【S-10】

Carcinogenicity : a Case Study of DA-8159

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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.

東亞製藥(株)

Brief review of DA-8159

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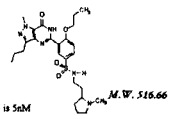
DA-8159

- **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 WO 00/27948
  - Process: PCT/KR01/00819
- **Developmental stage**
  - Phase 1 both in U.K. and Korea finished
  - P2 HEF at-home study (Korea) successfully completed
  - IND and P2 HEF (US) in preparation



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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: endotherial 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

-3/29-

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)	240	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 Carinogenicity 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**

-6/29-

Rat strain selection -1

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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, marrary 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

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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

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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.

-9/29-

Animal Supplier selection

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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

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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

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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

-12/29-

Route of administration

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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

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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.

-14/29-

Test substance (TSB)

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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

-15/29-

Dose selection – 1

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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

東亞醫藥(株)

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

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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

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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

-20/29-

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 ☞ 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-Walliss(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)

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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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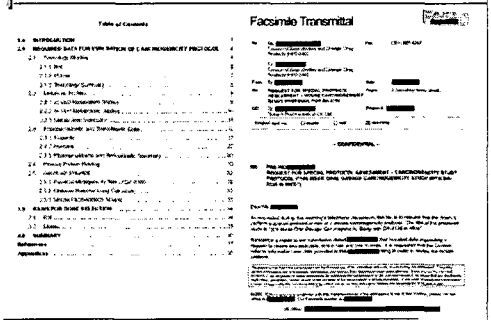
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Response & recommendation of CAC

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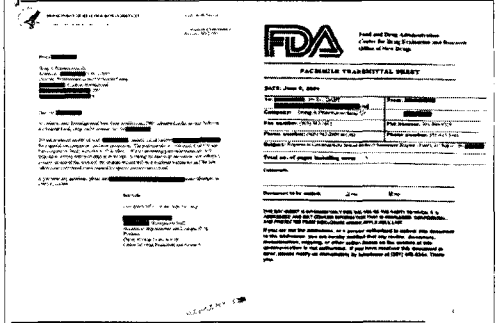

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General considerations - 1

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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

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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

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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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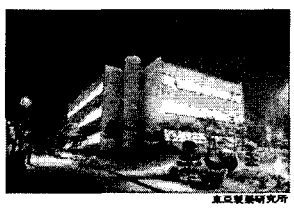

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Thank you !

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