American Board of Toxicology, Inc.
Recertification Literature Review 2021

Article 1: Nonclinical safety assessment of a human interleukin-22FC IG fusion protein demonstrates in vitro to in vivo and translatability.

Lee, DW, Zhong, S, Pai, R, et al. Pharmacol Res Perspect. 2018; e434.
https://bpspubs.onlinelibrary.wiley.com/doi/full/10.1002/prp2.434

Article 1 Questions

1) According to studies cited by the authors, what property of IL-22 is essential for host defense against various pathogens and leads to modulation of the colonic microflora?

   a. IL-22 increased production of antimicrobial peptides
   b. IL-22 induced expression of mucus-associated molecules
   c. IL-22 ability to exacerbate the inflammatory process
   d. IL-22 ability to decrease epithelial proliferation and repair

2) A GLP toxicity study was conducted in rats with doses of 50, 150, or 1500 µg/kg UTTR1147A administered IV twice weekly for 11 weeks. During that study what were the effects of fibrinogen and CRP elevations?

   a. Indirect downstream pharmacological effect of IL-22R activation
   b. Direct downstream pharmacological effect of IL-22R activation
   c. Indirect upstream pharmacological effect of IL-22R activation
   d. Direct upstream pharmacological effect of IL-22R activation

3) In the chronic toxicity study in cynomolgus monkeys, what pharmacodynamic (PD) biomarker confirmed the pharmacologic activity of UTTR1147A?

   a. CRP
   b. ADA
   c. STAT3
   d. REG3A

4) In support of species selection of toxicology studies, in vitro results demonstrated similarities in UTTR1147A-induced response of STAT3 and provided justification for what in vivo species to translate the pharmacologic activation of the IL-22 pathway in humans?

   a. Diseased Mouse model
   b. Minipig
   c. Dog
   d. Ferret

5) According to the authors what model showed minimal systemic absorption or bioavailability through topical administration?

   a. Nude Mouse
   b. Minipig
   c. Rabbit
   d. Dog

Article 2: Long-term evaluation of AAV-CRISPR genome editing for Duchenne muscular dystrophy.

Nelson CE, Wu Y, Gemberling MP, et al. Nat Med. 2019;25(3):427-432.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6455975/

Article 2 Questions

1) Duchenne Muscular Dystrophy (DMD) is a debilitating and prematurely fatal genetic disease caused by mutations in the DMD gene leading to the absence of dystrophin. CRISPR-based gene therapeutics are being investigated as a long-term treatment. What potential issues should be considered for this type of therapeutic?

    a. Establish genome editing as a curative approach for DMD and proceed with human development.
    b. Demonstrate dystrophin protein restoration in a mouse model of disease.
    c. Establish *in vitro* and *in vivo* efficacy for permanent gene correction of DMD and identify the potential for off-target effects, immunosuppression and genotoxicity.
    d. Determine whether local or systemic delivery of the AAV-CRISPR methodology is most appropriate for restoration of dystrophin expression.

2) The frequency of adeno-associated virus (AAV) integrations into the CRISPR-induced double-strand break was higher than the intended deletion. What could be the subsequent consequences?

    a. Induction of a novel DNA break by any genome editing construct could potentially change the integration landscape and safety profile suggesting that AAV will have no additional safety impact.
    b. Long-term expression of certain transgenes from an AAV vector may lead to genotoxic effects due to the sustained activity of an active nuclease.
    c. Repair enzymes correct the integration of the vector such that there is low risk of adverse effects.
    d. No consequence as AAV is known to integrate at a high frequency throughout the genome and especially at CRISPR cut sites.

3) What next step should be considered in developing AAV-CRIPSR genome editing as a therapeutic for DMD?

    a. Conduct additional animal studies to better characterize efficacy and safety of the AAV and CRISPR combination including host response and any pre-existing immunity.
    b. Demonstrate efficacy in children diagnosed with DMD.
    c. Develop non-invasive imaging biomarkers to allow longitudinal efficacy and safety monitoring.
    d. Design a marketing strategy to evaluate costs and profit for a curative therapeutic dosed once.

4) What is an example of an early clinical success measure that helps demonstrate dystrophin protein restoration?

    a. Protein restoration as evidenced by immunofluorescence staining of skeletal and cardiac muscles.
    b. Muscle biopsies for Western Blotting demonstrating sustained dystrophin protein restoration.
    c. Resolution of clinical signs of including frequent falls, trouble getting up or running, waddling gait, big calves, and learning disabilities.
    d. Decreased number of activated macrophages as well as a decrease in serum creatine kinase levels.

5) What is a benefit of utilizing a Nextera-transponson-based library preparation method?

    a. Although there is considerable sample loss and limited throughput, transposed-based Nextera provides a method for fragmenting DNA (sonication, nebulization or shearing), followed by DNA repair and end polishing (blunt end or A overhang) and platform-specific adaptor ligation.
    b. Transposed-based Nextera can comprehensively map all possible genome editing outcomes with an unbiased, high throughput approach.
    c. Transposed-based Nextera is a method for preparing fragmented and tagged DNA libraries specific to the generation of only select sequencing platform libraries.
    d. When compared to the traditional ligation-based methods, transposed-based Nextera can be completed over 72 hours with only a slight insertion bias.

Article 3: Short-term toxicogenomics as an alternative approach to chronic in vivo studies for derivation of points of departure: A case study in the rat with a triazole fungicide.

Jessica LaRocca, Eduardo Costa, Shreedharan Sriram, Bethany R. Hannas, Kamin J. Johnson.
Regulatory Toxicology and Pharmacology, Volume 113, 2020
https://www.sciencedirect.com/science/article/pii/S0273230020300817

Article 3 Questions

1) According to the authors, what information can *in vivo* transcriptomic studies provide for the purposes of chemical risk assessment?

    a. Identification of modes of action (MoA) and generation of biological effect points of departure (BEPOD).
    b. Correlation between BMD and NOAEL values from standard guideline toxicity studies.
    c. Principle component analysis (PCA) and correlation-based dendrograms based on normalized gene expression datasets.
    d. The response level associated with an adverse change in gene expression.

2) For the purposes of the toxicogenomic BMD analysis of Myclobutanil, the final BEPOD value reported was the median gene BMDL value of the GO-BP term with the smallest median gene BMD. What data were included in this analysis?

    a. Genes with modeled BMD values greater than 400 mg/kg/day
    b. GO-BP terms with less than 2 genes having BMD values
    c. Genes with BMDU/BMDL ratios greater than 40
    d. GO-BP terms with a Fisher's Exact Two Tail Test p-value less than (<) 0.05

3) According to the authors, the lowest testis POD based on an apical endpoint was 1.4 mg/kg/day, and the testis transcriptome POD was 25.4 mg/kg/day. What biological effects were these PODs based on, respectively?

    a. Decreased testis weight from a two-year rat carcinogenicity study and steroidogenic gene expression in a 14-day rat study.
    b. Seminiferous tubule atrophy from a two-year rat carcinogenicity study and gene set expression for neurotransmitter uptake / monoamine transport in a 14-day rat study.
    c. Seminiferous tubule atrophy from a two-year rat carcinogenicity study and steroidogenic gene expression in a 14-day rat study.
    d. Decreased testis weight from a two-year rat carcinogenicity study and gene set expression for neurotransmitter uptake / monoamine transport in a 14-day rat study.

4) According to the authors, the lowest liver POD based on an apical endpoint was 2.5 mg/kg/day, and the liver transcriptome POD was 22.2 mg/kg/day. What biological effects were these PODs based on, respectively?

    a. Hepatocyte hypertrophy in a 90-day rat study and gene set expression for positive regulation of sprouting angiogenesis in a 14-day rat study.
    b. Parental relative liver weight in a two-generation reproduction study and gene set expression for positive regulation of sprouting angiogenesis in a 14-day rat study.
    c. Hepatocyte hypertrophy in a 90-day rat study and *Cyp* gene expression in a 14-day rat study.
    d. Parental relative liver weight in a two-generation reproduction study and *Cyp* gene expression in a 14-day rat study.

5) The primary hypothesis evaluated by the authors was that liver and testis toxicogenomic PODs following a short-term exposure would closely approximate the most sensitive apical POD from a two-year carcinogenicity study. What conclusion was made by the authors based on the data presented?

    a. Liver and testis BEPODs from the short-term study were an order of magnitude higher than the most sensitive apical POD from a two-year carcinogenicity study.
    b. Liver and testis BEPODs in the short-term study were similar, corresponding to concurrent treatment related apical effects in both the liver and testis.
    c. A BEPOD derived from short-term exposure can be used to derive a reference dose that is protective of human health with the inclusion of an additional uncertainty factor of 10.
    d. Liver toxicogenomics should not be used to derive a BEPOD protective of human health for molecules that have liver toxicity as the most sensitive apical endpoint.

Article 4: Use of the kinetically-derived maximum dose concept in selection of top doses for toxicity studies hampers proper hazard assessment and risk management.

Minne B. Heringa, Nicole H.P. Cnubben, Wout Slob, Marja E.J. Pronk, Andre Muller, Marjolijn Woutersen, Betty C. Hakkert. Regulatory Toxicology and Pharmacology, Volume 114, 2020.
https://www.sciencedirect.com/science/article/pii/S0273230020300854

and

OECD Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies

https://www.oecd-ilibrary.org/environment/test-no-453-combined-chronic-toxicity-carcinogenicity-studies_9789264071223-en

Article 4 Questions

1) According to the Kinetically-derived Maximum Dose (KMD) concept, what is expected to occur when kinetics are saturated?

    a. systemic exposure of the parent substance or ultimate toxic metabolite does not change with increasing external dose
    b. systemic exposure of the parent substance or ultimate toxic metabolite decreases with increasing external dose
    c. systemic exposure of the parent substance or ultimate toxic metabolite no longer increases proportional to external dose
    d. systemic exposure of the parent substance or ultimate toxic metabolite increases proportional to increasing external dose

2) What is a dose-adjusted Area Under the Curve (AUC)?

    a. ratio of AUC to external dose
    b. ratio of AUC to peak concentration (C_{max})
    c. ratio of AUC to internal dose
    d. ratio of AUC time of peak concentration (T_{max})

3) According to the authors, why are toxic effects from animal studies at doses that produce high saturation relevant for human risk assessment?

    a. at the same external dose, animals may have a higher internal dose than humans
    b. at the same external dose, humans may have a higher internal dose than animals
    c. an adverse effect is not determined by the shape of the relationship between internal and external dose
    d. human exposure will be too low to reach saturation

4) What can be concluded by plotting log-AUC vs log-dose?

    a. The internal-to-external dose relationship is linear if the slope is <1
    b. The internal-to-external dose relationship is linear if the slope is =1
    c. The internal-to-external dose relationship is non-linear if the slope is =1
    d. The internal-to-external dose relationship is linear if the slope is >1

5) In a Letter to the Editor defending the scientific validity of the KMD methodology, Terry et al, refer to OECD Guideline 453, where recommendations are made with respect to selecting doses for chronic toxicity testing as outlined in OECD Guidance Document 116. What three scientific Weight-of-Evidence parameters align with these recommendations for dose level selection?

    a. linear range of systemic exposures in the animal model, apical toxicity data at doses where key aspects of mode of action occur, human exposure data
    b. maximum-tolerated dose (MTD) in the animal model, No Observed Adverse Effect Level (NOAEL) in previous animal studies, human exposure data
    c. linear range of systemic exposures in the animal model, NOAEL in previous animal studies, human exposure data
    d. linear range of systemic exposures in the animal model, Mode-of-Action of the test substance, NOAEL in previous animal studies