국립암센터 암과학포럼

항암신약개발 A-Z

Part 4. mRNA vaccine: from COVID-19 to cancer

날짜 : 2022. 5. 27.(금)

시간 : 13:00 ~ 17:05

국립암센터
NATIONAL CANCER CENTER

국립암센터 암과학포럼

항암신약개발 A-Z

Part 4. mRNA vaccine: from COVID-19 to cancer

2022. 5. 27.(금) 13:00 ~ 17:05

장소 | 경기도 고양시 국립암센터 검진동 8층 국제회의장
대상 | 의료인, 의과학자, 제약 및 바이오 기업 등 관련 종사자
참여방법 | 온라인 강연 ZOOM (사전등록 필수), 오프라인(국립암센터 내부 직원 대상)
등록비 | 무료

사전등록 바로가기

* 강의 일정은 운영상 변경될 수 있습니다.

13:00~13:10	**개회 및 축사**	**개회사** | **김영우** 국립암센터 연구소장 **축 사** | **서홍관** 국립암센터 원장
13:10~15:40	**1부 | mRNA 백신 개발 어디까지 왔나?**	**좌 장** | **백순명** (주)테라젠바이오 연구소장
13:10~13:50	mRNA COVID-19 vaccine	• **김혜영** | 한국화이자(Pfizer) 상무
13:50~14:30	Integrated mRNA CDMO service: learning from COVID-19 mRNA vaccine development	• **양주성** | 에스티팜㈜(ST Pharm) 상무 mRNA사업개발실장 / 바이오텍연구소장
14:30~15:10	Development of RNA therapeutics and lipid nanoparticle (LNP) formulation for in vivo delivery	• **이혁진** | 이화여자대학교 약학대학 교수
15:10~15:40	Clinical studies for mRNA cancer vaccine	• **김학균** | 국립암센터 항암신약신치료기술개발사업단 부단장
15:40~16:00	**휴식**	
16:00~17:00	**2부 | 패널토론 : 우리나라에서 mRNA 암백신을 어떻게 임상개발할 것인가?**	**좌 장** | **김영우** 국립암센터 연구소장
	• **강태진** | ㈜레나임(㈜Renhaim Therapeutics) 대표이사 [겸직]아이진(㈜EYEGENE Inc.) 임상팀장 • **백순명** | ㈜테라젠바이오(Theragen Bio) 연구소장 • **양주성** | 에스티팜㈜(ST Pharm) 상무 mRNA사업개발실장 / 바이오텍연구소장	• **오일웅** | 식품의약품안전평가원 바이오생약심사부 세포유전자치료제과장 • **이병희** | 과학기술정보통신부 생명기술과장 • **한상균** | 보건복지부 질병정책과장
17:00~17:05	**폐회**	**폐회사** | **김영우** 국립암센터 연구소장

- 의사연수평점 : 3평점(수강시간에 따른 평점 인정, 온라인 강의 입·퇴실 시간으로 출석체크)
- 온라인 강의 접속링크 및 참여방법은 사전등록 시 작성하신 메일 주소 및 연락처로 안내드릴 예정입니다.
- 신청방법 : 우측 링크 혹은 QR코드를 통한 신청 접수 (접수마감: 5월25일) https://ncc.re.kr/nsymposium_list.ncc

주관 | 국립암센터 **문의 및 안내** | 국립암센터 인재개발팀 **TEL** | 031-920-0037 **E-MAIL** | chungga96@ncc.re.kr
*기타 자세한 사항은 홈페이지(https://ncc.re.kr/)를 참고하시거나, 인재개발팀으로 문의하시기 바랍니다.

국립암센터 NATIONAL CANCER CENTER

국립암센터 암과학포럼 프로그램

○ 일　　시: 2022년 5월 27일(금) 13:00~17:05

○ 주　　제: 항암신약개발 A-Z, Part 4. mRNA vaccine; from COVID-19 to cancer

○ 장　　소: 국립암센터 검진동 8층 국제회의장

시 간		주 제	발 표 자	
13:00~13:05	5′	개 회	김영우	국립암센터 연구소장
13:05~13:10	5′	축 사	서홍관	국립암센터 원장
1부: mRNA 백신 개발 어디까지 왔나?			좌장: 백순명 (㈜테라젠바이오 연구소장)	
13:10~13:50	30′	mRNA COVID-19 vaccine	김혜영	한국화이자(Pfizer) 상무
	10′	질의응답		
13:50~14:30	30′	Integrated mRNA CDMO service: learning from COVID-19 mRNA vaccine development	양주성	에스티팜(㈜) (ST Pharm) 상무 mRNA사업개발실장 /바이오텍연구소장
	10′	질의응답		
14:30~15:10	30′	Development of RNA therapeutics and lipid nanoparticle (LNP) formulation for in vivo delivery	이혁진	이화여자대학교 약학대학 교수
	10′	질의응답		
15:10~15:40	20′	Clinical studies for mRNA cancer vaccine	김학균	국립암센터 항암신약신치료기술개발사업단 부단장
	10′	질의응답		
15:40~16:00	20′	휴 식		
2부: 패널 토론　우리나라에서 mRNA 암백신을 어떻게 임상개발할 것인가?			좌장: 김영우 (국립암센터 연구소장)	
16:00~16:10	10′	패널토론	강태진	㈜레나임(㈜Renhaim Therapeutics) 대표이사 [겸직]아이진㈜ (EYEGENE Inc.) 임상개발팀장
16:10~16:20	10′	패널토론	백순명	㈜테라젠바이오(Theragen Bio) 연구소장
16:20~16:30	10′	패널토론	양주성	에스티팜(㈜) (ST Pharm) 상무 mRNA사업개발실장 /바이오텍연구소장
16:30~16:40	10′	패널토론	오일웅	식품의약품안전평가원 바이오생약심사부 세포유전자치료제과장
16:40~16:50	10′	패널토론	이병희	과학기술정보통신부 생명기술과장
16:50~17:00	10′	패널토론	한상균	보건복지부 질병정책과장
17:00~17:05	5′	폐 회	김영우	국립암센터 연구소장

mRNA COVID-19 vaccine

한국화이자(Pfizer) 상무 / 김 혜 영

[붙임] 2022년도 암과학포럼 연자 프로필 및 강의 초록

성 명	김혜영	메일 주소	Hye-young.kim@pfizer.com bauz@naver.com
소 속	한국화이자제약	직위/직함	상무/Vaccines Medical Lead
주요 경력	내 용		
	⇦ 학력 • 2000.03-2006.02 학사, 서울대학교 의과대학 • 2008.03-2010.08 석사, 서울대학교 의과대학원 의학과 • 2011.03-2013.02 박사, 서울대학교 의과대학원 의학과 ⇦ 경력 • 2012.03-2013.09 서울대학교병원 마취통증의학과 진료교수 • 2013.09-2014.11 먼디파마 의학부 부장 • 2014.11-2019.09 화이자제약 백신의학부 이사 • 2019.09-현 재 화이자제약 백신의학부 총괄		
연구업적	• 주요/최근 연구논문 또는 저서 • Kim HY, Park SB, Kang ES, Lee SM, Kim HJ, Wasserman M. Cost-effectiveness of a national immunization program with the 13-valent pneumococcal conjugate vaccine compared with the 10-valent pneumococcal conjugate vaccine in South Korea, Human Vaccines & Immunotherapeutics 2021; 17(3): 909-918.		
5월 27일 포 럼 발표주제	mRNA COVID-19 vaccine		
발표내용	Pandemic 상황에서 화이자-BioNTech의 mRNA COVID-19 vaccine의 개발 과정(platform, 임상, 허가 등)및 RWE, 변이 대응 백신 개발 등 향후 계획 등을 간략하게 소개하는 내용		

Integrated mRNA CDMO service: learning from COVID-19 mRNA vaccine development

에스티팜(주) (ST Pharm) 상무
mRNA사업개발실장 / 바이오텍연구소장
양 주 성

[붙임] 2022년도 암과학포럼 연자 프로필 및 강의 초록

<table>
<tr><td>성 명</td><td>양주성</td><td>메일 주소</td><td>joosung.yang@stpharm.co.kr</td></tr>
<tr><td>소 속</td><td>에스티팜(주)</td><td>직위/직함</td><td>상무/연구소장</td></tr>
<tr><td>주요 경력</td><td colspan="3">내 용

○ 학력
• 1981.03-1985.02 학사, 서강대학교 생명과학과
• 1990.09-1992.08 석사수료, North Carolina St. Univ, 미생물학과
• 1992.09-1996.05 박사, North Carolina St. Univ, 미생물학과

○ 경력
• 1996.07-2002.02 Univ. of Pennsylvania 박사후연구원
• 2002.03-2011.02 성균관대학교 생명공학부 교수
• 2011.03-2016.12 비이오니아 연구소장
• 2017.01-2020.10 ㈜플럼라인생명과학, ㈜케어사이드 연구소장
• 2020.11-현 재 에스티팜(주) mRNA사업개발실장, 바이오텍연구소장</td></tr>
<tr><td>연구 업적</td><td colspan="3">○ 주요/최근 특허
• 뎅기 바이러스 특이적 siRNA, 그러한 siRNA를 포함하는 이중나선 올리고 RNA 구조체 및 이를 포함하는 뎅기 바이러스 증식 억제용 조성물 (2014.07.04. 출원/ 대한민국, 미국, 호주 등록)
• 핵산 전달용 지질 나노입자 및 조성물 (2021.03.11. 출원)
• 생분해성 결합을 포함하는 이온화 지질 및 이를 포함하는 지질나노입자 (2022.01.17. 출원)
• 신규 핵산 분자 (2022.02.07. 국내우선권주장출원, PCT국제출원)</td></tr>
<tr><td>5월 27일 포 럼 발표주제</td><td colspan="3">Integrated mRNA CDMO service: learning from COVID-19 mRNA vaccine development</td></tr>
<tr><td>발표내용</td><td colspan="3">Monomer, oligonucleotide CDMO 사업으로부터 확보한 합성 경험과 CMC, RA 역량, 풍부한 GMP 생산역량을 기반으로 5‘ cap 유사체인 SMARTCAP® 을 자체개발하였으며, 안전성과 유효성이 검증된 지질나노입자(LNP)를 기술이전받아 국내 최초 mRNA GMP 제조소에서 생산된 COVID-19 mRNA 백신의 임상시험 승인을 받았음. mRNA 백신의 원료물질 생산부터 IND 승인에 이르는 일련의 경험을 기반으로 국내외 고객사를 대상으로 mRNA CDMO사업 추진 내용에 대해서 소개함.</td></tr>
</table>

ST PHARM

국립암센터 NATIONAL CANCER CENTER

Integrated mRNA CDMO service : learning from COVID-19 mRNA Vaccine Development

May 27, 2022

Joo-Sung Yang, Ph.D.

VP. Head of mRNA R&BD / Head of Biotechnology R&D

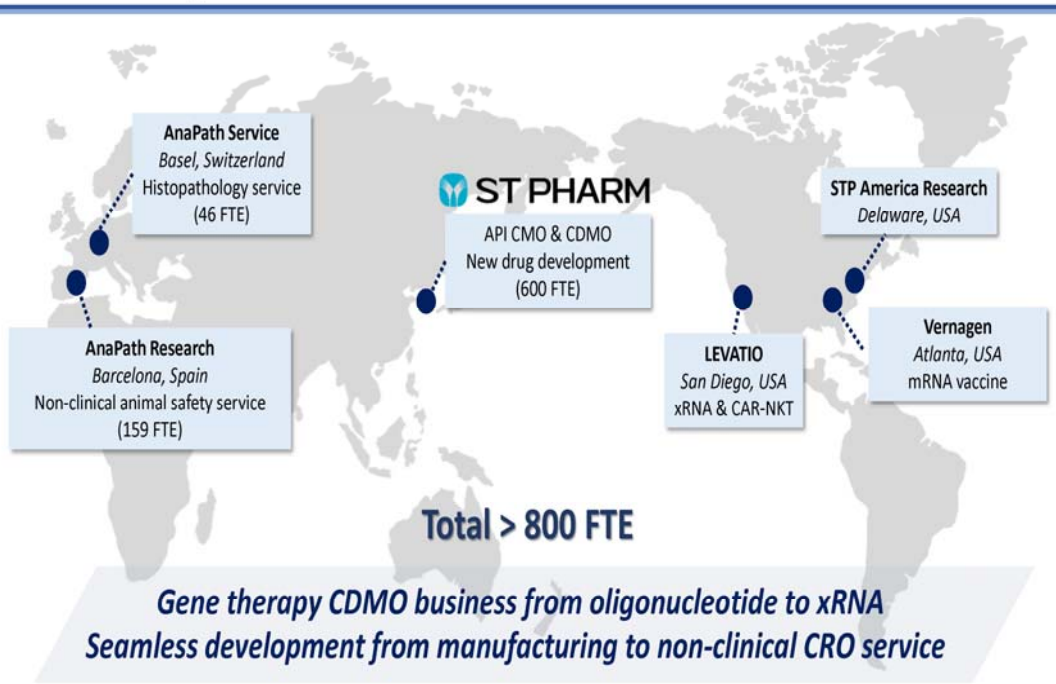
Disclaimer

This presentation is information purpose only and does not replace independent professional judgment. It contains forward-looking statements that are based on the current expectations and beliefs of ST Pharm Co. Ltd. All statements, other than statements of historical fact, are statements that could be deemed forward-looking statements. These statements relate to future events and involve known and unknown risks, uncertainties and other factors which may cause our actual results, performance or achievements to be different from any future performances or achievements expressed or implied by the forward-looking statements. Each of these statements is based only on current data, information, results, assumptions and expectations that may subject to change.

Forward-looking statements can be identified by the use of terminology such as 'intend', 'aim', 'project', 'anticipate', 'estimate', 'plan', 'believe', 'expect', 'may', 'should', 'will', 'continue', 'annualised' or similar words. These statements discuss future expectations concerning the results of operations or financial condition, or provide other forward-looking statements.

These forward-looking statements are not guarantees or predictions of future performance, and involve known and unknown risks, uncertainties and other factors, many of which are beyond our control, and which may cause actual results to differ materially from those expressed in the statements contained in this presentation. Attendees are cautioned not to put undue reliance on forward-looking statements.

ST PHARM Family



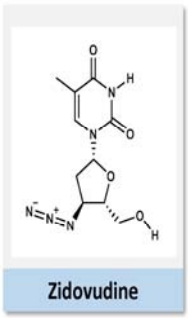
Total > 800 FTE

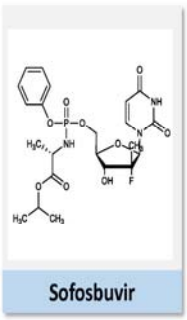
Gene therapy CDMO business from oligonucleotide to xRNA
Seamless development from manufacturing to non-clinical CRO service

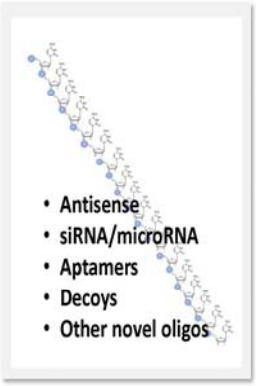
Journey from monomer to polynucleotide CDMO business

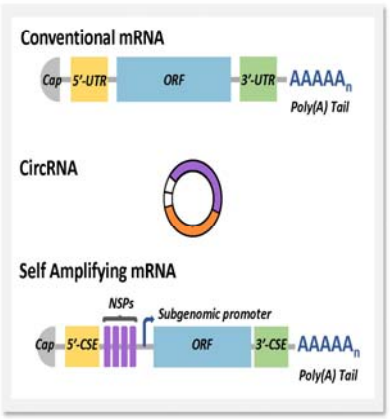
- *1983* Samchully Pharm
- *2008* Oligo GMP Manufacturing
- *2018* mRNA R&D

Nucleoside Nucleotide → *Oligonucleotide* → *Polynucleotide*


Zidovudine


Sofosbuvir


- Antisense
- siRNA/microRNA
- Aptamers
- Decoys
- Other novel oligos

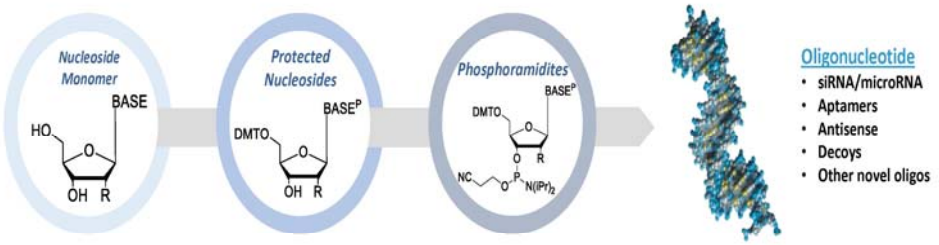


Integrated oligonucleotide CDMO services

Fully Integrated In-House Production of Monomer and Amidite Manufacture Capability
"One-Stop Service" from Nucleosides to Oligonucleotides

- **Innovative technology & manufacturing system**
  - ✓ Oligonucleotide GMP manufacturing with *very low endotoxin limit*
  - ✓ *Minimizing PO impurity formation* in the PO/PS mixed backbone oligonucleotides
  - ✓ Oligonucleotide purification process with *on-column detritylation*
- **Strong Track Record**
  - ✓ Selected as *Asia-Pacific's Top Oligonucleotide API Manufacturer*
  - ✓ World's third largest oligonucleotide manufacturing capacity
  - ✓ *Roche CDMO Award 2019:* Oligo New Drug (First in global), Small Molecule New Drug (First in Asia)

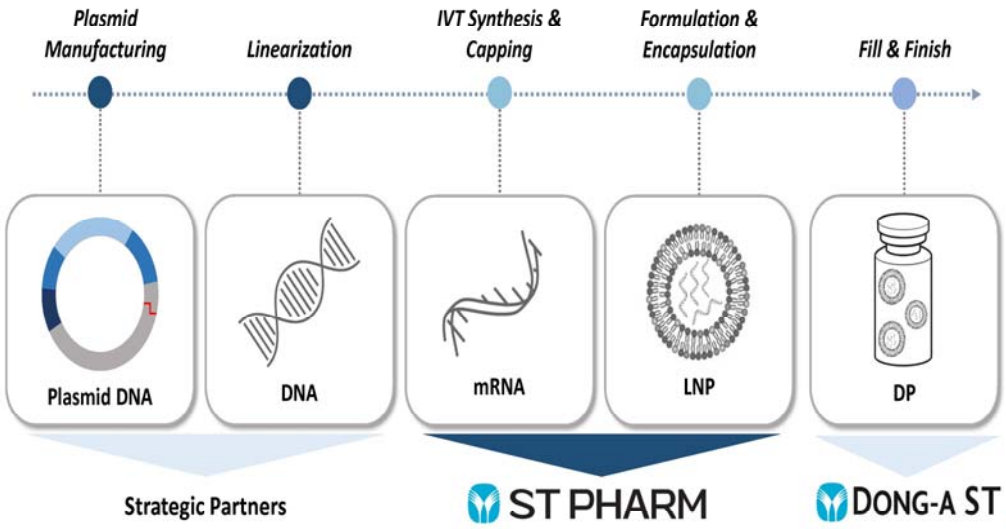
- ✓ Cost competitive
- ✓ Supply continuity
- ✓ Quality stability
- ✓ Expedition of process



Oligonucleotide
- siRNA/microRNA
- Aptamers
- Antisense
- Decoys
- Other novel oligos

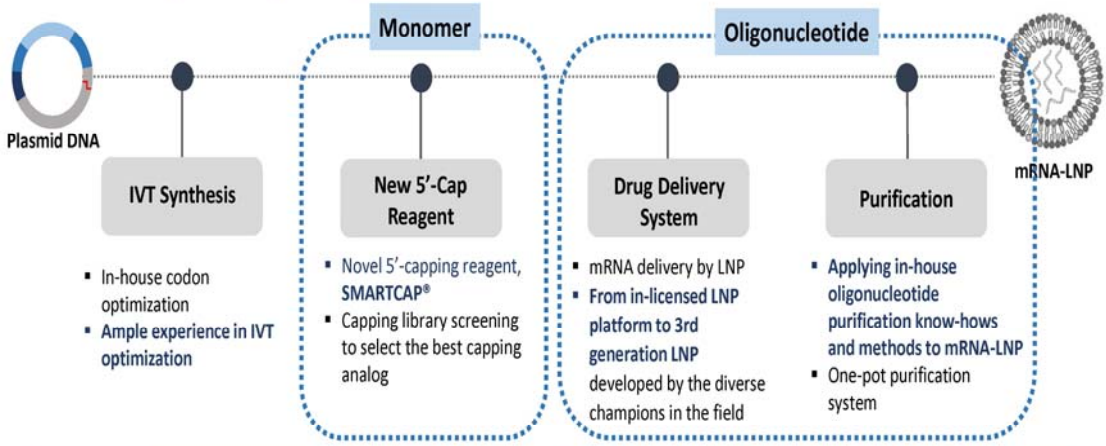
ST PHARM's mRNA CDMO workflow

- Platform technology from *in vitro* transcription (IVT) mRNA to lipid nanoparticle (LNP) encapsulation
- In-house 5'-capping reagents and LNPs in strong IP position



ST PHARM's mRNA platform technology

- **mRNA and Lipid Nanoparticle (LNP) Platform at ST PHARM**



CMC & Regulatory	▪ Analytical and biophysical characterization & analytical method development ▪ Regulatory SOP & guideline preparation
GMP Manufacturing	▪ Strong track record of nucleotide/oligonucleotide GMP manufacturer ▪ All steps controlled under GMP conditions

SMARTCAP® and Capping Library

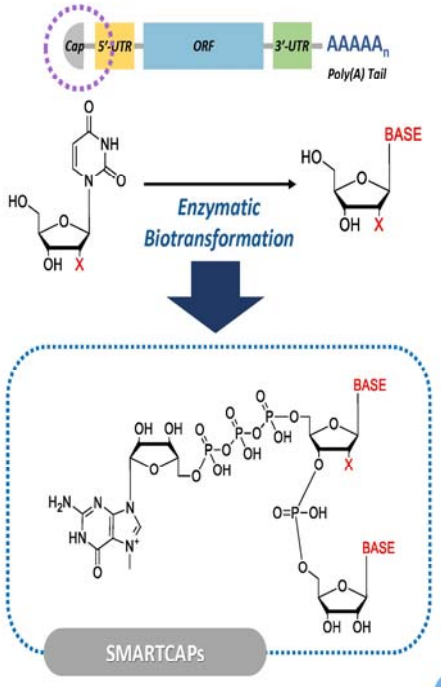
From Nucleoside to 5'-Capping Reagent

SMARTCAP®

- In-house 5'-capping analogs (ca. 30) with different ribose and base combination
- Utilizing the know-hows & experience from oligonucleotide RSM synthesis
- Strong IP position
- **Updating stability data**
  → Both powder and solution form are stable at room temperature (> 6 months)

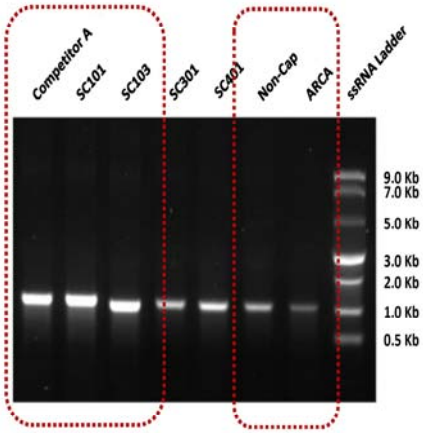
Capping Library

- Screening capping library to identify the most suitable 5'-cap analog with highest efficiency
- ORF-specific screening and selection

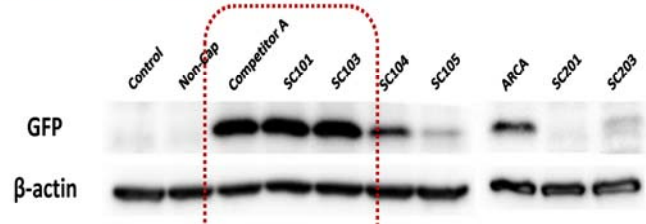


Western blot analysis of eGFP mRNA

- Four different SMARTCAPs compared with competitor's cap, ARCA cap and non-cap in IVT efficiency
- SC101 and SC103 have shown better results than non-cap or ARCA cap, and very comparable to competitor's cap
- Western blot analysis of eGFP mRNA with different 5'-cappings in transfected Hela cell
- Results confirmed in GFP-β-actin semi-quantitative analysis



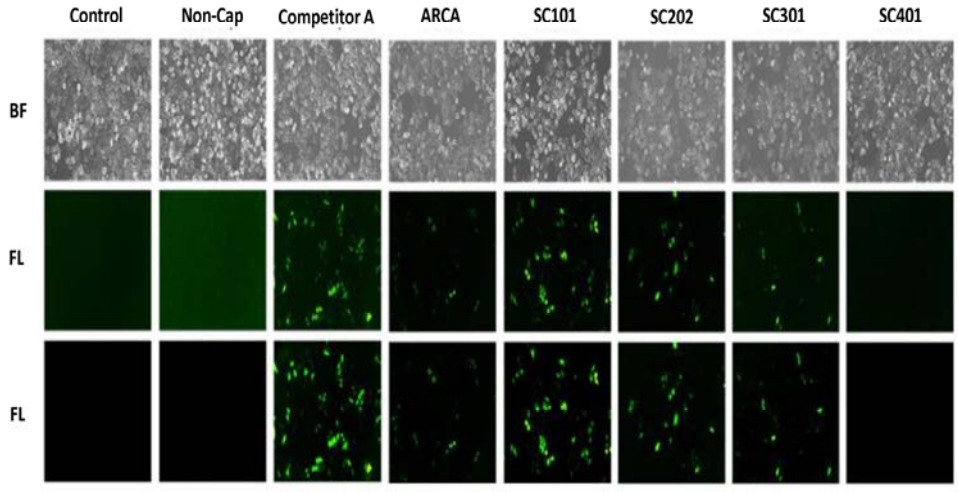
❖ GFP-β-actin Quantitative Analysis



	Con	NC	A	SC101	SC103	SC104	SC105	ARCA	SC201	SC203
Fold Change	1.0	1.11	8.66	8.67	8.30	4.51	2.00	4.09	0.95	1.83

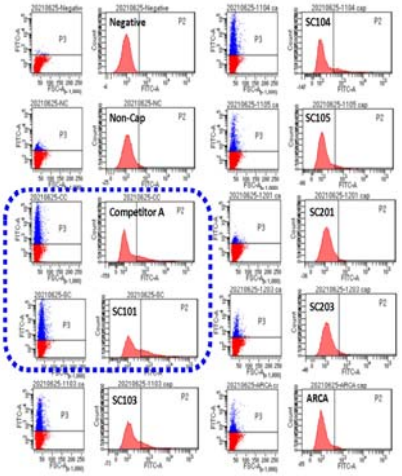
Fluorescence images of eGFP mRNA

- Fluorescence images of eGFP mRNA with different 5'-cappings in transfected Hela cells
- Compared to ARCA cap, SC101 has significant expression level with high fluorescence intensity



Flow cytometry analysis of GFP signals

- SMARTCAP® has shown significantly high GFP signal values with comparable results to competitor's cap
- SC101 has higher GFP signal value than other 5′-capping reagents

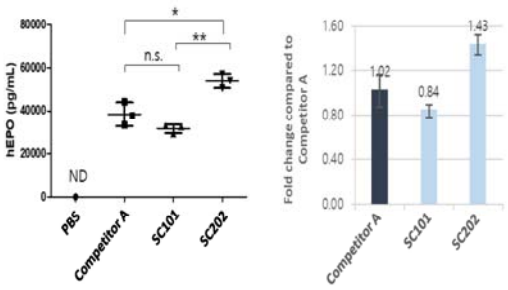


Materials	% parent-P3	Relative
Negative	0.4	1.0
Non-Cap	4.9	12.3
Competitor A	**29.9**	**74.8**
SC101	**35.6**	**89.0**
SC103	23.2	58
SC104	24.3	60.8
SC105	12.2	30.5
ARCA	**9.4**	**23.5**
SC201	4.1	10.3
SC203	8.3	20.8

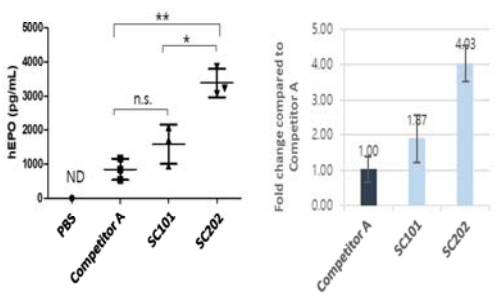
Cell-dependent transfection efficiency

- In vitro hEPO transfection efficiency was tested in two cell lines using three different 5′-capping reagents
- SMARTCAPs and competitor's cap were transfected in HEK293T and Huh7, and measured the protein expression level
- **Protein expression levels of three capping reagents were similar in HEK293T but SC202 had significantly higher expression level in Huh7**

❖ hEPO expression in HEK293T



❖ hEPO expression in Huh7



ST PHARM's Lipid Nanoparticles (LNP) – STLNP® & SMARTLNP®

From Conventional to 3rd Generation LNP

- **ST PHARM is running three different LNP platform strategies**

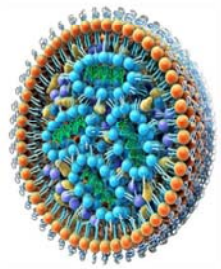


Image Source from *Precision Nanosystems*

In-licensing LNP

- In-licensing LNP technology
- Proven, unsurpassed and best LNP technology
- Applied to COVID-19 mRNA vaccine development

STLNP®

- ST PHARM's first generation LNP
- Used for mRNA CDMO business
- Further application to cancer and autoimmune disease vaccines

SMARTLNP®

- ST PHARM's second generation LNP
- Developed in collaborations with academies in KOREA
- Focused in increasing stability and improving immune response

Physicochemical properties of STLNP®

- eGFP encapsulated in STPLNP3003 shows comparable physicochemical properties with high encapsulation efficiency, compared to competitors'
- eGFP encapsulated in STLNP3003 is more stable than in lipofectamine and shows comparable stability to competitors'

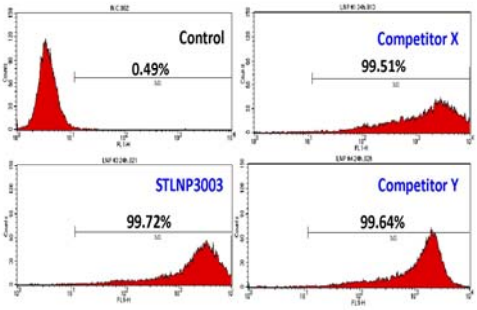
Sample	Competitor X	Competitor Y	STLNP3003
Z-Average (nm)	81.16	94.95	83.47
PDI	0.096	0.191	0.076
Intensity(%)	100	98.7	100
Encapsulation efficacy (%)	**94.8**	**95.5**	**95.3**

eGFP expression (%)	Non-transfected cell	SC-Lipofectamine	Competitor X	Competitor Y	STLNP3003
0 week	0.45	55.14	94.18	93.17	90.99
3 week, 4℃			93.82	92.96	93.57
3 week, - 20 ℃	0.52	36.69	93.87	91.91	93.32
3 week, - 80 ℃			94.97	94.18	92.98

Comparison of eGFP in mRNA-LNP transfected in 293T Cells

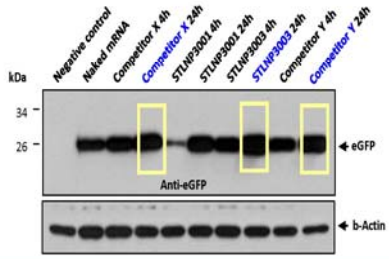
- STLNP3003 and competitors' LNP have shown similar activity and eGFP expression level in both flow cytometry and Western blot analysis

❖ Flow cytometry analysis of eGFP mRNA-LNP transfection (after 24 hrs)



Control	Competitor X		Competitor Y		STLNP3003	
	4h	24h	4h	24h	4h	24h
0.49	99.71	99.51	99.09	99.64	99.72	99.72

❖ Western blot analysis of eGFP mRNA or eGFP-LNP transfected 293T cells



Sample	Competitor X		Competitor Y		STLNP3003	
	4h	24h	4h	24h	4h	24h
Relative Expression Level	57.88	100	71.94	133.46	59.89	111.11

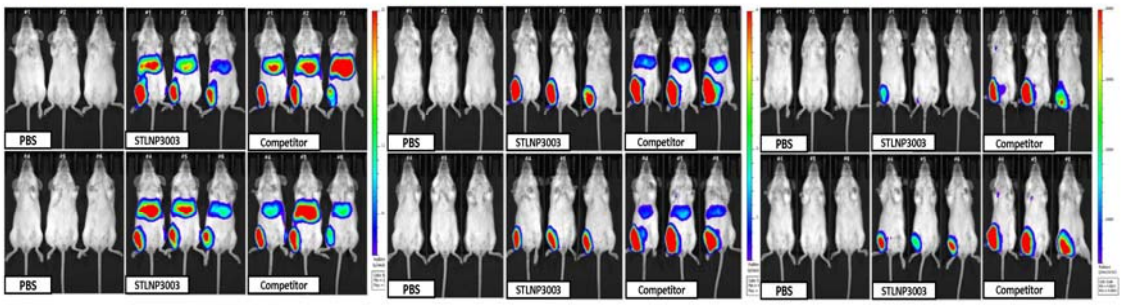
In Vivo Biodistribution of STLNP® (STLNP3003)

- FLuc mRNA with SMARTCAP® was encapsulated in STLNP3003 and competitor's LNP, and biodistribution observed up to 6 days
- STLNP3003 shows faster clearance from the liver than competitor's LNP

LNP Type	Antigen	Dose (ug)/50uL
Competitor	Luciferase	5
STLNP3003	Luciferase	5
PBS	PBS	-

	Competitor	STLNP3003
Z-Average (nm)	85.08	67.30
PDI	0.064	0.147
Intensity(%)	100.0	100.0
Encapsulation efficiency (%)	94.8	94.8

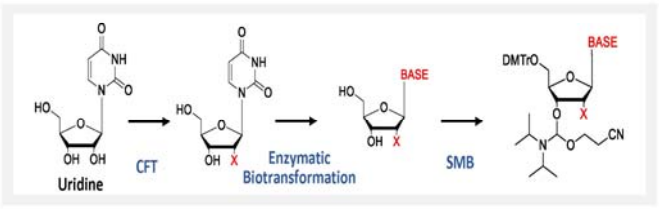
▪ After 0.5 hr ▪ After 6 hr ▪ After 72 hr

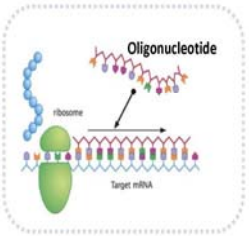


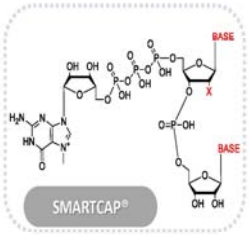
Capping reagent mass production

- Incorporating oligonucleotide CFT and SMB technology for capping *mass production*
- Mass production for diverse capping reagents including capping reagents of key companies, BioNTech-Pfizer & Moderna, and ST PHARM's SMARTCAP® from key raw materials (> multi-kilograms/year)
- *Both non-GMP and GMP-grade intermediate and product available*

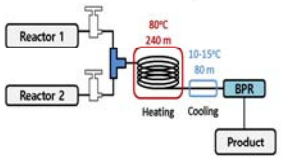
- 5'-capping reagent mass production scheme



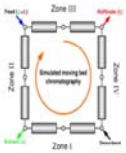




Continuous Flow Technology (CFT)



Simulated Moving Bed (SMB) Technology



LNP lipid mass production

GMP or GMP-like LNP Manufacturing Service

Raw materials from strategic partners

Tightly controlled under GMP-like condition

N-1 or N-3 GMP mass manufacturing

- Ionizable lipids
- PEG-lipids

❖ Mass production of lipids in LNP

- RMs are supplied by strategic domestic partners that are reliable, qualified and cost-effective
- ST PHARM is manufacturing both ionizable and PEG-lipid, required for LNP formulation
- Production of key lipids will be available upon client's request

		BioNTech-Pfizer		Moderna	
LNP components	Ionizable lipid	ALC-0315	>3 MT/yr*	SM-102	>3 MT/yr*
	PEG lipid	ALC-0159	>1 MT/yr*	PEG2000-DMG	>1 MT/yr*

** ST Pharm's manufacturing capacity in Sihwa campus*

ST PHARM mRNA GMP facility

mRNA synthesis from milligram to kilogram scale production

1. **R&D and small-scale production (non-GMP)**
   - mRNA Plant (3F): Established in August 2020
   - mRNA R&D and small-scale production for non-clinical animal study
2. **Mid-scale production (GMP)**
   - mRNA Plant (1F & 4F): Expansion for ST PHARM's COVID mRNA vaccine manufacturing in May 2021
   - Production Capacity: Multi-gram/month
3. **Commercial-scale production (GMP)**
   - mRNA Plant (3F-6F): Further expansion of mRNA manufacturing facility equipped with MF system
   - Expected expansion capacity: Multi-kgs/month
   - *Customized or dedicated facility available as per client's request*

Commercial-scale GMP Manufacturing Facility

7,000 ft²

Mid-scale GMP Manufacturing Facility

2,237 ft²

Summary

ST Pharm provides seamless GMP manufacturing service from LNP-encapsulated mRNA to key materials of caps & lipids in LNP

mRNA CDMO Business

- Manufacturer of key raw materials in GMP or non-GMP standard
- Provding one-stop service from CDMO to non-clinical GLP studies

LNP Technology and Lipid Mass Production

- In-licensed LNP technology, STLNP® and 3rd generation LNP, SMARTLNP®
- Mass production of key lipids for LNP formulation – ionizable and PEG-lipids

ST PHARM

5'-Capping Technology and Mass Production

- In-house 5'-capping analog, SMARTCAP®
- Total 30 capping analogs for capping library screening
- Cost effective large-scale production of diverse 5'-capping reagents

Post-COVID Strategy

- Expanding CDMO business of RNA therapeutics through strategic partnerships with various RNA companies
- Building up of xRNA projects through Levatio (circRNA) and Vernagen (mRNA)

Acknowledgements

Special thanks to

- Korea National Institute of Health
- KPBMA
- Dong A ST
- Hanmi Pharmaceuticals
- GC and Mogam
- Ajou University
- Ewha Women University
- Sogang University
- Catholic University of Korea

And to all ST PHARM family members

KDCA
Korea Disease Control and Prevention Agency

KPBMA 한국제약바이오협회

DONG-A ST

Hanmi GC

아주대학교 AJOU UNIVERSITY 1973

이화여자대학교 EWHA W.U.

서강대학교 SOGANG UNIVERSITY

가톨릭대학교 THE CATHOLIC UNIVERSITY OF KOREA

Development of RNA therapeutics and lipid nanoparticle (LNP) formulation for in vivo delivery

이화여자대학교 약학대학 교수 / 이 혁 진

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	내 용
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연구 업적	○ **주요/최근 연구논문 또는 저서** "In vivo delivery of CRISPR-Cas9 using lipid nanoparticles enables antithrombin gene editing for sustainable hemophilia A and B therapy." **Sci Adv.** 2022 Jan 21;8(3):eabj6901. "Engineered ionizable lipid nanoparticles for targeted delivery of RNA therapeutics into different types of cells in the liver." **Sci Adv.** 2021 Feb 26;7(9):eabf4398. "Combined hybrid structure of siRNA tailed IVT mRNA (ChriST mRNA) for enhancing DC maturation and subsequent anticancer T cell immunity", **J Control Release.** 2020 Nov 10;327:225-234. "Adjuvant incorporated lipid nanoparticles for enhanced mRNA-mediated cancer immunotherapy", **Biomater Sci.** 2020 Feb 21;8(4):1101-1105.
5월 27일 포 럼 발표주제	Development of RNA therapeutics and lipid nanoparticle (LNP) formulation for in vivo delivery
발표내용	다양한 RNA 치료제의 체내 적용을 위한 지질나노입자 제형에 대한 소개 및 새로운 이온화지질 발굴을 위한 연구내용에 대해 발표를 하고자 한다. mRNA 전달을 위한 지질나노입자 활용에 대한 예시로 covid mRNA vaccine 전달 및 암 백신 개발 활용에 대한 내용을 포함하고 있다.

[초 록]

발표 제목:

Development of RNA therapeutics and lipid nanoparticle (LNP) formulation for in vivo delivery

In recent years, RNA therapeutics have received tremendous attention as a tool to regulate gene expression in patients. These approaches include the regulation of abnormal gene expression by short interfering RNA (siRNA) and messenger RNA (mRNA). There have been two US FDA approved siRNA therapeutics (Patisiran and Givosiran) for rare disease treatments. These drugs target the selective degradation of mRNA to reduce the expression of disease associated proteins in polyneuropathy and acute hepatic porphyria. In addition, synthetic mRNA can produce insufficient proteins (e.g. Factor IX, VEGF) in patients to treat various diseases such as hemophilia A and ischemic heart disease. To fully realize the potential of RNA therapeutics, an efficient in vivo delivery system is of the utmost importance. Ionizable lipid nanoparticles (LNPs) have been widely utilized for the systemic delivery of RNA therapeutics. LNPs are mainly composed of ionizable lipid or lipid like materials with helper lipid, cholesterol, and polyethylene glycol (PEG)-lipid. Although LNPs are particularly advantageous for in vivo delivery, systemic delivery of RNA therapeutics other than liver hepatocytes remains highly challenging. Ionizable lipid nanoparticles (LNPs) have been widely utilized for in vivo delivery of RNA therapeutics into the liver. However, a main challenge remains to develop LNP formulations for selective delivery of RNA into certain types of liver cells, such as hepatocytes and liver sinusoidal endothelial cells (LSECs). Here we report the engineered LNPs for the targeted delivery of RNA into hepatocytes and LSECs.

NanoBiomaterial NanoTechnology Lab.

Development of RNA therapeutics and lipid nanoparticle (LNP) formulation for in vivo delivery

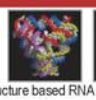
Prof. Hyukjin Lee
College of Pharmacy
Graduate School of Pharmaceutical Sciences
Ewha Womans University

Research Topics

Core research I. Nucleic Acid Nanotechnology Based Gene Therapy

N.A. nanostructure based gene therapy

- Development of biocompatible Bio-/Nano-fusion technology based gene carrier
- Optimization of disease specific nanocarrier
- Technique of building up DNA structure based gene carrier
- Manufacturing of disease targeted DNA structure
- Manufacturing technique Lipid/Peptide/Polymer hybrid particles
- Gene delivery technique with biocompatible materials


Structure based RNA carrier candidates

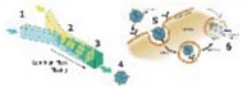

Large scale production

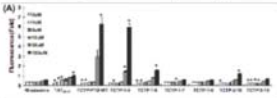

Biodistribution

Core research 2. Formulation of LNP for Enhance in vivo Delivery of Therapeutics

Lipid nanoparticle for in vivo delivery

- Screening of amine molecules for ionizable lipid synthesis
- In vivo screening of ionizable lipid for gene delivery
- Delivery of therapeutic mRNA
- Development of in vivo platform for Cas9 gene editing system
- Development of core technology for LNP formulation and optimization

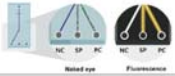


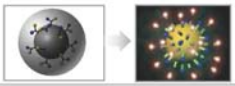


Core research 3. Molecular Diagnostics and Biosensing Technology

Molecular diagnostic for infectious diseases

- Development of molecular diagnostic test for evaluating infectious diseases
- Diagnosis technique based on isothermal amplification methods
- Development of appropriate molecular imaging technique for valid disease models
- Development of core technology for evaluating efficacy of siRNA carrier
- DNA nanotechnology based biosensor

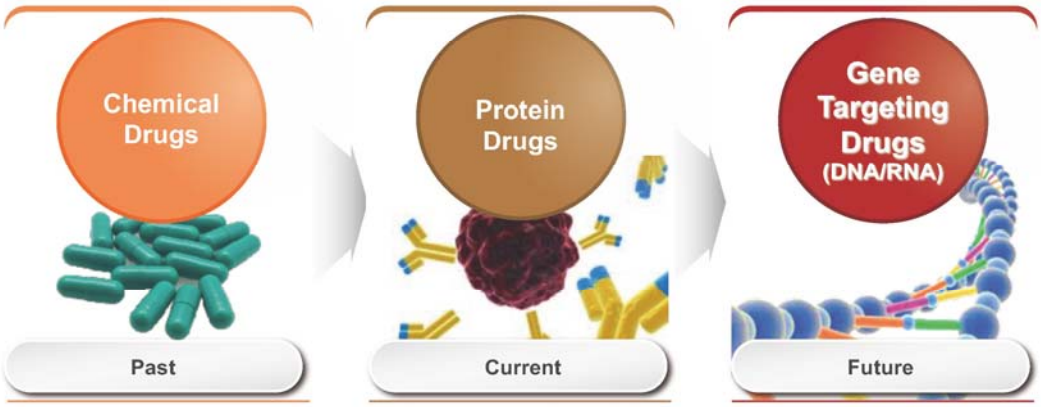




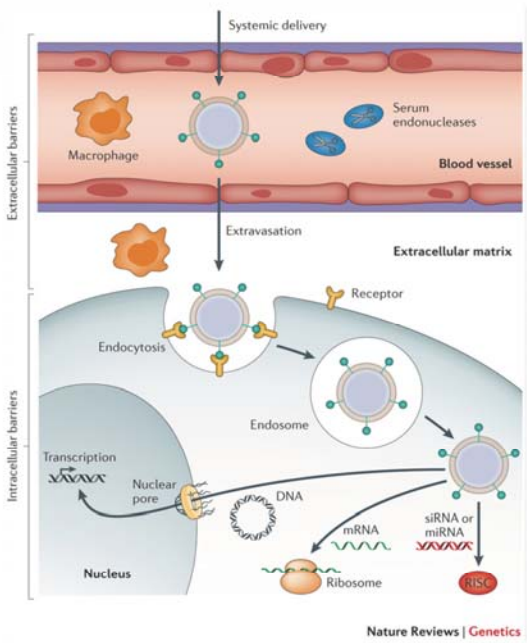
Background and Significance

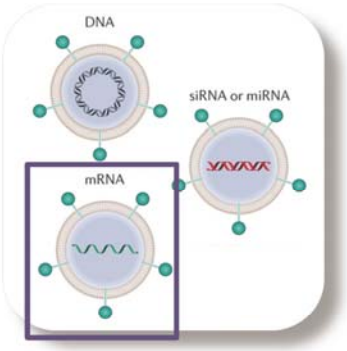
Recent Trends in the Drug Discovery Area

- Future of medicines is targeted and personalized therapeutic & diagnostic solutions



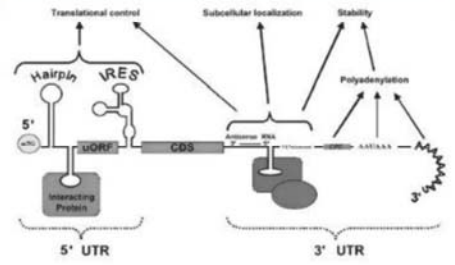
Background and Significance: Types of Gene Therapy



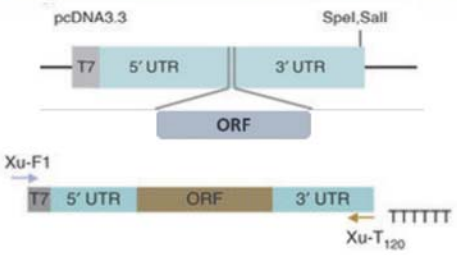


Synthesis of IVT mRNA

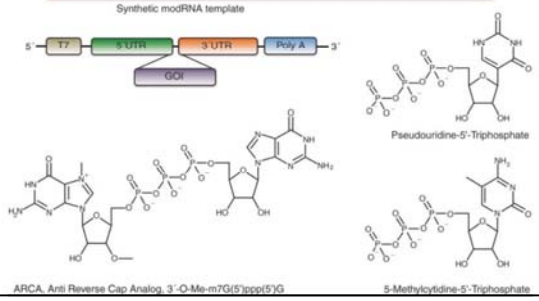
Selection of UTRs with high translation efficiency and stability



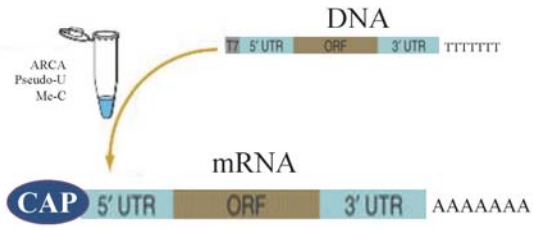
Addition of poly-T tail at DNA level



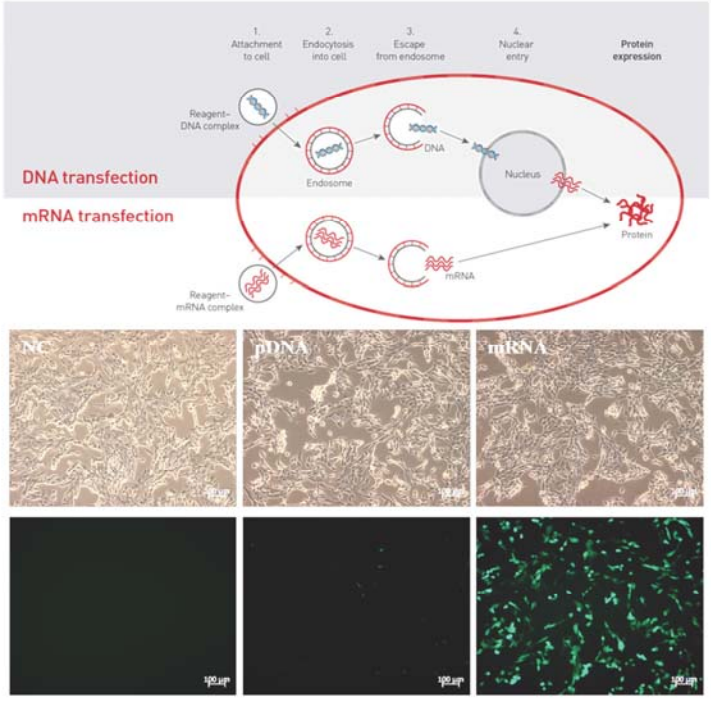
Capping and modification



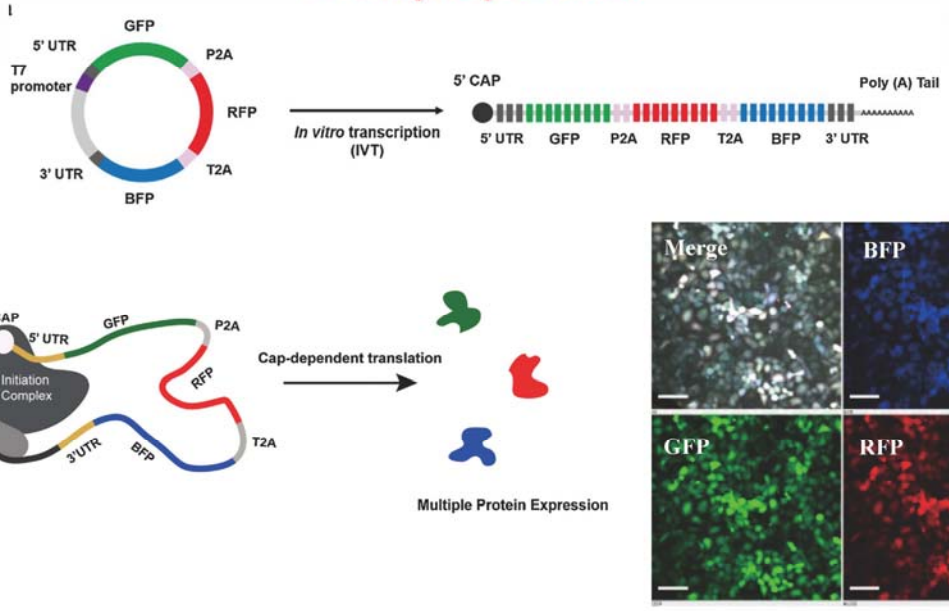
Preparation of synthetic mRNA by IVT



Comparison of IVT mRNA vs. pDNA

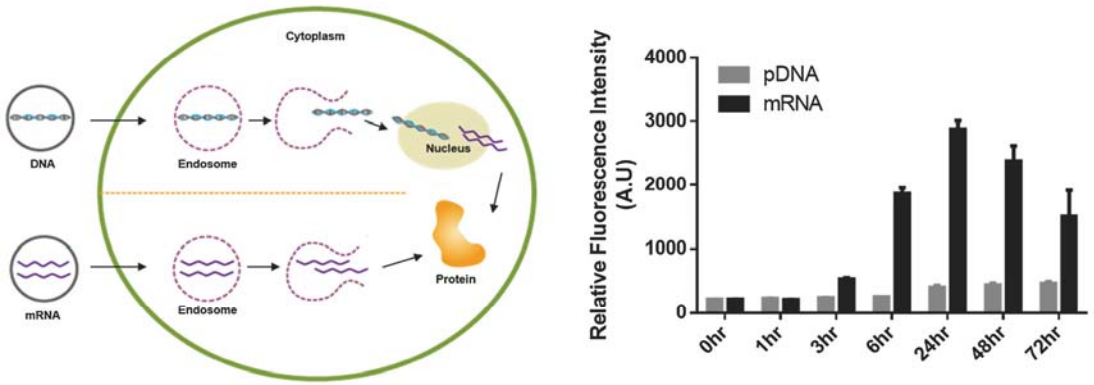


IVT mRNA for the simultaneous induction of multiple proteins



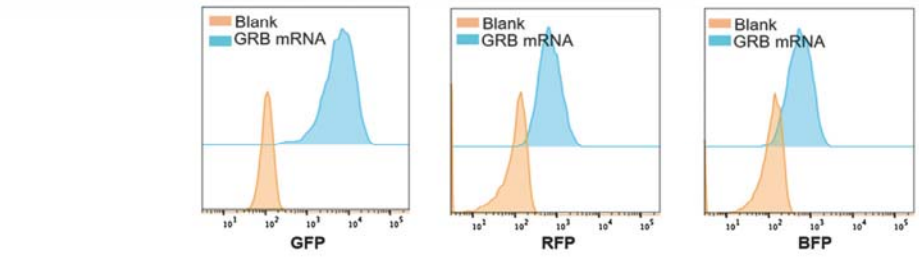
Lee et al. JIEC 2019

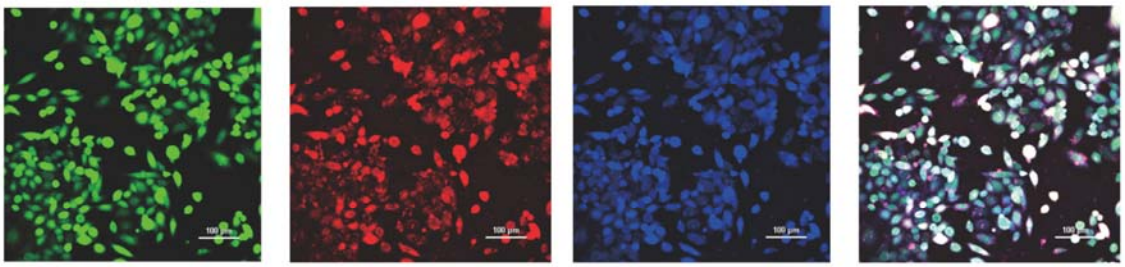
IVT mRNA for the simultaneous induction of multiple proteins



Lee et al. JIEC 2019

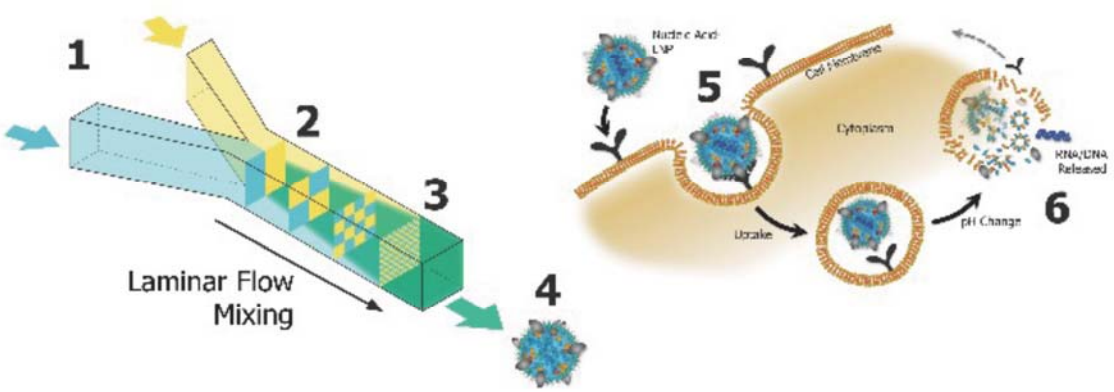
IVT mRNA for the simultaneous induction of multiple proteins



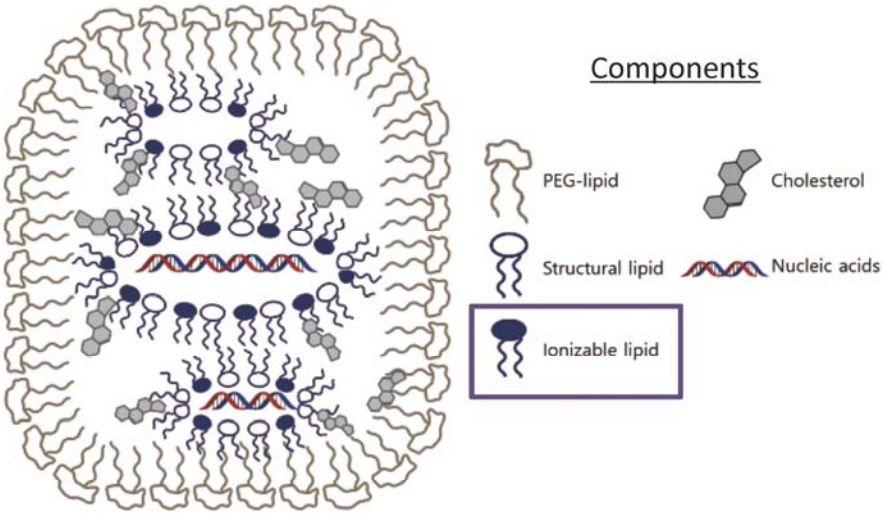


Lee et al. JIEC 2019

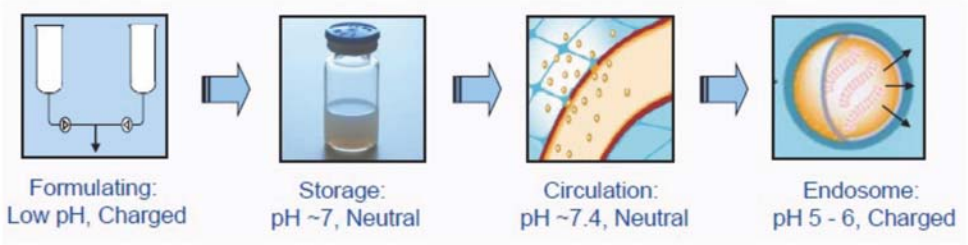
Lipid nanoparticle formulation for in vivo delivery of NA therapeutics

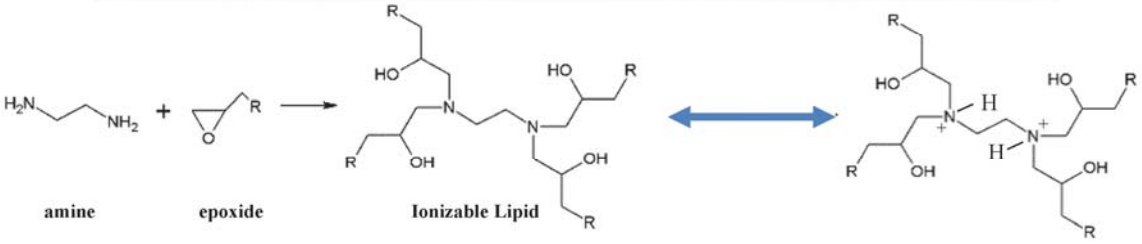


Lipid Nanoparticle Technology



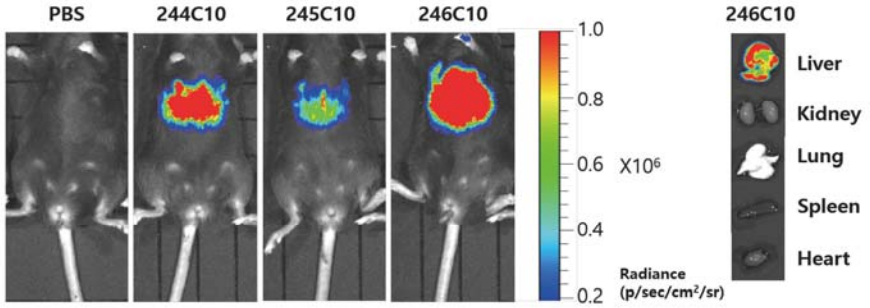
Function of Ionizable Lipid





Ionizable amine allows lipid to take advantage of surrounding pH

In vivo liver uptake screening using different ionizable lipid candidates

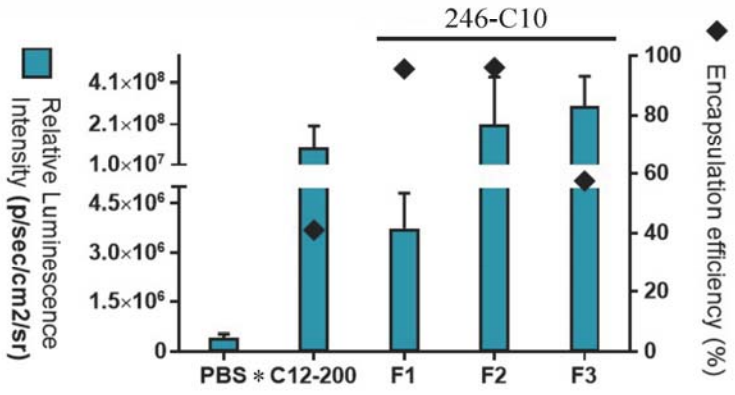


Ionizable lipid:mRNA	Ionizable lipid	DOPE	Chol.	PEG-lipid
10:1	26.5%	20%	52%	1.5%

	244C10	245C10	246C10
Diameter (nm)	66	70	68

Luc mRNA 0.1mg/kg, IV

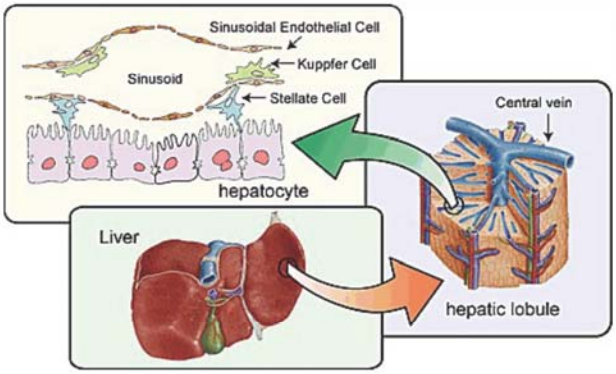
Optimization of encapsulation efficiency



Luc mRNA 0.1mg/kg, IV

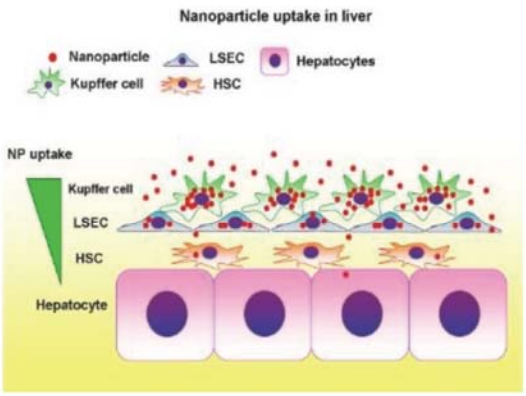
*C12-200 : Previously reported lipidoid for siRNA and mRNA delivery

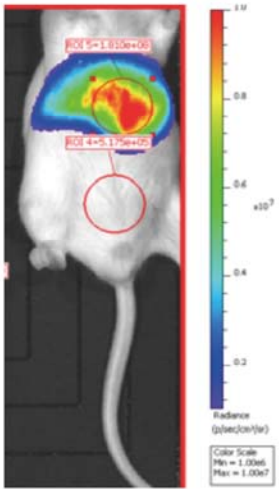
Systemic Delivery of LNP - Target Liver diseases



- **Hepatocyte**
  - Hepatitis B, viral cccDNA
  - Hypercholesterolemia, pcsk9
- **Liver Sinusoidal Endothelial Cell, LSEC**
  - Hemophilia A, Factor 8

Problem of liver specific delivery by LNPs

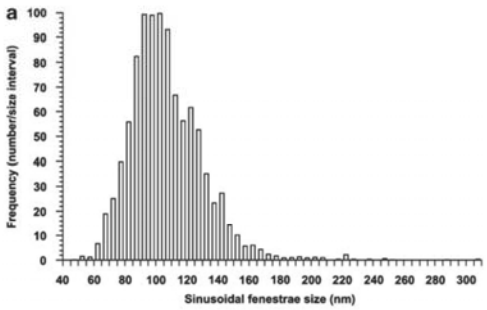


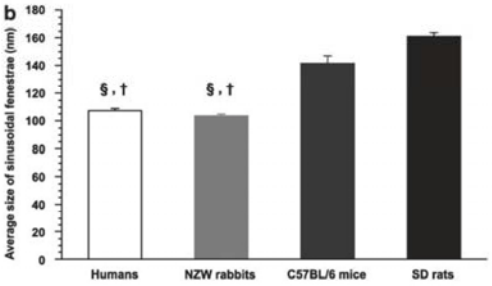


Effect of LNP size and their biodistribution

ORIGINAL ARTICLE

The size of endothelial fenestrae in human liver sinusoids: implications for hepatocyte-directed gene transfer



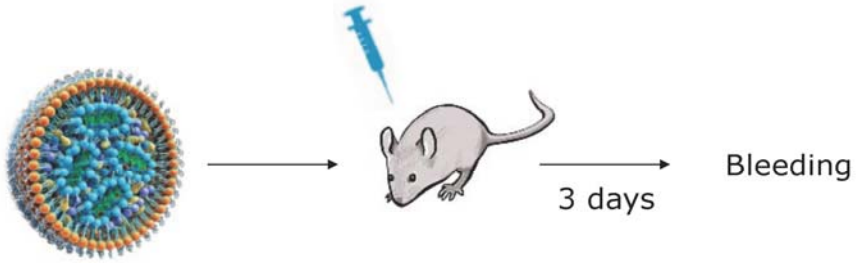


Gene Therapy (2008) 15, 1193–1199

www.nature.com/gt

Liver cell type specific delivery of siRNA

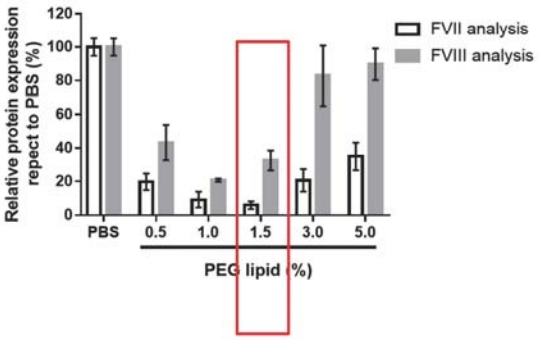
- Influence of particle size on the liver cell type specific delivery
- siRNAs targeting coagulation factor 7 & factor 8 tested to demonstrate liver cell type specific delivery
- siRNA dose of 0.2mpk
- C57BL/6



siFVII & siFVIII

Influence of particle size on the liver cell type specific delivery of siRNA

PEG (%)	Mean particle diameter	
	siFVII-LNPs	siFVIII-LNPs
0.5	120	166
1.0	78	87
1.5	52	60
3.0	44	42
5.0	40	41

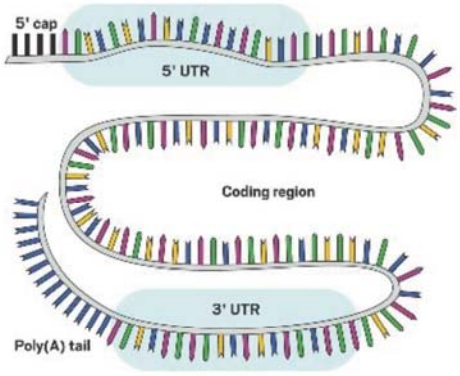


C67BL/6 6-8 weeks female mice
siFVII 0.2mg/kg, siFVIII 0.5mg/kg
2d post i.v injection (FVII)
3d post i.v injection (FVIII)

The difference between siRNA vs mRNA

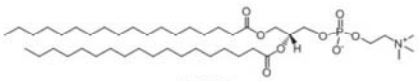
siRNA

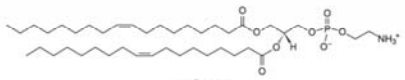
VS.



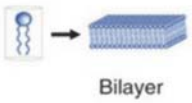
mRNA

Optimization of mRNA loaded LNPs

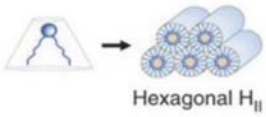


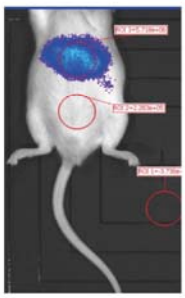


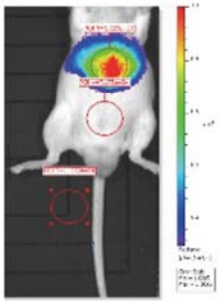
- Stable lamellar phase
- Stronger complexation of mRNA to lipid in LNPs (Encapsulation efficiency : > 90%)



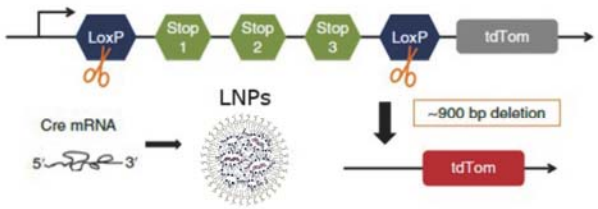
- Less stable hexagonal phase

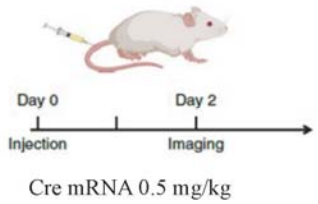




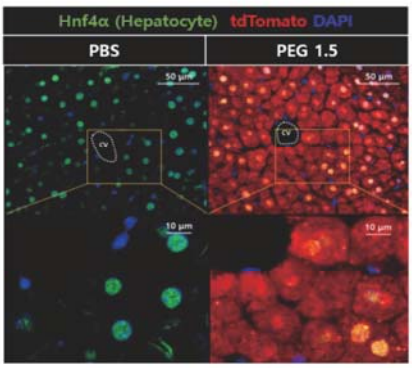


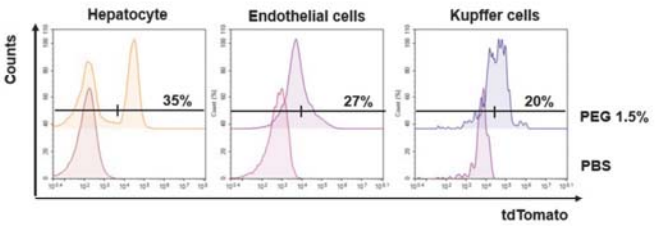
246C10 LNPs induced tdTom fluorescence specifically in the liver





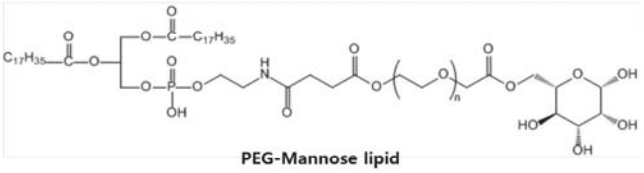
Cre mRNA 0.5 mg/kg





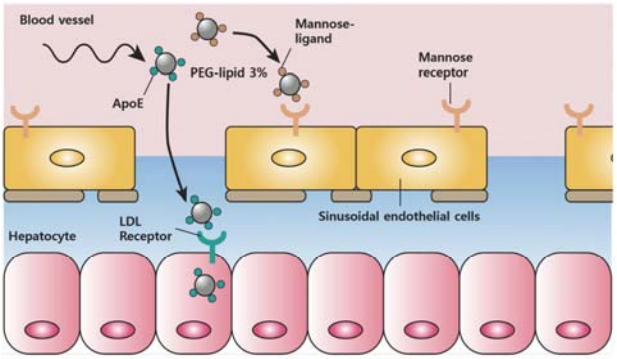
Sci. Adv. 2021

Ligand engineered LNPs for liver cell type specific delivery



PEG-Mannose lipid

A ligand for Mannose receptor expressed on LSECs



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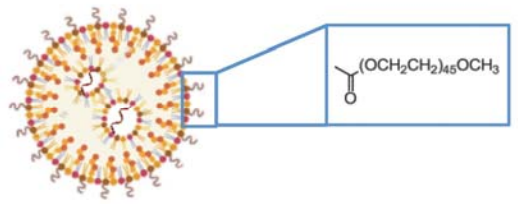
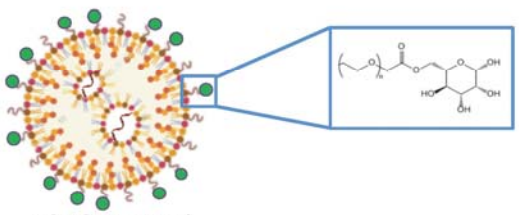
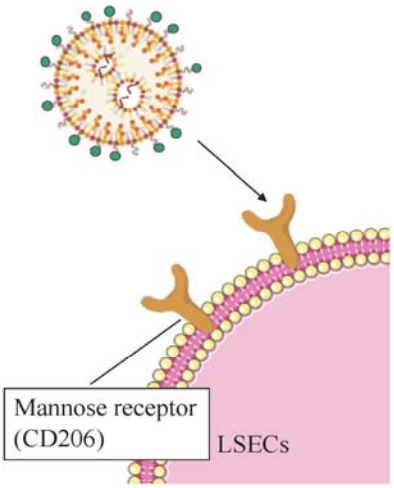


Non-targeted LNPs



LSECs targeted LNPs



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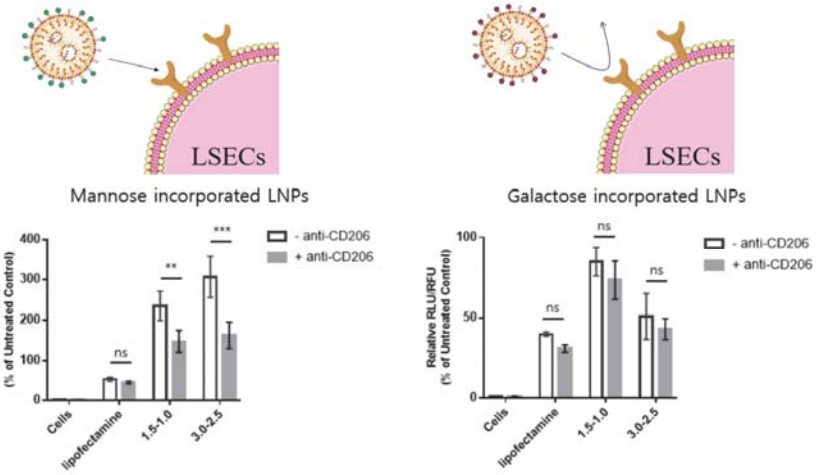

- HepG2 cell, Luciferase mRNA
- 1.5-1.0: Total PEG-lipid 1.5% with 1.0% of Mannose-PEG lipid or Galactose-PEG lipid
- 3.0-2.5: Total PEG-lipid 3.0% with 2.5% of Galactose-PEG lipid or Galactose-PEG lipid

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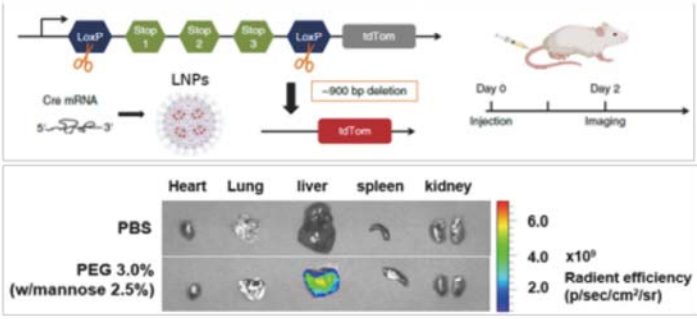
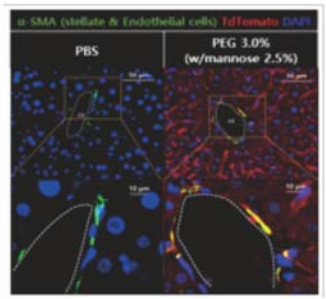
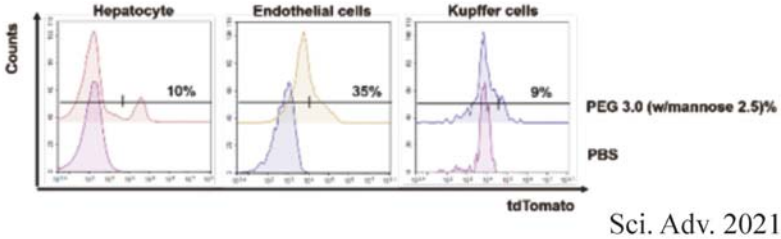
Ligand engineered LNPs for liver cell type specific delivery







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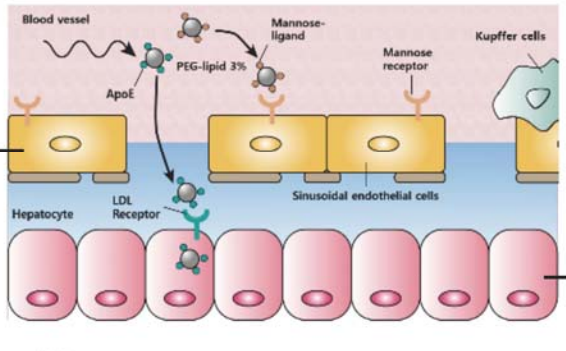

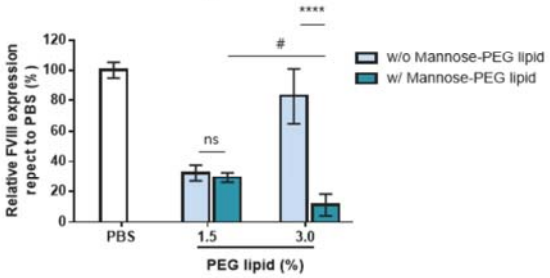

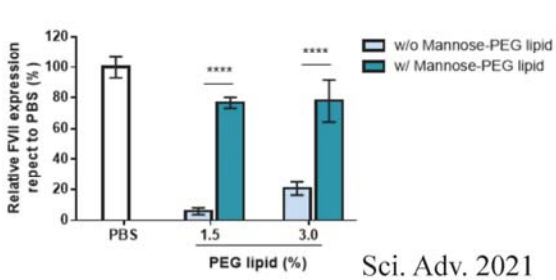

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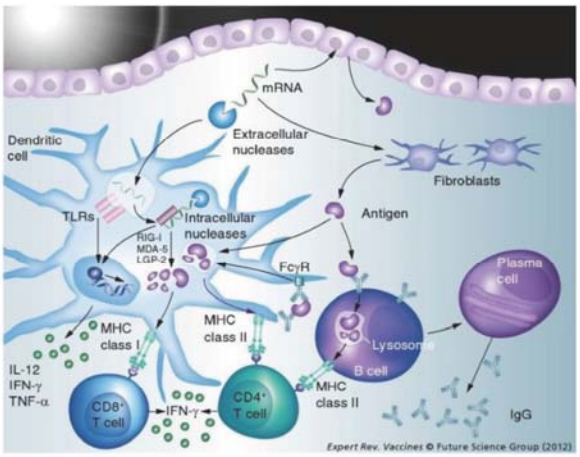
Immune therapy using IVT mRNA



moderna
messenger therapeutics

Over 60 projects, clinical trials planed for 2016

In collaboration with AstraZeneca, Merck, Alexion

€ 776m in cash in October 2015, € 157m invested, a raise of $ 462m in Dec 14 (The biggest private round in Biotech ever)

CUREVAC
the RNA people®

Already two in Phase II and three in Phase I plus many at a preclinical stage

In collaboration with Novartis, GSK, Boehringer, Sanofi, J&J

Approximately € 300m in equity raise

Ultimate Review: How Could mRNA Overtake all other Biologicals in Medicine? Jerome et al. 2016

Unmodified mRNA vs Modified mRNA

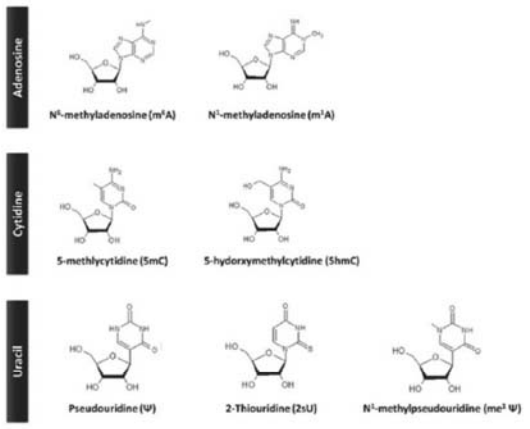
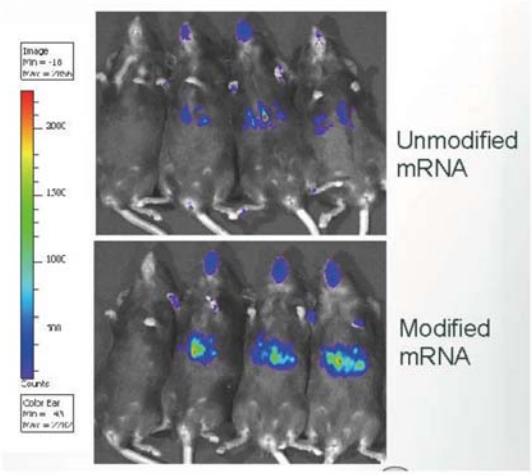
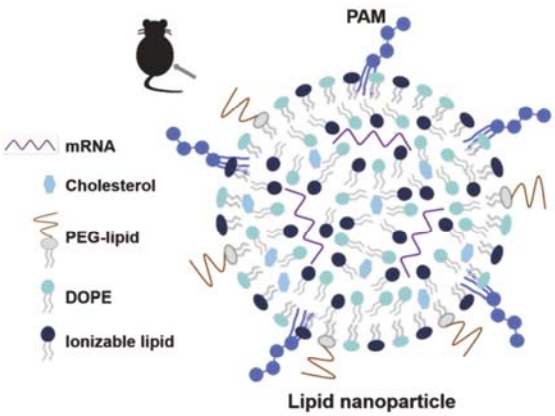


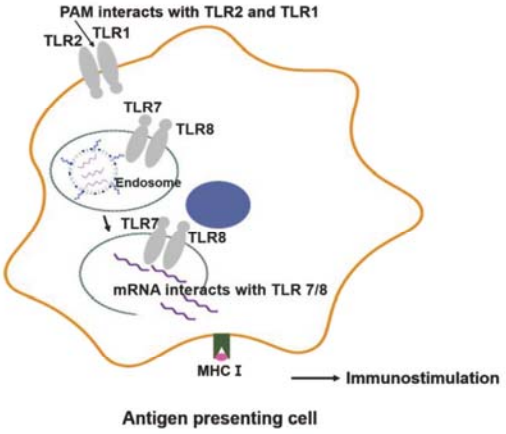
Fig. 5. Various types of modified nucleosides utilized in the preparation of *in vitro* transcribed (IVT) mRNA.



Emergence of synthetic mRNA : *In vitro* synthesis of mRNA and its applications in regenerative medicine. Kwon et al. Biomaterials. 2017

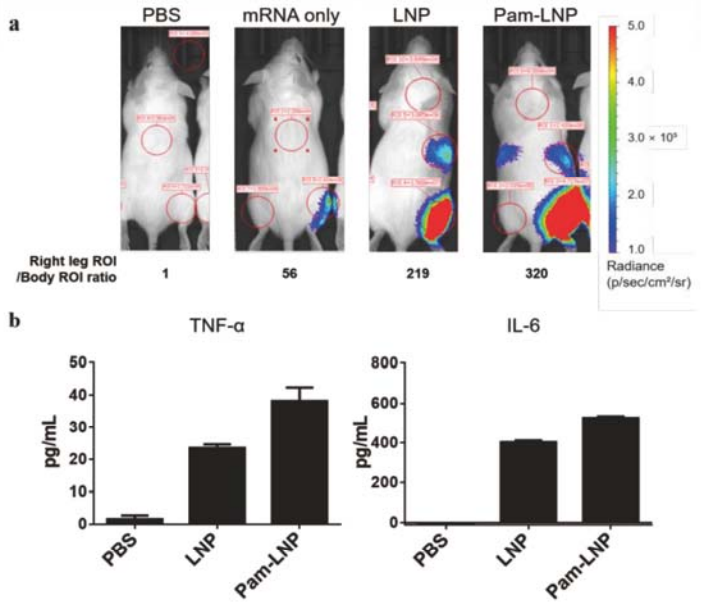
Co-delivery of modified luciferase mRNA - adjuvant





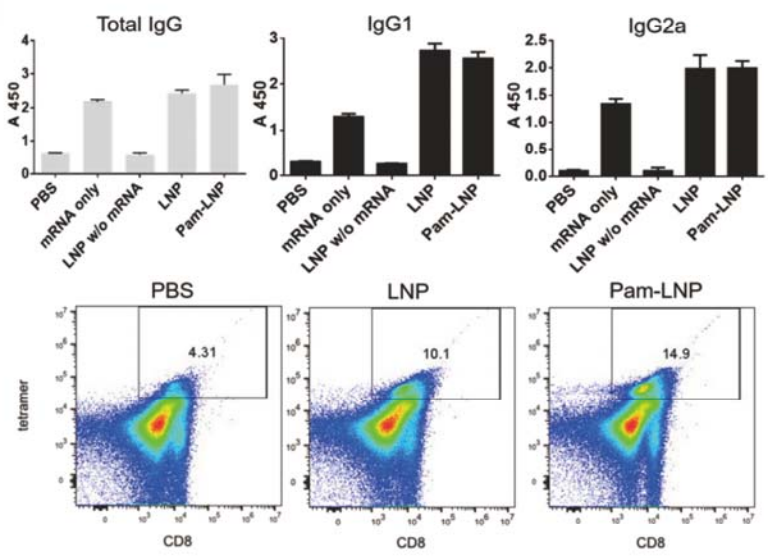
Lee et al. Biomaterial Sciences 2019

Co-delivery of modified luciferase mRNA - adjuvant



Lee et al. Biomaterial Sciences 2019

Co-delivery of modified luciferase mRNA - adjuvant



Lee et al. Biomaterial Sciences 2019

ChriST mRNA for enhancing DC maturation

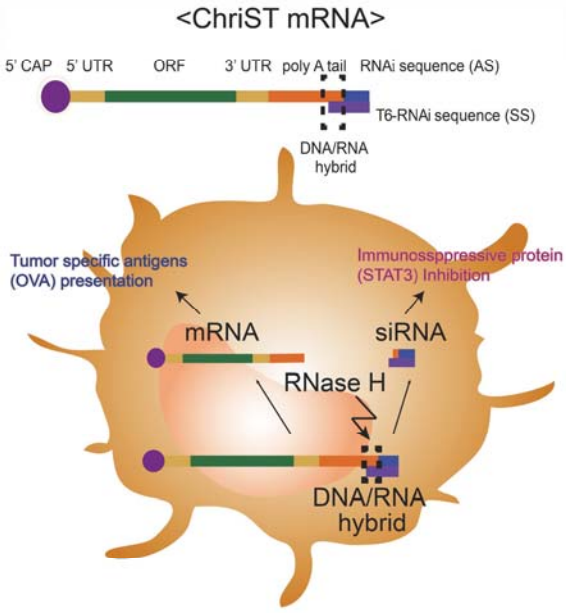
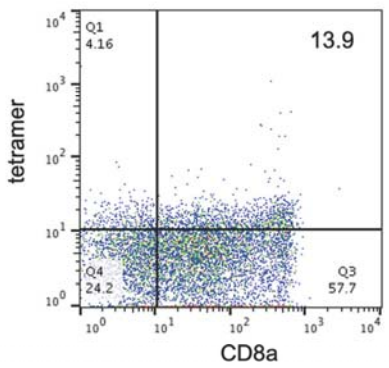
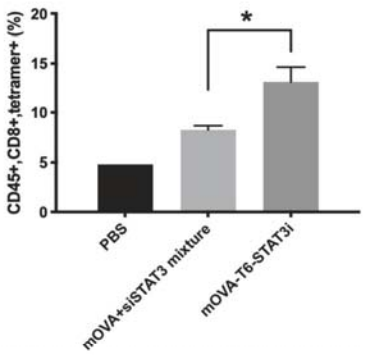


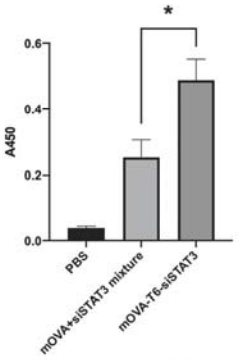
Fig. 1. Schematic illustration of the preparation of the ChriST mRNA and their application in the DCs-based cancer immunotherapy.

Lee et al. JCR 2020

ChriST mRNA for enhancing DC maturation







Lee et al. JCR 2020

Summary

- **Synthetic mRNA** is ideal for rapid onset of protein expression as compared to the conventional DNA based vectors
- Delivery of IVT mRNA is achieved using **lipid nanoparticles (LNPs)** for efficient in vivo expression of target proteins
- **Potential applications** include 1) therapeutic protein replacement, 2) mRNA based vaccines, 3) genome editing

Clinical studies for mRNA cancer vaccine

국립암센터 항암신약신치료기술개발사업단 부단장 / 김 학 균

[붙임] 2022도 암과학포럼 연자 프로필 및 강의내용(초록)

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소 속	항암신약신치료기술개발사업단	직위/직함	부단장

	내 용
주요 경력	○ 학력 • 1991 학사, 서울대학교 의과대학 • 2000 박사, 서울대학교 의과대학 • 2000 전공의 및 전임의, 서울대학교병원 혈액종양내과 ○ 경력 • 2001- 국립암센터
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Clinical studies for mRNA cancer vaccine

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Clinical trials of personalized approach, are active outside Korea for neoantigen-targeted, customized mRNA cancer vaccines. Neoantigens, such as somatic mutations, indels, and gene fusions, are more immunogenic than self-antigens. Targeting neoantigens has exhibited clinical efficacy and advantages; 1.there is no need for clinically-actionable molecular targets; 2.treatment is very safe, enabling the combination with other immunotherapeutic agents.

While public neoantigen can be developed as a universal, off-the-shelf therapeutic agent, it is extremely rare. For example, *TP53*^{R175H}, which is the most common mutation of the most commonly mutated gene, is present less than 2% of solid tumor, and restricted to HLA-A2 allele. Only 24 of 911,548 private neoantigens are common in more than 5% of patients with cancer[1]. Therefore, custom-synthesized mRNA vaccine is developed to target multiple private neoantigens.

In a phase 1a/1b trial (NCT03289962), a neoantigen-based mRNA vaccine RO7198457 (BioNTech), encoding ~20 patient-specific neoantigens based on neoantigen prediction, was injected intravenously to previously heavily-treated patients with non-small cell lung cancer, colorectal cancer, melanoma, and breast cancer, the majority of which express low levels of PD-L1. RO7198457 monotherapy achieved an objective response rate (ORR) of 4 % (1/26, a gastric cancer), and RO7198457 in combination with anti-PD-L1 antibody atezolizumab achieved ORR of 8 % (9/108)[2].

A phase 1 trial (NCT03313778) tested the intramuscular injection of mRNA lipid-encapsulated neoantigen vaccine mRNA-4157 (Moderna), alone or in combination with pembrolizumab (anti-PD-1). Of 63 patients treated with mRNA-4157/pembrolizumab, there were 3 complete remissions (CRs) (a head and neck squamous cell carcinoma (HNSCC), a microsatellite instability-high (MSI-H) colorectal cancer, and a MSI-H prostate cancer) and 8 partial remissions (PRs) (a bladder cancer, 4 HNSCC, 2 small cell lung cancers, and a MSI-H endometrial cancer), with ORR of 39%. Notably, in the 10 anti-PD-1/PD-L1-naive HPV-negative HNSCC patients, ORR was 50 % (1 CR, 4 PR, 4 stable disease (SD)) and median progression-free survival (PFS) was 9.8 months [published ORR of pembrolizumab monotherapy is 14.6 % with median PFS of 2.0 months][3].

In summary, mRNA vaccines enable ~66% of private neoantigens to prime naïve T cells to memory T cells, augmenting the clinical efficacy of immune checkpoint blockade in many tumor types. Neoantigen mRNA vaccines are safe and well tolerated in patients with advanced cancer, with needle-to-needle time, which is required for design and manufacture, being less than 2 months in these trials.

In collaboration with MFDS, National Cancer Center plans to launch neoantigen vaccine clinical trials by creating a nationwide cooperative group, and support the development of neoantigen prediction algorithms, adjuvant therapies, and vaccine manufacturing. Since we are lagging far behind in core mRNA cancer vaccine technologies, which may offer breakthrough therapeutic options, unprecedented collaboration among basic scientists, clinicians, and industry, as well as generous government support, will be crucial to benefit Korean patients without alternative treatment options.

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- 주요 면역항암제의 허가사항 및 임상시험들에 대한 비교분석을 통한 면역항암제 병용 임상시험에 대한 제언

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MEMO

MEMO

항암신약개발 A-Z

Part 4. mRNA vaccine; from COVID-19 to cancer

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