【S-10】

Carcinogenicity : a Case Study of DA-8159

Byoung Ok Ahn, DVM, Ph.D.
Dong-A Pharmaceutical Research Laboratories

DA-8159 is a potent and selective phosphor-diesterase 5 (PDE5) inhibitor, being developed as a new erectile treatment. Phase 1 studies coducted in both U.K. and Korea shows favorable pharmacokinetic and safety properties. The P2 IIEF study in Korea successfully completed at the dose of 100 and 200mg tablets. The IND and P2 IIEF study in US and phase 3 study in Korea are in preparation. DA-8159 has possibility to expand its indications such as endotherial dysfunction, pulmonary hypertension, hypertension, BPH, PE, FSAD etc. Carcinogenicity bioassays of DA-8159 are needed for successful development in global market and expansion of indications. However, Dong-A planed to perform carcinogenicity studies about one and half years ago, the main studies was started right now because of no experience for FDA as well as carcinogenicity, no clear understandings for the carcinogenicity, lack of background data of DA-8159 and other unexpected many problems to solve. I would like to introduce the preparation process of DA-8159 carcinogenicity to assist to other domestic companies and CROs who are planning to perform carcinogenicity studies.

This presentation focuses on CRO selection, test system and duration, strain selection, animal supplier, number of Animals to use, age of the onset, route of administration, environment, test substance, dose selection (DRF studies and CAC recommendation), toxicokinetics, statistics, some example documents etc..

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2004 11 5

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Brief review of DA-8159

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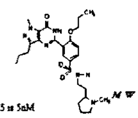
DA-8159

- **A new molecule (pyrazolopyrimidinone derivative) synthetized by Dong-A Research Lab**
- **A potent and selective phosphodiesterase 5 (PDE5) inhibitor**
- **Patent status**
  - Material & medical use PCT [illegible]
  - Process PCT [illegible]
- **Developmental stage**
  - Phase 1 both in US and Korea finished
  - P2 [illegible] (Korea) successfully completed
  - IND [illegible] (US) in preparation



Isozyme Fold Selectivity vs. PDE5 IC50 of DA 8159 for PDE5 is 5nM

Isozyme	DA-8159	Sildenafil (Viagra)	Vardenafil (Levitra)	Tadalafil (Cialis)
PDE 1	>174	80	500	>4 450
PDE 2	20 200	>8 570	44 290	>14 800
PDE 3	10 400	4 630	>7 140	>14 800
PDE-4	>1 760	2 057	47 000	>4 000
PDE 6	10	10	10 30	190
PDE 11	>3 000	780	1 160	5

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Objective of carcinogenicity study

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Treatment of ED PIII clinical study
PII IND filing in FDA
Expansion of indications endothelial dysfunction, pulmonary hypertension, hypertension, BPH, PE, FSAD etc.

⇓

Carcinogenicity is required for expansion of indications
If needed for NDA submission, globally acceptable data should be submitted
High quality enough not to perform additional tests

⇓

Successful development to a Blockbuster

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CRO selection - 1

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Evaluation items	KIT	Global Major CROs	mid-graded oversea CROs
Price (30)	H (90)	L (30)	M (60)
Experience (20)	M (40)	H (60)	M (40)
Quality (20)	M (40)	H (60)	M (40)
Communication (20)	H (60)	M (40)	L (20)
Reputation (10)	L (10)	H (30)	M (20)
Evaluation (100)	210	220	180

H=3, M=2, L=1

☞ Dong-A selected KIT as CRO for DA-8159 Carcinogenicity

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CRO selection - 2

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Assuring KIT's weakness

Quality organizing TFT, protocol review by CAC FDA,
step by step confirmation from global consulting company

Experience increase the number of animals (n=60),
including untreated control group instead of historical data
(finally not included as CAC recommendation)

Reputation Can not control by ourselves
Government invest institute

5/29

Test System and duration

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ICH
One long-term rodent Carcinogenicity study
The rat is more sensitive than mouse in carcinogenicity
One other study
- Short or medium-term rodent study
- models of initiation promotion in rodents
  hepatocarcinogen model, multi-organ carcinogenesis model
- models of carcinogenesis using transgenic or neonatal rodents
  p53+/- deficient model,the Tg.AC model, the TgHras2 model,
  the XPA deficient model, etc
- A long-term carcinogenicity study in a second rodent species (mouse or guinea pig)

OECD prefer rats (24-30 months) and mice (18 -24months)

FDA would like to review the 24 month mouse and rat study from consulting

☞ 24-month rat and mouse Carcinogenicity studies

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Rat strain selection -1

Species	Advantages	Disadvantages
F344	KIT experience High survival rate (≥ 60%) *Low B W gain (small TSB)* Many background data	High incidence of leukemia Abnormal Bone marrow change
SD	Single, 1 & 6 month tox PK/PD/ADME/TK *Many background data* Sildenafil	No experience Low survival rate (40%) *High B W gain* Pituitary, mammary tumor
Wister	Vardenafil, tadalafil High survival rate(≥ 70%) Low B W gain	No KIT experience Less Background data

☞ F344 rat was selected

7/29

Rat strain selection - 2

Considerations

FDA SD rat are acceptable until now

1980 ~ late 1990 usually used SD rat → raised longevity problem

Dep Of Health in England only 3/18 tests using SD rat are showed ≥ 50% of survival rate at 24 month → not acceptable

After late 1990 F344(Fisher) or Wister rat are generally employed world wide

Three tests performed KIT was all F344 rats used.

We concluded that F344 is the best strain for rat carcinogenicity study

We had to perform additional tests such as 2 and 13 week study with TK.

8/29

Mouse strain selection

species	Advantages	Disadvantages
ICR	*Single & 1w tox.* PK/ADME Sildenafil, Vardenafil, tadalafil	High mortality in case of sildenafil, Vardenafil, tadalafil (50 ~ ≥ 80%)
B6C3F1	Many case in NTP, EPA data for *FDA registration* High survival rate	No toxicology data

☞ B6C3F1 mice was selected

We had also few ICR data for carcinogenicity study

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Animal Supplier selection

supplier	Advantages	Disadvantages
CRJ	Close distance	Limited Supply Dpp-4 gene deletion in F344 (Japan & German)
CRL	Abundant supply	Long distance
Discussion	Check the animal supply capacity Use same supplier with DRF and main study Other global player can be accepted Korean supplier also acceptable with background data and capacity – less shipping stress, similar environments	

☞ CRJ animal were used (no deep consideration)

capacity problem : difficult to supply on time

10/29

Number of animals

Target ≥ 15 animals at planed sacrifice (2 years)
No interim sacrifice, additional 20 rats/group/sex for 24-month TK

OECD ≥ 50 animals/group/sex
sufficient number for statistics at the end of the study
study terminated if the survival rate ≤ 25% in the LD or Control

US EPA survival rate should be Rat ≥ 50% (18 months), 25% (24 months)
Mice ≥ 50% (15 months), 25% (18 months)

WHO study terminated if the survival rate ≤ 20% in HD

KIT's historical survival rate F344 ≥ 70%, B6C3F1 – no historical data
Few experiences for Carcinogenicity
Capacity problems

☞ 60 animals/group/sex for main + 20 rats/group/sex for TK

11/29

Age of the onset

OECD

Use weanling or post-weanling animals

The neonate usually is more sensitive than the adult.

Dosing of the rodents should begin as soon as possible

after weaning and acclimatisation, and preferably ≤ 6 weeks old.

☞ Animals will be acquired at 4 weeks and dosing started at 5-week old

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Route of administration

Route	Advantage	Disadvantage
Oral gavage	Large existing data Exact dosing Similar to human dosing Easy to TSB control	Difficult to dosing Low survival rate Dosing stress Required many TK animals
Mixing in food or water	Low costs Easy to perform High survival rate One point TK	Stability No existing data Test substance loss Impossible exact dosing

☞ We selected oral gavage unintentionally at DRF stage

Should consider many factors with advantage and disadvantage to select Route

13/29

Environments

KIT environment ☞ Checked by qualified consultant
- KIT poised to meet the US GLP regulations
- some minor recommendations

Cages

wire cage low costs, easy to exp , high stress to animals, FDA recommend

polycarbonate(PC) cage high costs, difficult to exp , low stress to animals

Housing

individual housing high costs, longevity, low social contact

group housing low costs, struggling, cannibalism, social contract

☞ individually (mouse) or 2 animals/cage (rat) in wire cages

individually housed in PC cages when indicated by health conditions.

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Test substance (TSB)

Quality GMP or GLP ?

non-clinical study GLP quality

What is GLP quality? GLP means QAU approved

How can QAU in KIT approve? ☞ Analysis in Dong-A and KIT

Supply large amount of TSB

Should have production plan

Can be supply separately but quality guaranteed

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Dose selection – 1

General consideration for DRF study protocol

ICH guideline

the same conditions as main study

mode of administration, diet, rodent strain etc

Mode of administration oral gavage/ TSB in feed or water

☞ Oral gavage

Diet PMI-5002

low protein(18%) diet – NIH-07, PMI-5002 etc

☞ Used PMI-5002 lab diet

Same rodent strains need for 2- and 13- week DRF study

☞ Used F344 rat & B6C3F1 mice

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Dose selection – 2

Rat DRF studies

2- week repeated dose toxicity study in rat

Dose 500, 250, 125, 62.5 mg/kg

Results 500 mg/kg – Death (M 2/5, F 1/5), salivation, body weight ↓,
food Consumption ↓, ↑ of ALT, AST & liver weight

250 mg/kg - Salivation, liver weight ↑

125 mg/kg - NOAEL

13- week repeated dose toxicity study in rat

Dose 240, 120, 60 mg/kg, HD is 73-fold greater than MRHD (W/W)

Results . HD - ↑ of Salivation, BUN, T-Chol, liver, spleen & adrenal gland
myelostromal proliferation, Hepatocellular hypertrophy

MD - ↑ of Salivation, BUN, T-Chol, liver & spleen
myelostromal proliferation, Hepatocellular hypertrophy

LD - NOAEL

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Dose selection – 3

Rat dose selection for main study - Dong-A

120 mg/kg MTD in 13-week DRF study

60 mg/kg relevance to human systemic exposure (AUC)

20 mg/kg MRHD comparable dose adjusted for body surface area (BSA)

Rat dose selection - CAC recommendation

Recommended dose 40, 80, 160 mg/kg day

Criteria based on MTD

- mortality and Decreased body Weight gain at 500 mg/kg in 2-week study

18/29

Dose selection – 4

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Mouse DRF studies

2- week repeated dose toxicity study in mice

Dose 1000, 500, 250, 125 mg/kg

Results 1000 mg/kg – Death (M 3/5, F 0/5), salivation, loss of fur,
↑ of motor activity, AST, ALP & liver weight

500 mg/kg - ↑ of ALT, TCHO & liver weight

250 mg/kg - NOAEL

13- week repeated dose toxicity study in mice

Dose 240, 80, 30 mg/kg, HD is 73-fold greater than MRHD (W/W)

Results 240mg/kg – No toxicological findings → NOAEL

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Dose selection – 5

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Mouse dose selection for main study (Dong-A)

500 mg/kg MTD in 2-week study

350 mg/kg 10 fold higher than the MRHD adjusted to body surface

80 mg/kg relevance to human AUC

35 mg/kg MRHD comparable dose adjusted for BSA

Mouse dose selection - CAC recommendation

recommended dose 50, 150 500 mg/kg/day for female
30 100, 300 mg/kg/day for male

Criteria based on MTD - mortality (M 3/5), decreased motor activity,
liver/general toxicity at 1000mg/kg/day in a 2-week study
Based on AUC - high dose in females (500mg/kg) gives
an approximately 25-fold AUC to human plasma exposure ratio

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Toxicokinetics (rat only)

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Number of animals

expected mortality rate less then 50%

sampling times 6, 12 & 24 month (reuse the animals)

sampling points 6 points (0.5, 1.5, 3, 5, 8, 24hr, same as DRF study)

No of animals/point 3 heads

No of Bleeding/animal twice/animal

= minimum 18 animals/group or 20 rats/group + 6 rats for control TK

Considerations

- TK sampling time
  ICH S3A No essential to continue beyond 6 months
  Consultant and FDA 6 12 & 24 month TK
  confirming that the TK profile has not changed in older animals
- Control TK To confirm no contamination to control samples (EMEA 2003)
- Major metabolites ≥ 25% of parent compound → should be analyzed
- FDA recommended that NO TK is needed in mouse study

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Statistics

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Numerical data

Multiple comparison tests for different dose groups

Bartlett test no sig. → ANOVA multiple comparison test & Dunnett's test
sig. → non-parametric Kruskal-Wallis(H) Test & Dunn's Rank Test

Frequency data

Chi-square Test & Fisher's Exact Probability Test

Survival analysis

Intercurrent mortality data Kaplan-Meier product-limit method

Each group compared with the control group log-rank test

Tumor incidence data

The unadjusted test Cochran-Armitage trend test & Fisher's exact test

The survival adjusted test the prevalence/mortality methods (Peto analysis)

** Refer to the FDA CDER draft guidance Statistical aspects of the design analysis and interpretation of chronic rodent carcinogenicity studies of pharmaceuticals (May 2001)

22/29

Changes of proposed study designs

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	First proposed	FDA submission	CAC recommendation
Strain	F344 rat / B6C3F1 mouse	F344 rat/ B6C3F1 mouse	F344 rat/ B6C3F1 mouse
Cage	*Polycarbonate cage*	*Stainless steel cage*	*Stainless steel cage*
Main study Number of animals	60/70 + untreated control	60/60	60/60
Toxicokinetic study	26 week rat & mouse	26, 52, 104 week, Rat & mouse	26, 52, 104 week rat only
Hematology	Y/Y	Y/Y	Y/N
Clinical Chemistry	Y/Y	Y/Y	Y/N
Cancer marker	Y/Y	N/N	N/N
Urinalysis	Y/Y	N/N	N/N
Organ weight	Y/Y	Y/Y	Y/N
Interim sacrifice	Y/N	N/N	N/N

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TOC & submission for CAC review

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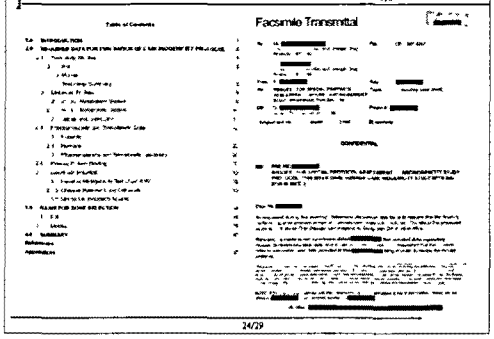
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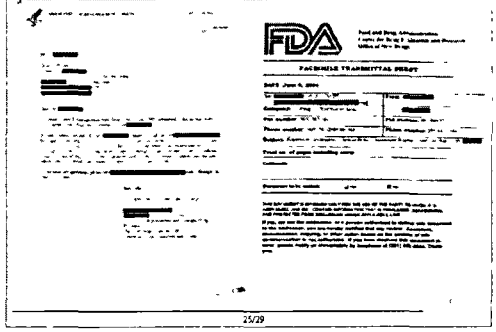
Response & recommendation of CAC



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General considerations - 1

The time for considering carcinogenicity

Carcinogenicity main study takes 2 5 to 3 years

If DRF study and FDA review are needed, 4 or more year needed

Carcinogenicity data are needed before NDA submission

US FDA recommends protocol review at the meeting before end of PII

CRO selection

Price, experience, quality, communication, reputation etc

Test System & duration

SD , F344 or Wister rat (24 or 30 months) and

ICR or B6C2F1 mice (18 or 24 months) or

Short term mice model (initiation promotion model, transgenic or neonatal model)

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General considerations - 2

Number of animals

minimum target number of survival at the end of study, interim sacrifice, TK

animal supplier Capacity to supply on time

age of onset at 4 to 6 week old

Route of administration gavage or mixing with diet or water

Environment general environment, housing, cage, diet etc

TSB check quality & supply

Dose selection by DRF study refer to the ICH guideline

TK sampling times and points, control TK, major metabolites

Statistics Cochran-Armitage trend test, Peto analysis

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General considerations - 3

Interpretation of DRF study

2 w & 13 w 2w also important for dose selection

MTD body weight gain (10% ↓)) mortality) general condition) others

AUC expected AUC by regression analysis

CAC review period 30 days notice, 45 days review

All data prepared, 30 days notice period can be removed

Cause of late on set

investigation period

CAC review

animal supply

stand-by period etc

FDA response on minor change less than 45 days review period

methods of review by FDA raw data first

Close contact with consultant and FDA

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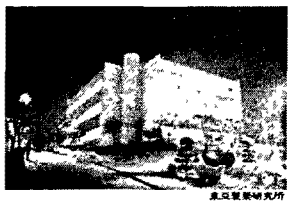

Thank you !

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