



비임상 독성자료의 생성

원상범

Focused topic:

비임상 독성자료의 생성

2022. 11. 30

원상범

AIMS BioScience
where development meets strategy

Introduction

Study reports

FINAL REPORT

Testing Facility Study No. XXXXXXX

A 28-Day Oral Gavage Toxicity and Toxicokinetic Study of test article in Sprague Dawley Rats with a 14-Day Recovery Period

Based on the results of this study, oral administration of Test article to CrI:CD(SD) rats at dosage levels of 25, 50, and 100 mg/kg/day for 28 days resulted in adverse microscopic findings of ovarian hemorrhagic and cystic degeneration of the corpora lutea in females at 50 and 100 mg/kg/day. Additionally, adverse microscopic findings of bone marrow hypocellularity (with correlating decreased red blood cell and lymphocyte counts) and decreased lymphoid cellularity in the Peyer's patches, spleen, and thymus was noted in males and females at 100 mg/kg/day. Furthermore, test article-related decreased RBC, HGB (males), and HCT (males), increased MCV and RDW, and decreased WBC and lymphocyte counts in the 100 mg/kg/day group males and females persisted in recovery. Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 50 mg/kg/day for males and 25 mg/kg/day for females. Those dosages corresponded to mean AUC_{0-24h} values of 16,200 and 3810 ng·hr/mL and mean C_{max} values of 1040 and 322 ng/mL for males and females, respectively, on Day 28.

Investigator's Brochure (IB)

INVESTIGATOR'S BROCHURE

REPARIXIN

Solution for i.v. administration

Tablets for oral administration

5.3. TOXICOLOGY

5.3.1. Summary

Reparixin under the form of the L-lysine salt (DF1681B) or as free acid (DF1681Y) was tested for toxicity in rodent and non-rodent animal species after single and repeated dose administrations either by i.v. or oral, according to the human foreseen administration route.

The general toxicological profile of reparixin L-lysine salt, as for the studies conducted to date, is characterized by a low toxicity after single administration by i.v. or oral route to mice (LD₅₀ = i.v. 609 mg/kg; LD₅₀ = p.o. >3 g/kg) and to rats (LD₅₀ = i.v. 348 mg/kg; LD₅₀ = p.o. 1303 mg/kg).

Single ascending doses of reparixin L-lysine salt (DF1681B) and reparixin acid (DF1681Y) were administered orally (by gavage) to rats up to the dose of 1000 mg/kg bis in die. The daily doses up to 2000 mg/kg were very well tolerated and no mortality, clinical signs or body weight changes were observed.

The repeated dose administration to rats by continuous infusion for 28 days resulted in a determination of a safe dose of 1000 mg/kg/day (NOAEL), while the continuous infusion administration to dogs for 2 weeks resulted in a safe dose of 60 mg/kg/day, even if after 2 weeks infusion at the dosage of 50 mg/kg/day one male animal showed a mucosal ulceration in the fundic area of the stomach.

The repeated oral administration by oral gavage in rats for a period of 14 days up to the doses of 400 mg/kg bis in die resulted to be well tolerated, and only minor adaptive liver changes of metabolic nature were observed in females at the dose of 400 mg/kg/bid. They consisted of an increase of liver weight and hepatocellular hypertrophy. The NOEL could be determined at 400 mg/kg/bid in males. In females 400 mg/kg/bid represents the NOAEL.

Introduction

Drug discovery

Drug Development

Disease

Target identification

Nonclinical research

Preclinical testing

Phase I-III clinical study

Regulatory approval

Basic Research
Target identification

- Molecular pathways
- Biomarkers & Target Discovery (Multiomics, Systems Biology)
- Computational Biology

Hit to Lead Identification

- Structure-Based drug design
- Structural Biology (NMR & Xray, MS)
- HT-screening
- Structural Bioinformatics

Nonclinical Research

- Disease models
- Toxicity/metabolism
- PK/PD models - ADME
- In vitro/in vivo/ex vivo cellular models for drug testing

Pre-clinical Research

- Pre-clinical Research
- Disease animal models for efficacy testing
- In vivo models for Toxicity assessment

Translation

- Biopharma industry
- Pharma industry

<https://4nb-ia.pt/thematic-line-1-platforms-drug-discovery-and-development>

Discovery

- Mini Ames test (3 or 5 strains)
- hERG assay (@ 10 μM or IC₅₀)
- 2 weeks repeated dose toxicity study

Non GLP

Evaluating the wide therapeutic window

$$\frac{\text{Toxic concentration}}{\text{Efficacious concentration}}$$

⇒ PCC selection

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Introduction

Drug discovery

Disease → Target identification → Nonclinical research → Preclinical testing

Drug Development

Phase I-III clinical study → Regulatory approval

Basic Research
Target identification

- Molecular pathways
- Biomarkers & Target Discovery (Multiomics, Systems Biology)
- Computational Biology

Hit to Lead Identification

- Structure-Based drug design
- Structural Biology (NMR & Xray, MS)
- HT-screening
- Structural Bioinformatics

Nonclinical Research

- Disease models
- Toxicity/metabolism
- PK/PD models - ADME
- In vitro/in vivo/ex vivo cellular models for drug testing

Pre-clinical Research

- Pre-clinical Research
- Disease animal models for efficacy testing
- In vivo models for Toxicity assessment

Translation

- Biopharma industry
- Pharma industry

https://4b6-1a.pl thematic-line-1-platforms-drug-discovery-and-development

Development

- General Toxicity
- Genotoxicity Study
- Safety pharmacology
- Etc.

} **IND Enabling studies (GLP)**

CRO selection

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Introduction

Small Molecule IND Timeline

Given the increasing pressures, understanding how and when to plan a preclinical Investigational new drug (IND)-enabling program is an integral part of meeting specific milestones necessary for timely and efficient IND submission. Please note the Gantt Chart is only an estimate optimized for speed and should be customized based on your lead optimization data, specific modality, therapeutic area, and intended clinical use.

[Learn more](#)

+

FDA approval

Standard for Exchanges of Nonclinical Data

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1. General Toxicity Study

② Repeated dose toxicity

(a) 2 weeks Repeated dose

Legend: Test article Administration, Necropsy

Week 0, 1, 2

Non-GLP (KFDA, FDA, etc.)

- ✓ Dose range finding for 4w

(b) 4 weeks Repeated dose

Legend: Test article Administration, Necropsy

Week 0, 1, 2, 3, 4

GLP (KFDA, FDA, etc.)

- ✓ Target organ
- ✓ No Observed Adverse Effect Level

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1. General Toxicity Study

② Repeated dose toxicity

(a) 2 weeks Repeated dose

Legend: Test article Administration (in-life period), Body weight (twice a week), Food consumption (once a week), Clinical observation (in-life period), Necropsy

- ✓ 투여: 임상예정경로
- ✓ 일반증상: 매일 관찰 기록
- ✓ 체중: 주 2회
- ✓ 사료섭취량: 주 1회
- ✓ 종료 후 육안적 해부조건, 장기중량, 혈액학 및 혈액생화학검사
- ✓ 필요 시 병리조직학적 검사 (육안적 이상조건 장기)

독성동태 (Toxicokinetic)

[2W Repeated Tox. in rat]				[2W Repeated Tox. in dog]		
Group	Dose (mg/kg/day)	Main group (Male/Female)	TK group (Male/Female)	Group	Dose (mg/kg/day)	Main + TK group* (Male/Female)
G1	0	5/5	-	G1	0	3/3
G2	Low	5/5	3/3	G2	Low	3*/3*
G3	Middle	5/5	3/3	G3	Middle	3*/3*
G4	High	5/5	3/3	G4	High	3*/3*

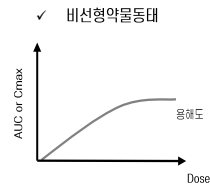
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1. General Toxicity Study

② Repeated dose toxicity

* Toxicokinetic study

- ✓ 독성시험에서의 용량단계 및 시험물질의 노출도와 시간경과와 상관성을 연구
- ✓ 독성시험 수행 시 시험물질의 전신 노출도를 평가하기 위하여 약물동태학적 자료 산출 (T_{max} , C_{max} , AUC)
- ✓ 고용량이 투여되므로 용해도 한계가 나타나 대부분 비선형 약물동태가 나타남



**용량상관성
(Dose proportionality)**

Day	1			14		
	Dose (mg/kg/day)	C_{max} (ng/mL)	AUClast (ng·hr/mL)	Dose (mg/kg/day)	C_{max} (ng/mL)	AUClast (ng·hr/mL)
	100	200	400	100	200	400
	1673.7	2132.0	3444.9	1543.0	2216.3	4230.3
	20812.4	35164.7	62966.6	18511.3	30164.1	65873.1
Dose ratio	1.0	2.0	4.0	1.0	2.0	4.0
C_{max} ratio	1.0	1.3	2.1	1.0	1.4	2.7
AUClast ratio	1.0	1.7	3.0	1.0	1.6	3.6

**축적성
(Accumulation)**

Day	1		Ratio
	Dose (mg/kg/day)	C_{max} (ng/mL)	
	100	100	
	1673.7	1543.0	0.9
	20812.4	18511.3	0.9
Dose (mg/kg/day)	200	200	
	2132.0	2216.3	1.0
	35164.7	30164.1	0.9
Dose (mg/kg/day)	400	400	
	3444.9	4230.3	1.2
	62966.6	65873.1	1.0

**성차
(Gender difference)**

Gender	Male		Ratio
	Dose (mg/kg/day)	C_{max} (ng/mL)	
	100	100	
	1673.7	2343.2	1.4
	20812.4	33259.8	1.6
Dose (mg/kg/day)	200	200	
	2132.0	3411.3	1.6
	35164.7	52747.0	1.5
Dose (mg/kg/day)	400	400	
	3444.9	5215.0	1.5
	62966.6	92254.7	1.5

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1. General Toxicity Study

② Repeated dose toxicity

(a) 2 weeks Repeated dose



December 2009
EMA/CPMP/ICH/286/1995

ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals

Figure 1: Recommended high dose selection for general toxicity studies:

1. MTD
2. Exposure saturation
3. MFD
4. Mean exposure margin 50x clinical*

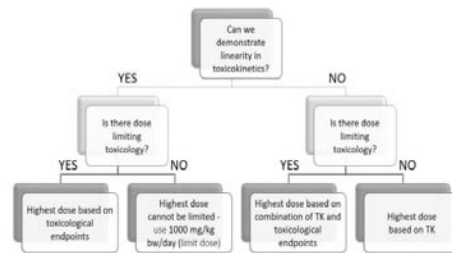
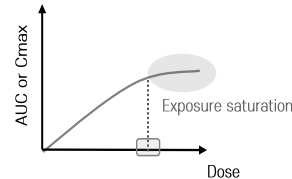


Fig. 1 Use of TK data in the design of toxicity studies. The decision flowchart depicts to what extent and in which cases TK information can be integrated in the decisions of the high dose for toxicity studies.

Fiona Sewell, et al. (2022). Recommendations on dose level selection for repeat dose toxicity studies. Archives of Toxicology 96:1921-1934

비선형약물동태

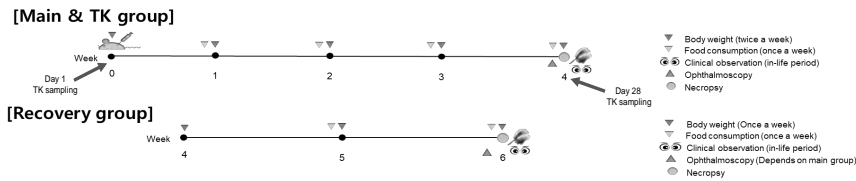


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1. General Toxicity Study

② Repeated dose toxicity

(b) 4 weeks Repeated dose



- ✓ 투여: 임상예정경로
- ✓ 일반증상: 매일 관찰 기록
- ✓ 체중: 주 2회
- ✓ 사료섭취량: 주 1회
- ✓ 종료 후 육안적 해부조건, 장기중량, 혈액학 및 혈액생화학검사
- ✓ 독성상태 평가
- ✓ 부형제, 고용량군 병리조직학적 검사 (필수)
- ✓ 안과학적 검사, 혈액응고 검사, 뇨 검사

회복군 (Recovery group)

[4W Repeated Tox. in rat]					[4W Repeated Tox. in dog]				
Group	Dose (mg/kg/day)	Main group (Male/Female)	TK group (Male/Female)	Recovery group (Male/Female)	Group	Dose (mg/kg/day)	Main + TK group (Male/Female)	Recovery group (Male/Female)	
G1	0	10/10	-	5/5	G1	0	3/3	2/2	
G2	Low	10/10	3/3	-	G2	Low	3/3	-	
G3	Middle	10/10	3/3	-	G3	Middle	3/3	-	
G4	High	10/10	3/3	5/5	G4	High	3/3	2/2	

- ✓ 독성 시험군과 동일한 항목 평가
- ✓ 안과학적 검사는 독성시험군에서 증상 발생 유무에 따라 의존적

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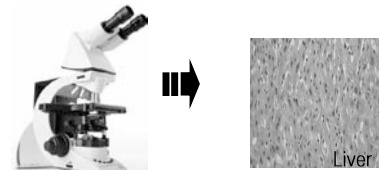
1. General Toxicity Study

② Repeated dose toxicity

[4w Repeated Tox. in rat]

군	용량 (mg/kg)	동물 수	체중	사료섭취량	임상증상	장기중량	혈액학 및 혈액생화학	혈액응고 및 안 검사	노검사	육안조건	체내노출도 (day 28)
G1	0	10/10	-	-	-	-	-	-	-	-	-
G2	50	10/10	15% ↑	10% ↑	-	-	-	-	-	간 증대	C _{max} : 843 ng/mL AUC: 9,511 ng·hr/mL
G3	100	10/10	10% ↑	5% ↑	-	절대 및 상대 간 중량 ↑	-	-	-	간 증대	C _{max} : 1,543 ng/mL AUC: 18,511 ng·hr/mL
G4	200	10/10	15% ↓	-10% ↓	시험물질 영향 증상 (혈동성저하, 응크린 자세)	절대 및 상대 간 중량 ↑	WBC ↓, LYM ↓, AST ↑, ALT ↑	-	-	간 증대 및 색변화	C _{max} : 2,216 ng/mL AUC: 30,164 ng·hr/mL

- ✓ 간은 표적장기로 판단되어 저, 중용량 모두 조직 병리 진행



- ✓ 50 mg/kg/day : 병리학적 조건 없음
 - ✓ 100 mg/kg/day : 단핵구 침윤소 (부형제군 동일)
 - ✓ 200 mg/kg/day : 염증세포 침윤, 섬유화 조건
- 시험물질 투여에 의한 형태학적 변화

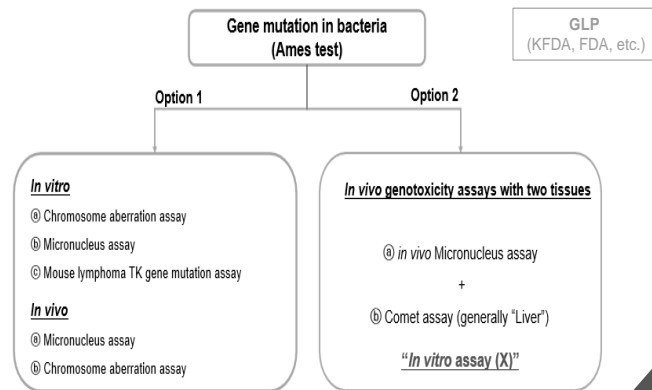
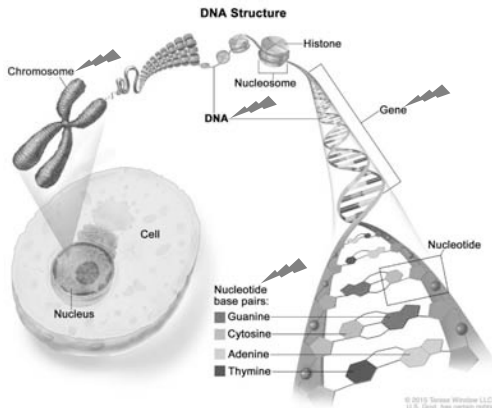
✓ No Observed Adverse Effect Level = 100 mg/kg/day

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2. Genotoxicity Study

Genotoxicity

DNA 염기서열, 유전자 및 염색체에 직접적으로 손상을 주어 형태학적 및 기능적 이상을 일으키는 현상



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2. Genotoxicity Study

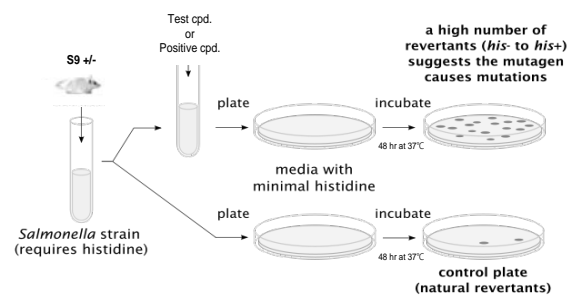
Bacterial reverse mutation (Ames test)

1) Strains

- ✓ *Salmonella Typhimurium* TA98 (Frame shift)
- ✓ *Salmonella Typhimurium* TA1537 or TA97 or TA97a (Frame shift)
- ✓ *Salmonella Typhimurium* TA100 (Base substitution)
- ✓ *Salmonella Typhimurium* TA1535 (Base substitution)
- ✓ *Escherichia coli* WP2 *uvrA* or *E. coli* WP2 *uvrA* (pKM101) or *Salmonella Typhimurium* TA102

2) Top concentration

- ✓ 5,000 µg/plate
- ✓ Colony 수 감소가 나타나지 않는 농도
- ✓ 침전물이 계수를 방해하지 않고, 평가를 제한하지 않는 농도
- ✓ 5단계 이상의 농도로 평가



3) Results

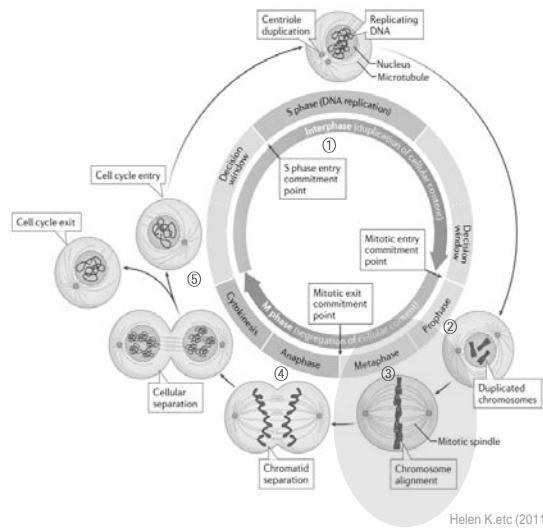
- ✓ 최소 1개 균주에서 농도 의존적인 증가와 재현성 있는 증가 (대사활성계 유,무 관계 X)
- ✓ 실험실 내 측정된 음성대조군 결과 범위 밖
- ✓ 대조군 대비 2 ~ 3 배 증가
- ✓ 통계학적 방법 → 보조 수단

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2. Genotoxicity Study

In vitro Chromosome Aberration

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Helen K. etc (2011)

① Interphase (간기)

- ✓ 핵막 존재, DNA 복제

② Prophase (전기)

- ✓ 핵막 사라짐, 염색사 → 염색체, 방추사 형성

③ Metaphase (중기)

- ✓ 염색체 중앙 배치, 방추사 동원체에 부착

④ Anaphase (후기)

- ✓ 방추사에 의해 염색분체 분리

⑤ Telophase (말기)

- ✓ 핵막 생성 (2개의 핵), 세포질 형성

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2. Genotoxicity Study

In vitro Chromosome Aberration

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1) Cell

- ✓ Chinese hamster lung (CHL)
- ✓ Chinese hamster ovary (CHO)
- ✓ Human peripheral blood lymphocyte

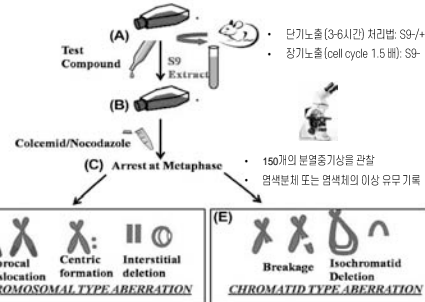


FIGURE 4.3 In vitro mammalian chromosome aberration test (Test Guideline [TG] 473). The cells are plated and treated with the metabolic activator (A), along with the test compound (B). This is followed by exposure of cells to colcemid/nocodazole (C), which allows arrest at metaphase. Various chromosomal type aberration (D) and chromatid type aberration (E) are observed.
Dixit, Manisha (2018)

2) Top concentration

- ✓ 1 mM or 0.5 mg/mL 중 낮은 농도
- ✓ 50%의 세포성장 감소를 초과하지 않는 농도
- ✓ 침전물이 보이는 최저농도 / pH, 삼투압 고려
- ✓ 3단계 이상의 농도로 평가

3) Result

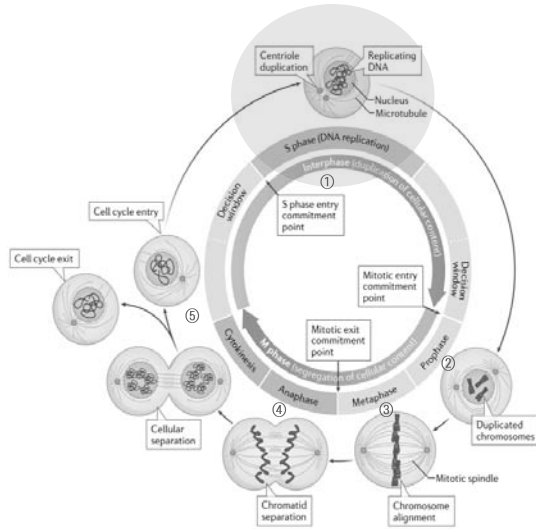
- ✓ 염색체 이상(구조 및 수적이상)을 가진 분열종기상의 수가 적어도 1개 이상의 농도에서 음성대조군과 비교하여 통계학적으로 유의적 증가
- ✓ 농도의존성인 증가
- ✓ 실험실 내 축적된 음성대조군 결과 범위 밖

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2. Genotoxicity Study

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In vitro Micronucleus Assay



Helen K. et al (2011)

① Interphase (간기)

- ✓ 핵막 존재, DNA 복제

② Prophase (전기)

- ✓ 핵막 사라짐, 염색체 → 염색체, 방추사 형성

③ Metaphase (중기)

- ✓ 염색체 중앙 배치, 방추사 동원체에 부착

④ Anaphase (후기)

- ✓ 방추사에 의해 염색분체 분리

⑤ Telophase (말기)

- ✓ 핵막 생성 (2개의 핵), 세포질 형성

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2. Genotoxicity Study

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In vitro Mouse lymphoma TK gene mutation

1) Cell

- ✓ Chinese hamster ovary (CHO)
- ✓ Human peripheral blood lymphocyte
- ✓ Human TK6

2) Top concentration

- ✓ 1 mM or 0.5 mg/mL 중 낮은 농도
- ✓ 50%의 세포성장 감소를 초과하지 않는 농도
- ✓ 침전물이 계수를 방해하지 않는 농도 / pH, 삼투압 고려
- ✓ 3단계 이상의 농도로 평가

3) Result

- ✓ 소핵을 가진 세포의 수가 적어도 1개 이상의 농도에서 음성대조군과 비교하여 통계학적으로 유의적 증가
- ✓ 농도의존성인 증가
- ✓ 실험실 내 축적된 음성대조군 결과 범위 밖

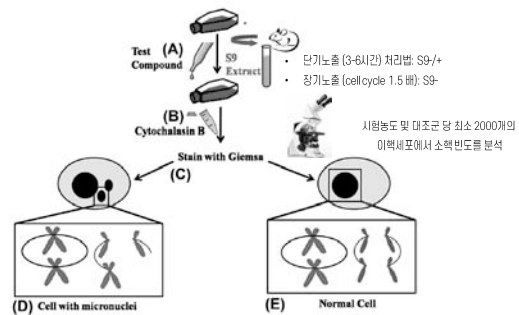


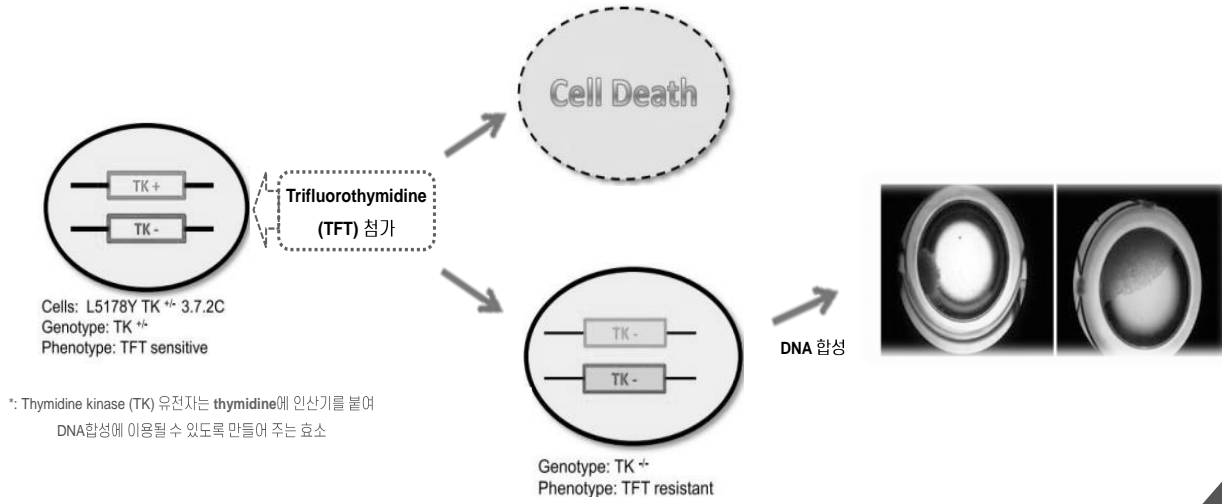
FIGURE 4.5 In vitro mammalian cell micronucleus test (Test Guideline [TG] 487). The cells are plated and treated with the metabolic activator (A), along with the test compound. The cells are then treated with cytochalasin B (B) to prevent cytokinesis. The cells are then stained with Giemsa stain (C) and the micronuclei are visualized (D) under microscope and compared to the normal cells (E).

Dixit, Manisha (2018)

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2. Genotoxicity Study

In vitro Mouse lymphoma TK gene mutation



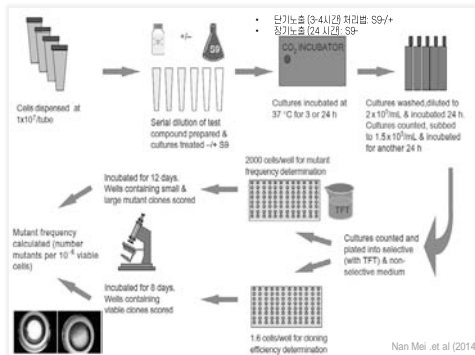
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2. Genotoxicity Study

In vitro Mouse lymphoma TK gene mutation

1) Cell

- ✓ Mouse Lymphoma Cell (L5178Y TK^{+/+} - 3.7.2.C)



2) Top concentration

- ✓ 80 ~ 90% 세포독성을 나타내는 농도
- ✓ 세포독성이 나타나지 않는 경우, 1 mM or 0.5 mg/mL 중 낮은 농도
- ✓ 침전물이 계수를 방해하지 않는 농도/ pH, 삼투압 고려
- ✓ 4농도 이상의 농도로 평가

3) Results

- ✓ 돌연변이 발생빈도가 통계학적으로 유의성 있게 농도의존적으로 증가
- ✓ 적어도 하나 이상의 용량 단계에서 재현성 있게 양성반응을 나타내는 경우
- ✓ Global evaluation factor (GEF)를 초과하고 농도의존적으로 증가

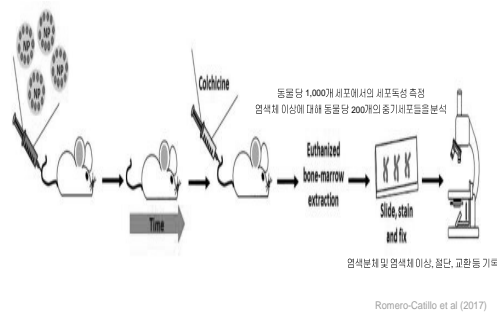
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2. Genotoxicity Study

In vivo Chromosome Aberration assay

1) Animals

- ✓ Mouse or Rat
- ✓ 일반적으로 수컷 사용



2) Dose selection

- ✓ 골수에 독성의 징후가 나타난 용량
- ✓ 독성이 관찰된다면 최대 내성용량 (MTD)
- ✓ 14일 이내: 2,000 mg/kg/day
- ✓ 14일 이상: 1,000 mg/kg/day
- ✓ 3단계 이상의 용량군을 설정

3) Result

- ✓ 염색체 이상을 가진 분열중기상 수가 1개 이상의 농도에서 음성대조군과 비교하여 통계학적으로 유의성 있게 증가하고, 용량의존적인 증가
- ✓ 실험실내 축적된 음성대조군 결과 범위를 벗어나는 경우

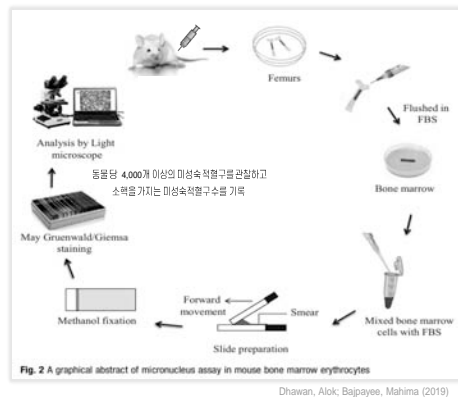
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2. Genotoxicity Study

In vivo Micronucleus assay

1) Animals

- ✓ Mouse or Rat
- ✓ 수컷이 감수성 높아 일반적으로 수컷을 사용



2) Dose selection

- ✓ 치사율이 나타나지 않은 최대 용량 (MTD)
- ✓ 14일 이내: 2,000 mg/kg/day
- ✓ 14일 이상: 1,000 mg/kg/day
- ✓ 골수 내 총적혈구 중 미성숙적혈구 비율 감소되는 용량
- ✓ 3단계 이상의 용량군을 설정

3) Result

- ✓ 소핵을 가진 미성숙 적혈구의 수가 1개 이상의 용량에서 음성대조군 대비 통계학적으로 유의성 있게 증가하고 용량의존적으로 증가
- ✓ 실험실내 축적된 음성대조군 결과 범위를 벗어나는 경우

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2. Genotoxicity Study

In vivo comet assay

1) Animals

- ✓ Rat (다른 종 사용가능)
- ✓ 성별 상관 없음

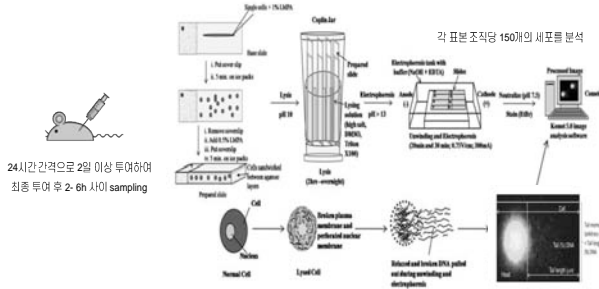


Fig. 1 A schematic representation of the Comet procedure. Mahima Bapayee et al (2019)

% tail DNA, tail 길이 및 tail moment를 통해 DNA 절단 확인

2) Dose selection

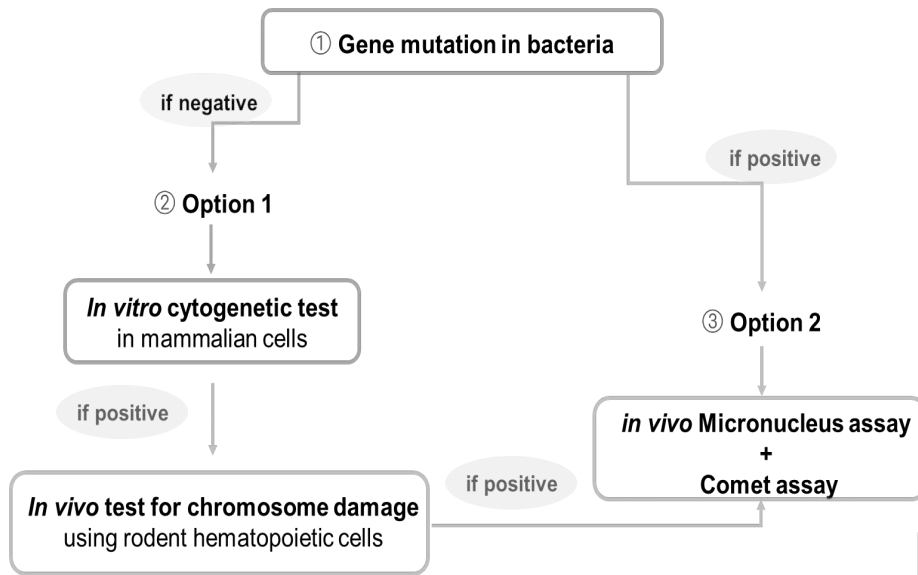
- ✓ 독성이 있는 시험물질은 최대내성용량 (MTD)
- ✓ 14일 이내: 2,000 mg/kg/day
- ✓ 14일 이상: 1,000 mg/kg/day
- ✓ 3단계 이상의 용량군을 설정

3) Result

- ✓ % tail DNA는 결과평가 및 해석용으로 권고
- ✓ 각 동물에 대한 중간치 % tail DNA 값의 평균을 계산할 것을 권고
- ✓ 세포 내 총 DNA 발현 정도 대비 % tail DNA의 발현정도에 의해 결정
- ✓ 음성대조군 대비 통계학적으로 유의한 증가, 용량의존적인 증가
- ✓ 실험실내 축적된 음성대조군 결과 범위를 벗어나는 경우 DNA 손상

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2. Genotoxicity Study



ICH S2 (R1) Genotoxicity testing and data interpretation for pharmaceuticals intended for human use

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3. Safety pharmacology

치료 범위 이상의 노출과 관련하여 생리 기능에 대한 잠재적인 바람직하지 않은 약력학적 효과를 조사



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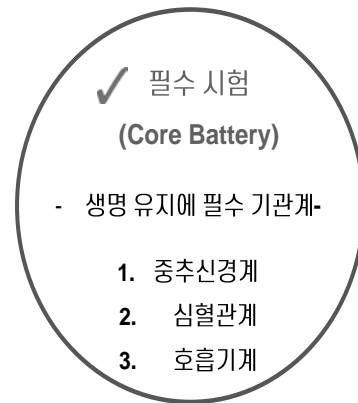
3. Safety pharmacology

Impact of adverse effects of drugs by organ system throughout the pharmaceutical life cycle

Phase	'Nonclinical'	Phase I	Phase I-III	Phase III Marketing	Post-Marketing	Post-Marketing
Information:	Causes of attrition	Serious ADRs	Causes of attrition	ADRs on label	Serious ADRs	Withdrawal from sale
Source:	Car (2006)	Sibile et al. (1998)	Olson et al. (2000)	BioPrint® (2006)	Budnitz et al. (2006)	Stevens & Baker (2006)
Sample size:	88 CD's stopped	1,015 subjects	82 CD's stopped	1,198 drugs	21,298 patients	47 drugs
CARDIOVASCULAR:	27%	9%	21%	36%	15%	45%
Hepatotoxicity:	8%	7%	21%	13%	0%	32%
Haematology/BM:	7%	2%	4%	16%	10%	9%
NERVOUS SYSTEM:	14%	28%	21%	67%	39%	2%
Immunotox. photosensitivity:	7%	16%	11%	25%	34%	2%
GASTROINTESTINAL:	3%	23%	5%	67%	14%	2%
Reprotox.:	13%	0%	1%	10%	0%	2%
Musculoskeletal:	4%	0%	1%	28%	3%	2%
RESPIRATORY:	2%	0%	0%	32%	8%	2%
RENAL:	2%	0%	9%	19%	2%	0%
Genetic tox.:	5%	0%	0%	0%	0%	0%
Carcinogenicity:	3%	0%	0%	1%	0%	0%
Other:	0%	0%	4%	16%	2%	2%

The various toxicity domains have been ranked first by contribution to products withdrawn from sale, then by attrition during clinical development.

Adapted from Radfern WS et al. SOT 2010; 2011



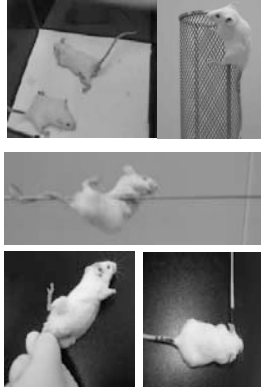
3. Safety pharmacology

3.1 Central Nervous System (CNS)

- 운동성, 행동변화, 운동 협조성, 감각/운동신경의 반사반응, 체온 등을 평가하여 중추신경계의 약력학적 유해반응 확인

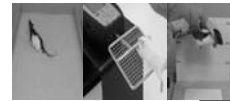
Modified Irwin's test

- 자발 운동량
- 꼬리들기 반응
- 떨림
- 음성면
- 복부 긴장상태
- 각진증
- 평형감각
- 정향반사
- 이개반사
- 입모
- 피부색
- 호흡수
- 안검
- 안구돌출
- 누액분비
- 타액
- 설사
- 사망



Functional Observation Battery (FOB)

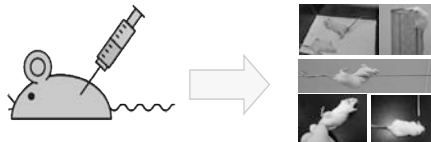
- ㉔ 사육상자 내 관찰
자세, 호흡, 간헐적 및 긴장성 비의도적 움직임, 이상 발성, 안성 폐쇄
- ㉕ 손으로 잡은 채로 관찰
꺼내기 및 다루기 쉬운 정도, 안검 폐쇄, 유류, 유연, 입모, 기타 (상태를 표시하여 기록)
- ㉖ 체온
- ㉗ 사육상자 밖에서의 운동성
공중정향반사, 호흡, 경련, 도약, 이상발성, 몸단장, 안검상태, 상동행동, 보행, 뒷다리로 일어서기, 배뇨, 설사, 이동성
- ㉘ 자극반응성
동공반응 검사, 접근반응 검사, 접촉반응 검사, 청각 반응 검사, 통각반응 검사, 정향반응 검사
- ㉙ 신경 및 근육 측정
자발운동량 측정, 약력측정, 착지보폭측정
- ㉚ 동공 반응



3. Safety pharmacology

3.1 Central Nervous System (CNS)

Group	Dose (mg/kg)	Number of Animal
G1	Vehicle	6
G2	Low	6
G3	Mid	6
G4	High	6



- ✓ Rat or mouse, 수컷, 단회투여 (임상에정경로)

- ✓ 관찰 항목:

치사율, 임상증상, 체중, 신경행동 관찰* 및 체온 측정

*사육상자 내 관찰, 손으로 잡은 채 관찰, 사육상자 밖에서의 운동성, 자극 반응성, 신경 및 근육 측정, 동공 반응

- ✓ 각 항목 점수화

- ✓ 음성대조군과 비교하여 통계학적으로 유의적 증가

- ✓ 용량 의존적인 증가

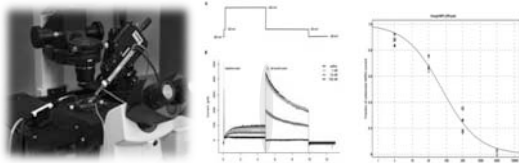
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3. Safety pharmacology

3.2 Cardiovascular System (CV)

In vitro hERG assay

hERG 칼륨 이온 채널을 발현시킨 세포에 시험물질을 처리하여
hERG channel currents에 미치는 영향을 평가



Timm Danker and Clemens Möller (2014)

In vivo (Dog or Monkey) Telemetry study

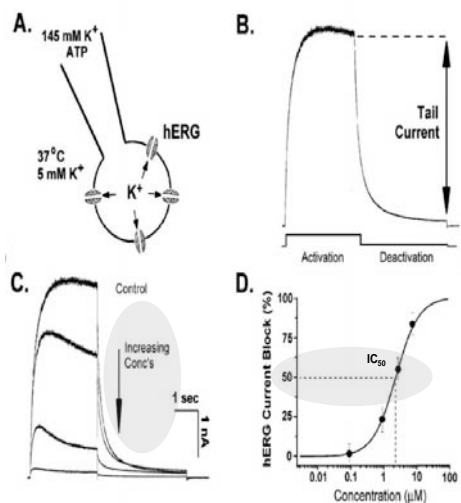
마취 및 동물을 구속하지 않은 상태에서 시험물질 투여 후 혈압,
심박수 및 심전도를 측정하여 심혈관계에 미치는 영향 평가



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3. Safety pharmacology

3.2 Cardiovascular System (CV)_In vitro hERG assay



Gary A Gintant et al (2006)

- ✓ Patch clamp
- Channel currents 억제 정도를 patch clamp로 조사
- ✓ 음성대조군 포함 5 농도 (음성대조군 포함 5 농도)
- ✓ IC₅₀ 산출
- ✓ **Criteria: IC₅₀ > 10 μM (No inhibition)**
- ✓ Total Safety Margin = $hERG\ IC_{50} / C_{max}$
- ✓ Free Safety Margin = $hERG\ IC_{50} / FreeC_{max}$
- ✓ 30 배 margin 적용

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3. Safety pharmacology

3.2 Cardiovascular System (CV)_In vivo Telemetry study

Animal No.	Dose 1 Treatment (mg/kg)	Dose 2 Treatment (mg/kg)	Dose 3 Treatment (mg/kg)	Dose 4 Treatment (mg/kg)
001	Vehicle	Mid	Low	High
002	Low	Vehicle	High	Mid
003	Mid	High	Vehicle	Low
004	High	Low	Mid	Vehicle

✓ Dog or monkey, 수컷

✓ Latin square 방법으로 진행

(동일한 개체에 각 용량을 순환 투여, 임상예정경로)

✓ 관찰 항목:

심혈관계 parameter (혈압, 심박수 및 심전도) 및 체온

✓ 음성대조군과 비교하여 통계학적으로 유의적 증가

✓ 용량 의존적인 증가

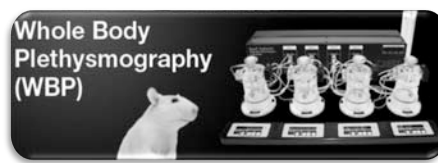


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3. Safety pharmacology

3.3 Respiratory (RP)

✓ 약물에 의한 호흡기계 기능 변화 평가



<https://www.primetech.co.jp>

• Double chamber (비강 chamber, 흉강 chamber)

• 호흡 평가 parameter 정확성 높음

• Chamber에 동물고정 → 스트레스 발생 (긴 시간 측정 x)

• Chamber 내 압력 측정 → 공기 흐름을 간접적 측정

• 무마취, 무구속 single chamber

• 스트레스 발생 낮음 → 긴 시간 측정 가능 ✓

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3. Safety pharmacology

3.3 Respiratory (RP)

Group	Dose (mg/kg)	Number of Animal
G1	Vehicle	8
G2	Low	8
G3	Mid	8
G4	High	8



<https://www.primetech.co.jp>

- ✓ Rat or mouse, 수컷
- ✓ Chamber 내 호흡에 의한 압력 변화 → 그래프로 나타냄
- 그래프의 높이와 면적으로 호흡수와 호흡량 계산
- ✓ 평가 항목: 호흡율, 일회 호흡량, 분당호흡량
- ✓ 용량 의존적인 증가 or 음성대조군과 비교하여 통계학적으로 유의적 증가 시 양성판정

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4. Determination of the Starting dose in FIH study

charles river

Small Molecule IND Timeline

Given the increasing pressures, understanding how and when to plan a preclinical investigational new drug (IND)-enabling program is an integral part of meeting specific milestones necessary for timely and efficient IND submission. Please note the Gantt Chart is only an estimate optimized for speed and should be customized based on your lead optimization data, specific molecule, therapeutic area, and intended clinical use.

[Learn more](#)

Full Timeline (Weeks 0-27)

Development and validation

- Analysis and validation
- Reporting and audit

General Toxicology

- Range-finding rodent toxicology
- 28-day study rodent toxicology
- Reporting and audit

Genetic Toxicology

- Ames test
- Chromosomal aberrations
- Rodent in vivo micronucleus
- Reporting and Audit

Safety Pharmacology

- hERG channel test
- Cardiovascular drug study
- Respiratory rodent study
- ONS safety pharmacology in rat
- Reporting and audit

✓ Small molecule: NOAEL
 ✓ Anticancer drug: STD10 or HNSTD

Guidance for Industry

Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

Guidance for Industry

S9 Nonclinical Evaluation for Anticancer Pharmaceuticals

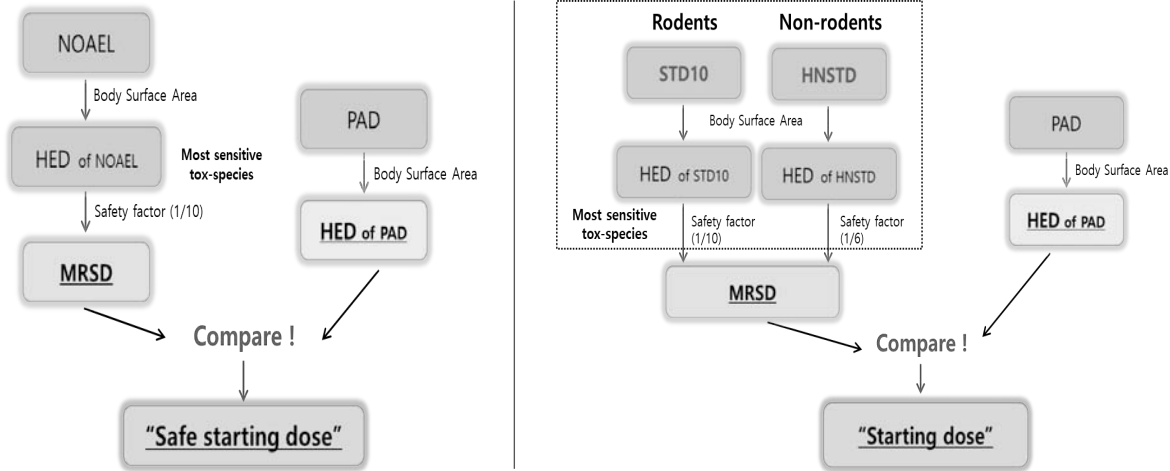
Consensus Toxicology Recommendation

values to be used for interspecies dose conversion for NOAELs. (These factors may also be applied when comparing safety margins for other toxicity end-points (e.g., reproductive toxicity and carcinogenicity) when other data for comparison (e.g., ADIC) are unavailable or are otherwise inappropriate for comparison.)

Species	Based on Body Surface Area	
	From Current Animal Dose to Starting Dose in Initial Clinical Trials (mg/kg)	From NOAEL to Starting Dose (mg/kg)
Human	10	10
Chimpanzee ^a	10	10
Monkey	10	10
Rat	6.3	6.3
Mouse	3.15	3.15
Guinea pig	6.3	6.3
Sheep	10	10
Pig	10	10
Primate	10	10
Monkey ^b	10	10
Monkey ^c	6.3	6.3
Monkey ^d	3.15	3.15
Monkey ^e	10	10
Monkey ^f	10	10
Monkey ^g	10	10
Monkey ^h	10	10
Monkey ⁱ	10	10
Monkey ^j	10	10
Monkey ^k	10	10
Monkey ^l	10	10
Monkey ^m	10	10
Monkey ⁿ	10	10
Monkey ^o	10	10
Monkey ^p	10	10
Monkey ^q	10	10
Monkey ^r	10	10
Monkey ^s	10	10
Monkey ^t	10	10
Monkey ^u	10	10
Monkey ^v	10	10
Monkey ^w	10	10
Monkey ^x	10	10
Monkey ^y	10	10
Monkey ^z	10	10

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4. Determination of the Starting dose in FIH study



- NOAEL: No observed adverse effect level
 - HED: Human equivalent dose
 - MRSD: Maximum recommended starting dose
 - PAD: Pharmacologically active dose
 (the lowest dose tested in an animal species with the intended pharmacologic activity)

- STD: Severely toxic dose
 - HNSTD: Highest non-severely toxic dose

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4. Determination of the Starting dose in FIH study

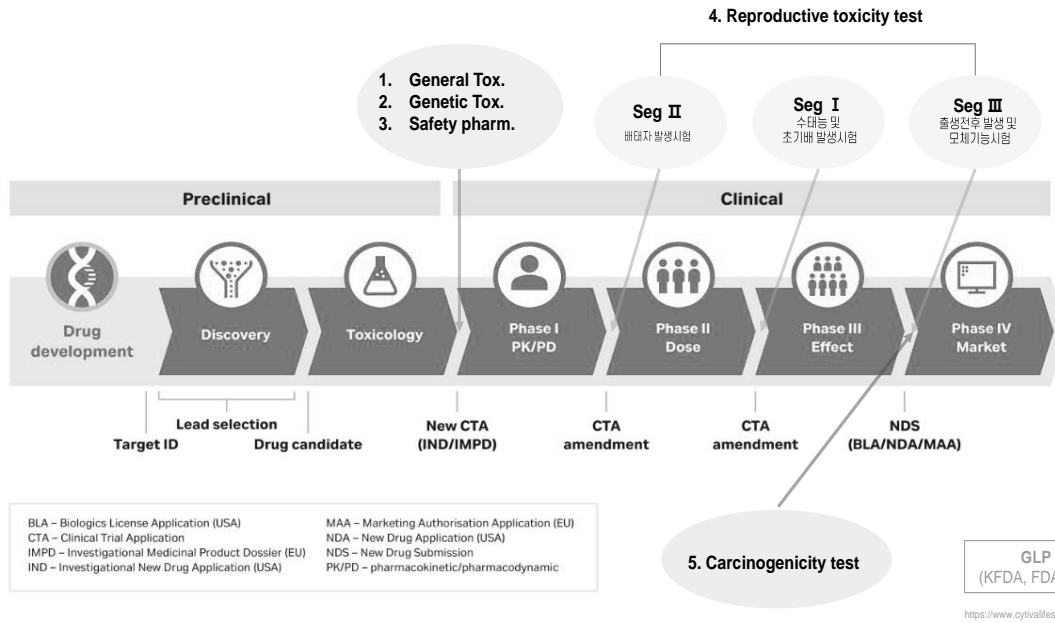
Example)

Test article	Test species	Calculations
Small molecule	Rat	<ul style="list-style-type: none"> If NOAEL = 20 mg/kg; • HED of NOAEL = 20 mg/kg * 0.16 * 60 kg = 192 mg
	Dog	<ul style="list-style-type: none"> If NOAEL = 20 mg/kg; • HED of NOAEL = 10 mg/kg * 0.54 * 60 kg = 324 mg
	Appropriate species	<ul style="list-style-type: none"> • More sensitive tox species = Rat • MRSD = HED of NOAEL * safety factor = 192 mg * (1/10) = 19.2 mg
Anticancer drug	Rat	<ul style="list-style-type: none"> If STD10 = 20 mg/kg; • HED of NOAEL = 200 mg/kg * 0.16 * 60 kg = 192 mg
	Dog	<ul style="list-style-type: none"> If HNSTD = 5 mg/kg; • HED of NOAEL = 200 mg/kg * 0.54 * 60 kg = 162 mg
	Appropriate species	<ul style="list-style-type: none"> • More sensitive tox species = Dog • MRSD = HED of NOAEL * safety factor = 162 mg * (1/6) = 27 mg

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5. And other things

AIMS BioScience
where development meets strategy



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AIMS BioScience
where development meets strategy

경청해주셔서 감사합니다.

Question: wonsb2@aimsbiosci.com