# Discussion Points for Panel Discussion

## <Carcinogenicity Studies on Biopharmaceuticals>

Shigeru Hisada
ASKA Pharmaceutical

## Need for carcinogenicity studies on biopharmaceuticals in S1A guideline

- Not to be required :
  - Endogenous peptides or proteins and their analogues
    - Endogenous substances given essentially as replacement therapy
- To be considered :
  - Treatment duration, clinical indication, patient population
    - Providing neutralizing antibodies not produced to such an extent as to invalidate the results
  - Products where there are significant differences in biological effects to the natural counterparts
  - Products where modifications leads to significant changes in structure compared to the natural counterpart
  - Products resulting in humans in a significant increase over the existing local or systemic concentration

## Biopharmaceuticals : no concern about direct carcinogenicity

- Proteins and peptides : not permeable across cell membranes and degraded to amino acids
  ➔not interacted with DNA
  ➔no concern about direct carcinogenicity
  ➔basically no need for carcinogenicity testing
- Exception: bioconjugates with organic linker
  - Evaluation of carcinogenicity of such compounds ➔ to follow S1A and S1B guidelines thinking about the bioassay of, for example, a linker-conjugated fragment because of the difficulty of testing of a whole molecule

## Biopharmaceuticals with concern about tumor promoter activity

- Some biopharmaceuticals show epigenetic carcinogenesis
  - In many cases, mediated by exaggerated pharmacological effects
    - e.g. PTH-induced osterosarcomas in rodent 2-year bioassay
  - Unknown epigenetic mechanism
    - e.g. calcitonin-induced pituitary tumors in a rodent 2-year bioassay
- Causes for concern of epigenetic carcinogenesis
  - Drugs with growth promotive effects
    - Growth factors
    - Hormones
      - given at supraphysiological levels
    - Agonistic monoclonal antibodies
  - Drugs with immunosuppressive effects
    - Therapeutic monoclonal antibodies

## Feasibility of rodent two-year bioassay

- Preclinical toxicity studies of biopharmaceuticals should be conducted using pharmacologically relevant species
- Three patterns of relevant species
  - Rodents and non-rodents including non-human primate (NHP)
    - In a 2-year rodent carcinogenicity study, neutralizing antibodies may be produced to reduce blood concentration of the drug, or to induce anaphylaxis or renal lesions
  - NHP only
    - Lifetime carcinogenicity study is impracticable
  - None (except for chimpanzees)
    - Lifetime carcinogenicity study is impossible

## Chronic toxicity study / additional study

- In case that a relevant species is present, proliferative lesions may be induced in rodent and/or NHP chronic toxicity studies on biologics with promoter activity
- However, proliferative lesion by itself is insufficient to conclude the tumor promoter activity at present
- Additional studies are to be considered:
  - Stimulation of target cell proliferation
    - PCNA immunostain of target tissue in chronic toxicity study
    - RDS (replicative DNA synthesis) if rodent is a relevant species
    - In vitro assay using human target cells
  - Two-step carcinogenicity study in case that rodent is a relevant species

## Examples of carcinogenicity evaluation of biopharmaceuticals

- Recombinant insulin
  - Rat 1-year repeated dose toxicity study
  - Growth promotive effects on breast cancer cells
  - Relationships between insulin / IGF receptors and mammary gland tumor development
- Insulin analogues
  - Rat 52-week repeated dose toxicity study
  - Growth promotive effects on human breast cancer fibroblast cell line, human osteosarcoma cell line, hamster CHO cells
- Recombinant FSH
  - 52-week rat and dog repeated dose toxicity studies
  - Growth promotive effects on ovarian cancer cells

## Examples of carcinogenicity evaluation of biolopharmaceuticals

- Recombinant basic fibroblast growth factor
  - Mouse : relevant species
    - Two-step skin carcinogenesis study in mice
    - Skin tumor promotion study in nude mice
  - Chronic toxicity studies in rats and monkeys
    - Renal lesions due to antibody production
- Recombinant human α-L-iduronidase
  - 26-week chronic toxicity study using Cynomolgus monkeys
    - No proliferative lesions
- Biologics without carcinogenicity testing
  - Treatment period is less than 6 months
  - No genotoxic and pharmacological effects indicative of carcinogenicity

## In case of no relevant species

- Conventional preclinical toxicity studies are meaningless
- Human risks of toxicity including carcinogenesis may be considered based on its pharmacology and clinical data are to be collected from deliberate clinical trials
- Another approaches to evaluate toxicity in preclinical studies:
  - Homologous proteins or surrogate antibodies
    - Rodent repeated dose toxicity studies
  - Humanized animals
    - Human target molecule DNAs are transfected
    - Repeated dose toxicity studies using
  - Knockout or transgenic mice
    - Incidence of spontaneous tumors compared with wild type mice

## Use of homologous protein

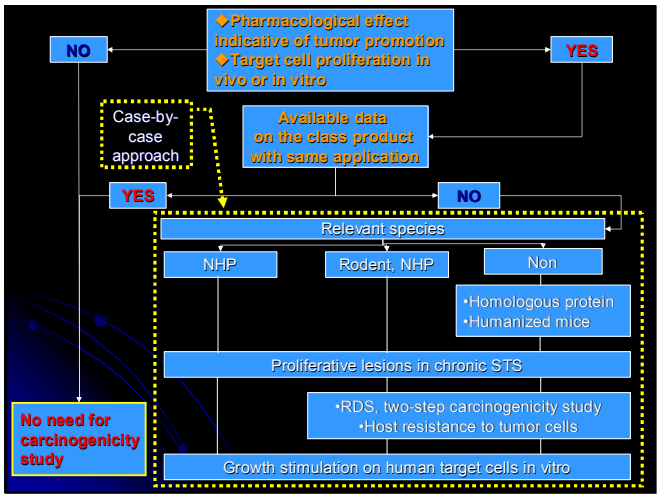
- Repeated dose toxicity study using homologous protein or surrogate antibodies
  - Growth hormone
    - Rat and mouse recombinant growth hormones
    - Two-year carcinogenicity study in rats and mice
    - No carcinogenicities were noted
  - Infliximab (chimeric monoclonal antibody to human TNF-α)
    - Only cross-reactive with chimpanzee TNF-α
    - 6 month toxicity study in mice treated with anti mouse TNF α monoclonal antibody
      - No treatment-related changes
      - Toxicological significance is low because of neutralizing antibody production
    - (No increased incidence of spontaneous tumors in TNF α-knock-out mice)

## Use of transgenic animals

- Keliximab (primatized anti-human CD4 monoclonal antibody)
  - Only cross-react with chimpanzee CD4
  - Toxicity studies were carried out using humanized mice (HuCD4/Tg mice, human CD4-transgenic mice)
  - Knock-out/knock-in mice: endogenous mouse CD-4 gene is depleted and transfected human CD-4 gene is functioned to reconstitute immunocompetence
  - Micronucleus test : negative
  - Host resistance to B16 melanoma cells is not altered in the Keliximab-treated HuCD4/Tg mice

## Use of transgenic mice

- Growth hormone
  - GH-transgenic mice with GH over-expression
  - Liver tumors are induced within one year
  - DEN-induced hepatocarcinogenesis
  - Dramatically accelerated only in young Tg mice
- Infliximab (chimeric monoclonal antibody to human TNF-α)
  - TNF α-knock-out mice
    - No increased incidence of spontaneous tumors




## Conclusion: Discussion points for up-dating S6 and S1A guidelines

- No need for the evaluation of direct carcinogenicity of biopharmaceuticals regardless of:
  - Structural modification of endogenous compounds except for bioconjugates with organic linker
  - Duration of administration
- Need for the evaluation of tumor promotive effect of biopharmaceuticals with growth stimulative or immunosuppressive effects
  - Rodent 2-year bioassays are generally meaningless
  - Case-by-case approach depending upon the characteristics of the compound and its relevant species
  - Proliferative lesions in chronic toxicity studies and additional studies may be useful:
    - Stimulated proliferation of target cells in vitro or in vivo (RDS or PCNA immunostain)
    - Two-step carcinogenicity models
    - Rodent studies using homologous proteins or surrogate antibodies
    - Use of humanized mice