



Hit to lead 개발 및 최종개발 후보물질 선정

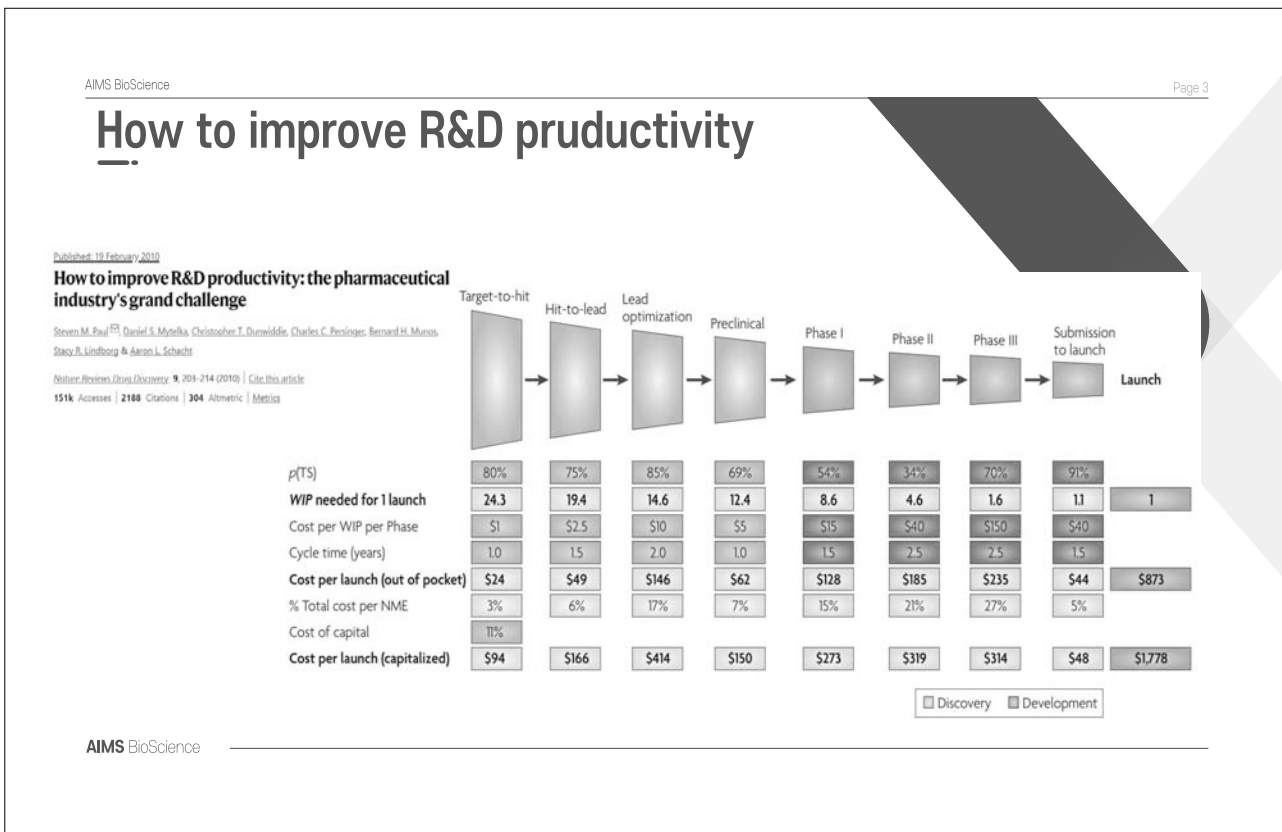
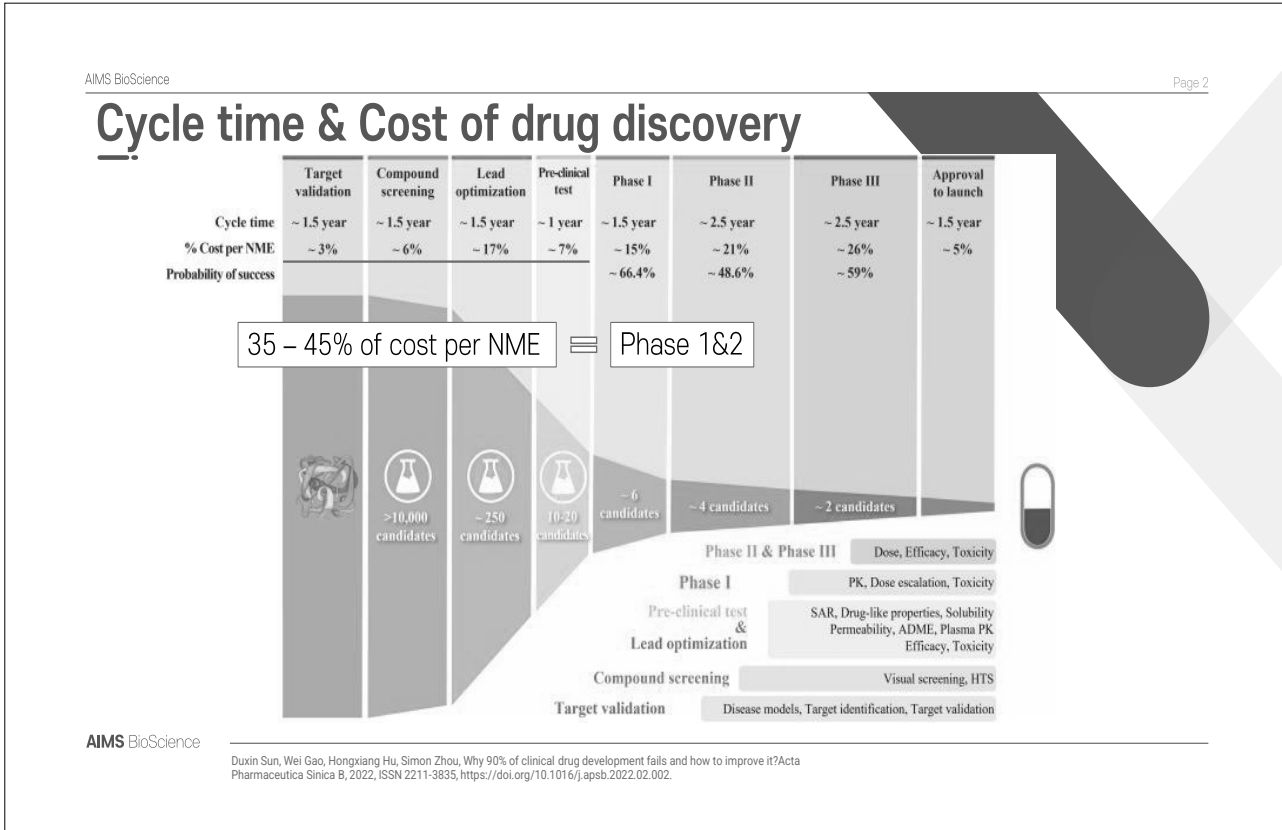
김정호

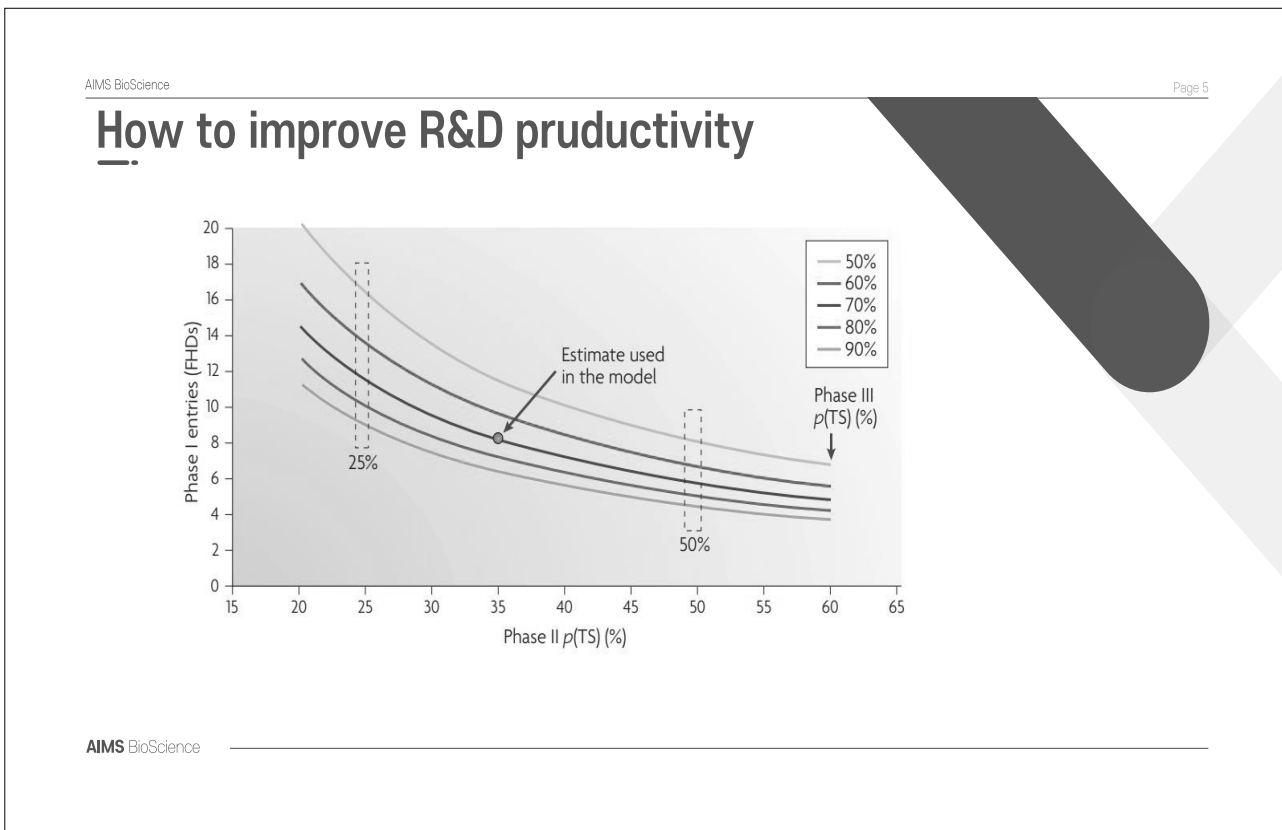
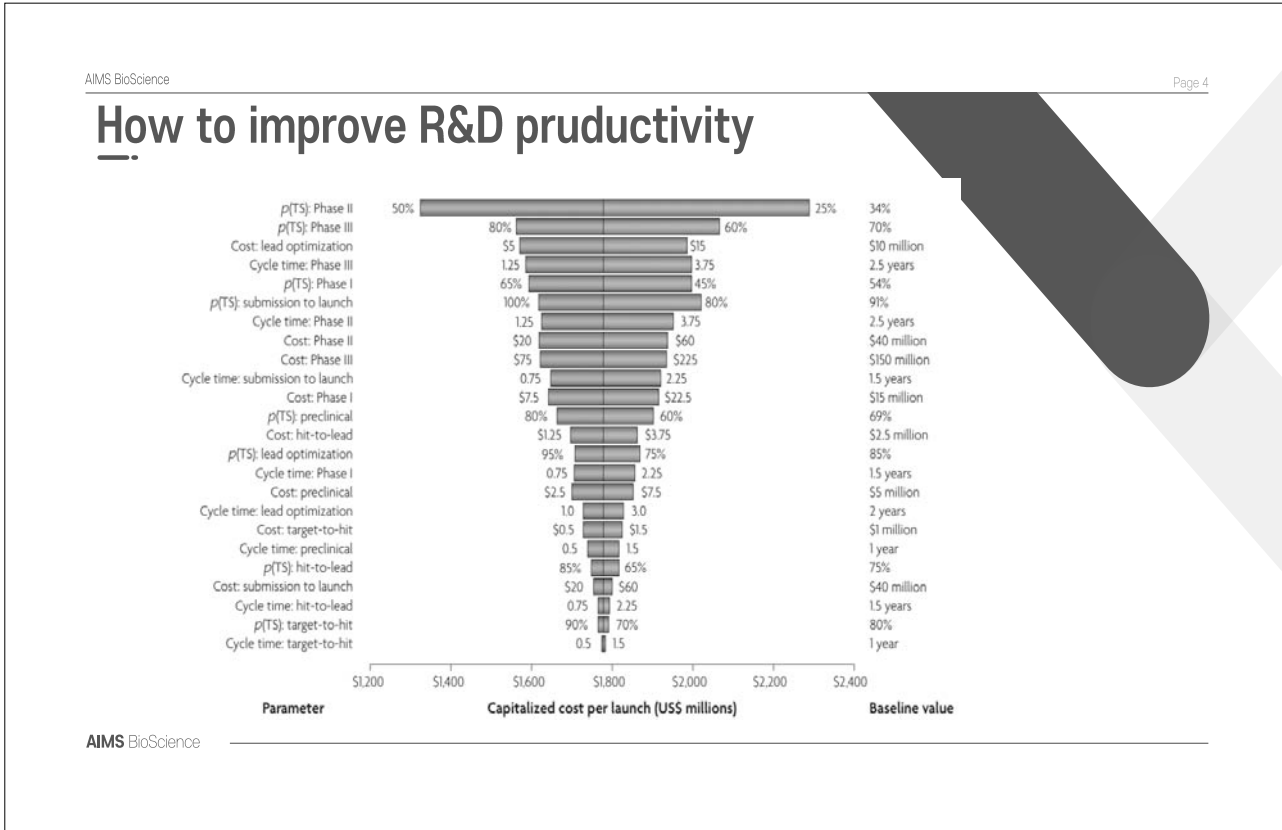
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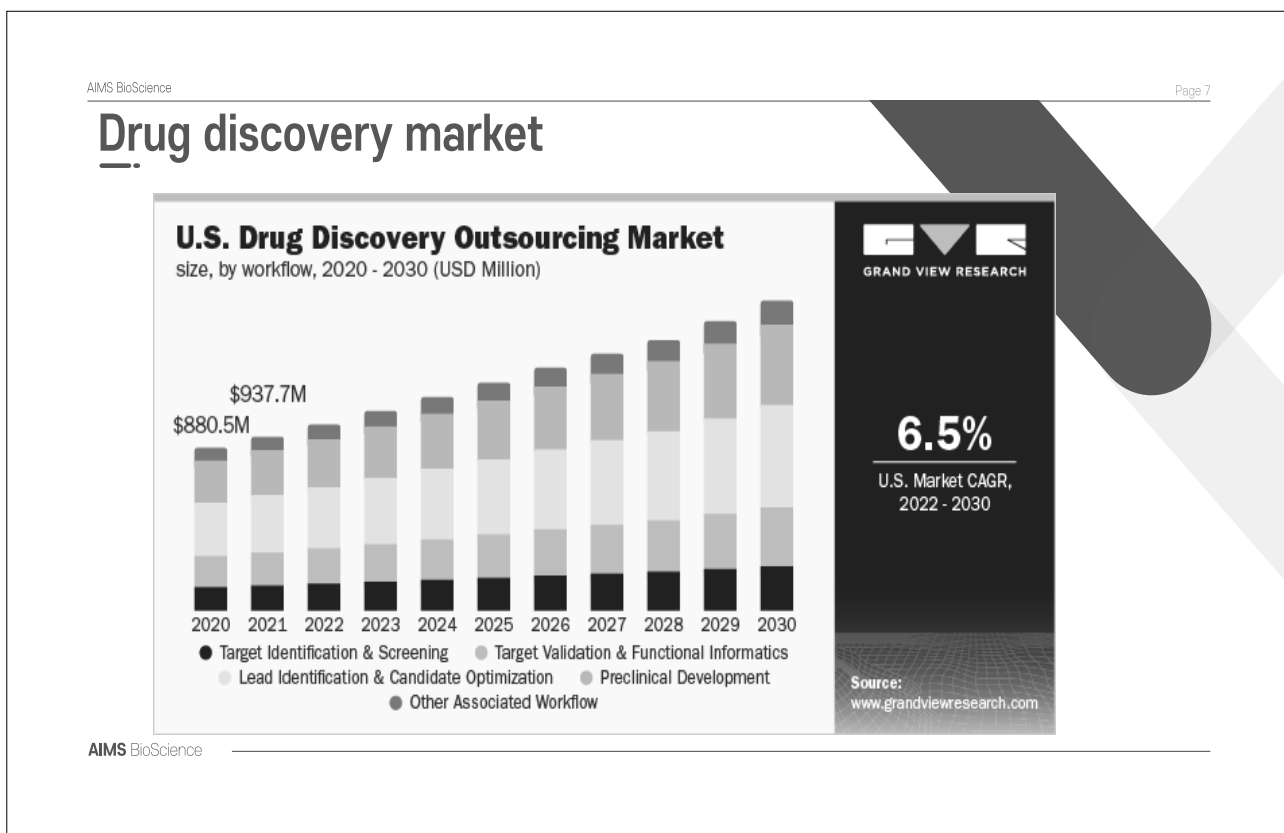
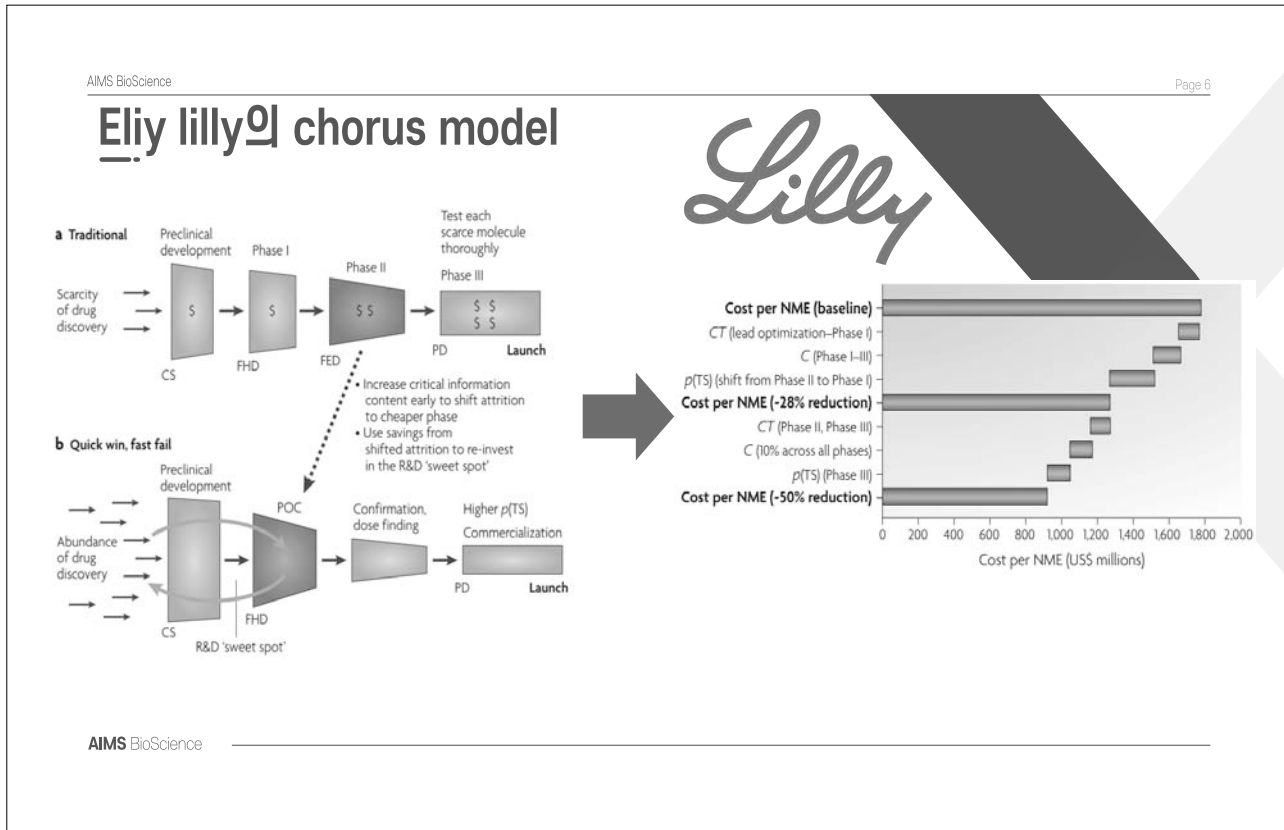
Hit to lead 개발 및 최종개발 후보물질 선정

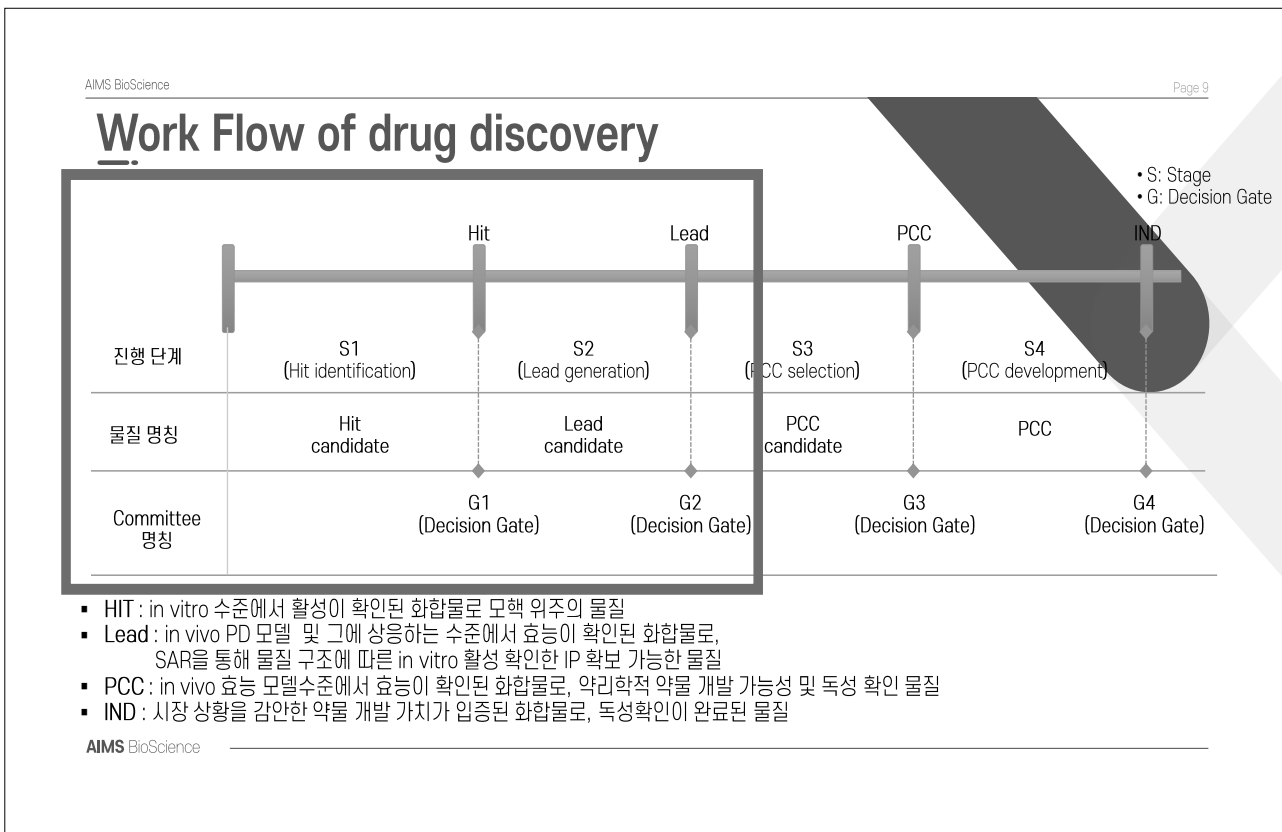
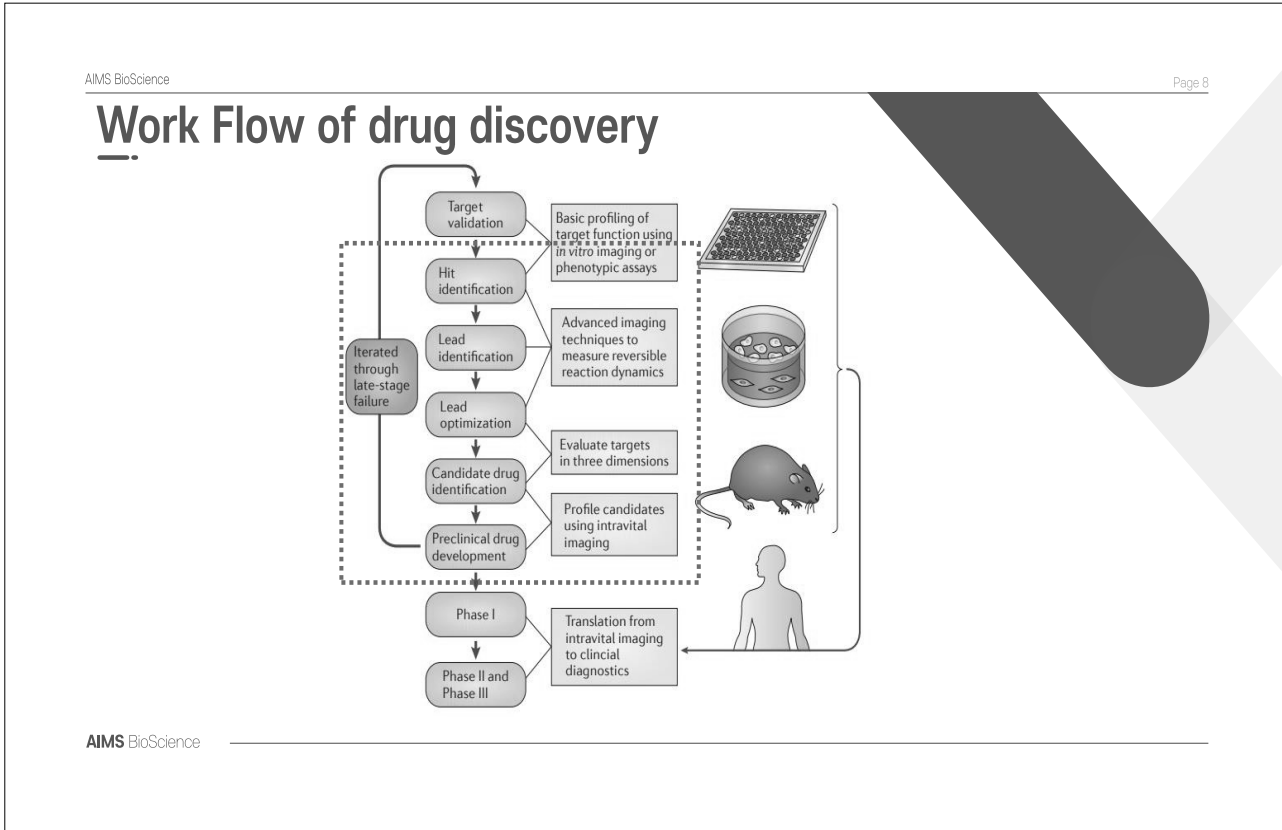
애임스바이오사이언스
개발전략팀 김정호 PM

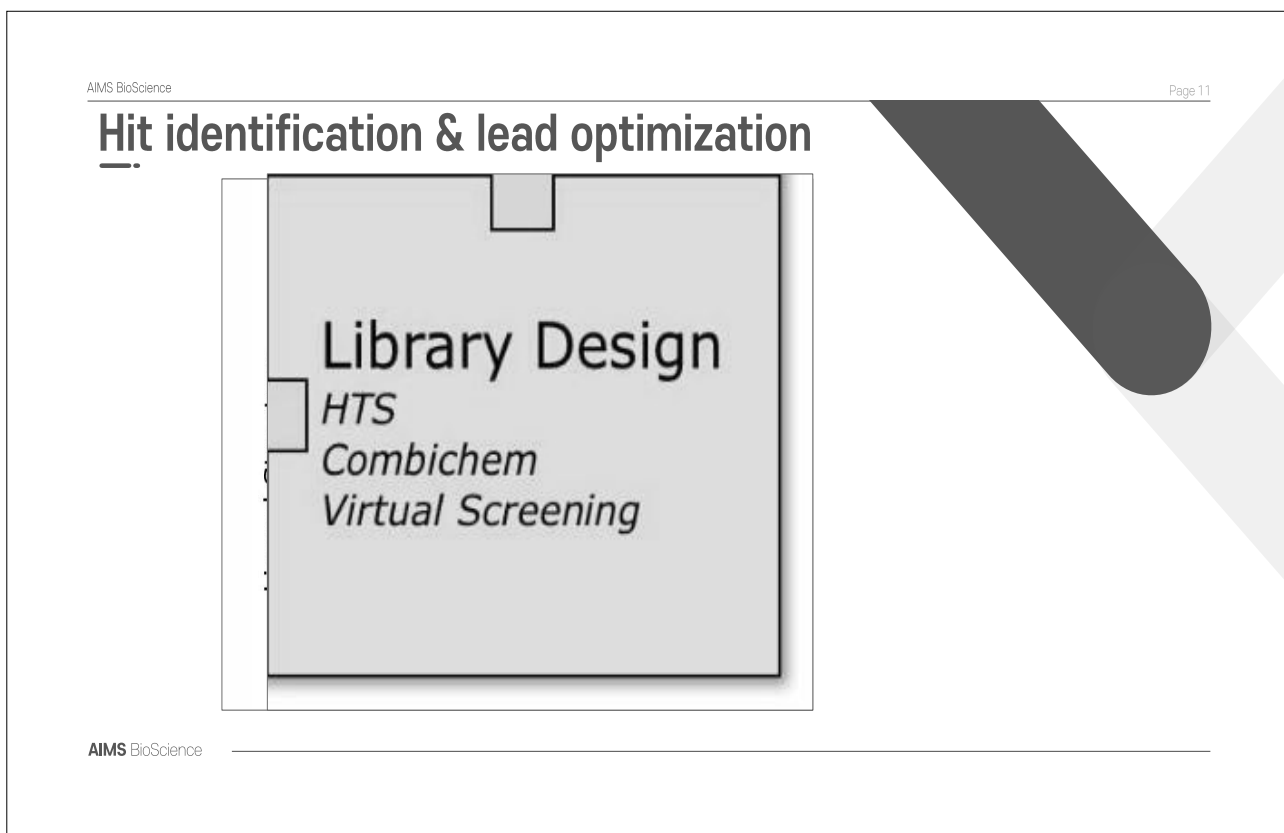
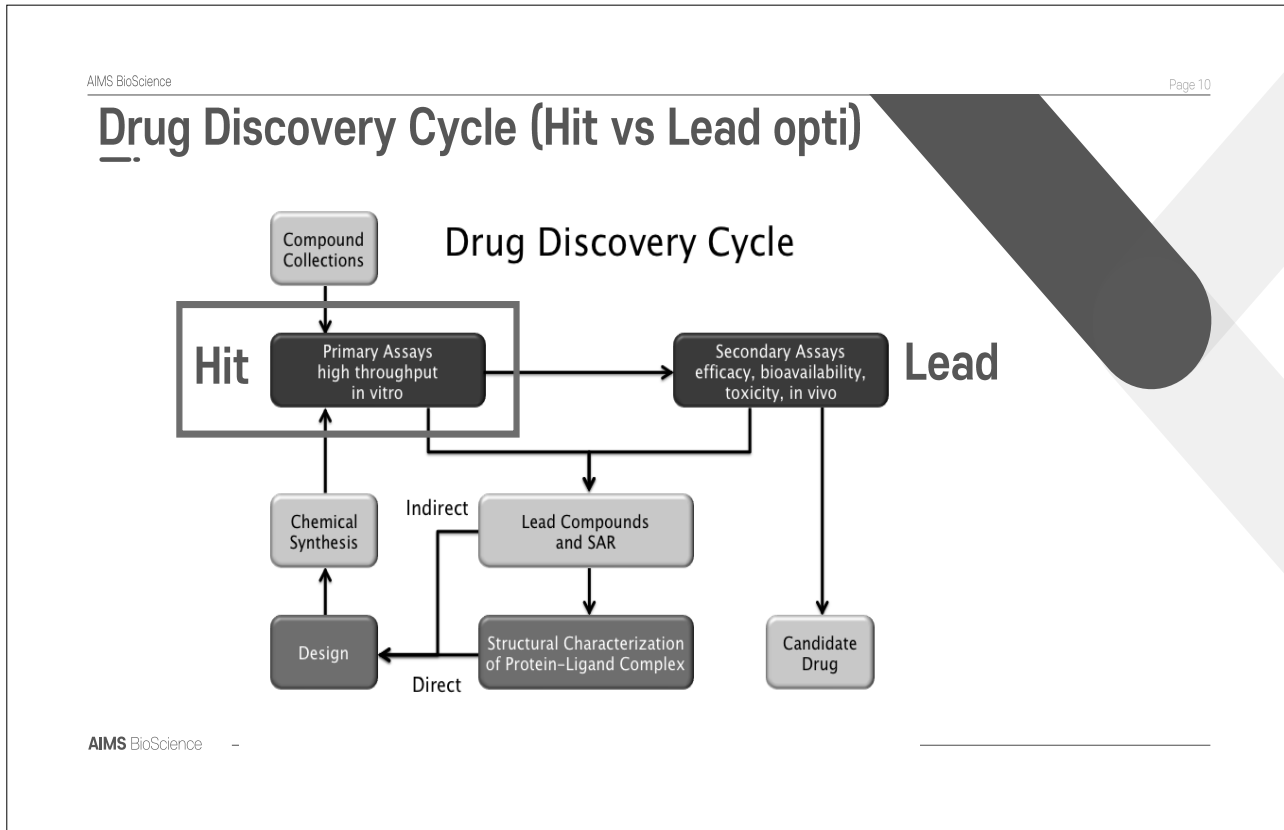












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HTS

General Screening

- Diverse
- Drug/lead/fragment-like
- 100K compounds

Targeted Library

- Knowledge based
- SBD
- Ligand based

Synthetic Targets

Design Models

- Virtual libraries
- QSAR
- Pharmacophore
- Docking
- Shape
- ADMET filtering

Search for off-the-shelf analogues

Primary Assay

potency

Secondary Assays

- IC₅₀/EC₅₀
- Selectivity
- Solubility
- Stability

Analysis of HTS Data

Actives

Lead Criteria

- potency
- selectivity
- ADMET
- patentability

Clinical Trials

Lead Molecule

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Gap of library

Johnson & Johnson **Pfizer**

Roche **abbvie**

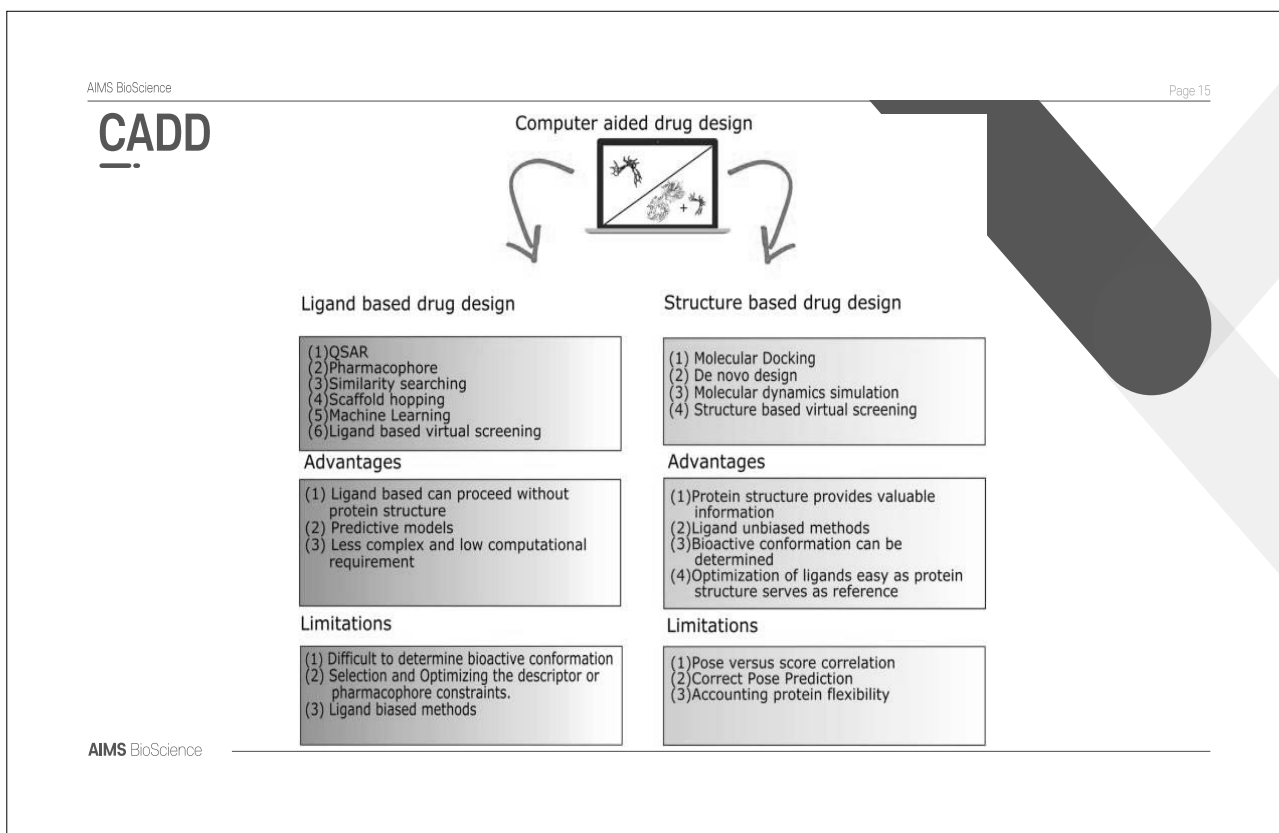
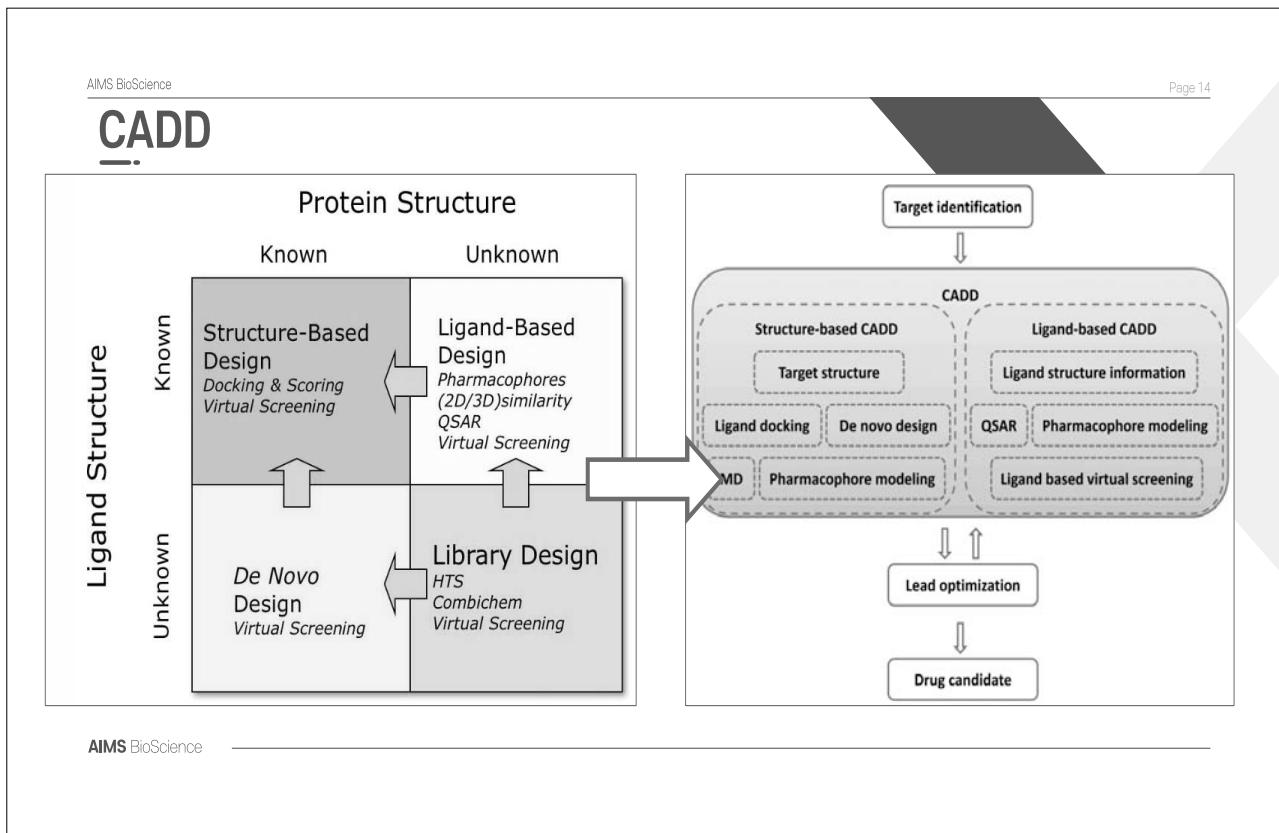
NOVARTIS **MERCK**

Bristol Myers Squibb

gsk **sanofi** **AstraZeneca**

KPBMA
한국제약바이오협회
Korea Pharmaceutical and Bio-Pharma Manufacturers Association

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SBDD (structure based drug discovery)

A Link/grow strategy

a Place fragments

b Place first fragment

Link

Grow

$K_i = 16 \mu\text{M}$

Initial state

Level 1

Level 2

...

End state

Designed molecule

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SBDD (structure based drug discovery)

Success rate (ability to find longer structures)

Initial state

End state

Y50A W60D S195 N58 W215 G216 G219 D189

3.1 Å

Y50A W60D N58 W215 G216 G219 D189

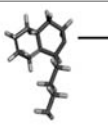
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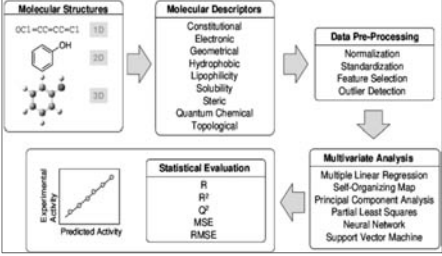
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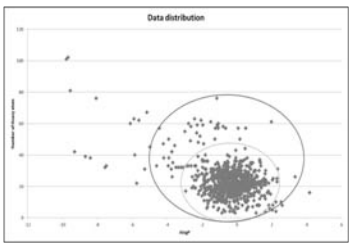
LBDD (Ligand based drug discovery)

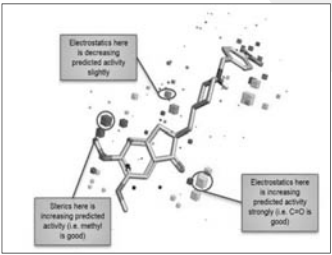


1. 원자 개수
2. 결합 개수
3. 분자량
4. HOMO
5. LUMO
6. 생성열
7. 반데르발스 표면적
8. 반데르발스 부피
- ...



No.	Structure	Calcut No. ($\log_{10} \text{IC}_{50}$)	Binding ($\log_{10} \text{IC}_{50}$)	LF	Crystal rank	1:1	Class
1		107	228	0.26	37	1726	0.36
2		76	-	0.24	416	3367	0.23
3		87	0.31	0.36	488	3867	0.24
4		77	25.1	0.25	137	3367	0.23
5		85	13.8	0.28	488	5027	0.32

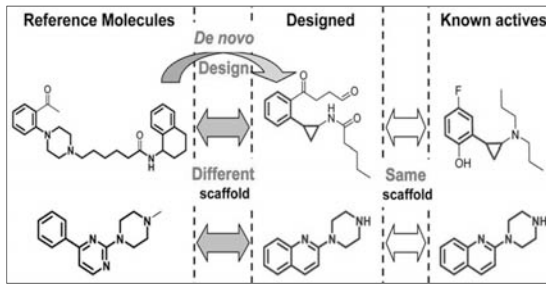


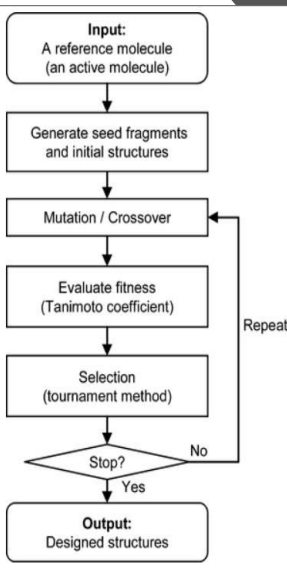


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FBDD (fragment based drug discovery)





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1. De Novo Design of Drug-Like Molecules by a Fragment-Based Molecular Evolutionary Approach
 2. Kentaro Kawai, Naoya Nagata, and Yoshimasa Takahashi
 3. Journal of Chemical Information and Modeling 2014 54 (1), 49-56
 4. DOI: 10.1021/ci400418c

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FBDD (fragment based drug discovery)

Generation of seed fragments

A reference molecule $\xrightarrow{\text{Fragmentation}}$ Seed fragments

Generation of initial structures

A seed fragment + Add a fragment from fragment library \rightarrow An initial structure

\downarrow Fragmentation

Ring	Linker	Side-chain
 <chem>[*]c1ccc(cc1)[*]</chem> <chem>[*]c1cc([*])nc([*])n1</chem> <chem>[*]c1ccccc1[*]</chem>	 <chem>[*]CNC([*])=O</chem>	 <chem>C[*]</chem> <chem>N[*]</chem> <chem>CCC[*]</chem>

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FBDD (fragment based drug discovery)

Allowed connections

a) ring-ring connections

b) ring-linker connections

c) ring-sidechain connections

Prohibited connections

d) linker-linker connections

e) sidechain-sidechain connections

f) linker-sidechain connections

Mutation

a) Add a fragment

b) Remove a fragment

c) Replace a fragment

Crossover

d) Crossover

Parent molecules \rightarrow Child molecules

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FBDD (fragment based drug discovery)

Reference molecules

- $K_d = 3.8 \text{ nM}$ (CHEMBL389718; 2)
- $K_d = 613 \text{ nM}$ (CHEMBL45158; 12)
- $K_d = 17 \text{ nM}$ (CHEMBL1161087; 15)

Designed molecules

- Tanimoto = 0.83 (8)
- Tanimoto = 0.48 (10)
- Tanimoto = 0.87 (13)
- Tanimoto = 0.77 (16)

Known active molecules

- $K_d = 6.4 \text{ nM}$ (CHEMBL158850; 9)
- $K_d = 99 \text{ nM}$ (CHEMBL539497; 11)
- $K_d = 230 \text{ nM}$ (CHEMBL18772; 14)
- $\log K_d = 8.43$ (CHEMBL26525; 17)

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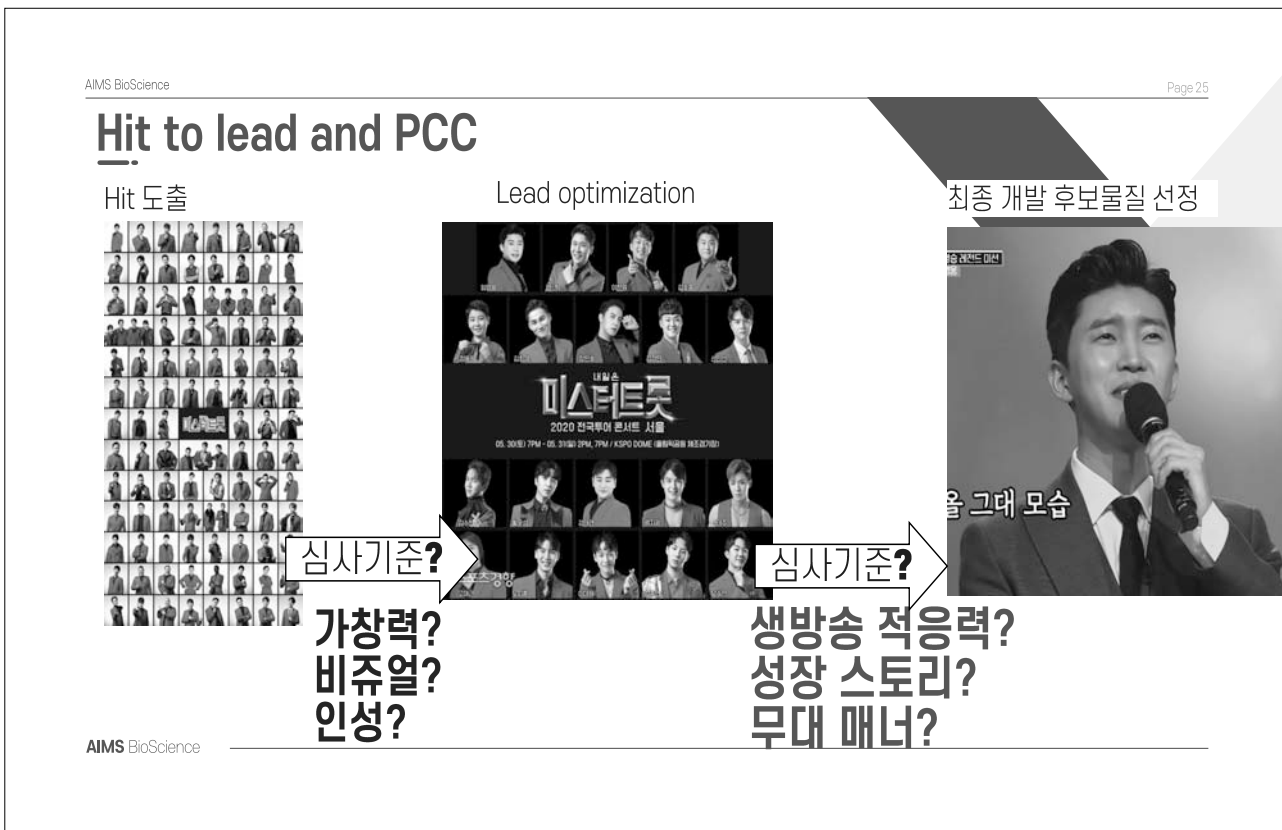
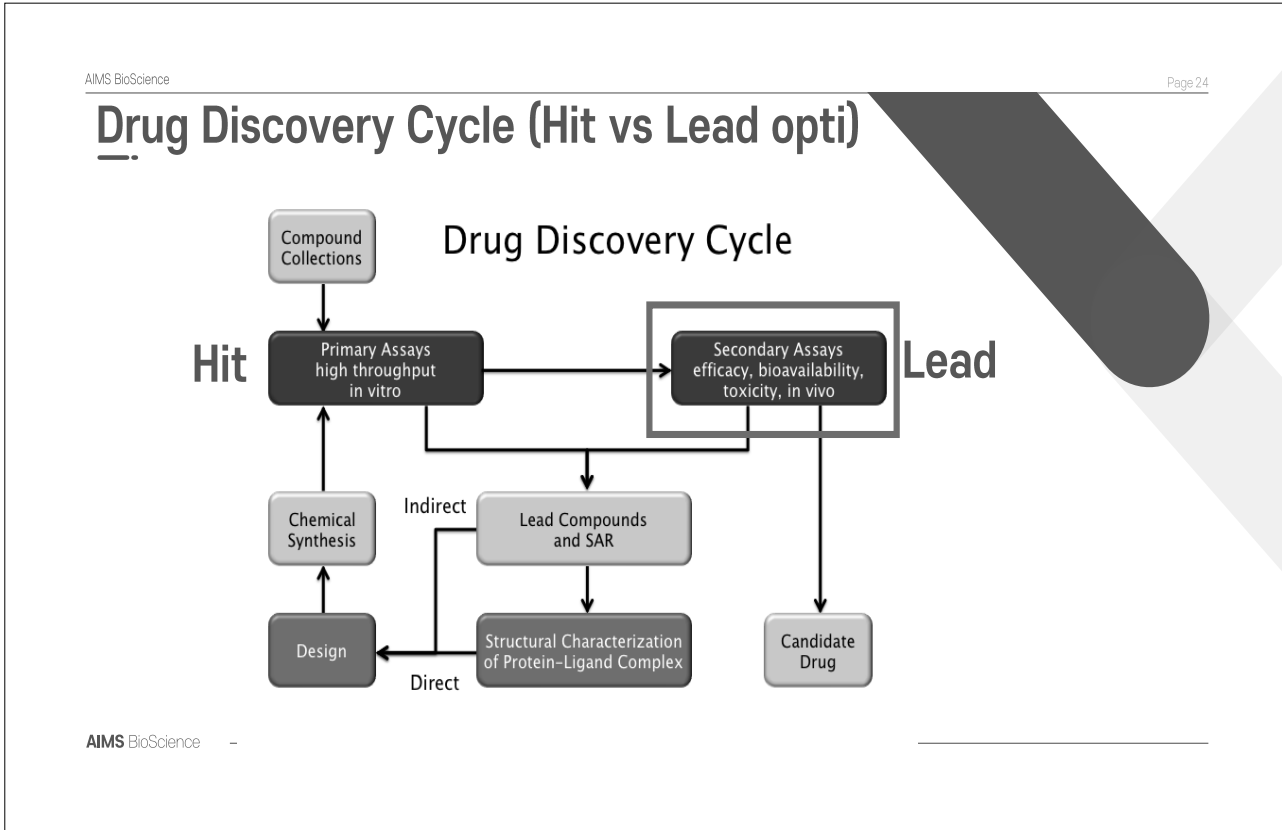
Work Flow of drug discovery

진행 단계	S1 (Hit identification)	S2 (Lead generation)	S3 (PCC selection)	S4 (PCC development)
물질 명칭	Hit candidate	Lead candidate	PCC candidate	PCC
Committee 명칭		G1 (Decision Gate)	G2 (Decision Gate)	G3 (Decision Gate)
		Hit	Lead	PCC
				G4 (Decision Gate)
				IND

- S: Stage
- G: Decision Gate

- **HIT**: in vitro 수준에서 활성이 확인된 화합물로 모핵 위주의 물질
- **Lead**: in vivo PD 모델 및 그에 상응하는 수준에서 효능이 확인된 화합물로, SAR을 통해 물질 구조에 따른 in vitro 활성 확인한 IP 확보 가능한 물질
- **PCC**: in vivo 효능 모델수준에서 효능이 확인된 화합물로, 약리학적 약물 개발 가능성 및 독성 확인 물질
- **IND**: 시장 상황을 감안한 약물 개발 가치가 입증된 화합물로, 독성학인이 완료된 물질




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심사기준 = Target product profile

Characteristic	Comparator				Candidate
	A	B	C	D	
Indication					
Active ingredient					
MoA					
Efficacy					
Dosage/ Administration					
Adverse reactions					

Target product profile – Ex 위산분비 억제제

Characteristic	Comparator			Candidate
				
Indication	GERD NERD	GERD NERD	GERD NERD	GERD NERD
Active ingredient	Famotidine	Esomeprazole	Anti acid	-
MoA	H2 blocker	PPI	Anti acid	P-CAB
Dosage/ Administration	1일 2회 식후	1일 1회 식전	1일 3포 식간	1일 1회
Weakness	짧은 지속시간 Anti-androgen effect DDI 가능성	낮은 on-set time 식전 복용 필요 DDI 가능성	짧은 지속시간	-
Strength	저렴함 높은 복용순응도	오랜 지속시간 강력한 효과	빠른 on-set time	오랜 지속시간 빠른 on-set time 낮은 DDI

- 긴 반감기를 가지는 약물 (대사안정성)
- 단회 투여로도 지속되는 효과 확인 (in vivo efficacy)
- 낮은 DDI potential (CYP inhibition study)

Target product profile - IPF

Category	****	First mover (discontinued in IPF phase 3)	Ongoing in IPF phase 1	Ongoing in IPF phase 1
		GLPG-1690 (Ziritaxestat)	BLD-0409 (PAT-409, Cudetaxestat)	BBT-877
Developer	-	Galapagos (collaboration with Gilead)	Blade therapeutics (Originator: Pharmakea)	Bridgebio (Boehringer Ingelheim 권리 반환)
Indication		IPF, systemic sclerosis (Orphan drug in USA)	IPF, systemic sclerosis (Orphan drug in USA) NASH (Preclinical)	IPF
Efficacy		-	-	-
Adverse event		Dose-dependent death (Phase 3)	-	Genotoxicity (Comet assay (+)) (Comet 추가 자료 및 전자현미경 자료 제출 요구 From US FDA)
Administration route		Oral	Oral	Oral
Dosing/Duration (phase 2)		600 mg QD, 3 month	-	100-200 mg BID (Predicted human effective dose)
Market		-	-	US

- GLPG-1690, BBT-877은 목표 적응증이 다르나, 모두 개발 중 독성 문제가 있었기 때문에 이에 대해 개선된 데이터 확보 시 개발 물질의 가치 향상이 기대됨.
- BLD-0409는 동일한 적응증을 목표로 하는 경쟁 물질이므로, 이와 비교하여 최소한 동등 이상의 효능을 보이는 것이 필요함.

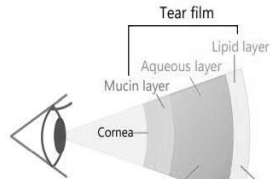
Work Flow of drug discovery



- In vitro efficacy study
- In vivo efficacy study
- ADME study
- Physical chemistry assays
- In vitro Tox study
- TPP에 부합되게, 예산과 소요시간을 고려하여 물질들을 선별할 study의 tier를 선정하여 Lead optimization된 물질들에 대한 다양한 평가 수행
- 물질 선정 시 중요도가 떨어지거나, 비용이 높고 시간이 오래걸리는 study는 최종 후보 물질 선정 후 개발시 수행

안구건조증 치료제 개발 사례

Two Major Categories of DED



ADDE (Aqueous Deficient Dry Eye)		EDE (Evaporation Dry Eye)	
SSDE Sjögren Syndrome Dry Eye	NSDE Non-Sjögren Syndrome Dry Eye	Lipid-related EDE (MGD: meibomian gland dysfunction)	Ocular surface related EDE

One-third of patients have a combination of both types

Unmet Needs and Opportunities

- Additional pharmacotherapies with novel MOAs
- Favorable tolerability profile by improving,
 - ocular pressure & epithelial cell damage
 - burning sensation & ocular discharge
- Simplified dosing regimen (<3 times a day dose)

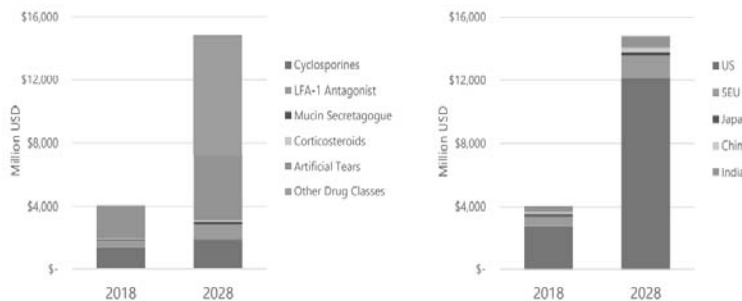


**“Novel therapy with
a preferable tolerability profile
& long-acting formulation”**

안구건조증 치료제 개발 사례

DED Market Overview

- ☑ The global DED market was valued at \$4.1B in 2018 and it is expected to reach \$14.8B in 2028 (CAGR of 13.8%)
- ☑ Artificial tears made up the biggest market share, with sales of \$2.1B (2018)
- ☑ Geographically, US accounted for 68.5% of the total market value(2018)



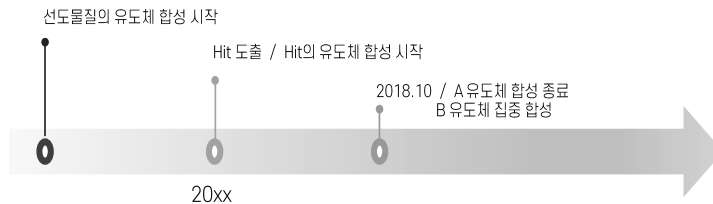
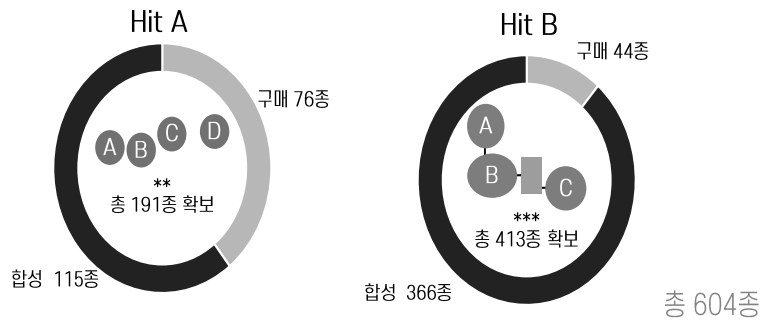
안구건조증 치료제 개발 사례

Category	Hyaluronic acid	Restasis (Allergan)	Diqas (Santen)	Xiidra (Novartis)	Tyrvaya (Oyster Point)	
Indication	Keratoconjunctival epithelial disorder	Inflammation associated with keratoconjunctivitis sicca	Keratoconjunctival epithelium disorders that accompany lacrimal fluid abnormality	Dry eye disease	Dry eye disease	Dry eye disease w/w/o inflammation
Active Ingredient	Hyaluronic acid 0.1~0.3%	Cyclosporine 0.05%	Diquafosol sodium 3%	Lifitegrast 5%	Varenicline	xxx
Dosage Form	Eye drop	Eye drop	Eye drop	Eye drop	Nasal spray	Eye drop
MoA	Lubricant	Immunomodulation increase tear production	Stimulate water & mucin secretion by acting on P2Y2 receptors	Prevent immunological response by blocking LFA-1 and ICAM-1 interaction	Stimulate trigeminal nerve to produce natural tear film	xxx
Efficacy	Sign	X	O	O	O	O
	Symptoms	O	X	X	O	O
Eyedrop dosage	5~6 times daily	2 times daily	3 times daily	2 times daily	2 times daily	QD
Adverse reaction & weakness	<ul style="list-style-type: none"> Blepharitis (0.1~5%) Preservative-associated allergic or toxic reactions 	<ul style="list-style-type: none"> Ocular burning (~17%) Poor tolerance, slow-onset of action (6 months) 	<ul style="list-style-type: none"> Eye irritation (≥5%), blurred vision, eye discharge Not approved in US 	<ul style="list-style-type: none"> Irritation, decreased visual acuity (5-25%) Withdrawn in EU with efficacy issue in 2020 	<ul style="list-style-type: none"> Sneezing (82%), cough, throat /instillation-site irritation (5-16%) 	TBD (low irritation)

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안구건조증 치료제 개발 사례



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안구건조증 치료제 개발 사례 - SAR

A 유도체 총 115종 합성

- D part benzene 에 hydrophobic group, hydrophilic group 도입한 결과 hydrophilic group이 도입 되었을 경우 현저한 활성감소를 확인.

Potency (nM)	100	220	250	>30,000
LogD	3.48	3.91	2.30	-6.81

Classification

8종 A $EC_{50} \le 1000nM$
3종 $EC_{50} \le A$

1 uM Inactive ($EC_{50} \mu M$)

Potency optimization

- Aromatic ring에 다양한 R group 도입
- Salit formation → 용해도 증가

Potency (nM)	18	4.3	5.3
Solubility (nM)	<212	ND	>1961

Property optimization

- Cycloalkane 도입 → 용해도 개선
- Bridged cycloalkane 도입 → 활성 개선

Solubility (nM)	300	150
	>470000	383005

B 유도체 366종 합성

98종	100nM $EC_{50} \le 1000nM$
90종	$EC_{50} \le 100nM$
39종	$EC_{50} \le BC-3$

Classification

139종 / 227종

Solubility (uM) vs Potency ($EC_{50} \mu M$)

Bridged Cycloalkane, Benzene, Pyridine

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안구건조증 치료제 개발 사례 - efficacy

xxx assay

- CHO-K1 cells: xxx xxx 단백질 발현 stable cell line
- Test compound를 10분간 처리 시 알린 yyy를 통해 요오드 이온이 세포 내로 이동하여 xxx 형광을 감소시키는지 여부를 확인.
- 형광 감소가 많을수록 test compound의 활성이 뛰어나음을 의미함.

xxx assay 재실험 물질 결정

총 691개 data

Data quality 이상 없음 363개 328개 Data 재검토 필요 (<math>EC_{50} < 1 \mu M</math> 280여종)

23종 305종

32nM 활성 / 재실험 필요 A 모핵 or 활성 X / 재실험 불필요

xxx assay data review

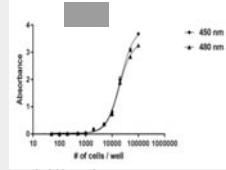
- xxx raw value : Forskolin 20uM (xxx activation 시키는 농도) 에 의한 yyy의 활성을 100%로 했을 때 각 화합물의 환산 활성 값 ($N \ge 3$)
- Raw data 및 EC_{50} 산출 data quality 확인
- Raw data CV check 초기 시험 최대 1600%.
- 10차 이후 B모핵 EC_{50} 4~32nM로 안정 CV 30% 이하

	제 6차	제 10차	제 24차
CV value (%)	57.9	15.6	20.7
B모핵 EC_{50} Range (nM)	4~32		

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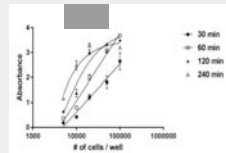
안구건조증 치료제 개발 사례 – in vitro tox

xx cell에 대한 Reagent incubation 흡광 파장대별 최적화 실험



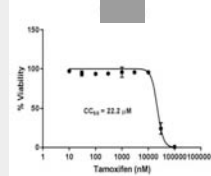
→ 최적화 조건: $A_{450} > A_{480}$

xx cell에 대한 yy 처리 시간대별 최적화 실험 (A_{450} 조건)



→ 최적화 조건: yy reagent 1 시간 처리 시 가장 우수한 linearity 보임

최적화 조건에서 독성평가법 확립을 위한 구배 대조물질 1종에 대한 세포독성을 평가함 (0.5% DMSO 조건, 약물처리 농도: 10 nM-100 μ M)



Chemical	xx CC ₅₀
	18.8 μ M (문헌치)
Tamoxifen	22.2 μ M (실정치)

→ 대조약을 평가 결과: Tamoxifen의 CC₅₀가 문헌치 (18.8 μ M) 와 유사 수준 (22.2 μ M) 으로 평가됨

아사 대조약을 1종에 대한 세포독성을 평가함

(0.5% DMSO 조건, 약물처리 농도: 10 nM-100 μ M)

Chemical	Structure	CC ₅₀ (μ M)
xxx		> 100

전체 처수	
CV value (%)	< 30%
Comments	1-30차

→ 100,000 nM 이상 안전성 확보, No toxicity until 10,000 nM

안구건조증 치료제 개발 사례 – solubility

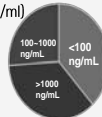
Solution : PBS 용해도

- PBS (pH 7.5) 선정 근거
 - 1) 주성분의 약리 작용을 간섭하지 않음.
 - 2) 안구 내 무자극성을 위해 눈물의 생리학적 특성 및 pH와 유사.
 - 3) 점안제에 사용되는 완충제: 인산염, 붕산염, 시트르산염, 아황산염 등

- PBS 용해도 시험

화합물 204종의 시험 결과, PBS에서 난용성을 나타낸 (target conc 10 μ g/ml)

 - 78종: <100 ng/mL
 - 53종: 100 ~ 1000 ng/mL
 - 73종: >1000 ng/mL



- 용해도 개선 방안
 - 1) Salt 추가
 - 2) Polymorphism
 - 3) 미립자화
 - 4) emulsion, nano-micelle 등 제형 (계면활성제, 용해제 추가)

제형 추가 연구

Emulsion vs. Nano-micelle

Emulsion	Nano-micelle
<ol style="list-style-type: none"> 1) Restasis® 제형 (2003, FDA approved) 2) 오일 함유 <ul style="list-style-type: none"> - 점안 시 시야 흐릿 - 점안 후 눈물 다 - 복약 순응도 ↓ 3) 큰 droplet size (<200 nm) <ul style="list-style-type: none"> - 평균 필터 사용 불가능 → 전 공정 멸균 시설 제조. 	<ol style="list-style-type: none"> 1) Cequa® 제형 (2018, FDA approved) 2) 오일 미함유 <ul style="list-style-type: none"> - Emulsion 제형의 부작용 경감 (Restasis의 제형으로 인한 단점 극복 위해 출시) - 복약 순응도 ↑ 3) 작은 droplet size (<100 nm)

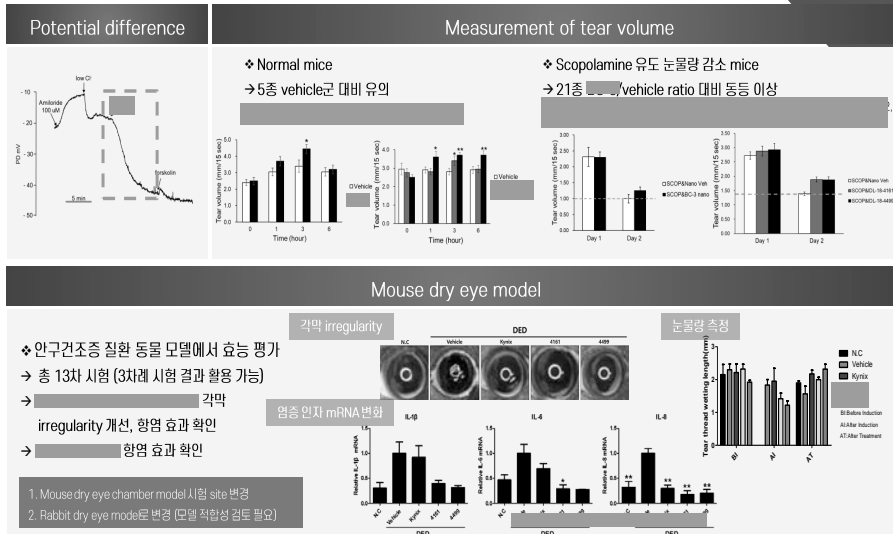
Nano-micelle 용해도

- Nano-micelle 용해도 시험 결과

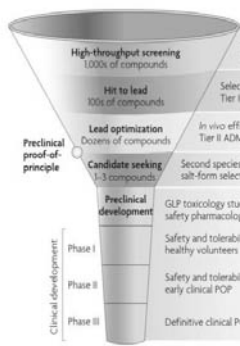
평가된 시험 물질 30종 중, 29종의 용해도는 PBS 대비 2.4 - 6880배 증가됨.

Compound	Solubility (μ g/mL)		Solubility ratio (Nano-micelle/PBS)
	PBS	Nano-micelle	
BC-3	0.0247	18	728.7
OCA-3	0.034	74	2176.5

안구건조증 치료제 개발 사례 – efficacy



안구건조증 치료제 개발 사례 – 정리



	기준	기준만족/평가된 물질
활성	yyy pot ≤ 0.1 μM	150/624
	0.1 μM ≤ yyy pot ≤ 1 μM	130/624
	0.1 μM ≤ xxx pot ≤ 1 μM	34/90
세포독성	100 μM < CC ₅₀ HeLa cell	257/342
용해도 평가	PBS 1000 ng/ml 이상	73/204
	PBS 100~1000 ng/ml	53/204
in vivo 효능	Nanomicelle	30
	정상 동물평가	5/17
	Scopolamine 동물평가	21/50
	건성안 질환동물 평가	3/12

안구건조증 치료제 개발 사례 – Lead to PCC

	IDL code	MW	yyy EC ₅₀ (μM)	Nanomicelle solubility (μM)	Nanomicelle sol /PBS sol	CC50 (μM)	Scopolamine mice (/vehicle)	Dry eye mouse model
			0.008	43.0	728.7	100	1.25	0
1			0.09	5209.1	1886.0	100	2.1	
2			0.18	1024.0	734.9	30	1.95, 1.83	
3			0.012	170.0	6880.0	30	1.58	
4			0.033	33.0	1478.7	95.7	1.53	
5			0.049	18.3	85.6	100	1.48	
6			0.0071	3044.0	1478.2	100	1.43	
7			0.16	1600.0	789.4	100	1.43	
8			1.1	165.0	0.2	100	1.41	0
9			0.034	1877.0	29.2	100	1.58, 1.22	
10			0.13	168.0	6400.0	100	1.4	
11			0.067	4144.2	7410.3	100	1.4	
12			0.13	10137.0	28.3	100	1.38	
13			0.087	1091.0	22.2	100	1.36	0
14			0.057	664.0	3.0	100	1.35	
15			0.2	8165.0	9.5	47.4	1.35	
16			0.063	28.5	12.3	100	1.35	
17			0.053	1961.0	1000.0	100	1.35	
18			0.045	145.8	656.6	100	1.34	
19			0.065	400.0	21860.0	100	1.32	
20			0.015	165.0	103.1	44.8	1.29	
21			0.02	1807.0	828.2	30	1.29	

안구건조증 치료제 개발 사례 – 후보물질선정

	A	B	C
<i>in vitro</i> yyy EC ₅₀ (μM)	0.008	0.087	1.1
<i>in vitro</i> xxx EC ₅₀ (μM)	0.044	4.8	3.3
Solubility, PBS (μM)	0.059	53.60	702.6
Solubility, Nanomicelle (μM)	43.02	1,091	165
세포독성 CC ₅₀ (μM)	100	100	100
Scopolamine 눈물량 감소 모델 (vehicle 대비 눈물 증가비율)	1.25	1.36	1.41
Dry eye 모델 평가	0	0	0