposed to do this in collaboration with some investigators out at Stanford who were using homology-directed repair to target both gene Calleles. So, the problem is hitting both alleles. If you have a X-linked disorder, you're lucky in that you can just target one. But to make sure you're hitting both alleles, I think the homology directed repair where you're putting in two different selectable markers or markers that you can sort on (through flow) is the best approach or strategy to do. This way, you can be sure that you are dealing with a fully targeted stem cell. Once confirmed, you could infuse from there. But to my knowledge has not been done with any amount of great efficiency. Investigators (in particular, Derrick Rossi https://www.cell.com/cell-stem-cell/pdfExtended/S1934-5909(14)00455-X) have initially looked and used this approach to target CCR5 in T cells for looking at resistance to HIV infection. That was a bit easier because each mouse didn't have to have a fully homozygous setting, they could just look at the T cells in which they were correctly targeted on both sides. The strategies are there at least on paper. Execution is tough.

Compare how the microbiome impacts the outcome in the chemically induced models (DSS, TNBS).

Chassaing: These are two questions. If we compare DSS treatment for GF vs. conventional mice, there is not much of an effect in GF. The DSS GF model seems to be microbiota independent. But in terms of within conventional mice, if you compare only mice with microbiota, the differences in the microbiota composition affects the outcome of DSS and TNBS. This is well known. Therefore, I think it is very important to report the provider or facility you receive your mice from when you publish the logistics of your study because the microbiota and some of the effects are different based on the facility. For example, if we take mice that are really clean from a very clean provider or facility, we have much less of an effect. And if we take mice from a "dirty" facility, we have much more of an effect. So, there is a difference even in the microbiota and how clean or diverse it is in the presence of the symbiotic and host microbiome as well as various other bacterial strains which can drive inflammation in these models.

Interactive Panel

(CONTINUED)

What advice or suggestions would you offer in deciding which animal models to pursue in developing drugs and biologics for IBD?

Smith: I think what the message is from a lot of the speakers today at the workshop is that you need to use the model that's appropriate to the mechanism of action for which you are targeting. (This includes targeting with a compound, focusing on a low molecular weight target, or a biologic.) If it is an epithelial target, the DSS model is your best option. If you are looking at T cell migration, the T cell transfer (model) will suffice.

Can you speak to some of the challenges you've encountered in your IBD research?

Chassaing: I think something that is a downside of the IBD model, but a big advantage is the reproducibility of the model. For example, in some IBD information models where you focus on (or deviate from) a specific gene, the data is not reproducible and difficult to see because you don't know if the data is specifically related to that gene (consider differences in gene vs. protein expression for data with the model, etc.). However, when you think about it, this discrepancy is a very big advantage for the model. You can use those discrepancies to identify some new mechanisms of information. So, I think there are both downsides and advantages for reproducibility of the IBD model.