Carcinogenicity Assessment of Biologics

June 13, 2013

Provided by:
The American College of Toxicology (ACT) and The German Society of Toxicology

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Carcinogenicity Assessment of Biotechnology-derived Pharmaceuticals

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ACT Webinar
June 13, 2013
Outline

1. Introduction
2. Regulatory Guidance
3. Carcinogenicity Assessment of Biologics
4. Carcinogenicity Assessment Strategy
5. Take Home Messages
Small versus Large Molecules

◆ Small molecule pharmaceuticals
  – Chemically-synthesized small molecules (up to 500 – 800 Da), which are capable of crossing cell membranes / entering the nucleus
  – May be metabolized to active / genotoxic intermediates
  – DNA interaction possible
  – Toxicities are mostly due to off-target effects

◆ Biotechnology-derived pharmaceuticals
  – Protein therapeutics manufactured in living cells
  – Large molecules (around 3 KDa up to 150+ KDa) which require specific transport mechanisms to enter cells
  – Direct interaction with DNA / other chromosomal material is highly unlikely and metabolic degradation pathway is of no concern
  – Toxicities are primarily on-target effects (“exaggerated pharmacology”)
Carcinogenicity versus Genotoxicity

◆ Carcinogenicity
  – The ability of a carcinogen to cause cancer
  – A carcinogen is an agent whose administration to animals leads to a statistically significant increased incidence of neoplasms compared to untreated controls (Casarett & Doull)
  – Neoplasm is a heritably altered, relatively autonomous growth of tissue (Casarett & Doull)

◆ Genotoxicity
  – The ability of an agent to damage or alter the genetic information (DNA)

◆ Carcinogenicity can be a result of a genotoxic insult, but can also be induced by nongenotoxic mechanisms
“The assessment of a carcinogenic potential or the ability to promote tumor growth are among the most challenging areas in the nonclinical assessment of bio-therapeutics.

In the initial development of these therapies, there appeared to be a perception that bio-therapeutics were exempt from carcinogenicity concerns. This perception was largely based on the fact that two-year rodent studies were often not possible and genotoxicity concerns typically do not exist for biologics.

With the rapid expansion of new bio-therapeutics and targets, increased attention has been directed toward carcinogenicity assessments.”

Is There a Cause for Concern for Biologics?

- There is little to no concern that bio-therapeutics may induce a genotoxic insult or act as complete carcinogens.

- But there is concern that bio-therapeutics may increase the incidence of existing neoplasms by secondary mechanisms related to their pharmacology, e.g.:
  - Promotion of growth / cell differentiation / proliferation
    - Enhanced cell proliferation can increase the probability of neoplastic progression
  - Immunomodulation
    - Chronic immune activation (inflammation) enhances the risk of neoplastic progression
    - Suppression of anti-tumor immune responses can foster carcinogenicity
    - Immune suppression may activate latent oncogenic viruses (e.g. HPV, EBV)
Primarily focused on small molecules

Exclusively focused on biotherapeutics
The need for a product-specific assessment of the carcinogenic potential for biopharmaceutical should be determined with regard to the intended clinical population and treatment duration

- Expected clinical use is continuous for at least 6 month or frequently intermittent to treat chronic / recurrent conditions (ICH S1A)
- “Case-by-case approach” based on scientific justifications guided by the specific product characteristics (ICH S6R1)
- Consider target biology, clinical indication / medical need / life-expectancy and special risk factors in the target population (ICH S1A)
When an assessment is warranted, the sponsor should design a strategy to address the potential hazard…This strategy could be based on a weight of evidence approach

- Review of data from various sources
  - Published data, e.g.; tg / KO models, animal disease models, human genetic diseases, epidemiology data
  - Information on class effects
  - Data on target biology / mode of action including down-stream signaling
  - Available in vitro / in vivo (especially chronic toxicity) and clinical data
ICH S6 – Totality of Evidence Review

Totality of Evidence Review

Sufficient data
- Cause for concern
  - Address potential hazard in product labeling / risk management practice

Unclear / insufficient knowledge
- More extensive assessment
- No concern / low risk
  - No additional nonclinical testing recommended

Propose addition nonclinical studies to mitigate the concern
The product-specific assessment of carcinogenic potential is used to communicate risk and provide input to the risk management plan along with labeling proposals, clinical monitoring, post-marketing surveillance, or a combination of these approaches.

- Localization of carcinogenicity information in the label

<table>
<thead>
<tr>
<th>US Label (21 CFR 201.56 /57)</th>
<th>EU SmPC (EMA Guideline on Summary of Product Characteristics)</th>
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<tbody>
<tr>
<td>Boxed Warning</td>
<td>4.4 Special Warnings and Precautions for Use</td>
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<tr>
<td>5 Warnings and Precautions</td>
<td>4.8 Undesirable Effects</td>
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<tr>
<td>6 Adverse Events</td>
<td>5.3 Preclinical Safety Data</td>
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<tr>
<td>13 Nonclinical Toxicology</td>
<td>Annex II C Other Conditions and Requirements of the Marketing Authorization</td>
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<tr>
<td>17 Patient Counseling Information</td>
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Infliximab (Remicade®) Label Information

- Monoclonal antibody against TNF-α for the chronic treatment of various autoimmune type diseases, e.g. Crohn’s disease, RA, psoriasis

5.2 Malignancies
Malignancies, some fatal, have been reported among children, adolescents and young adults who received treatment with TNF-blocking agents (initiation of therapy ≤ 18 years of age), including REMICADE. Approximately half of these cases were lymphomas, including Hodgkin’s and non-Hodgkin’s lymphoma. The other cases represented a variety of malignancies, including rare malignancies that are usually associated with immunosuppression and malignancies that are not usually observed in children and adolescents. The malignancies occurred after a median of 30 months (range 1 to 64 months) after the first dose of TNF blocker therapy. Most of the patients were receiving concomitant immunosuppressants. These cases were reported post-marketing and are derived from a variety of sources, including registries and spontaneous postmarketing reports.

Lymphomas
In the controlled portions of clinical trials of all the TNF-blocking agents, more cases

5.3 NONCLINICAL TOXICOLOGY
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
The significance of the results of nonclinical studies for human risk is unknown. A repeat dose toxicity study was conducted with mice given CV1q anti-mouse TNFα to evaluate tumorigenicity. CV1q is an analogous antibody that inhibits the function of TNFα in mice. Animals were assigned to 1 of 3 dose groups: control, 10 mg/kg or 40 mg/kg CV1q given weekly for 6 months. The weekly doses of 10 mg/kg and 40 mg/kg are 2 and 8 times, respectively, the human dose of 5 mg/kg for Crohn’s disease. Results indicated that CV1q did not cause tumorigenicity in mice. No clastogenic or mutagenic effects of infliximab were observed in the in vitro mouse micronucleus test or the Salmonella-Escherichia coli (Ames) assay, respectively.

17 PATIENT COUNSELING INFORMATION
See FDA-Approved Patient Labeling (Medication Guide)

17.1 Patient Counseling
Patients or their caregivers should be advised of the potential benefits and risks of REMICADE. Physicians should instruct their patients to read the Medication Guide before starting REMICADE therapy and to reread it each time they receive an infusion. It is important that the patient’s overall health is assessed at each treatment visit and that any questions resulting from the patient’s or their caregiver’s reading of the Medication Guide be discussed.

- Immunosuppression
Inform patients that REMICADE may lower the ability of their immune system to fight infections. Instruct patients of the importance of contacting their doctors if they develop any symptoms of an infection, including tuberculosis and reactivation of hepatitis B virus infections. Patients should be counseled about the risk of lymphoma and other malignancies while receiving REMICADE.
Theoretical concern of unwanted stimulation of growth / neoplastic progression of tumor cells

- Growth factor receptors are constitutively expressed / up-regulated on tumor cells
- Blockade of pathways important for tumor growth (e.g.: angiogenesis / bevacizumab: anti VEGF-A mAb) is used as anti-cancer therapy

Potential risk mitigation strategies (staggered approach)

- Analysis of target expression in various tumor tissues
- *In vitro* mitogenicity assay (clinical relevance of in vitro cell proliferation to be determined)
- *In vivo* analysis of cell proliferation (not warranted, if no finding suggestive of cell proliferation seen in repeat dose toxicity studies)
- 2 year rodent bioassay (if feasible)
There is increasing epidemiologic evidence that chronic immunosuppressive therapy is associated with increasing incidences of certain tumor types

- T cells, NK cells, dendritic cells and macrophages play a major role in tumor immunosurveillance
- Several immunosuppressive biologics are associated with increased risk of lymphoma (and other malignancies), e.g.: anti-TNF-α mAb’s, abatacept (CTLA-4-Ig fusion)

Potential risk mitigation strategies (staggered approach)

- Understand the immunological consequences of target engagement
- Predictive value of rodent bioassay uncertain *
- (Pre)neoplasia observed in NHP studies following reactivation of viral infection (difficult to standardize / interpret)

Monoclonal antibodies are the major fraction of biologics in clinical development
- 35 mAbs currently approved in EU / US and more than 300 in clinical development *
- A broad variety of indications is targeted and a various administration paradigms / routes are employed

* US PhRMA Pipeline Report 2013
Genotoxicity and carcinogenicity data of approved mAb’s

- No rodent bioassay data submitted
- All performed in vitro and/or in vivo genotoxicity assays negative
- 4 mAb’s (all anti-TNF-α) have boxed warning for “lymphoma and other malignancies”

<table>
<thead>
<tr>
<th>Genotoxicity</th>
<th>Carcinogenicity</th>
<th>Black Box</th>
<th>Warning &amp; Precautions</th>
<th>Nonclin Toxicology</th>
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<tr>
<td>30% (9 / 30)</td>
<td>0% (0 / 30)</td>
<td>13% 4 /30</td>
<td>27% (8 / 30)</td>
<td>100% (30 /30)</td>
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In general, impact of nonclinical data on labeling minimal

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<tr>
<th>Natalizumab (Tysabri®)</th>
<th>Ustekinumab (Stelara®)</th>
<th>Ofatumumab (Arzerra®)</th>
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<tr>
<td>No effects in in vitro assays of α4-integrin positive human tumor line proliferation/cytotoxicity. Xenograft transplantation models in SCID and nude mice with two α4-integrin positive human tumor lines (leukemia, melanoma) demonstrated no increase in tumor growth rates or metastasis resulting from natalizumab treatment.</td>
<td>Published literature showed that administration of murine IL-12 caused an anti-tumor effect in mice that contained transplanted tumors and IL-12/IL-23p40 knockout mice or mice treated with anti-IL-12/IL-23p40 antibody had decreased host defense to tumors... The relevance of these experimental findings in mouse models for malignancy risk in humans is unknown.</td>
<td>In a repeat-dose toxicity study, no tumorigenic or unexpected mitogenic responses were noted in cynomolgus monkeys treated for 7 months</td>
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Antibody-Drug-Conjugates (ADC)

- ADC are developed for targeted tumor therapy in oncology indications
  - 2 ADC approved recently (Brentuximab vedotin / Adcetris®; T-DM1 / Kadcyla®) and 15+ molecules in clinical development
  - Consist of a small molecule (warhead) attached by a linker to an antibody
  - Small molecule is typically an anti-mitotic (MMAF, MMAE, DM1, DM4) or DNA breaking agent (calicheamicin)

Antibody-Drug-Conjugates (ADC)

- **In vitro** and **in vivo** genotoxicity studies were conducted with warhead
  - Both ADC were concluded as clastogenic / aneugenic

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<thead>
<tr>
<th>Test System</th>
<th>Adcetris® (MMAE) *</th>
<th>Kadcyla® (DM1) **</th>
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<tbody>
<tr>
<td>Ames Assay</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Mouse Lymphoma Assay</td>
<td>Negative</td>
<td>NA</td>
</tr>
<tr>
<td>Rat Micronucleus Assay <strong>in vivo</strong></td>
<td>Positive</td>
<td>Positive</td>
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- No carcinogenicity studies were conducted **/**
  - Consistent with ICHS1A

- No impact on label / prescribing information **/**
  - Results were summarized in nonclinical toxicology section

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* Brentuximab vedotin EPAR 2012 / US Prescribing Information
** Ado-trastuzumab emtansine FDA Pharmacology Review 2013 / US Prescribing Information
Non-Antibody Bio-therapeutics

Non-antibody protein therapeutics include recombinant (fusion) proteins / peptides of various sizes but also antisense / gene / cell therapy, and vaccines

- Huge variety in nonclinical assessment strategies ranging from lack of rodent bioassay data (“mAb approach) over standard 2 year bioassays in one or two species (consistent with ICHS1B) up to addition of extensive mechanistic studies to mitigate a risk / finding (e.g. GLP-1 analogues)
- For more “drug-like” molecules (e.g.: GLP-1 analogues), the nonclinical assessment strategy tend to follow the “standard”” (small chemical) approach
Exenatide (Byetta® / Bydureon®) and liraglutide (Victoza®) are approved for the treatment of type 2 diabetes

- MoA: Mimicking the anti-hyperglycemic activity of endogenous GLP-1, mainly enhance glucose-dependent insulin secretion by pancreatic beta-cell

A concern for a potential carcinogenic potential were raised by the FDA / EMA based on nonclinical data

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<th>Exenatide</th>
<th>Liraglutide</th>
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An extensive package of mechanistic studies was performed to investigate the human relevance

- Analyses of GLP-1R on C-cells of human / toxicology species revealed a higher number of C-cells and GLP-1 expression in normal rat tissue compared to human tissue samples and a higher number of receptors per cell in rat cell lines compared to human suggest a greater sensitivity of rodents.

- Analyses of downstream signaling showed that liraglutide massively induced cAMP / calcitonin secretion in rat but only marginal in human cell lines.

Conclusion was that C-cell hyperplasia / tumors observed in the carcinogenicity studies are caused by a non-genotoxic mechanism for which rodents are particularly sensitive, i.e.: continuous release of calcitonin due to persistent activation of C-cell GLP-1 receptors and the accompanying increased demand for calcitonin synthesis.
GLP-1: Case Example for Risk Mitigation Strategy

- Nonclinical data did not impact exenatide (Byetta®) label
- Mechanistic studies ensured approvability but current label for liraglutide (Victoza®) contains boxed warning

**WARNING: RISK OF THYROID C-CELL TUMORS**

Liraglutide causes dose-dependent and treatment-duration-dependent thyroid C-cell tumors at clinically relevant exposures in both genders of rats and mice. It is unknown whether Victoza® causes thyroid C-cell tumors, including medullary thyroid carcinoma (MTC), in humans, as human relevance could not be ruled out by clinical or nonclinical studies. Victoza® is contraindicated in patients with a personal or family history of MTC and in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2). Based on the findings in rodents, monitoring with serum calcitonin or thyroid ultrasound was performed during clinical trials, but this may have increased the number of unnecessary thyroid surgeries. It is unknown whether monitoring with serum calcitonin or thyroid ultrasound will mitigate human risk of thyroid C-cell tumors. Patients should be counseled regarding the risk and symptoms of thyroid tumors [see Contraindications (4), Warnings and Precautions (5.1) and Nonclinical Toxicology (13.1)].
Carcinogenicity Assessment Strategy

- Identify potential / theoretical concerns early based on
  - Target biology, mode of action, published nonclinical evidence, prior clinical experience
  - Scrutinize relevance / validity of published data

- Adapt weight of evidence review as data emerge
  - Assess internal pharmacology / toxicology data for signals of concern
  - Monitor literature, external clinical data and regulatory interactions

- Agree Carcinogenicity assessment strategy with regulatory agencies
  - Pre-IND meeting (lack of chronic data problematic) or EoP2 meeting (may be too late in case additional investigations are recommended), CAC, EMA Scientific Advise
  - Carcinogenicity data usually required for BLA filing or even as PMC
    - Companies developing anti-PCSK9 mAb’s received FDA guidance to submit a “thorough carcinogenicity assessment” early (EoP2) *

* Gelzleichter T, NorCal SOT 2012
2 year rodent bioassays are generally conducted for small molecules, despite controversial discussion about predictive value

- Most bio-therapeutics are not cross-reactive to rodent targets due to exclusive species specificity (especially mAb’s)
  - Studies in non-relevant species generally not warranted (ICH S6)
- Even in case of rodent cross-reactivity, technical feasibility of traditional rodent bio-assays can be challenging
  - Lack of relevant pharmacology in rodent species
  - Immunogenicity consequences during long-term exposure
- Use of a surrogate (homologous) protein to the clinical candidate is discouraged
  - Translatability of results uncertain
  - The surrogate is a unique molecule and may differ in various attributes (e.g. sequence, binding affinity, manufacturing, PK)
Alternative Assessment in Standard Repeat Dose Studies

- Standard repeat dose toxicity studies can pick-up signals for a potential carcinogenicity risk, e.g.: preneoplasia (hyperplasia, cellular hypertrophy, and atypical cellular foci) or immune suppression *
  - The NHP as model for carcinogenicity testing is considered impractical **
    - Long life-span (approx. 25 – 30 yrs compared to approx. 2 yrs in rats)
    - Chronic study duration usually 6 month for biologics (approx. 2% of overall life-span compared to almost 100% coverage in rodents (absence of evidence ≠ evidence for absence)
    - Statistical power of NHP studies (n = 3 – 4 animals / dose / sex) low
  - Longer study duration in NHP to gain additional data on carcinogenicity?
    - Technical feasible extension period, e.g. 12 instead of 6 month would add only a few percent treatment duration relative to the overall life-span
    - Low background incidence of neoplastic lesions require treatment period of 5 – 10 yrs required to demonstrate detectable background tumor incidences ***/****

**** Schoeffler DJ & Thorgeirsson UP (2000) In Vivo 14: 149-56
Case Example #1: Antibody X

- **Human monoclonal antibody**
  - MoA: Antagonist of soluble target preventing target interaction with receptor
  - Indication: Chronic inflammatory diseases

- **Available data**
  - Internal data
    - NHP single toxicology species, no rodent cross-reactivity
    - No adverse findings or neoplastic/pre-neoplastic lesions in NHP repeat dose studies up to 6 months
  - External data
    - Hodgkin’s lymphoma associated with target blockade
    - However, there are also data to suggest blockade of target could have beneficial effects
    - Target may have anti-proliferative properties towards certain tumor cells and may also negatively impact anti-tumor immunity
Case Example #1: Antibody X

- Regulatory interaction at pre-IND meeting
  - Company position: No genotoxicity / carcinogenicity studies required
  - FDA position: Agreed on genotoxicity waiver but requested evaluation of carcinogenic potential in one species unless company is able to provide evidence that not possible; consider use of KO model or surrogate

- Options considered and ruled out for in vivo carcinogenicity assessment
  - Rodent bioassay: Not possible because of lacking rodent cross-reactivity
  - Target deficient mice: Literature suggests gene disruption may affect other nearby genes, so KO data may not be reflective of impact to only target
  - Surrogate molecule: Available reagent had acceptable in vitro potency but lacked in vivo potency (i.e. unlikely to provide clinically meaningful data)
  - Use of clinical product in humanized target tg mice: Species mismatch likely to result in immunogenicity, confounding data interpretation
Case Example #1: Antibody X

Further regulatory interactions

- Final company position: Given the lack of suitable reagent/model options to provide clinically meaningful data, proposed to use results from the completed repeat dose studies in cynomolgus monkeys to provide nonclinical risk assessment information regarding carcinogenic potential following chronic administration.

- Agency questioned that the absence of pre-neoplastic lesions in NHP after a 6 month treatment period ruled out carcinogenic potential and requested to robustly evaluate the target deficient (KO) mouse as potential model for in vivo assessment.

- Detailed assessment of gene deficient mice concluding that this model is not suitable for in vivo assessment submitted to agency.
Case Example #1: Antibody X

- Detailed assessment of gene deficient mice submitted to agency
  - Lack of regulatory, logistical, and study design precedence with use of target gene deficient mice
  - Gene deficiency represents an all or none and represents a life-time deficiency with adaptations and other phenomena not indicative of the clinical situation
  - Various examples of unexpected deficiency phenotypes and examples of the impact of strain on gene deficient mouse phenotype were reviewed
  - Differences in target pharmacology in mouse and man with potential to confound study interpretation were assessed
  - Final conclusion: Propose not to conduct 2 year study in gene deficient mice

- Final FDA response
  - 2 year study in gene deficient mice not required but monitor patients for potential development of tumors
Case Example #2: Antibody Y

◆ Humanized monoclonal antibody
  – MoA: Depletion of (non-T non-B) immune cells expressing target receptor
  – Indication: Chronic inflammatory diseases

◆ Available data
  – Internal data
    – NHP single toxicology species, no rodent cross-reactivity
    – No adverse findings or neoplastic/pre-neoplastic lesions in NHP repeat dose studies up to 9 months
  – External data
    – Target cells found in association with solid tumors (especially of epithelial origin but any role in tumor growth remains unclear)
    – Some clinical studies suggest target cell presence may be a positive prognostic indicator of cancer patient survival
    – Nonclinical data are mixed
Case Example #2: Antibody Y

Options considered and ruled out for *in vivo* carcinogenicity assessment

- Rodent bioassay: Not possible because of lacking rodent cross-reactivity
- Target deficient mice: KO mice have reduced levels of, but are not depleted of target cells – differs from product MOA so clinical relevance questionable
- Alternative target deficient mice: Deficient for other genes that results in depletion of the target cells, but the absence of those genes also has other pharmacological effects inconsistent with the targeted MoA
- Some other pharmacological differences in mice regarding target cell depletion are likely
- MoA-based cell depletion in humans can not be completely replicated in rodents due to differences between species in MoA, therefore surrogate molecule unlikely to provide clinically meaningful data
Case Example #2: Antibody Y

Regulatory interaction at EoP2 meeting

- Final company position: Given the lack of suitable reagent/model options to provide clinically meaningful data, proposed to use results from the completed repeat dose studies in cynomolgus monkeys to provide nonclinical risk assessment information regarding carcinogenic potential following chronic administration.

- EMA endorsed proposal but asked to pay attention to available information of target on cellular instability, cell division processes, cellular communication, apoptosis, as well as any impact on immune function, with a weight of evidence approach advised.

- FDA agreed that carcinogenicity risk assessments is sufficient to support initiation of the planned clinical trials but requested to monitor patients for potential development of tumors.

- FDA concluded that it is unlikely that additional nonclinical studies would be required for the filing of a BLA.
The expanding target diversity and increasing number biologics in development has focused attention toward carcinogenicity assessments.

- There is little to no concern that bio-therapeutics may induce a genotoxic insult or act as complete carcinogens but there is concern that bio-therapeutics may increase the incidence of existing neoplasms by secondary mechanisms, non-genotoxic mechanisms.

ICH S6(R1) require a weight of evidence approach to assess the carcinogenicity potential (if warranted).

The product-specific assessment of carcinogenic potential is used to communicate risk and provide input to the risk management plan along with labeling proposals, clinical monitoring, post-marketing surveillance, or a combination of these approaches.

Develop the nonclinical assessment strategy early and align with regulatory expectations.
Acknowledgement

- Scott Manetz, PhD DABT, Biologics Safety Assessment, MedImmune LLC Gaithersburg