nucleic

核酸藥物創新產品發展趨勢

與

4/22

審查法規考量經驗分享

指導單位

衛生福利部
Ministry of Health and Welfare

主辦單位

財團法人醫藥品查驗中心
Center For Drug Evaluation

acid

drugs

核酸藥物創新產品發展趨勢與審查法規考量經驗分享

會議邀請函

新冠肺炎加速 mRNA 技術的應用成功，開啟核酸藥物發展的新里程碑，預估西元 2032 年全球 mRNA 疫苗與藥物市場可達 4 億 2 千 5 百萬美元。核酸藥物主要是針對標的 mRNA 進行調控，在藥物研發上相對單純、快速，早期多應用於罕見疾病與遺傳性疾病，但近年有廣泛應用於癌症及慢性疾病的趨勢，有望成為重要標靶工具。由於不同類型核酸藥物在化學製造管制、臨床前試驗與臨床試驗皆有其在法規科學上的特殊考量與議題，故須以經過驗證、基於科學和風險的方法確保藥物研發之安全性。因此，財團法人醫藥品查驗中心擬邀請中央研究院生醫轉譯研究中心陶秘華執行長與法信諾生醫張嘉銘董事長分享核酸疫苗與核酸藥物開發經驗，並由查驗中心資深審查員針對研發前期最為關鍵之藥毒理部分進行核酸藥物審查經驗分享，期望能對於國內核酸藥物研究發展有所助益。**竭誠歡迎各界先進撥冗參加交流！**

■ 活動日期與時間：2022 年 4 月 22 日(星期五) 14:00~17:00

■ 活動地點：集思北科大會議中心感恩廳

■ 指導單位：衛生福利部

■ 主辦單位：財團法人醫藥品查驗中心

■ 活動議程：

時間	講題	講者
13:30-14:00	報到	
14:00-14:10	開場致詞	財團法人醫藥品查驗中心
14:10-14:50	核酸疫苗開發經驗分享	中央研究院生醫轉譯研究中心 陶秘華執行長
14:50-15:30	核酸藥物關鍵修飾技術開發 經驗分享	法信諾生醫股份有限公司 張嘉銘董事長
15:30-16:00	中場休息	
16:00-16:40	國際核酸藥物開發之藥毒理 法規考量與案例分享	財團法人醫藥品查驗中心 蔡岸圻資深審查員
16:40-17:00	綜合討論	

■ 分享會影片連結：

核酸疫苗開發經驗分享-陶秘華執行長

https://youtu.be/URHoaWSw-io

核酸藥物關鍵修飾技術開發經驗分享-張嘉銘董事長

https://youtu.be/xsvYGySd8Lg

國際核酸藥物開發之藥毒理法規考量與案例分享-蔡岸圻資深審查員

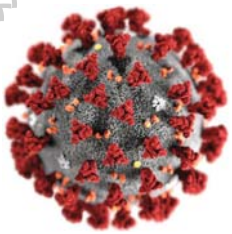
https://youtu.be/rMoxw1ZRHWA

核酸疫苗開發經驗分享

陶秘華執行長
中央研究院生醫轉譯研究中心

核酸疫苗開發經驗分享

陶秘華 博士
生醫轉譯研究中心/轉譯醫學專題中心 執行長
生物醫學研究所 研究員



2003/05 SARS專案研究小組

時間：民國九十
地點：本院行政
主席：李遠哲
出席：陳定信、
紀錄：陶秘華

主席報告：

李遠哲院長：

SARS 現在
SARS coronavir
SARS 的研究還
也很願意協助規
外一點報告的是
統召開國安會議
疫情的控制，有
各單位。

工作小組	召集人	任職機構
Virology	楊明語	Prof., Dept. of Molecular Microbiology & Immunology, USC
	陳培哲	台大醫學院教授
Immunology	江伯倫	國立台灣大學臨床醫學研究所教授
	黎煥耀	成大學醫學院微生物及免疫學研究所教授
Diagnostics	陳垣崇	中央研究院生醫所所長
	楊泮池	台大醫院內科主任
Drug Development	翁啟惠	中央研究院基因體研究中心主任
	許明珠	太景生物科技公司總經理
	鄧哲明	台大藥理所教授
Vaccine	何大一	Scientific Director & CEO, Aron Diamond AIDS Research Center
	陳定信	台大醫學院院長
	陳垣崇	中央研究院生醫所所長
	陶秘華	中央研究院生醫所副研究員
Clinical	陳定信	台大醫學院院長
	楊泮池	台大醫院內科主任
Epidemiology	陳建仁	衛生署署長
	蘇益仁	疾病管制局局長
	何美鄉	中研院生醫所副研究員

研究計畫，準備進行
的意願，但是國內對
入 SARS 的研究，他
和大家會面討論。另
亂，上星期已經由總
一未來國內對 SARS
明亮統一指揮，協調

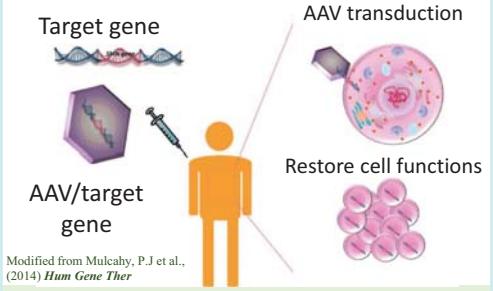
2022/02廖俊智院長在中研院召開第一次國內學研單位針對新冠病毒疫情的緊急會議

February, 2020

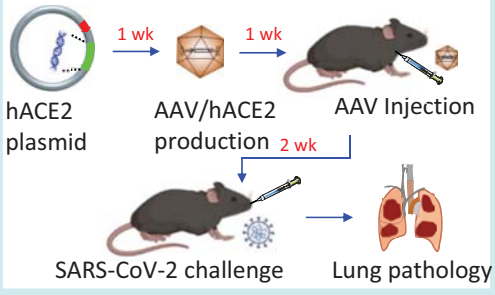
爭取盡速建立動物模式測試疫苗效力_1個月內完成建立AAV/hACE-2小鼠

詹家琮團隊
林宜玲團隊
陶秘華團隊

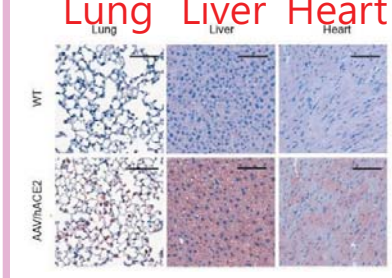
Adeno-associated viral vector



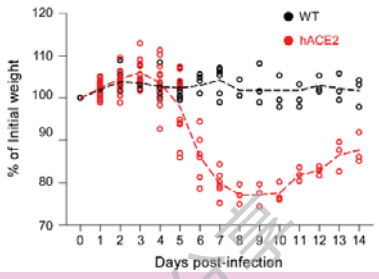
Strategy to make COVID-19 animal model



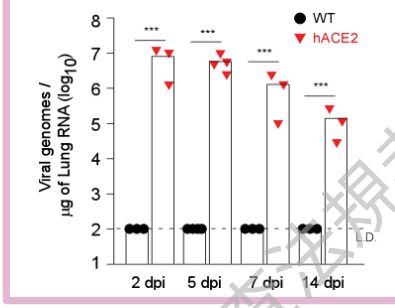
hACE2 expression



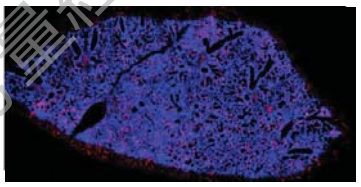
Weight Loss



Lung viral RNA by PCR



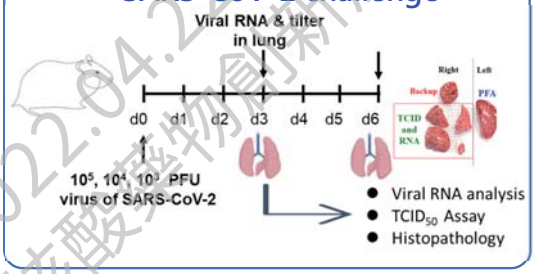
N protein expression in whole lungs



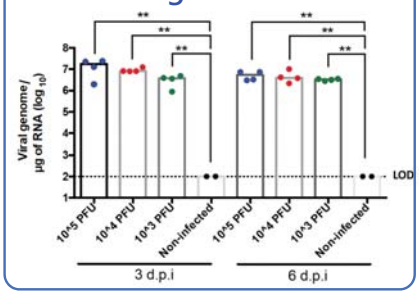
Sun et al, (2021) PLoS Pathog

新冠病毒倉鼠動物模式_諮詢法規單位(CDE)的要求

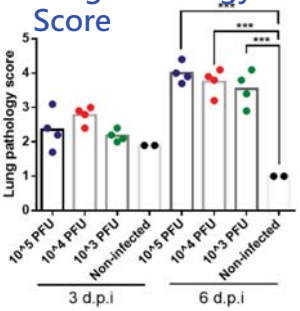
SARS-CoV-2 challenge



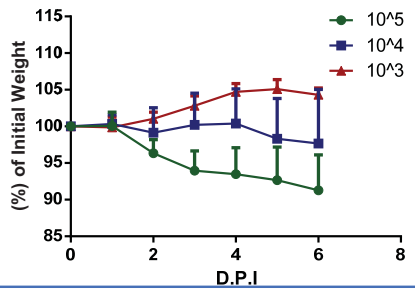
Lung viral RNA



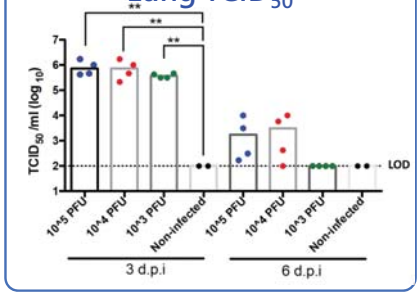
Lung Pathology Score

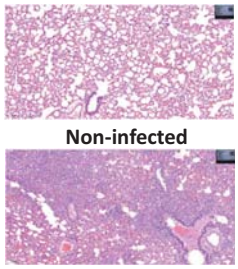


Weight Loss



Lung $TCID_{50}$





First reported by Sia et al, Nature in May, 2020

產品開發進程及重點項目

基礎研究 (研發時間 1-3年) → 轉譯研究 (2-3年) → 臨床前試驗 (2-3年) → IND 臨床試驗 (4-6年) → NDA 產品上市 (1-2年)

補助階段 / 重點項目

BRPT 申請條件

Hit-to-Lead
- in vitro/vivo activity
- MOA
- IP / Market (初階)

Candidate / Prototype
- in vitro/vivo activity
- Proof of Concept
- IP / Market (進階)

Drug
- CMC: non-GMP, GMP scale-up
- TOX: Genotox, Rat, Dog, Safety pharmacology
- PD/PK
- Pre-IND meeting (CDE, TFDA, FDA)
- IND application (CDE, TFDA, FDA)

Device
- Design Validation

- 科學家
- 臨床醫師
- CRO
- 法規諮詢

研發費用 1億 - 3億 /元 (NTD)

- 專業經理人
- CEO

院內外補助計畫

- 任務導向生技研究計畫 BRPT
- 科發計畫 (推薦申請)
- 科技部 產學計畫
- 科技部 Pre育苗計畫
- 科技部 育苗計畫
- 科技部 科研創業計畫-萌芽/拔尖
- 經濟部 科研成果價值創造計畫
- 私部門經費

NBRP National Biotechnology Research Park 國家生技研究園區

快速開發疫苗是防疫的關鍵

Traditional development

Design and exploratory preclinical studies (years) → Process development preclinical, toxicology studies (2–4 years) → IND submitted → Clinical trials (5–7 years total): Phase I (1–2 years) → Phase II (2 years) → Phase III (2–3 years) → BLA submitted → Regulatory review by FDA, EMA etc. (1–2 years) → Large-scale production and distribution

15 years or longer

SARS-CoV-2 vaccine development

Design and exploratory preclinical studies (months) → Process development preclinical, toxicology studies (months) → IND submitted → Clinical trials (months): Phase I, Phase II, Phase III → BLA submitted → Regulatory review by FDA, EMA etc. (1–2 months)

10 months to 1.5 years total

Pre-existing from SARS-CoV and MERS-CoV | Partially pre-existing and parallel development | Overlapping clinical phases | Production (at risk) | Review on a rolling basis?

- 保護性抗原的選擇/臨床前驗證
- 製造生產
- 法規
- 臨床試驗 I-III 資金

Krammdr, (2020) Nature

美國政府的曲速行動加速了新冠疫苗的開發

- BARDA, Biomedical Advanced Research and Development Authority,
- NIH, National Institutes of Health
- HHS, Department of Health and Human Services
- CDC, Centers for Disease Control and Prevention
- FDA, Food and Drug Administration
- DOD, Department of Defense
- 整合公私部門的合作

Table 1. Vaccine Candidates Supported by BARDA and Other Federal Agencies

Company	Type	Contract Value	Specifications	Doses per Person	Current Phase (Preliminary Effectiveness – U.S. Strain)[a]	Storage
Pfizer/BioNTech	mRNA[b]	$5.97B	300 million doses	2	Phase II/III (95%) EUA Issued	Ultra cold storage (-70° C)
Moderna	mRNA	$4.94B $954M	300 million doses Development	2	Phase III (94.5%) EUA Issued	Cold storage (6 mos, -20° C) Refrigerator (30 days, -2° to -8° C)
AstraZeneca/ Oxford Univ.	Viral Vector[c]	$1.2B	300 million doses	2	Phase II/III (70%)	Refrigerator (-2° to -8° C)
Johnson & Johnson (Janssen Pharmaceuticals)	Viral Vector	$1B $456M	100 million doses Development	1	Phase III (72%) EUA Issued	Refrigerator (3 mos, -2° to -8° C)
Novavax	Protein[d]	$1.6B	100 million doses	2	Phase III (95.6%)	Refrigerator (-2° to -8° C)
Sanofi/GSK	Protein	$2.04B $30.8M	100 million doses Development	2	Phase I/II	Refrigerator (-2° to -8° C)
Merck/IAVI[e]	Viral Vector	$38M	Development[f]	1	DISCONTINUED	N/A

Sources: https://www.medicalcountermeasures.gov/app/barda/coronavirus/COVID19.aspx?filter=vaccine; https://www.defense.gov/Explore/Spotlight/Coronavirus/Operation-Warp-Speed/.

Note: Current as of March 1, 2021.

全球Covid-19疫苗發展近況 (2021/12/08更新資料)

全球Covid-19疫苗發展數: 330

美國 – Cyan Vac 犬副流行性感冒病毒
義大利 – Takis & Rottapharm Biotech DNA
中國 – 三葉草生物製藥 病毒蛋白 (保護力79%)
中國 –安徽智飛龍科馬生物製藥 病毒蛋白 (保護力81.8%)
德國 – BioNTech/Pfizer mRNA (保護力91%)
日本 – Daiichi Sankyo mRNA
美國 –Vaxxinity 病毒蛋白
以色列 – Israel Institute for Biological Research 水疱性口炎病毒
古巴 – Finlay Vaccine Institute 病毒蛋白(保護力71%)
美國 – Moderna/NIH mRNA (保護力93.2%)
泰國 – Chula VRC mRNA
南韓 – GeneOne Life Science DNA
古巴 – Center for Genetic Engineering and Biotechnology 病毒蛋白(保護力92.3%)
英國 – AstraZeneca/Oxford 腺病毒 (保護力74%)
澳洲 – BioNet-Asia DNA
美國 – Vaxart 腺病毒口服錠
日本 – Shionogi/Kyushu Univ. 昆蟲細胞生產病毒蛋白
印度–Zydus Cadila DNA (保護力66.6%)
印度–Bharat Biotech 去活病毒 (保護力77.8%)
美國 – Johnson & Johnson 腺病毒 (保護力64~72%)
中國 – Stemirna Therapeutics mRNA
日本 – AnGes DNA
美國 – Arcturus Therapeutics /DukeNUS mRNA
加拿大 – Entos Pharmaceuticals mRNA
台灣 – 國光生技 病毒蛋白
台灣 – 聯亞生技 病毒蛋白
台灣 –高端疫苗 病毒蛋白

前臨床研究	臨床1期	臨床2期	臨床3期	批准限度使用	批准使用
184	53	46	43	16	9

英國 – Scancell DNA
南韓 – GeneOne DNA
美國 – Inovio DNA
意大利 – Reithera 腺病毒
越南 – Nanogen Biopharma 病毒蛋白
澳洲 – Vaxine 病毒蛋白
中國 –中國醫藥集團/Sinopharm 去活病毒 (保護力78.1%)
加拿大 – Symvivo DNA
印度 – Gennova/HDT DNA
美國 – City of Hope 牛痘修飾病毒
印度 – Bharat Biotech/ Washington Univ. 腺病毒 (鼻腔吸入)
法國 – Sanofi/GSK 昆蟲細胞生產病毒蛋白
伊朗 – Shafa Pharma Pars 去活病毒
中國 – 武漢生物/ Sinopharm-Wuhan 去活病毒 (保護力72.8%)
加拿大– Symvivo 生產病毒DNA及中和性抗體的轉殖基因益生菌
美國 – Elixirgen RNA
加拿大 – Providence Therapeutics mRNA
南韓 – Genexine DNA
中國 –沃森生物 mRNA
中國–康泰生物 腺病毒
中國 – 科興生物 Sinovac Biotech 去活病毒 (保護力50.7-83.5%)
南韓 – Eyegene mRNA
美國 – Meissa Vaccines 減弱呼吸道融合病毒 RSV
俄羅斯 - BIOCAD 腺病毒
泰國 – Mahidol University 新城病毒
日本 – KM Biologics 去活病毒
法國 – Valneva/ Dynavax 去活病毒
伊朗 – Razi Vaccine 病毒蛋白
中國 – 康希諾生物 CanSinoBIO 腺病毒 (保護力63.7%)
挪威 – Nykode DNA
德國 – University of Tübingen DNA
加拿大 – Medicago/GSK 煙草細胞萃取之類病毒顆粒(保護力75%)
美國 – Novavax 病毒蛋白微顆粒 (保護力89.7%)
俄羅斯 – Vector Institute 病毒胜肽
美國 - Kentucky BioProcessing 煙草細胞萃取之類病毒顆粒
印度 – Gennova Biopharmaceuticals 自行放大RNA
中國 –萬泰生物/香港大學 流感病毒 (鼻腔吸入)
中國 – 中逸安科生物 CHO細胞
韓國 – SK Bioscience/ GSK 病毒蛋白
俄羅斯 - Gamaleya Research Institute 腺病毒 (保護力91.6%)
德國 – German Center for Infection Research 牛痘修飾病毒
南韓 – Cellid/LG Chem 腺病毒
中國 –沃森生物病毒蛋白

資料來源: **The New York Times Coronavirus Vaccine Tracker** (Dec 08, 2021 更新); **WHO DRAFT landscape of COVID-19 candidate vaccines** (Dec 07, 2021 更新)

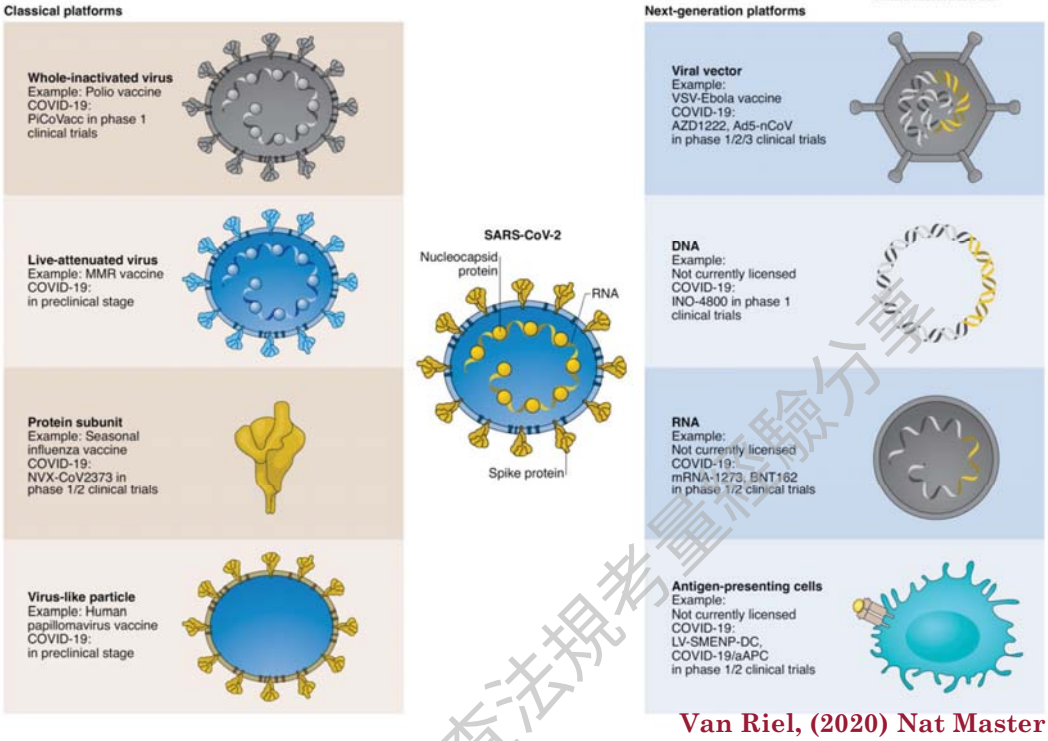
傳統疫苗技術 vs 次世代疫苗技術

傳統疫苗

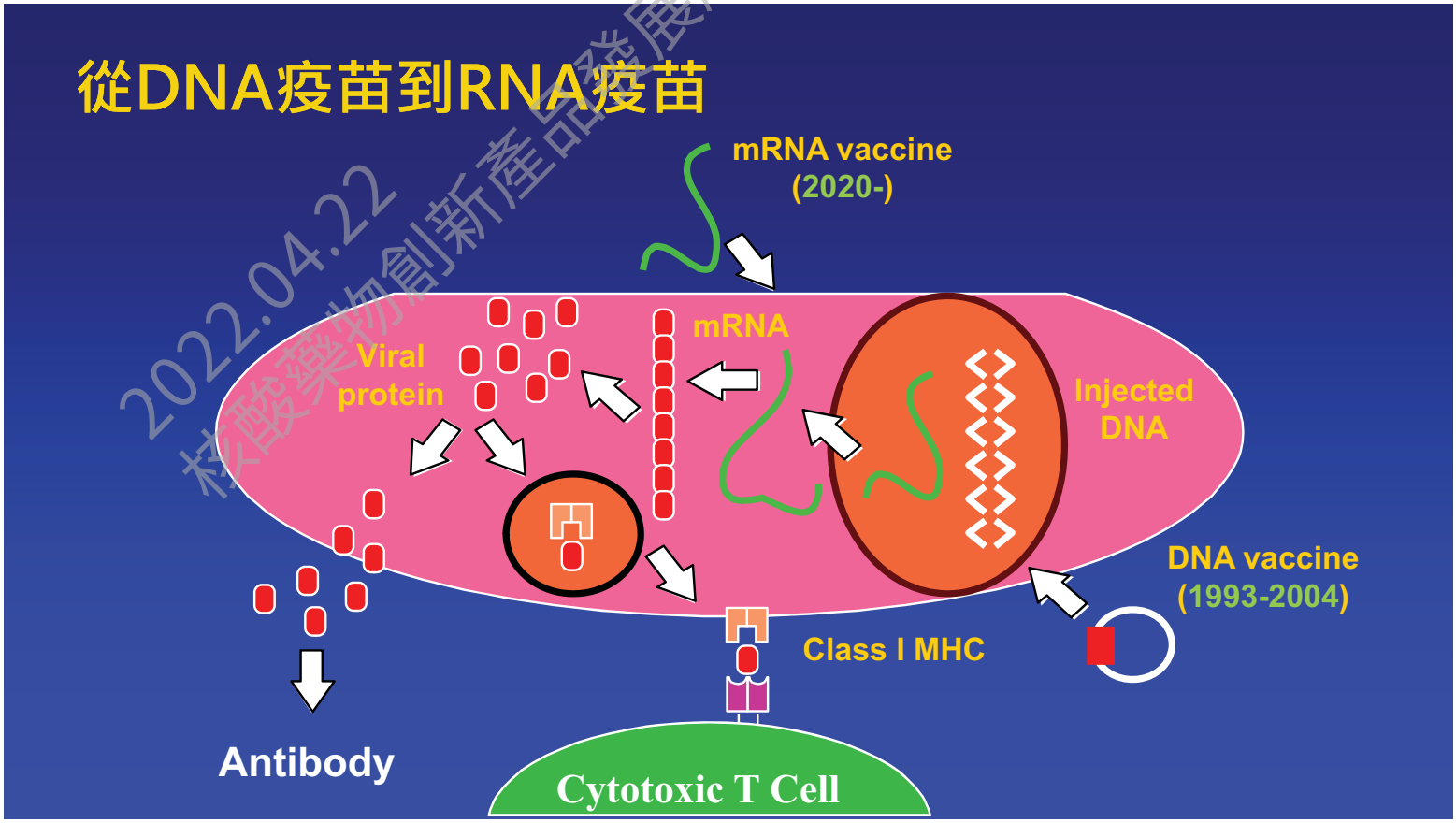
- Whole-inactivated virus
- Live-attenuated virus
- Protein subunit
- Virus-like particle

次世代疫苗

- Viral vector
- DNA
- RNA
- Dendritic cells

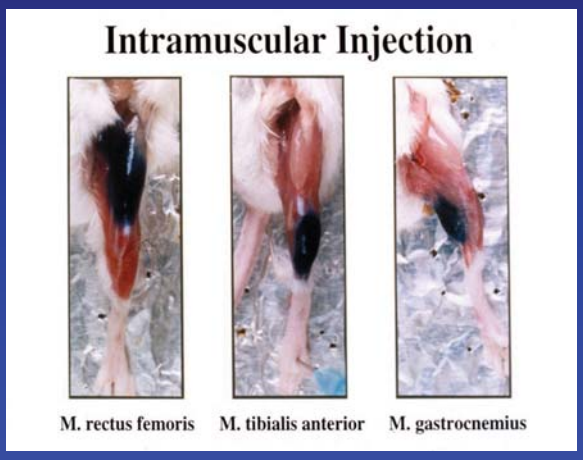


Van Riel, (2020) Nat Master

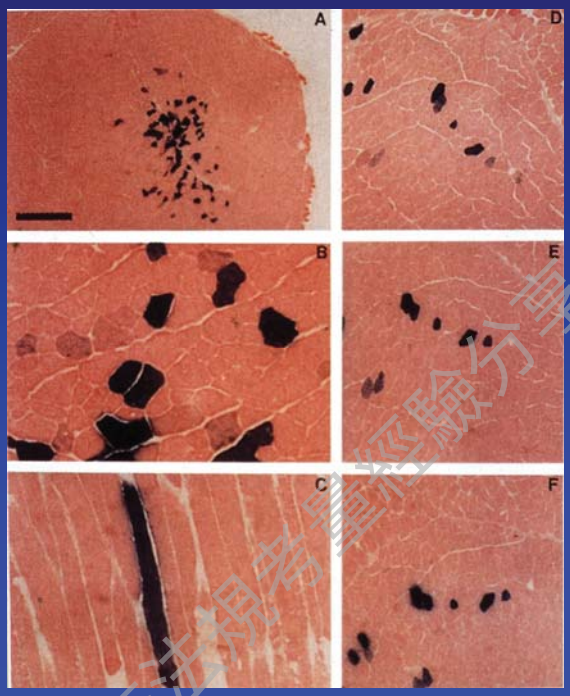


肌肉注射Plasmid DNA表現Luciferase Reporter

India Ink



Luciferase plasmid



DNA Vaccine Publications

nature medicine
ARTICLES
Immunoprophylaxis of allergen-induced immunoglobulin E synthesis and airway hyperresponsiveness *in vivo* by genetic immunization

Hepatology
DNA-Based Immunization Produces Th1 Immune Responses to Hepatitis Delta Virus in a Mouse Model

JOURNAL OF MEDICAL VIROLOGY
Co-Delivery of GM-CSF Gene Enhances the Immune Responses of Hepatitis C Viral Core Protein-Expressing DNA Vaccine: Role of Dendritic Cells

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology
Improvement of Hepatitis B Virus DNA Vaccines by Plasmids Coexpressing Hepatitis B Surface Antigen and Interleukin-2

Virology Journal
Limitations of *in Vivo* IL-12 Supplementation Strategies to Induce Th1 Early Life Responses to Model Viral and Bacterial Vaccine Antigens

Molecular Therapy
Therapeutic HER2/Neu DNA Vaccine Inhibits Mouse Tumor Naturally Overexpressing Endogenous Neu

The Journal of Immunology
This information is current as of December 14, 2018.
Development of Th1 and Th2 Populations and the Nature of Immune Responses to Hepatitis B Virus DNA Vaccines Can Be Modulated by Codelivery of Various Cytokine Genes
Yen-Hung Chow, Bor-Luen Chiang, Yueh-Lun Lee, Wei-Kuang Chi, Wen-Chang Lin, Yen-Teen Chen and Mi-Hua Tao

The Journal of Immunology
This information is current as of December 14, 2018.
Suppression of Immune Response and Protective Immunity to a Japanese Encephalitis Virus DNA Vaccine by Coadministration of an IL-12-Expressing Plasmid
Hsin-Wei Chen, Chien-Hsiung Pan, Hwei-Wen Huan, Ming-Yi Liau, Jen-Ron Chiang and Mi-Hua Tao

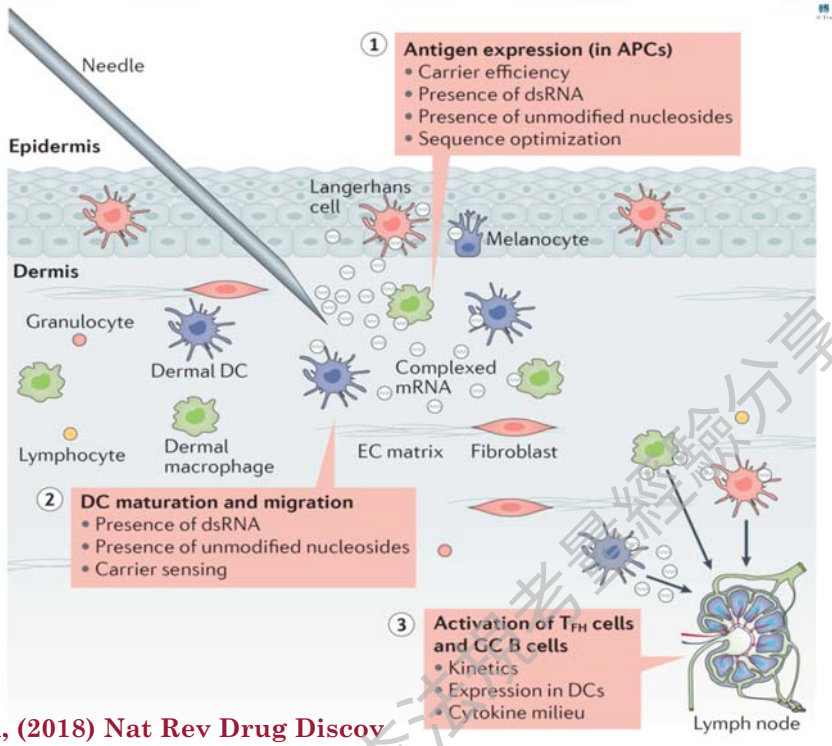
Vaccine
In vivo electroporation of skeletal muscles increases the efficacy of Japanese encephalitis virus DNA vaccine

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology
Screening of Protective Antigens of Japanese Encephalitis Virus by DNA Immunization: a Comparative Study with Conventional Viral Vaccines

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology
Protective Mechanisms Induced by a Japanese Encephalitis Virus DNA Vaccine: Requirement for Antibody but Not CD8+ Cytotoxic T-Cell Responses

核酸疫苗引發高價抗體反應的關鍵因素

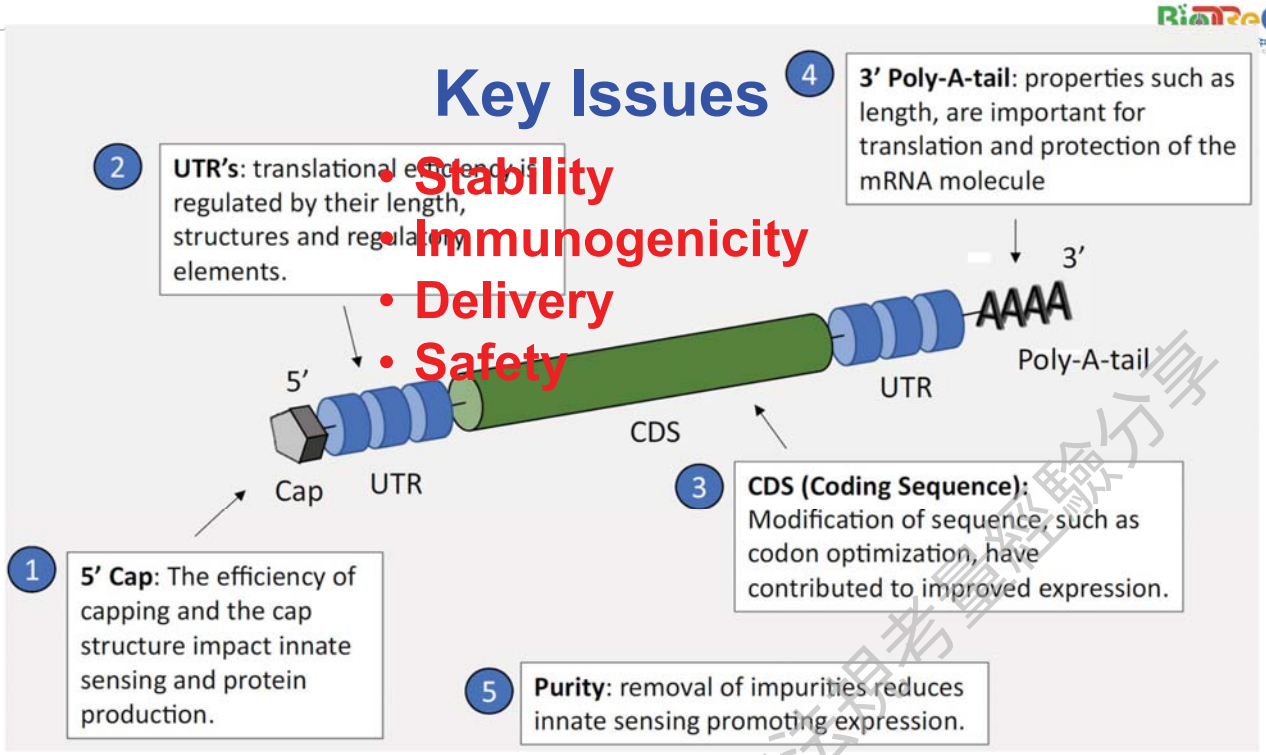
- 抗原(antigen)表現
- 抗原呈現細(dendritic cells)胞成熟、轉移到淋巴結
- 活化T Follicular Helper (T_{FH}) cells和Germinal Center (GC) B cells



Pardi et al, (2018) Nat Rev Drug Discov

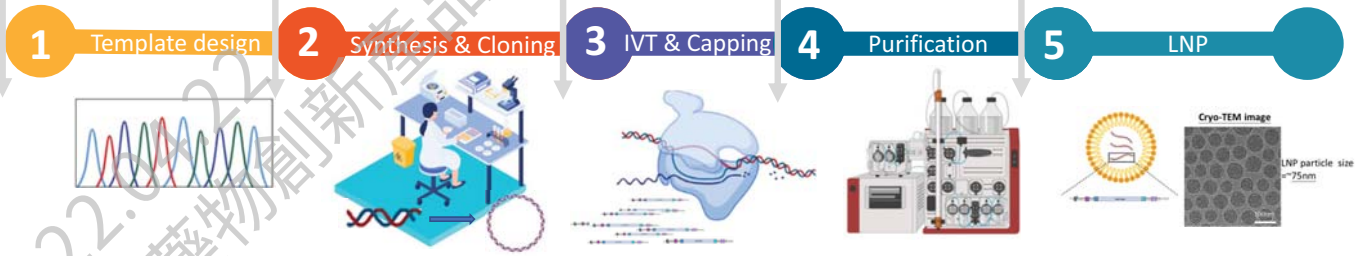
練兵 (2020.12 -2021.05)
武漢病毒株

影響mRNA疫苗效力的關鍵因素

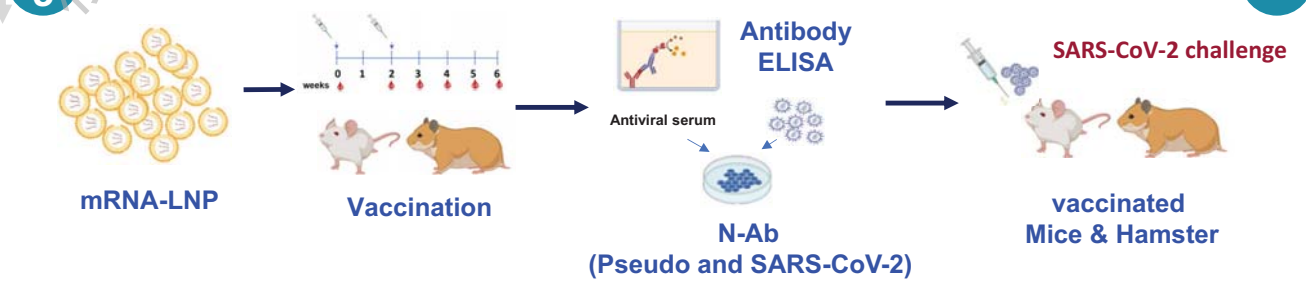


Jackson et al, NPJ Vaccines

在中研院建立mRNA疫苗技術

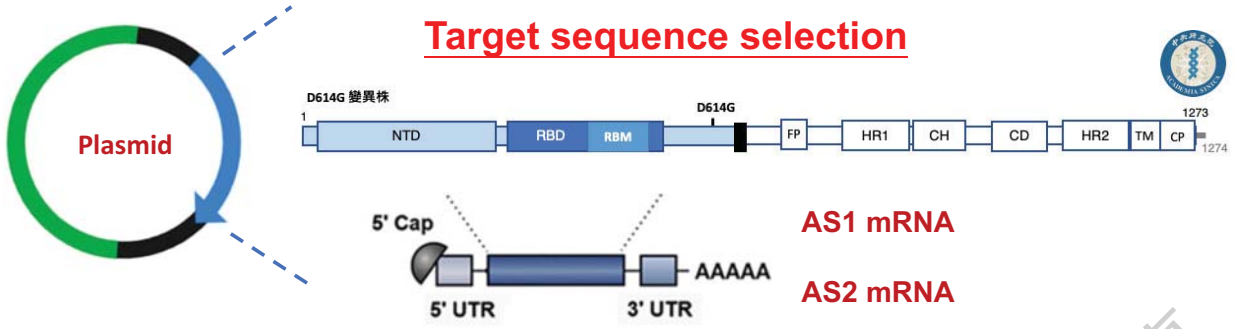


Proof of concept study : immunogenicity & protection

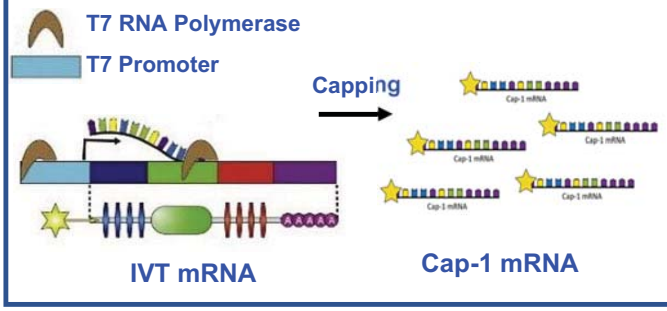


製造mRNA-LNP

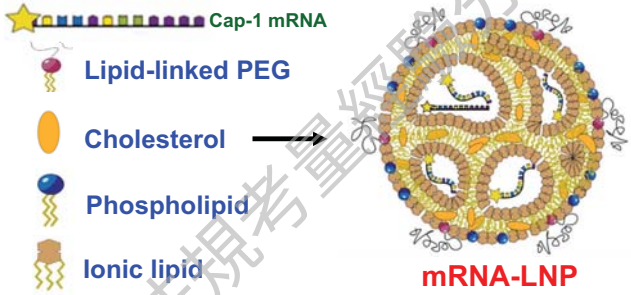
Target sequence selection



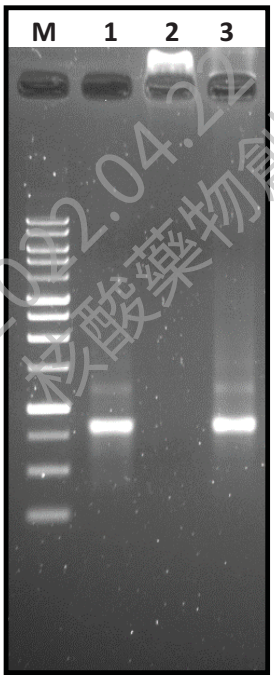
IVT mRNA production and capping



mRNA-lipid nanoparticle complex



中研院mRNA-LNP包覆率高且RNA結構完整



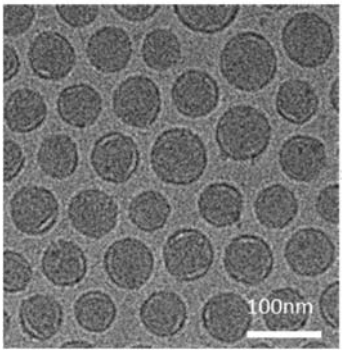
Agarose gel retardation assay of AS1 mRNA-LNP

1. ASMR1 mRNA

2. ASMR1 mRNA-LNP

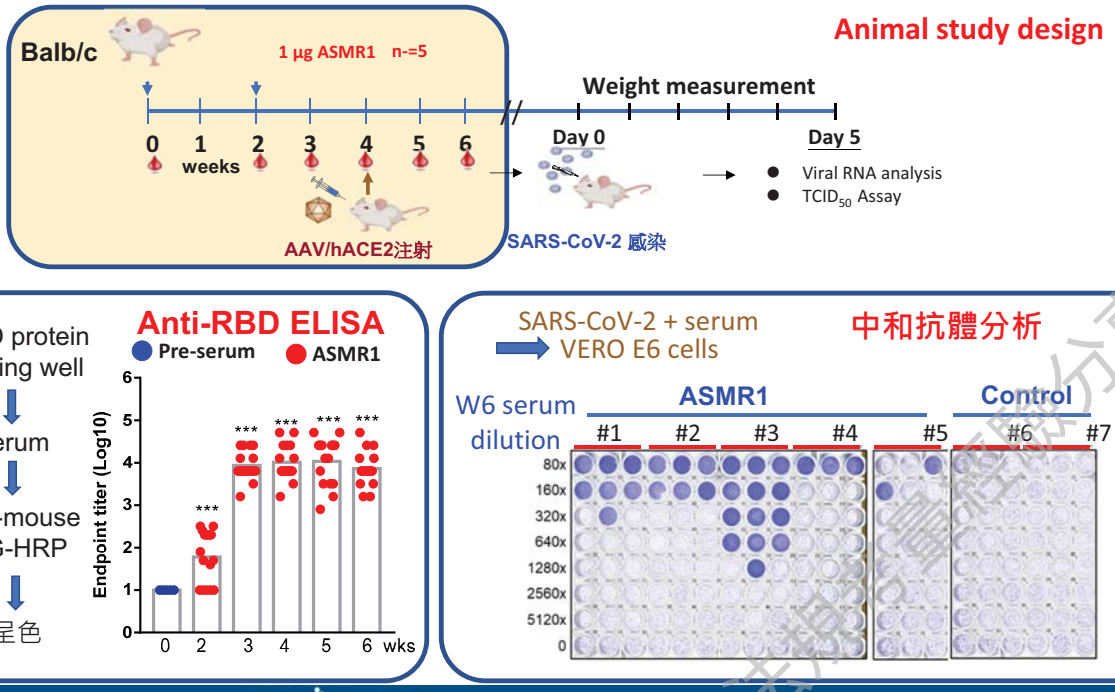
3. ASMR1 mRNA-LNP + triton-100

Cryo-TEM image

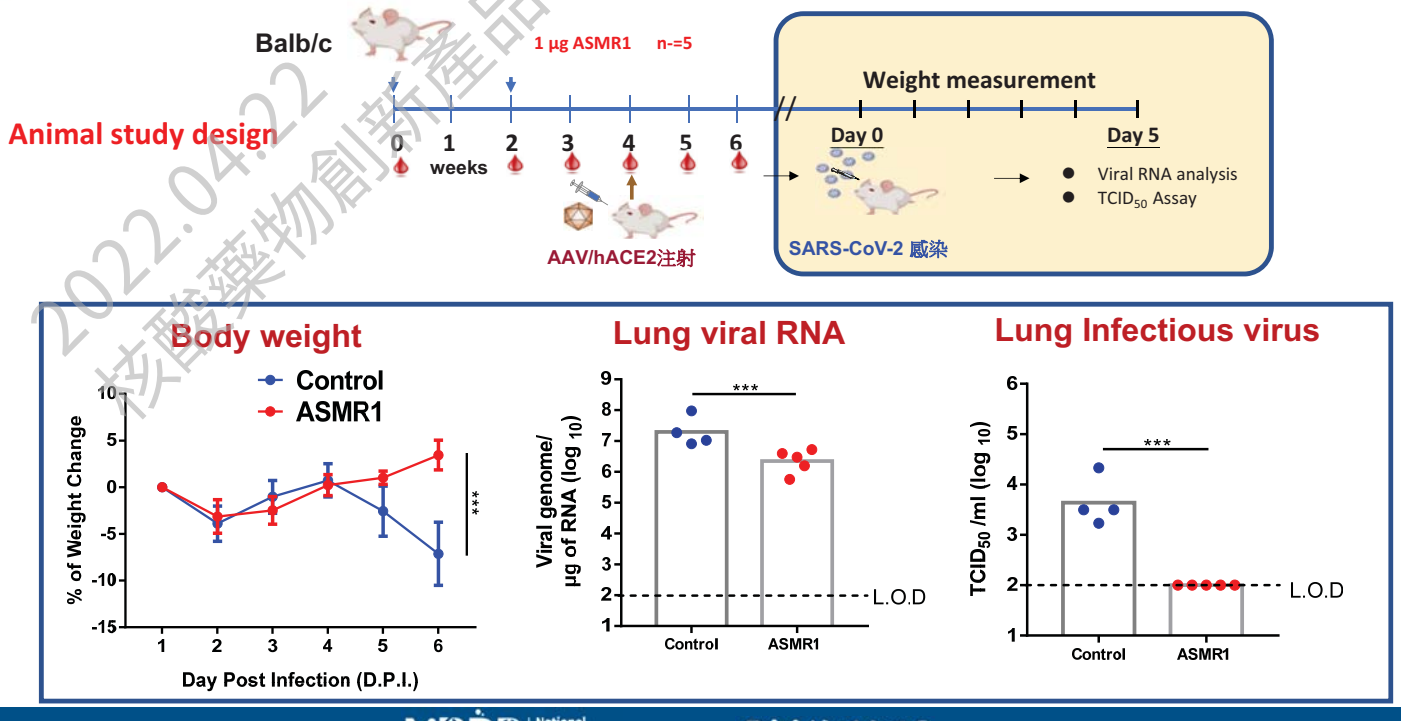


LNP Particle size =~ 75 nm

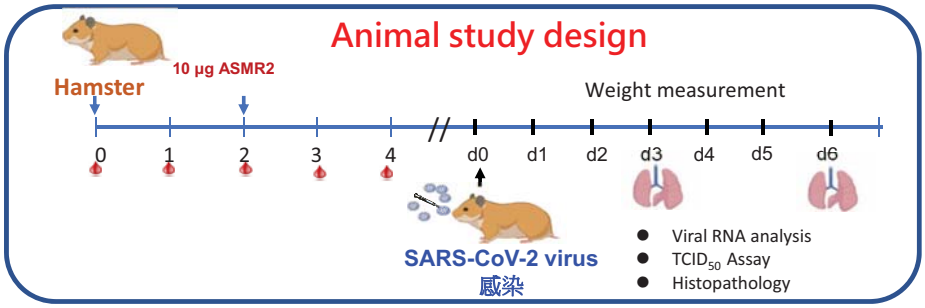
ASMR1 疫苗在小鼠產生高效價中和抗體

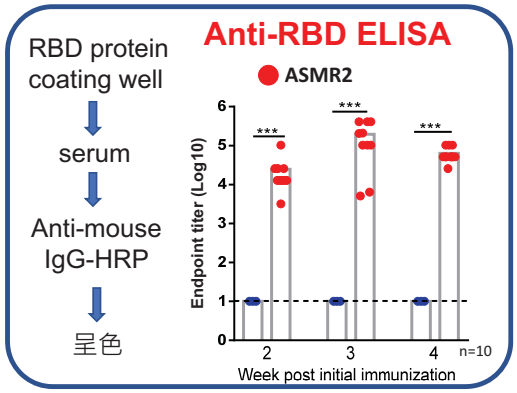


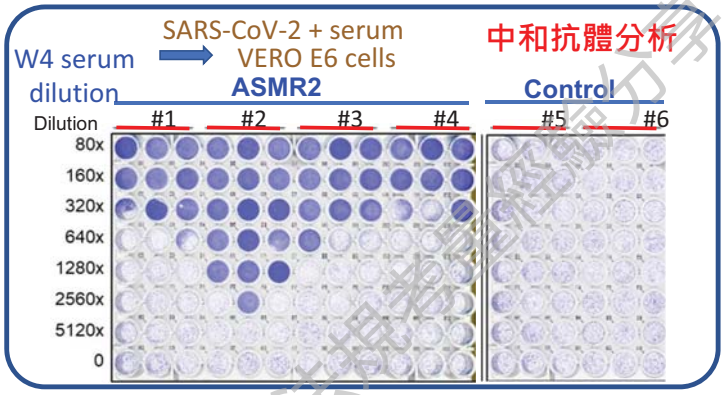
ASMR1 mRNA 疫苗保護小鼠抵抗新冠病毒感染



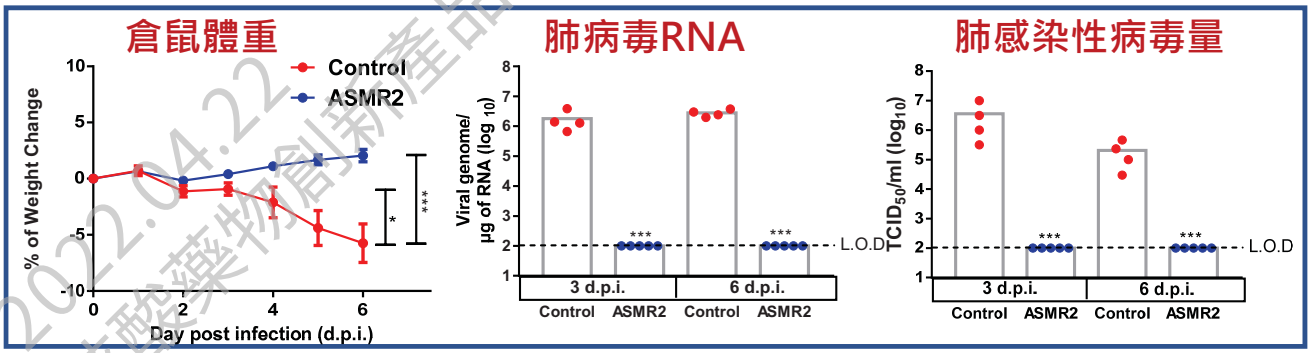
ASMR2 疫苗在倉鼠產生高效價中和抗體

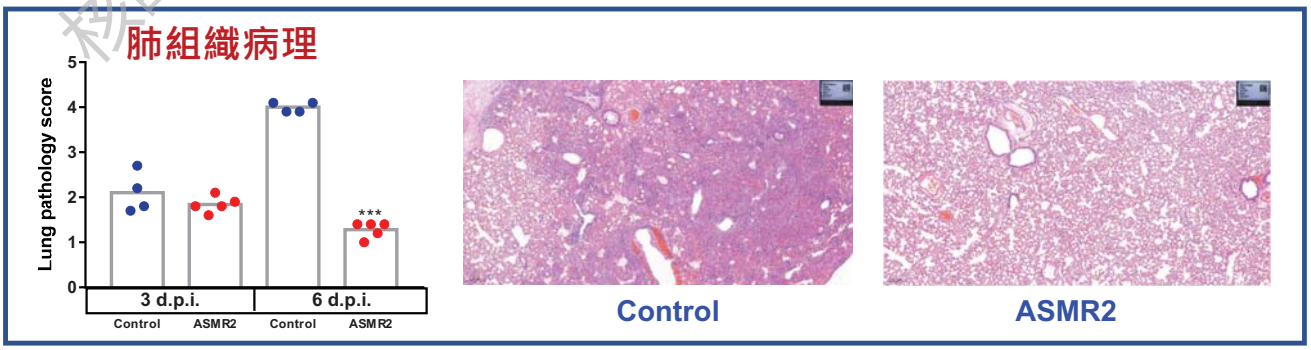






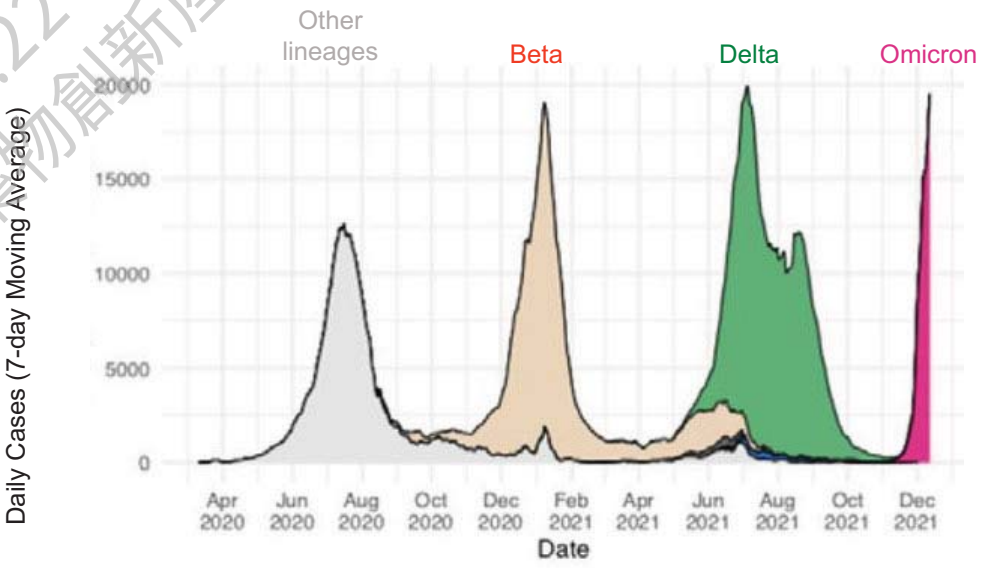
ASMR2 mRNA 疫苗保護倉鼠抵抗新冠病毒感染



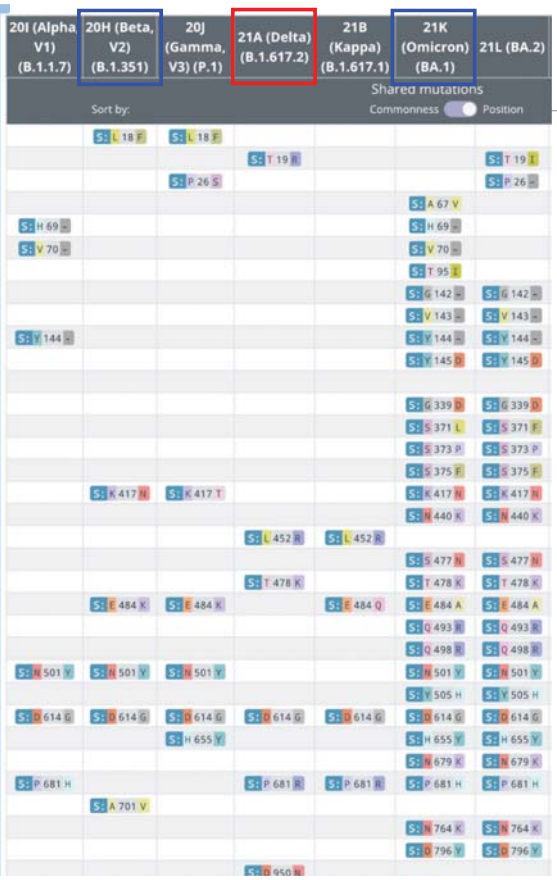


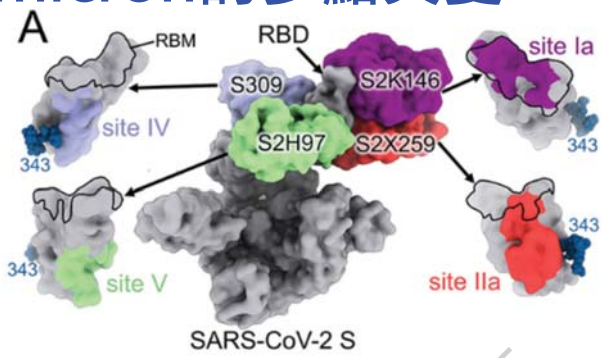
實戰演習(2021.12 -2022.01)
Omicron的挑戰

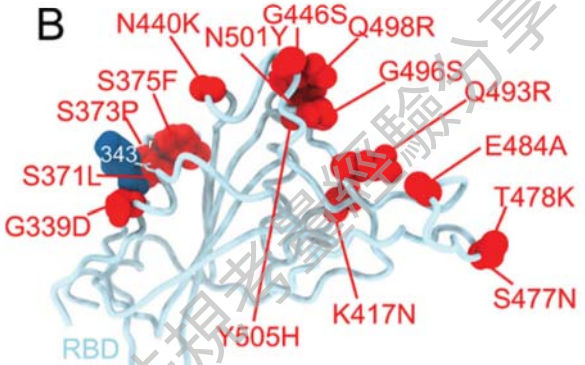
來自南非的Omicron橫掃全球



Omicron的多點突變

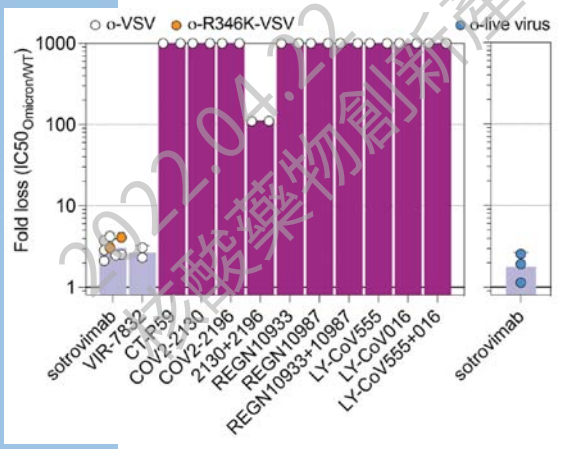


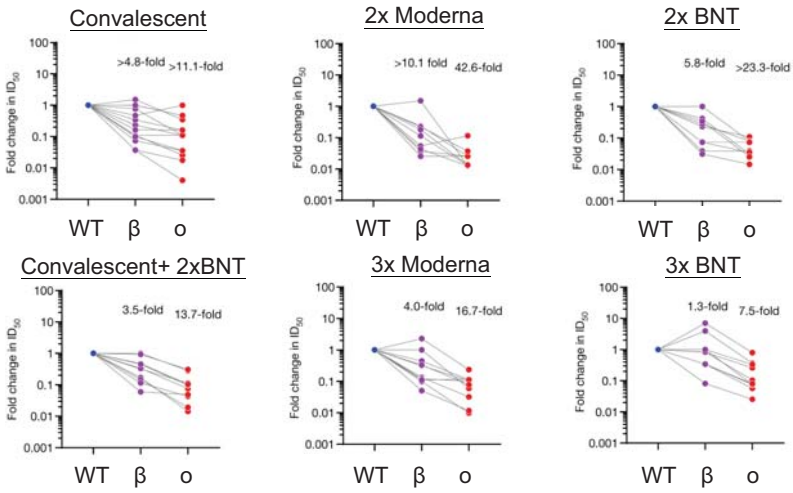




McCallum et al, (2022) Science

現有的抗體藥物和疫苗對Omicron效果大幅降低





(Carreno, et al., 2021, ***Nature***)

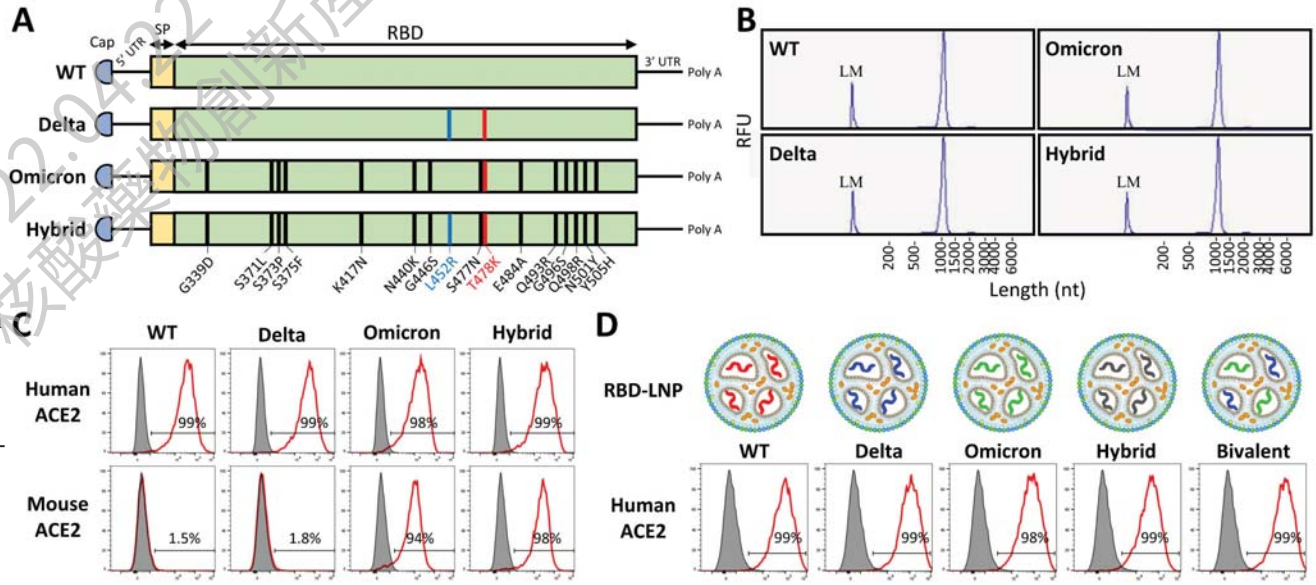
- Only Sotrovimab, a broadly neutralizing sarbecovirus mAbs recognizing antigenic sites outside the RBM retained neutralizing activity
- Omicron efficiently evades antibodies from infected or 2x mRNA vaccinated individuals
- 3x dose booster partially restored nAb titers

(Cameroni, et al., 2021, ***Nature***)

以Omicron變異株為標的演練中研院團隊應付未來緊急疫情時開發疫苗的能力和速度

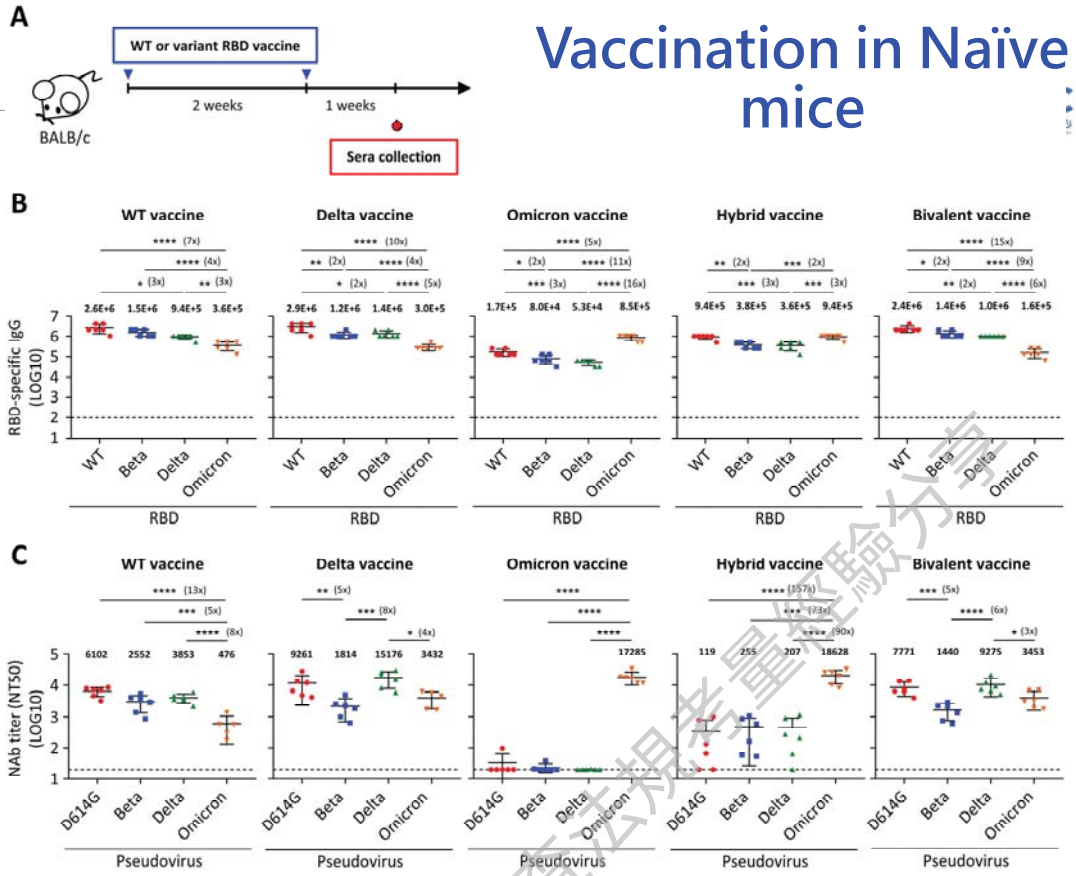
- 2021/11/26WHO宣布Omicron為新的VOC變種病毒，病毒基因體序列公布。
- 2021/12月初中研院團隊開始Omicron mRNA疫苗開發計畫。
- 2星期完成疫苗模板(plasmid template)的製作。
- 1星期完成mRNA疫苗(mRNA-LNP, 脂質奈米粒)生產。
- 2021/12/24在小鼠進行第一次疫苗注射。
- 2022/1/7在小鼠進行第二次疫苗注射。
- 2022/1/14採血分析中和抗體。
- 2022/1/28完成第一版的preprint manuscript，投稿bioRxiv。
- 2022/1/31搶先全球在bioRxiv發表第一例針對Omicron變異株的次世代mRNA疫苗。
- 2022/2/3 ~ 2/15搶陸續有6篇論文發表在bioRxiv
- 2022/2/14, 18 Nature (News), Nature Reviews Microbioogy引用我們的論文
- 2022/2/22完成實驗資料補充，投稿正式期刊。

RBD mRNA constructs and RBD-LNP vaccines

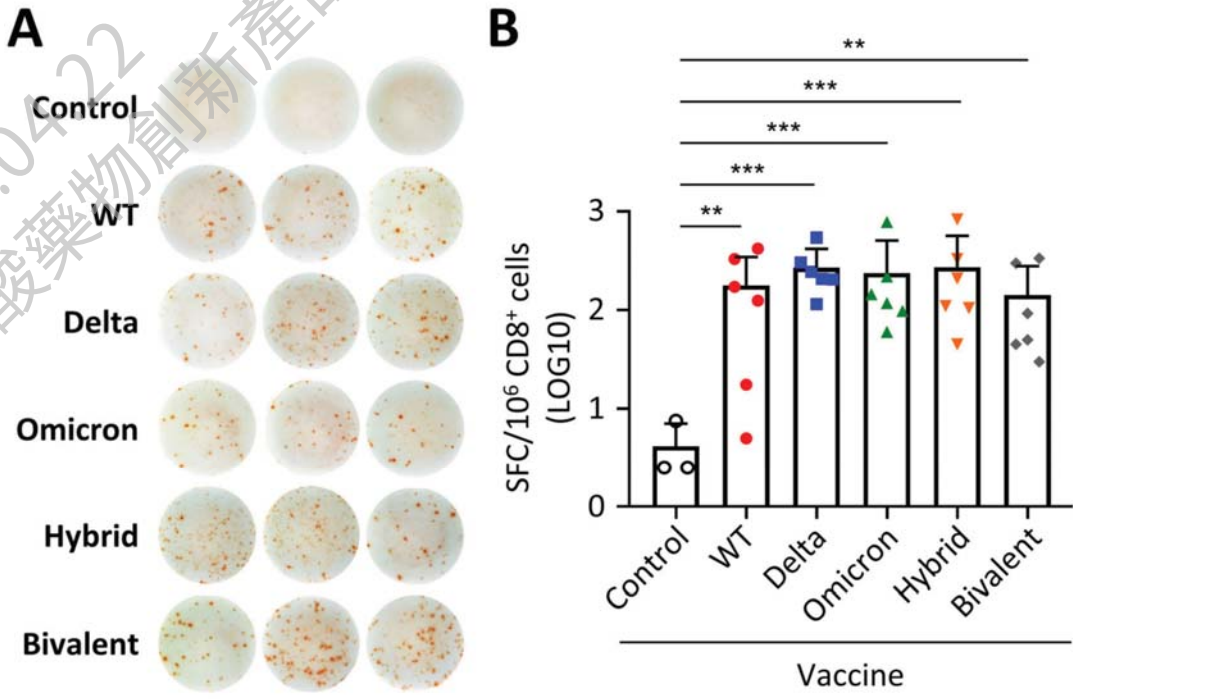


RNA transfection
↓
Collect sup.
↓
Applied to ACE2-expressing cells
↓
polyclonal Ab against all RBDs
↓
FACS

Vaccination in Naïve mice

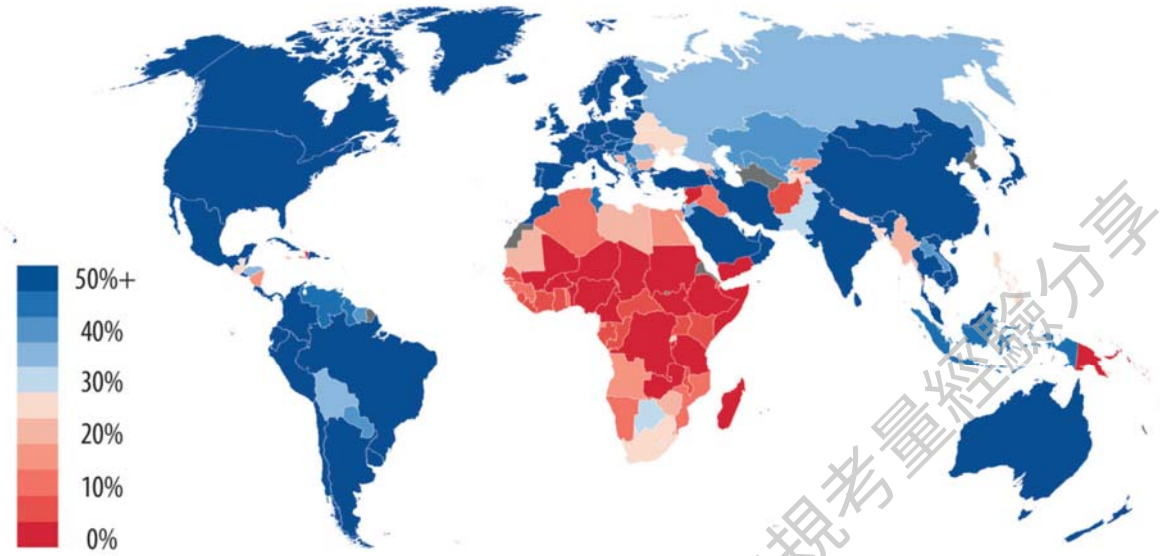


不同變異株mRNA疫苗都能引發T細胞免疫反應

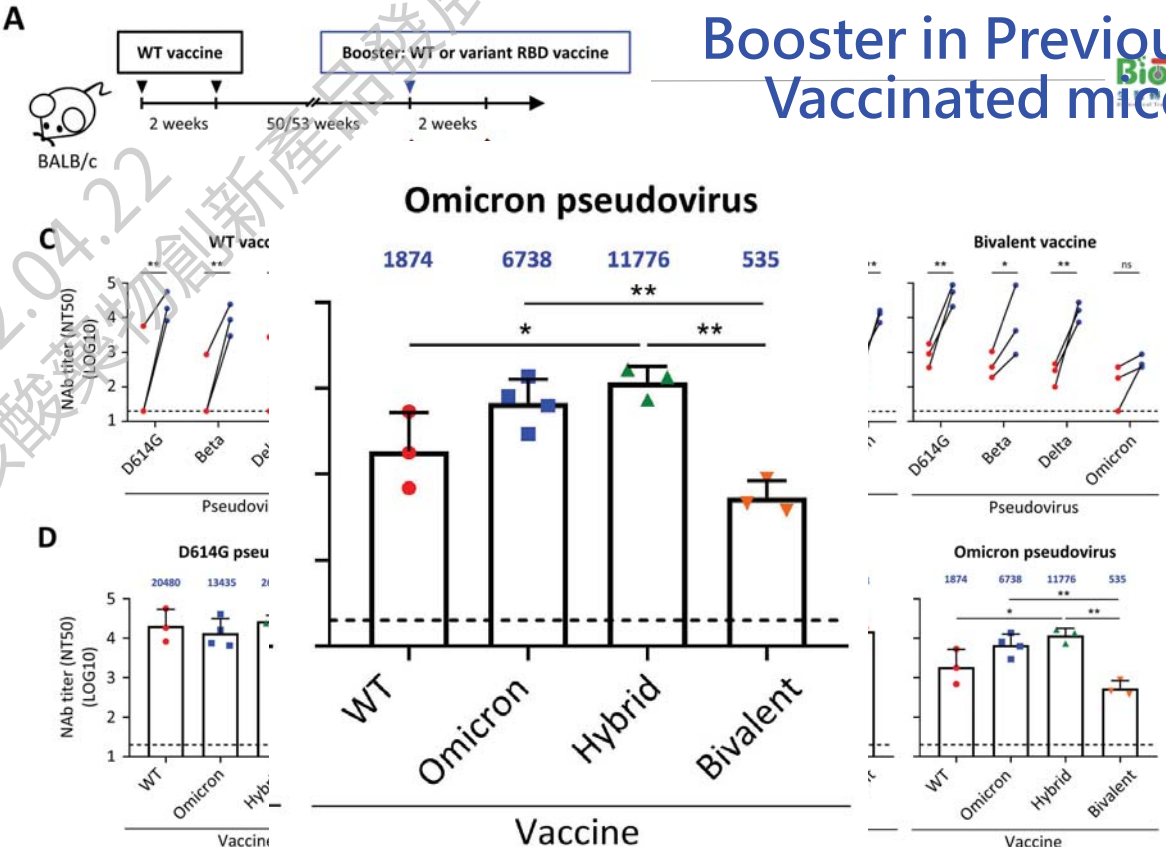


全球COVID-19疫苗覆蓋率(至少一劑) 2022.12

Picture 2: Vaccine coverage—at least one dose administered (percent of population)



Booster in Previously Vaccinated mice



Omicron mRNA疫苗的相關論文

Date	Authors	Institute	Major findings
1/31	Lee IJ/Tao MH	Academia Sinica	Omicron-specific mRNA vaccine induced potent neutralizing antibody against Omicron but not other SARS-CoV-2 variants https://doi.org/10.1101/2022.01.31.478406
2/3	Hawman D./Erasmus J.	NIH/ University of Washington School of Medicine	Replicating RNA platform enables rapid response to the SARS-CoV-2 Omicron variant and elicits enhanced protection in naïve hamsters compared to ancestral vaccine https://doi.org/10.1101/2022.01.31.478520
2/4	Gagne M./Seder R.	NIH/Moderna	mRNA-1273 or mRNA-Omicron boost in vaccinated macaques elicits comparable B cell expansion, neutralizing antibodies and protection against Omicron https://doi.org/10.1101/2022.02.03.479037
2/7	Zang J/Huang Z	Institute Pasteur of Shanghai, CAS	An mRNA vaccine candidate for the SARS-CoV-2 Omicron variant https://doi.org/10.1101/2022.02.07.479348
2/9	Ying B/Diamond M.	Washington University School of Medicine/Moderna	Boosting with Omicron-matched or historical mRNA vaccines increases neutralizing antibody responses and protection against B.1.1.529 infection in mice https://www.biorxiv.org/content/10.1101/2022.02.07.479419v1
2/14	Zhang N./Qin C.	Academy of Military Medical Sciences	Rapid development of an updated mRNA vaccine against the SARS-CoV-2 Omicron variant. LETTER TO THE EDITOR_Cell Research
2/15	Fang Z./Chen S.	Yale University School of Medicine	SARS-CoV-2 Omicron-specific mRNA vaccine induces potent and broad antibody responses in vivo https://www.biorxiv.org/content/10.1101/2022.02.14.480449v1

挑戰(2021.05-迄今)
mRNA製程/申請試驗中新藥IND / 開發自有專利技術

發展策略

- 發展新技術平臺開發核酸疫苗
- 引領台灣mRNA產業發展, 肩負國家重點科技布局之大任

●利用現有技術發展核酸疫苗

- 建立平台並且全盤部局，了解未來產業化各面向
- 應付未來不時之需(防疫及國安問題)
- 具技轉潛能

●開發新智財權

- 新脂質奈米微粒配方 (New cationic lipid)
- 新型核酸遞送系統 (New biomaterials)
- 新生產製程技術 (Microfluidic reactor system)
- mRNA新適應症開發:癌症治療, 傳染性疾病，其他疾病 …etc

●與業界商討新創公司或技轉

mRNA先導研究設施

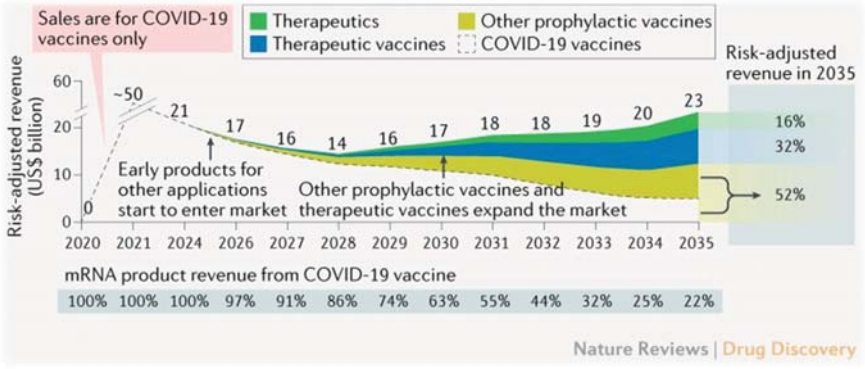
轉譯中心已著手於國家生技研究園區建置符合GMP標準與精神之mRNA先導研究設施，預計於111年完成硬體建置。

- 為發揮mRNA疫苗快速應變之特性，並解決以往新藥在研發端與製程端之技術斷層，於研發時期同步同步考量後續量產之法規規範與技術背景，突破製程關鍵技術。
- 建立以液相層析、微流道技術為主軸的製程，進行mRNA疫苗研發，使研發與產製銜接順暢，縮短應變期程。
- 供應mRNA疫苗臨床一期和二期試驗規模。

國家生技研究園區

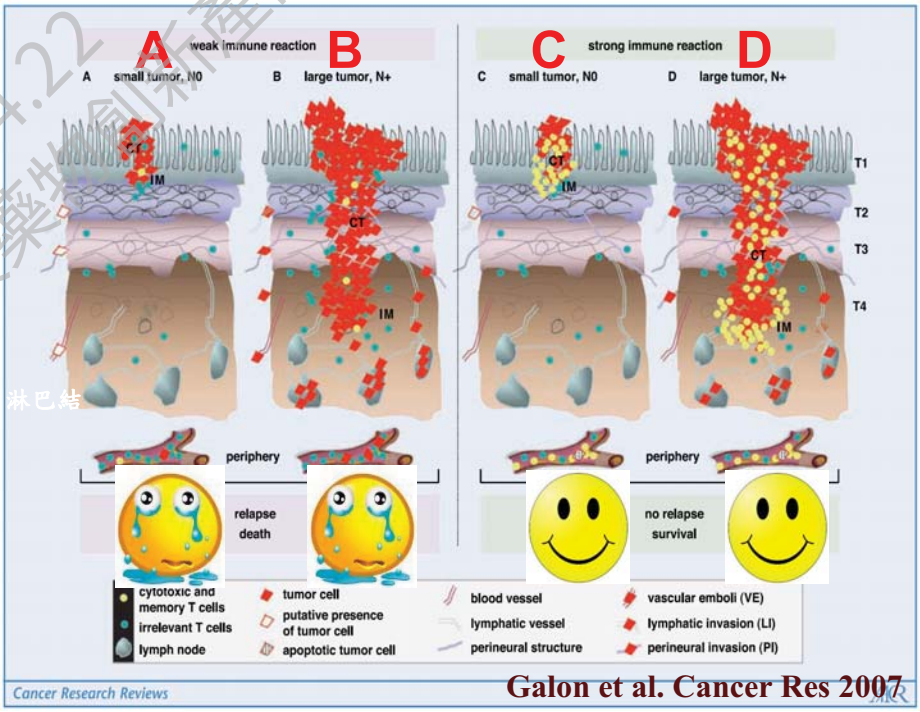
mRNA技術的市場估值逐年成長

- 只看短期，著重眼前的效益 – 僅評估Covid-19 mRNA Vaccine的效益
- 放遠眼光、掌握時機及堅持 – 評估mRNA技術是否為未來發展主軸



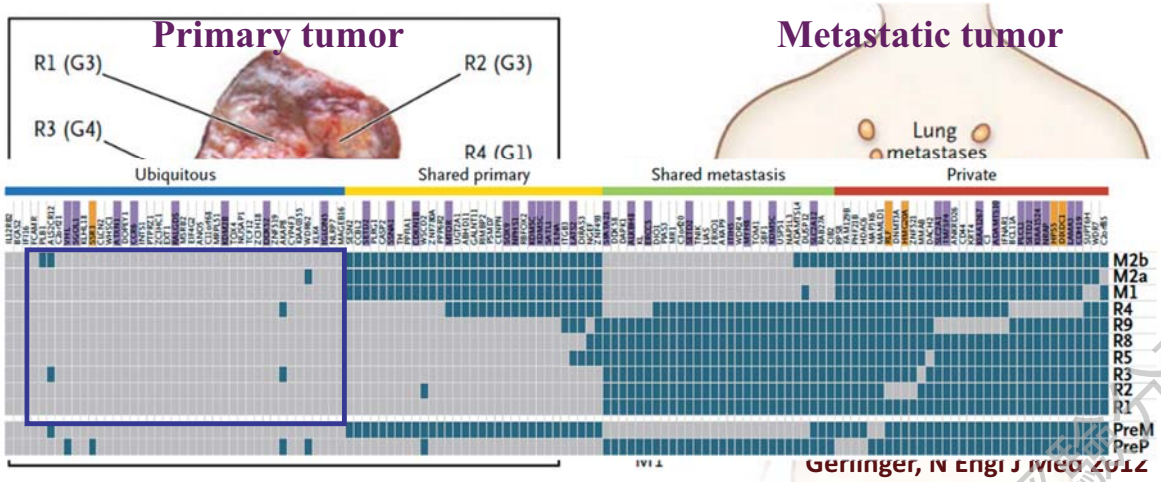
- 短期內，mRNA 產品主要為COVID-19 疫苗的銷售， 2021 年其價值超過 500 億美元。
- 2022-2024 全球廣泛進行加強注射，預計可達 200 億美元的銷售額，但 COVID-19 疫苗逐年需求減少，因此其銷售額預計逐年遞減。
- 預防性疫苗和治療性藥物預計於2028 年開始增長，到 2035 年達到 230 億美元。

大腸癌病人治療的效果，決定於治療前腫瘤內的免疫細胞(T細胞)數目



Galon et al. Cancer Res 2007

Primary and Metastatic Tumors Have Shared Mutations (Tumor Antigens)

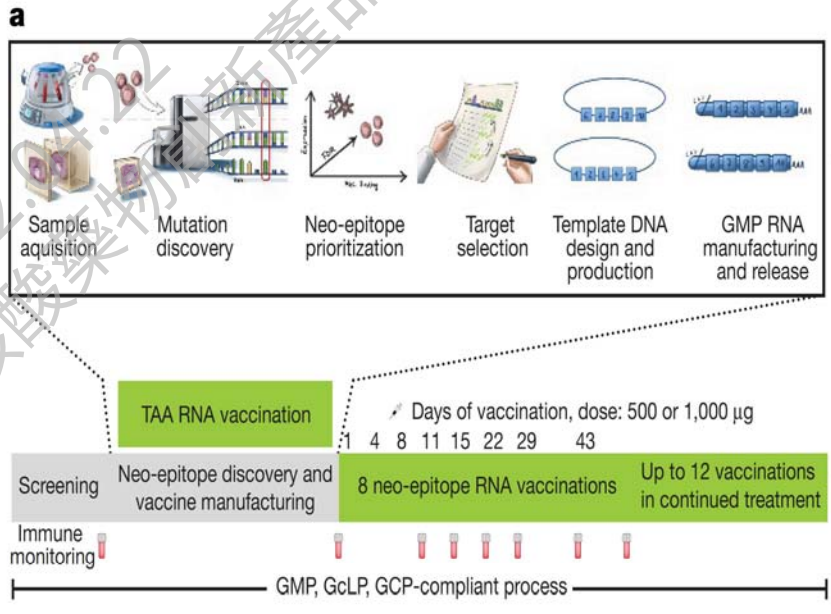


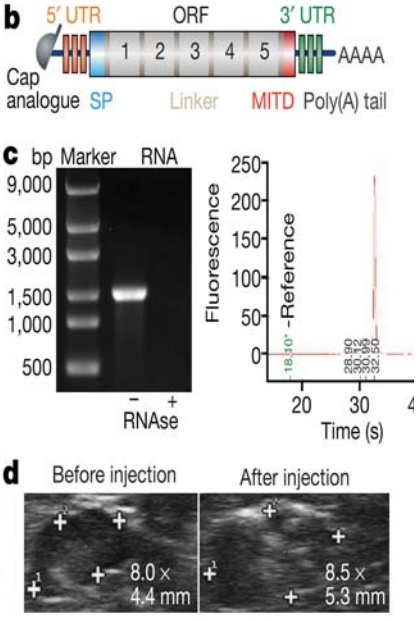
Gerlinger, N Engl J Med 2012

1. NGS was applied to sequence tumor tissues from primary renal cell carcinoma located at different regions as well as samples from their metastases.
2. In the 101 mutations, some shared by primary tumors; some shared by metastatic tumors; some are shared by both primary and metastatic tumors.
3. In theory, anti-tumor T cells induced by treatment of primary tumors may be able to recognize and control both primary tumors and their metastases.

Neoantigen mRNA Vaccine Design and Manufacturing

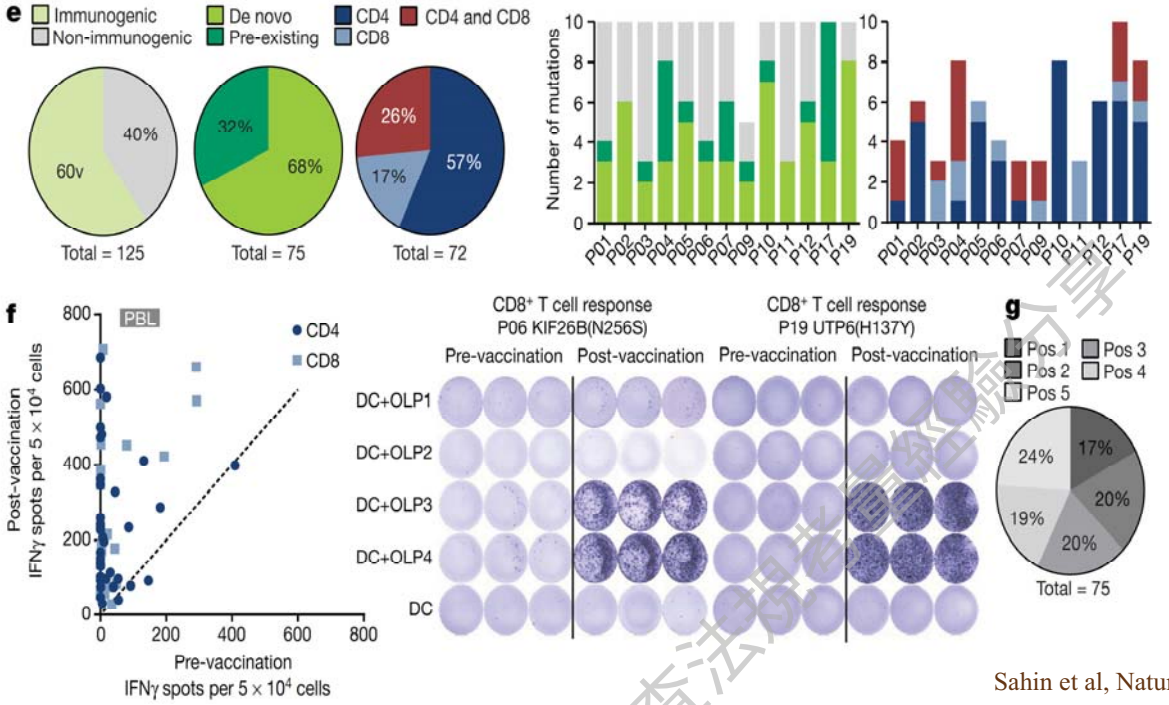
BioReC





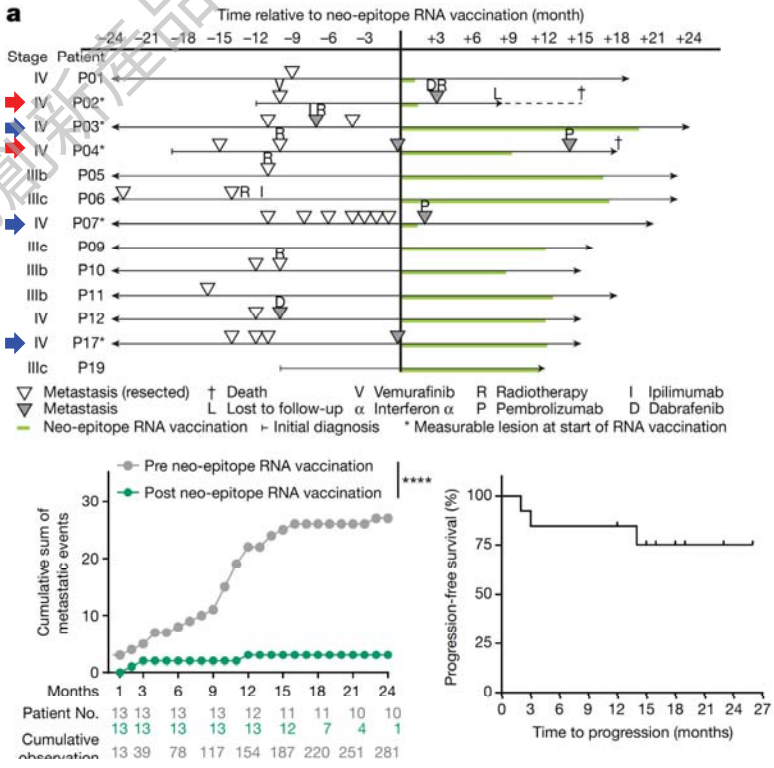
Sahin et al, Nature 2017

CD4+ and CD8+ T Cells induced by Neoantigen mRNA Vaccine



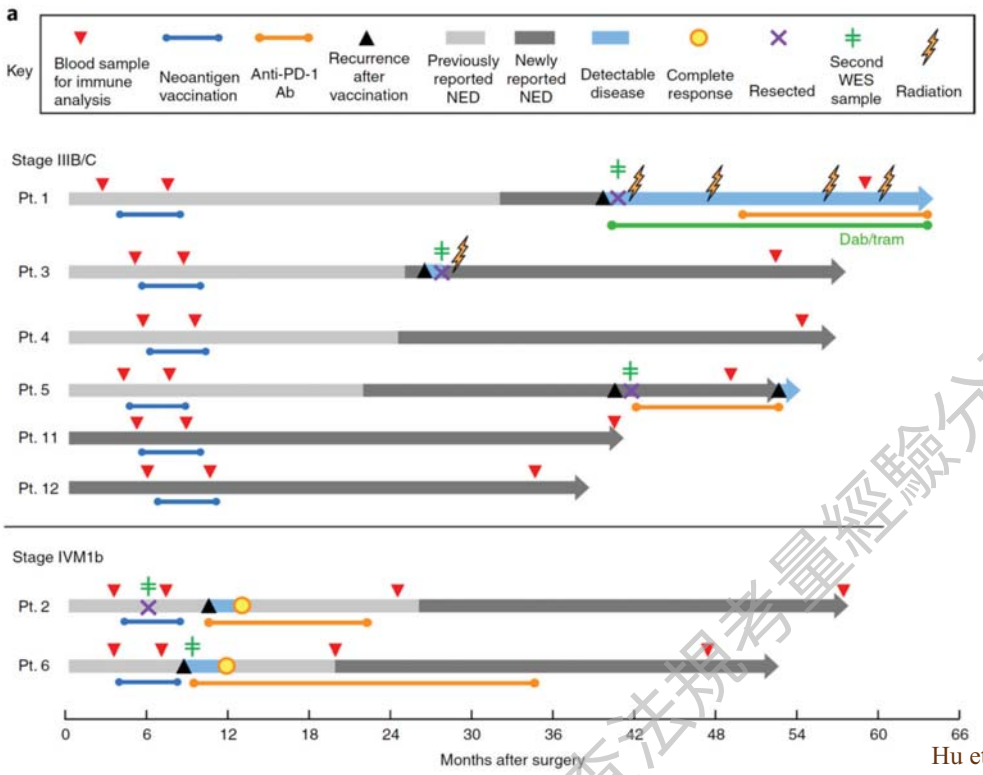
Sahin et al, Nature 2017

mRNA Neoantigen Vaccine Control Disease of High Risk Melanoma Patients.



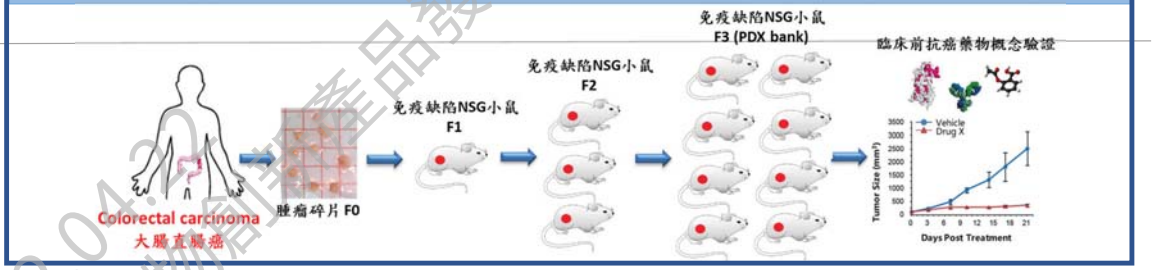
Sahin et al, Nature 2017

Neoantigen Vaccine Induced Long-term Clinical Effect



Hu et al, Nat Med 2021

人源化腫瘤異種移殖 (PDX) 動物模型

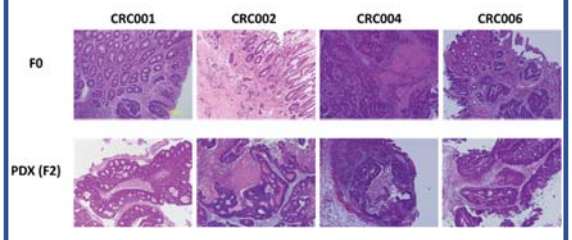


人源化大腸直腸腫瘤異種移植

病患基本資料及完整異種移植資料及精準畫基因定序

Case No.	Age	Location	Stage (AJCC7)	Tumor formation rate (%) F1	Tumor formation rate (%) F2	Analysis	F0	F1
CRC001 (M)	67	Sigmoid colon	3	60 (3/5)	100 (5/5)	WES	✓	✓
						RNAseq	✓	✓
CRC002 (M)	59	Descending colon	4	17 (1/6)	80 (4/5)	WES	✓	✓
						RNAseq	✓	✓
CRC003 (M)	60	Ascending colon	4	43 (3/7)	100 (3/3)	WES	✓	✓
						RNAseq	✓	✓
CRC004 (F)	52	Rectum	3	83 (5/6)	100 (7/7)	WES	✓	✓
						RNAseq	✓	✓
CRC005 (M)	77	Sigmoid colon	2	60 (3/5)	100 (7/7)	WES	✓	✓
						RNAseq	✓	✓
CRC006 (F)	63	Rectum	3	29 (2/7)	100 (4/4)	WES	✓	✓
						RNAseq	✓	✓
CRC007 (M)	84	Cecal	4	X	X	WES	✓	X
						RNAseq	✓	X
CRC008 (F)	41	Sigmoid colon	3	X	X	WES	✓	X
						RNAseq	✓	X
CRC009 (M)	65	Ascending colon	3	25 (2/8)	100 (4/4)	WES	✓	✓
						RNAseq	✓	✓
CRC010 (M)	70	Splenic flexure	2	20 (1/5)	100 (3/3)	WES	✓	✓
						RNAseq	✓	✓

專業病理切片



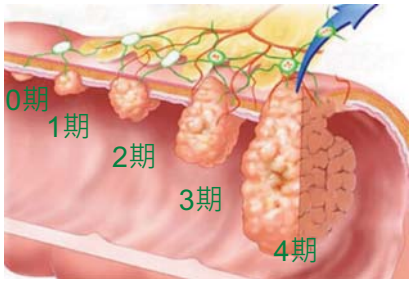
F2代PDX腫瘤病理切片保留了患者初代腫瘤組織之病理特徵

缺點/優點

- 具有人體腫瘤異質性
- 與原發腫瘤的分子、組織病理一致
- 需長時間建立癌症動物模式
- 可以預測臨床結果, 提供可靠臨床前資料

癌症疫苗用於預防原位癌和轉移癌復發

原位大腸癌



治療早期癌 → 局部治療

治療晚期癌 → 免疫組合療法 (ICI, CAR/TCR T cells)

+ 放射治療
+ 標靶治療
+ 手術切除
+ 化學治療

病人專屬癌症疫苗 → 腫瘤抗原專一性T細胞

每個曾經罹癌病人
都需要量身訂做癌症疫苗
預防轉移癌和原位癌復發

致謝

生醫所陶秘華老師實驗室
李逸容 小姐
孫承溥 博士
吳品逸 小姐
藍玉樺 小姐
曾聖哲 先生

中研院mRNA團隊
廖俊智 院長
吳漢忠 主任
李宗洲 總監
張育瑋 博士
林音秀 博士
鄭昭旻 博士

細生所吳漢忠老師實驗室
Kumari 博士

基因體中心暨P3動物實驗室
詹家琮 老師
馬秀華 小姐

生醫所P3實驗室
林宜玲 老師
廖俊哲 博士
梁健忠 博士

AAV核心設施
陳庭忻 小姐
陳亮諭 先生

BioTReC P2&P3核心設施
劉玟君 博士

Thank you for your attention!

敬請指教

國家生技研究園區
National Biotechnology Research Park

48

核酸藥物關鍵修飾技術開發經驗分享

張嘉銘董事長
法信諾生醫股份有限公司

張嘉銘 Jia-Ming Chang

現職:

法信諾生醫股份有限公司 **董事長/總經理**

經歷: 生物技術開發中心 藥物平台技術研究所 **所長**

新北市生物科技產業發展聯盟 **第二屆理事**

衛福部基改食品審查委員會 **諮議委員**

衛福部中華藥典 **第九版編修委員**

經濟部國家標準局 **標準制定委員**

TAF ISO 醫學實驗室 **技術審查委員**

國防醫學院 病理及寄生蟲研究所 **兼任助理教授**

國防醫學院 醫學科學研究所 **兼任助理教授**

國立交通大學 應用化學系 **兼任助理教授**

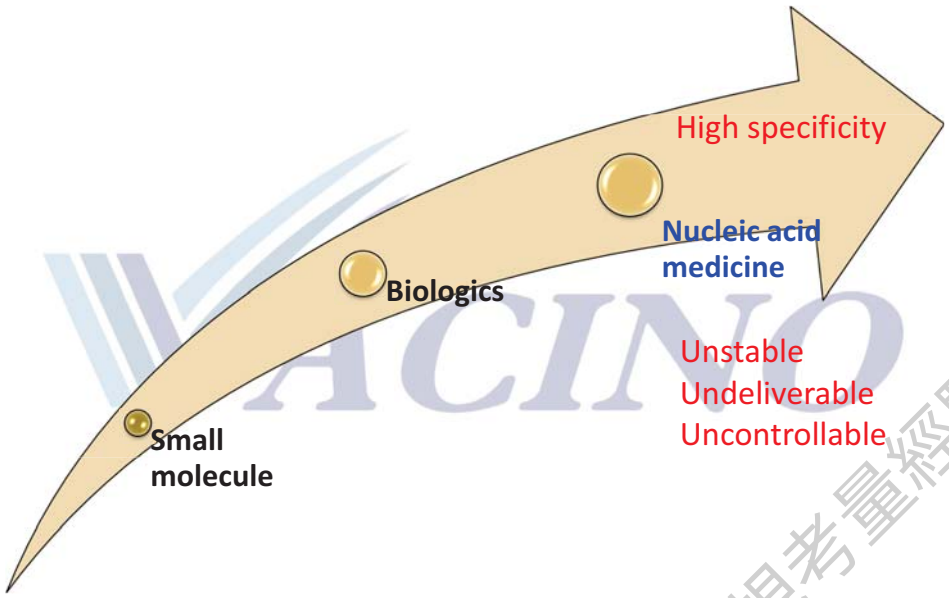
學術成就: 發表同儕審查國際論文 **32** 篇，**20** 項國內外專利核准，專書章節 **5** 篇，及 **20** 篇以上國內外研討會壁報論文。**美國癌症協會 active 會員** (1997)，**美國化學會會員**，**台灣生物化學及分子生物學會** 永久會員。第十七屆國家新創獎 學研新創獎

核酸藥物關鍵技術開發經驗分享

張嘉銘 博士
法信諾生醫 董事長

主題

藥物發展的歷史



核酸藥物的歷史

1990~

基因治療 Gene therapy
- DNA vaccine
- Adenovirus
- Retrovirus

2000~

細胞治療 Cell therapy
- Dendritic cell
- NKT

2010~

核酸藥物 Nucleic acid medicine
- RNAi
- Aptamer
- ASO

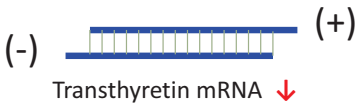
2020~

疫苗 Vaccine
- mRNA

已核准的核酸類藥物

Alnylam Pharmaceuticals

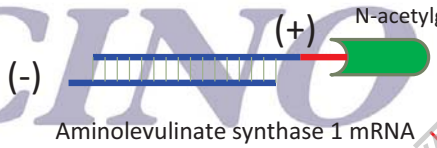
1. Patisiran, 2018, to treat hereditary transthyretin amyloidosis (hATTR)



14 KDa

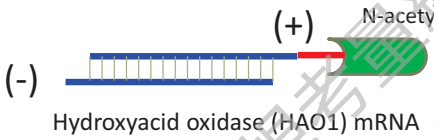
The FDA granted this application Fast Track, Priority Review and Breakthrough Therapy designations. Onpattro also received Orphan Drug designation, which provides incentives to assist and encourage the development of drugs for rare diseases. (US FDA)

1. Givosiran, 2019, to treat acute hepatic porphyria.



17 KDa

2. Lumasiran, 2020, to treat primary hyperoxaluria type 1.



17 KDa

藥物標靶的選擇

- **Huge market**
  - EGFR inhibitor
- **Clinical unmet need**
  - Market leading drug
- **Rare disease**
  - Orphan drug

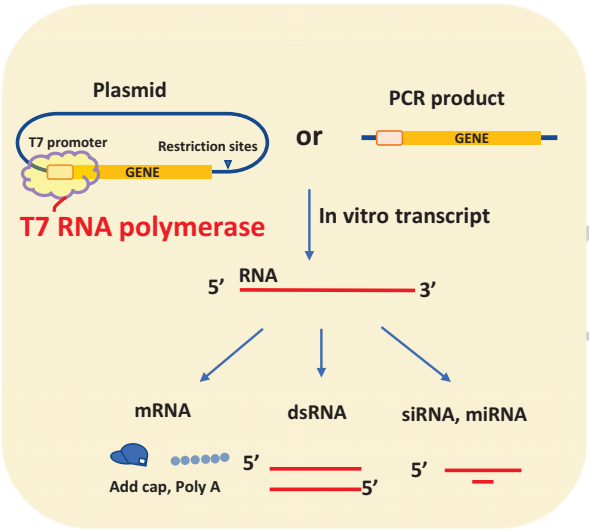
藥物開發死亡之谷

建構核酸藥物

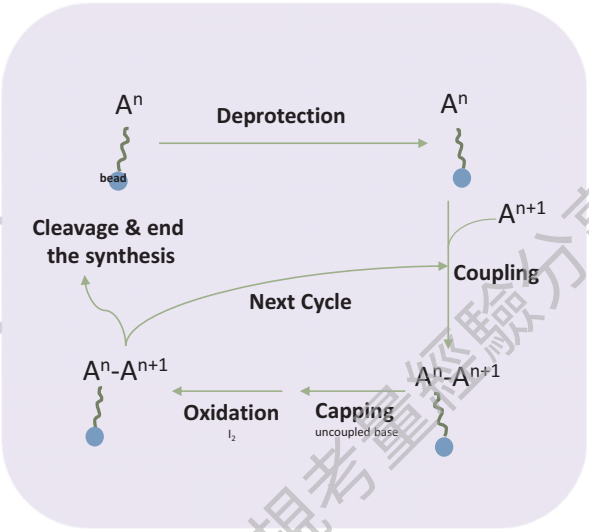
- **基礎材料**
  - A、T、C、T
- **特殊材料**
  - Methylation
  - Halogenation
  - Psudonucleotide
- **功能修飾**
  - Linker
  - Targeting
  - Stabilizer

生產技術的選擇

生物長鏈合成
In vitro transcription



直接短鏈合成
Solid phase synthesis



核酸藥物的配方設計要點

- **Stability**
  - Storage
  - Administration
- **Excipient**
  - Buffer
  - Surfactant
  - Salt
  - pH

已核准核酸藥物的配方設計

ONPATTRO (patisiran) (2018) **GIVLAARI (givosiran) (2019)** **OXLUMO (lumasiran) (2022)**

	ONPATTRO	GIVLAARI	OXLUMO	COMIRNATY (Purple cap)	COMIRNATY (Gray cap)	SPIKEVAX
RNA						
	2 mg Patisiran	189 mg Givosiran	94.5 mg Lumasiran	30 mcg of a nucleoside-modified messenger RNA (mRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2	30 mcg of a nucleoside-modified messenger RNA (mRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2	100 mcg of nucleoside-modified messenger RNA (mRNA) encoding the pre-fusion stabilized Spike glycoprotein (S) of SARS-CoV-2 virus.
Excipients	● 13.0 mg DLin-MC3-DMA ((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate) ● 1.6 mg PEG2000-C-DMG (α-(3'-{[1,2-di(myristyloxy)propanoxy]carbonylamino}propyl)-ω-methoxy, polyoxyethylene) ● 3.3 mg DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) ● 6.2 mg Cholesterol			● 0.43 mg ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) ● 0.05 mg 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) ● 0.09 mg 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) ● 0.2 mg Cholesterol	● 0.43 mg ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) ● 0.05 mg 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) ● 0.09 mg 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) ● 0.19 mg Cholesterol	1.93 mg Lipid ● SM-102 (heptadecan-9-yl 8-{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate) ● 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000 DMG) ● 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) ● Cholesterol
	2.3 mg Disodium hydrogen phosphate, heptahydrate	Sodium hydroxide (pH adjustment)	Sodium hydroxide (pH adjustment)	0.07 mg dibasic sodium phosphate dihydrate	0.06 mg Trometamol	0.31 mg Trometamol
	0.2 mg Potassium dihydrogen phosphate, anhydrous	Phosphoric acid (pH adjustment)	Phosphoric acid (pH adjustment)	0.01 mg monobasic potassium phosphate	0.4 mg Trometamol hydrochloride	1.18 mg Trometamol hydrochloride
						0.043 mg Acetic acid 0.20 mg Sodium acetate trihydrate
				6 mg sucrose	31 mg Sucrose	43.5 mg Sucrose
	8.8 mg Sodium chloride			2.52 mg sodium chloride, 0.01 mg potassium chloride		
	Water for injections	Water for injections	Water for injections	Water for injections	Water for injections	Water for injections
	pH ~ 7.0	pH ~ 7.0	pH ~ 7.0	pH: 6.9 - 7.9	pH: 6.9 - 7.9	pH: 7.0 – 8.0

法信諾生醫

Into the future

國際核酸藥物開發之藥毒理法規考量與案例分享

蔡岸圻資深審查員
財團法人醫藥品查驗中心

蔡岸圻

學歷

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經歷

財團法人醫藥品查驗中心 諮詢輔導中心　資深藥毒理審查員 (2020/01~)

財團法人醫藥品查驗中心 新藥科技組　藥毒理審查員 (2013/08~2019/12)

台北醫學大學　藥學系　博士後研究員 (2013/01~2013/07)

國家衛生研究院　生技與藥物研究所　博士後研究員(2010/02~ 2013/01)

國際核酸藥物開發之藥毒理法規考量與案例分享

諮詢輔導中心
藥毒理資深審查員　蔡岸圻

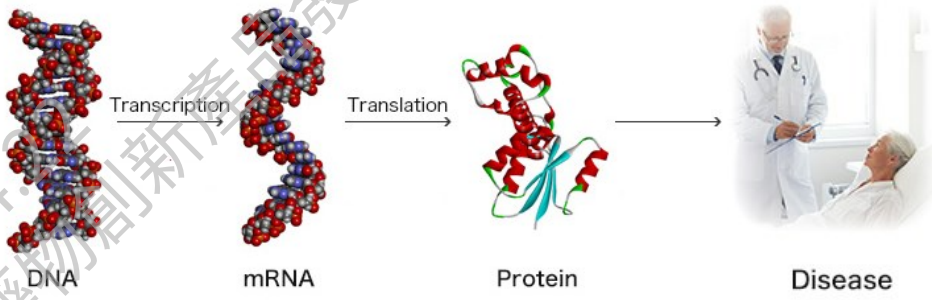
2022.04.22

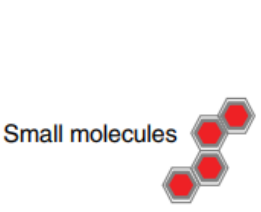
財團法人醫藥品查驗中心
Center For Drug Evaluation

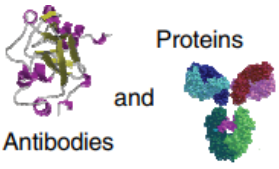
本次演講內容僅代表查驗中心之觀點，
凡涉及政策方向及法規解釋與適用，
應依衛生主管機關之指示為準。

Outline

The Central Dogma





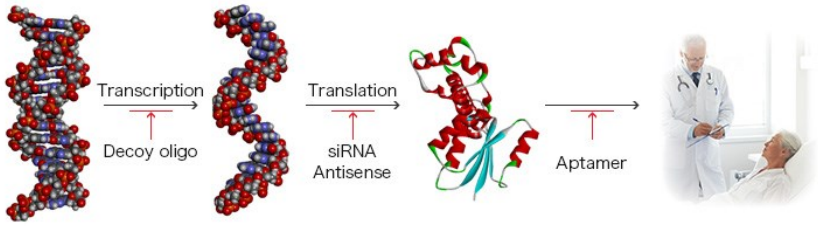


- Antagonism or agonism of target
- Extracellular and intracellular targets
- Not all target classes can be modulated selectively and potently
- Lead ID and optimization slow
- Easy to synthesize

- Antagonism or agonism of target
- Extracellular targets
- Highly selective and potent
- Lead ID and optimization slow
- Difficult to produce

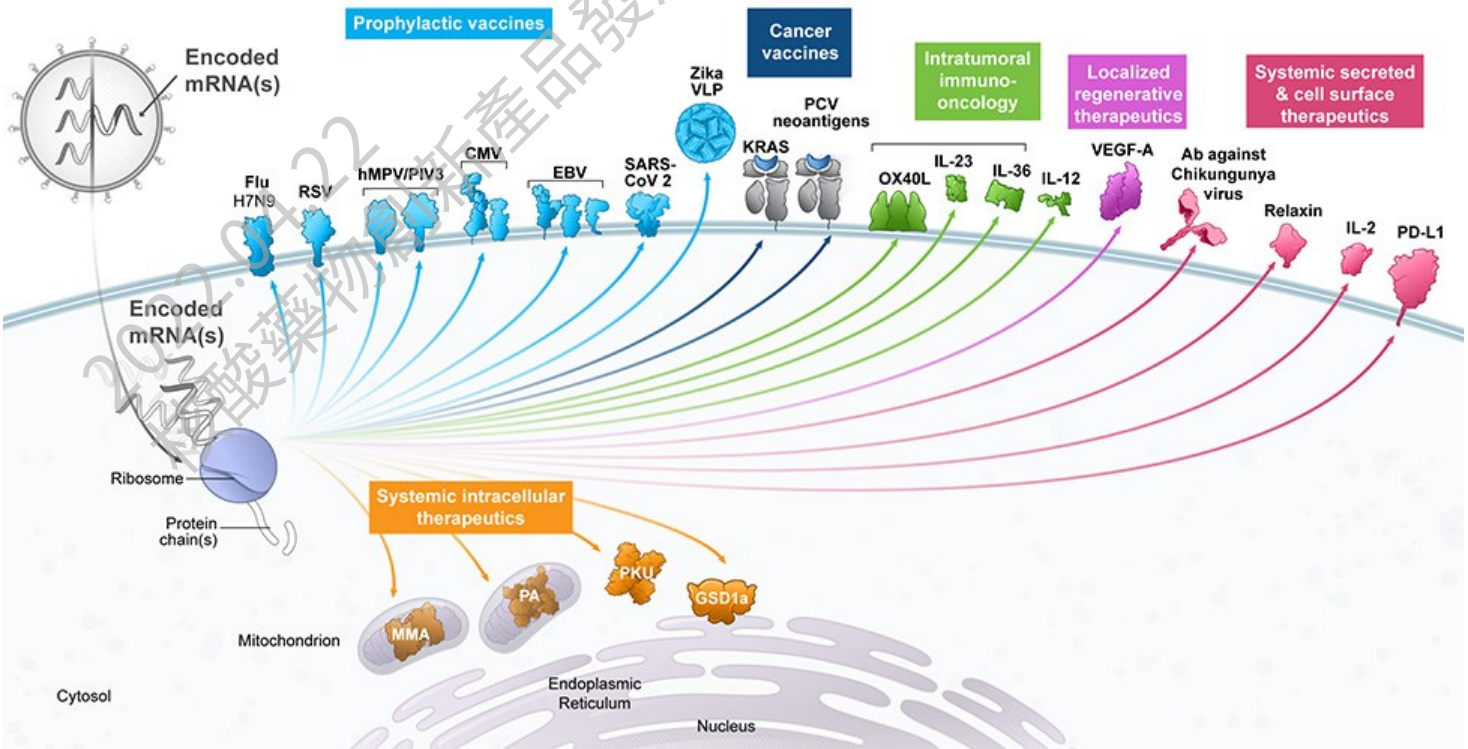
[PMID: 17108989]
https://www.bonac.com/global/en/nucleic/about/
https://www.sumitomo-chem.co.jp/oligonucleotide/oligonucleotides/

Nucleic Acid Drugs

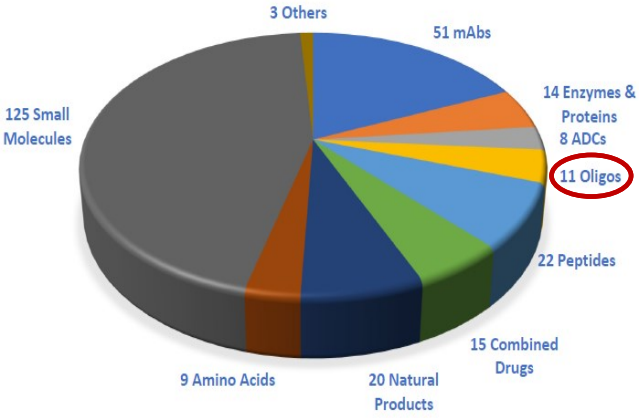


Type	Target	Site of action	Mechanism of action	Summary
siRNA	mRNA	Inside the cell (cytoplasm)	mRNA cleavage	Double-stranded RNA with cleavage of mRNA homologous to the sequence (siRNA), single-stranded hairpin RNA (shRNA), etc. with effect according to the principle of RNAi
miRNA	microRNA	Inside the cell (cytoplasm)	microRNA replacement	Double-stranded RNA, miRNA of single-stranded hairpin RNA or its mimic is used to strengthen the function of miRNA deteriorated by disorders
Antisense	mRNA, miRNA	Inside the cell (in the nucleus, cytoplasm)	mRNA and miRNA degradation, splicing inhibition	Single-stranded RNA/DNA which binds to the target mRNA and miRNA to cause degradation or inhibition, or acts to skip exon when splicing
Aptamer	Protein (extracellular protein)	Outside the cell	Functional inhibition	Single-stranded RNA/DNA which binds to the target protein in a similar manner to antibodies/DNA
Decoy	Protein (transcription factor)	Inside the cell (in the nucleus)	Transcription inhibition	Double-stranded DNA with identical sequence to the binding site for transcription factor, which binds to the transcription factor of the affected gene to suppress the target gene
Ribozyme	RNA	Inside the cell (cytoplasm)	RNA cleavage	Single-stranded RNA with enzyme function for binding and cleavage of target RNA
CpG oligo	Protein (receptor)	Cell surface	Immunopotentiation	Oligodeoxynucleotide with CpG motif (single-stranded DNA)

[PMID: 34638486]; https://www.bonac.com/global/en/nucleic/about/; https://www.sumitomo-chem.co.jp/oligonucleotide/oligonucleotides/

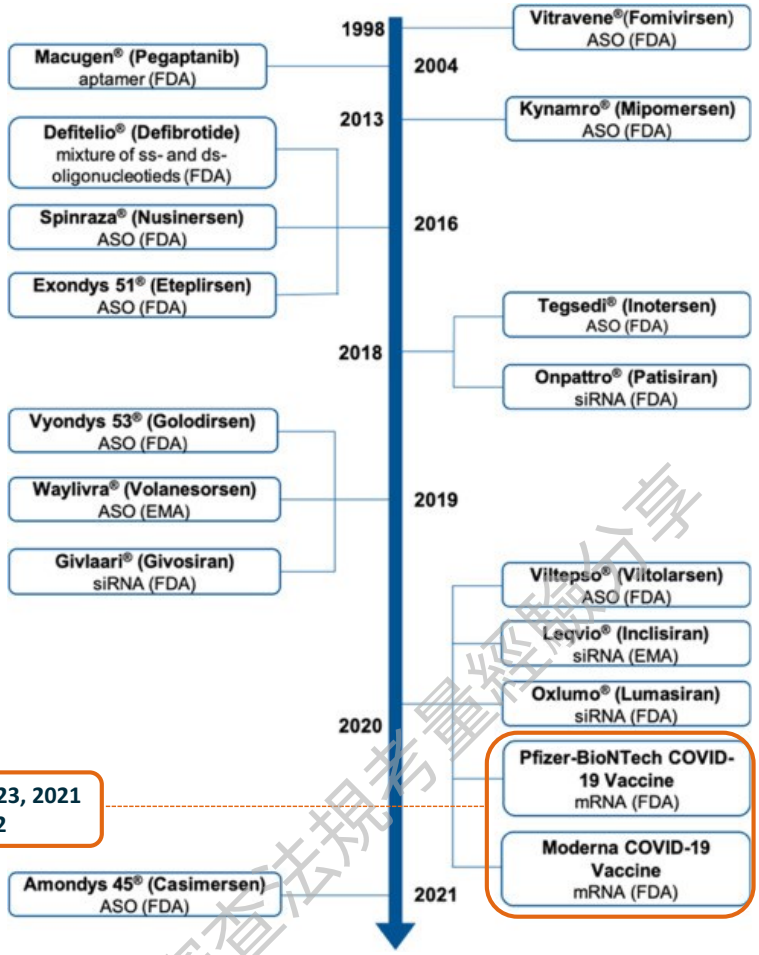


https://www.modernatx.com/blog/moderna-2020-shareholder-lette

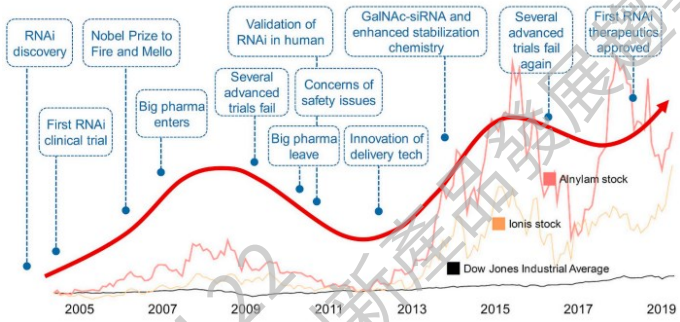


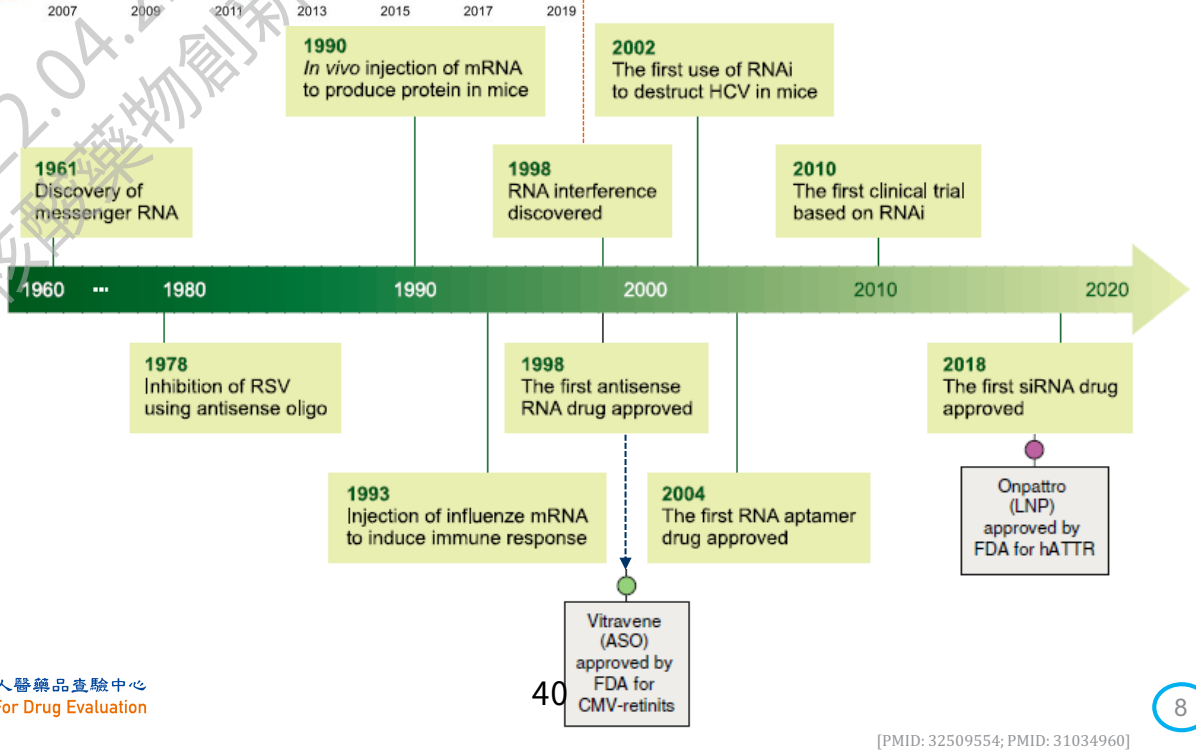
A total of **278** new drugs were approved by the Food and Drug Administration (FDA) from 2016 to 2021.

***Comirnaty (Pfizer-BioNTech COVID-19 Vaccine): FDA, August 23, 2021**
***Spikevax (Moderna COVID-19 Vaccine): FDA, January 31, 2022**



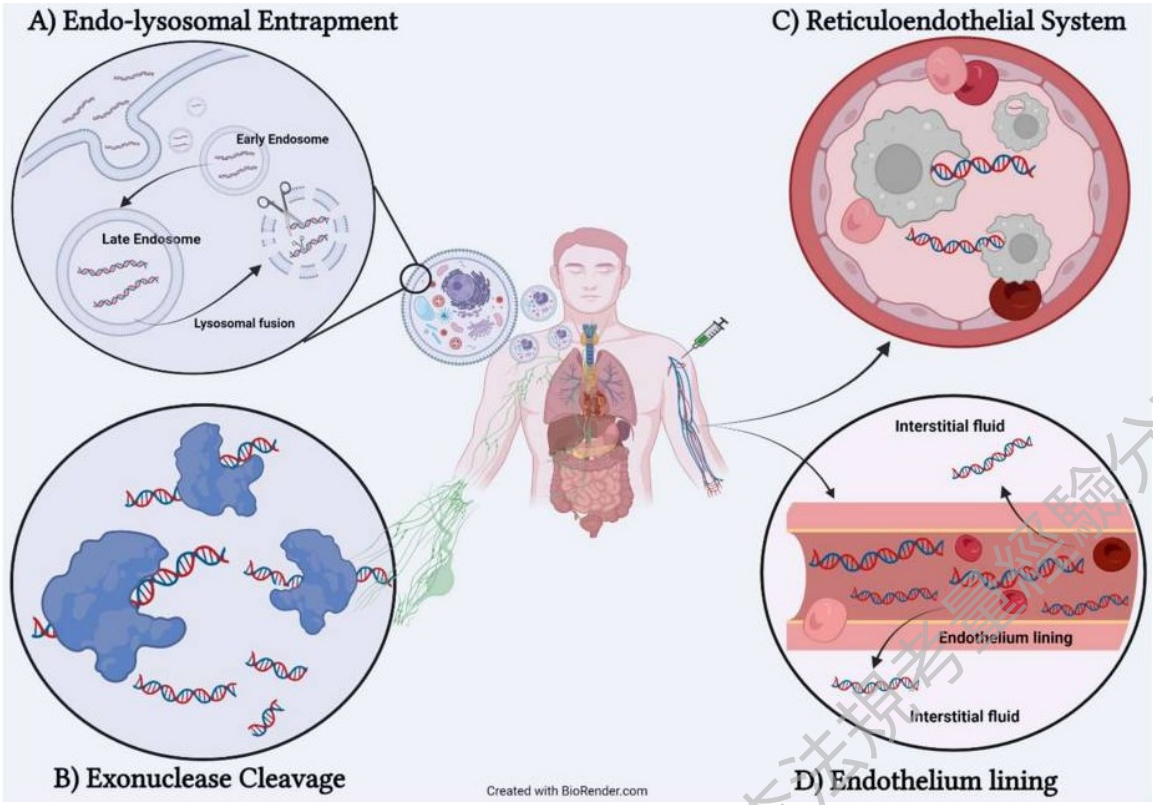
[PMID: 35215334]
https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/inotersen





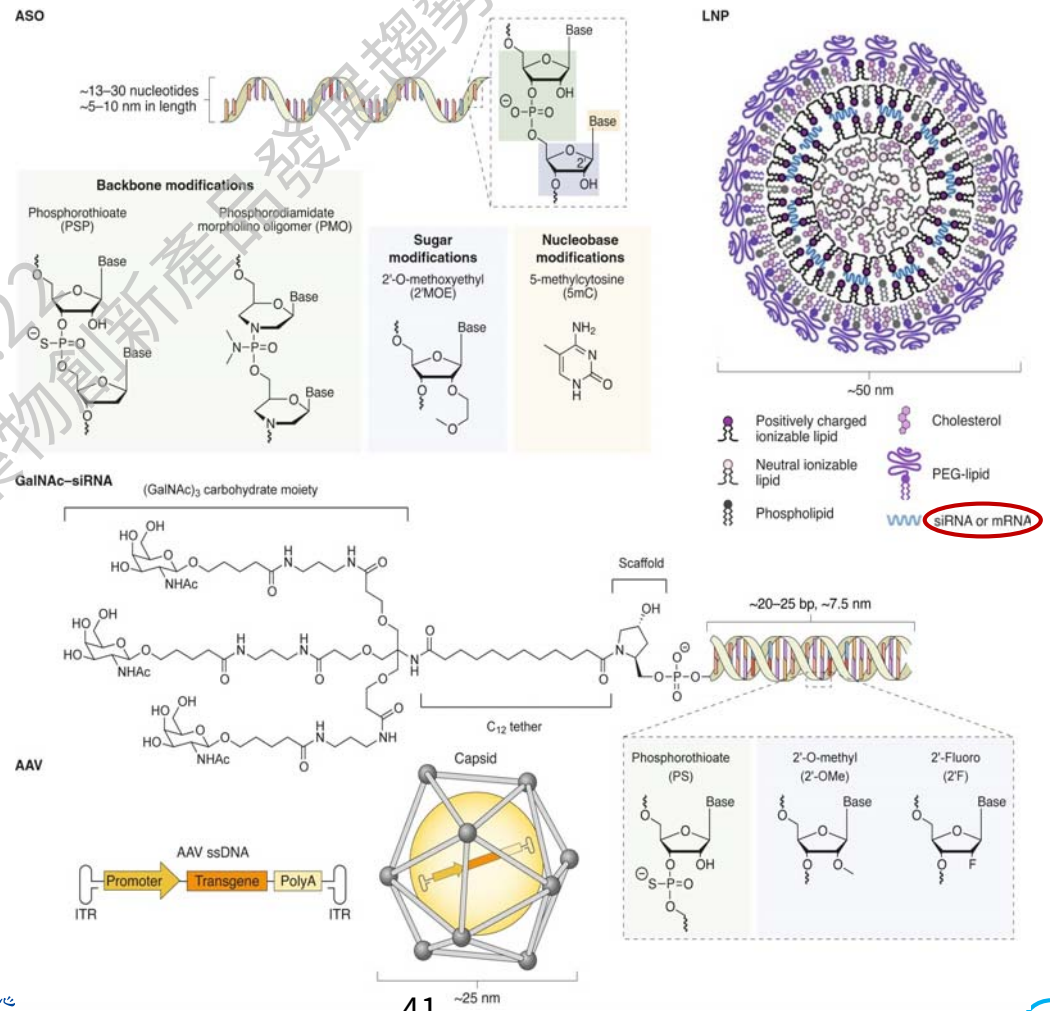
[PMID: 32509554; PMID: 31034960]

Biological Barriers

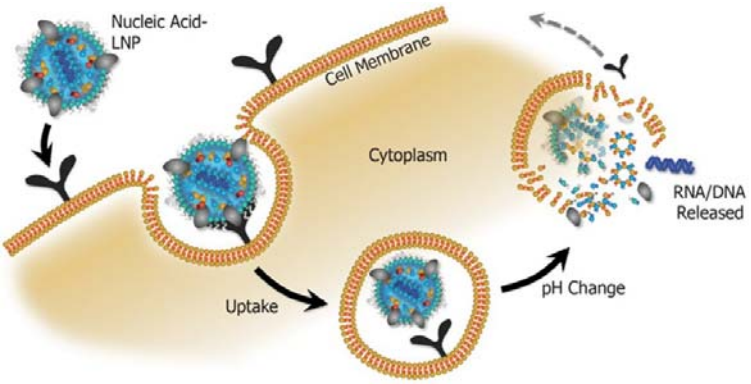


[PMID: 35214074; PMID: 26674130]

Solution

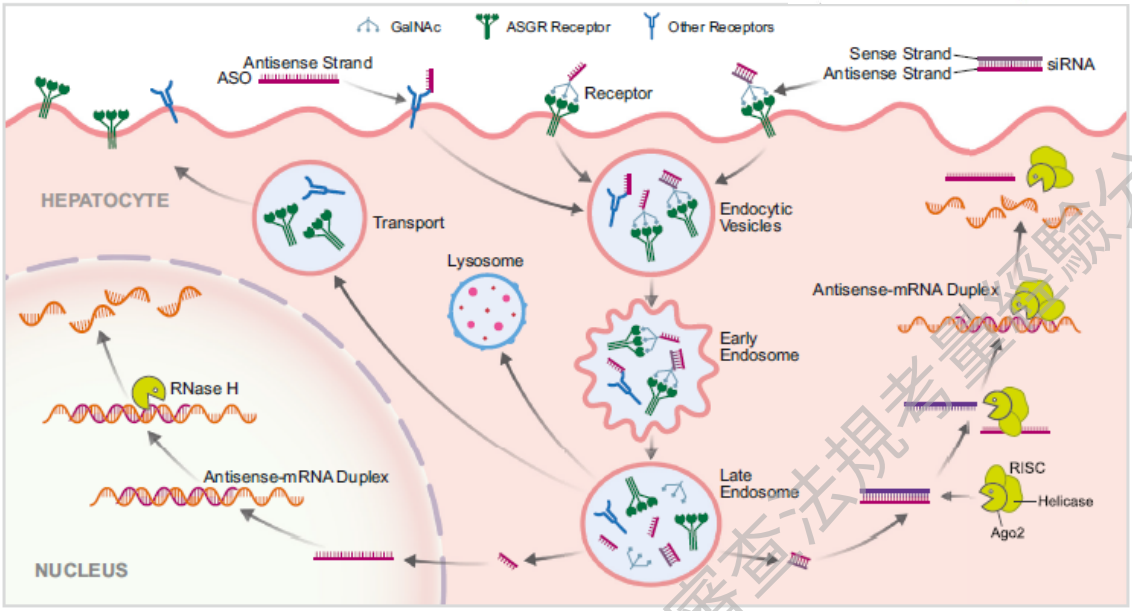


[PMID: 34059811]; https://www.creative-biolabs.com/gene-therapy/modified-oligonucleotides.htm



Asialoglycoprotein Receptor (ASGPR)

Highly expressed in hepatocytes
High turnover (recycling time ~15 min)
Conserved across species



[PMID: 34020790]

FDA Approved Oligonucleotide Drugs

Name	Type	Modification	Mechanism	Indication/*Target*	FDA Approval
Fomivirsen	ASO	21 nt PS DNA	RNase H1	Cytomegalovirus retinitis/*CMV UL123*	Aug 1998
Pegaptanib	Aptamer	27 nt 2'-F/2'-OME PEGylated	Blocking binding	Neovascular age-related macular degeneration/*VEGF-165*	Dec 2004
Mipomersen	ASO Gapmer	20 nt PS 2'-MOE	RNase H1	Homozygous familial Hypercholesterolemia/*APOB*	Jan 2013
Defibrotide	ssDNA and dsDNA	Mixture of PO	Non single sequence dependent based mechanism	hepatic veno-occlusive disease	Mar 2016
Nusinersen	Steric block ASO	18 nt PS 2'-MOE	Splicing, intron 7	Spinal muscular atrophy/*SMN2 exon 7*	Dec 2016
Eteplirsen	Steric block ASO	30 nt PMO	Splicing, exon 51	Duchenne muscular dystrophy/*DMD exon 51*	Sep 2016
Milasen	ASO	22 nt PS 2'-MOE	Splicing	Batten disease/CLN7	Jan 2018
Patisiran	siRNA LNP formulation	19 + 2 nt 2'-OME	Ago2	Hereditary transthyretin amyloidosis, polyneuropathy-*TTR*	Aug 2018
Inotersen	Gapmer ASO	20 nt PS 2'-MOE	RNase H1	hereditary transthyretin amyloidosis, polyneuropathy/*TTR*	Oct 2018
Givosiran	Dicer substrate siRNA	21/23 nt- GalNAc conjugate	Ago2	Acute hepatic porphyria *ALAS1*	Nov 2019
Golodirsen	Steric block ASO	25 nt PMO	Splicing, exon 53	Duchenne muscular dystrophy/*DMD exon 53*	Dec 2019
Viltolarsen	ASO	21 nt-PMO	Splicing	Duchenne muscular dystrophy/*DMD exon 53*	Aug 2020
Casimersen	ASO	22-PMO	Splicing	Duchenne muscular dystrophy/DMD Exon 45	Feb 2021
Inclisiran	siRNA	21/23 nt- GalNAc conjugate	Ago2	Hypercholesterolaemia/PCSKK9	Dec 2021

LNP-RNA	GalNAc–siRNA conjugates
Onpattro, patisiran (Alnylam Pharmaceuticals)	Givlaari, Givosiran (Alnylam Pharmaceuticals)
Comirnaty, tozinameran (BioNTech/Pfizer)	Leqvio, inclisiran (Novartis/Alnylam Pharmaceuticals)
mRNA-1273 (Moderna/NIAID/BARDA)	Oxlumo, lumasiran (Alnylam Pharmaceuticals)

[PMID: 35214074]

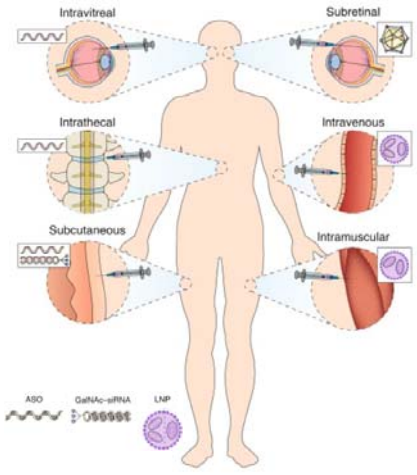
Outline

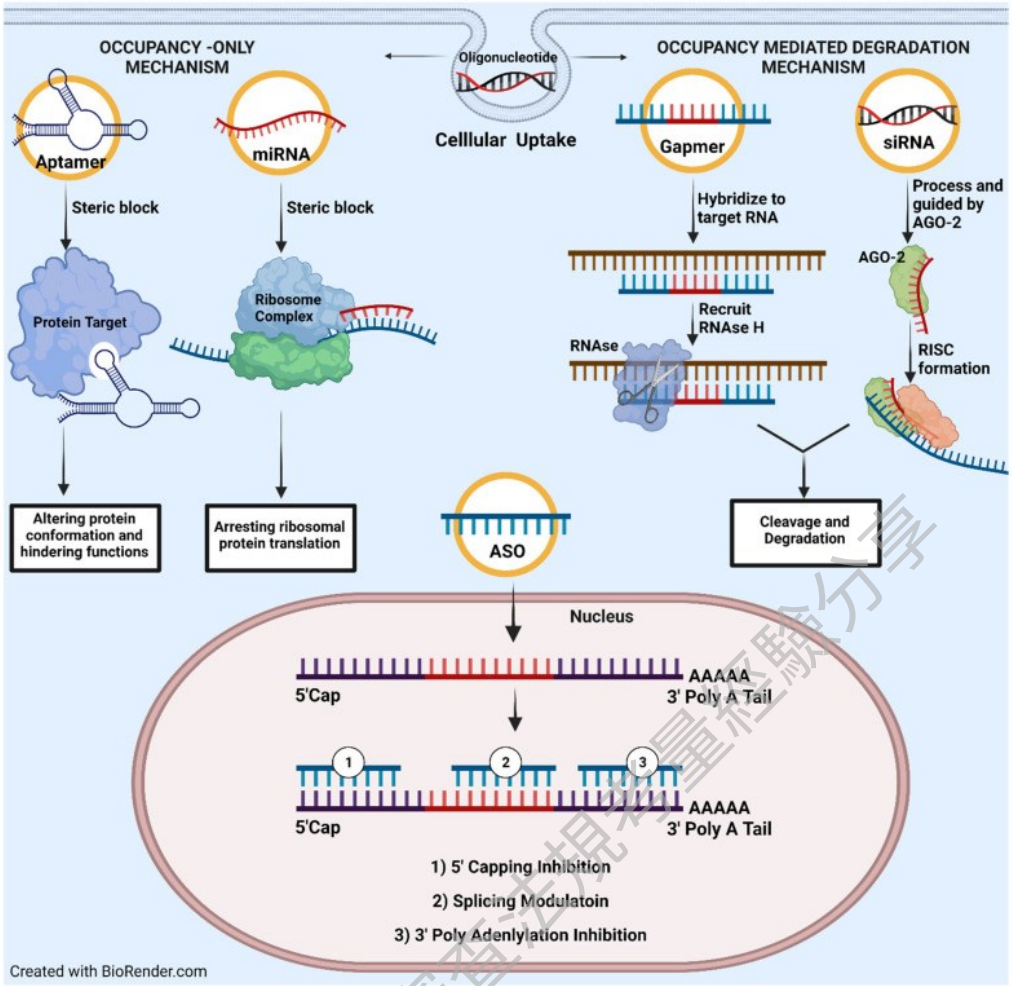
法規參考

- 藥品臨床試驗計畫-技術性文件指引 (104年11月02日)
- 藥品非臨床試驗安全性規範 (第五版) (103年07月07日)
- ICH Guidelines
- 核酸医薬品の非臨床安全性評価に関するガイドラインについて

  (厚生労働省薬生薬審発0330第1号，令和 2 年 3 月 30日)

Major ONTs Mechanism of Action





財團法人醫藥品查驗中心
Center For Drug Evaluation

15

[PMID: 35214074]

- Have the properties of both NCEs and biopharmaceuticals
  - **Highly target-specific**: similar to biopharmaceuticals
  - Most ONTs are composed of nucleotides with **various chemical modifications**: have the toxicological characteristics of new chemical entities (NCEs)

SIMILARITIES AND DIFFERENCES BETWEEN OLIGONUCLEOTIDE THERAPEUTICS AND EXISTING DRUGS

	Chemically synthesized drugs	*Oligonucleotide therapeutics*	*Biopharmaceuticals*
Molecular weight	Generally <1 kDa	Generally ≤10 kDa	Generally >30 kDa
Production method	Chemical synthesis	Chemical synthesis	Biotechnology
Target	Intracellular and/or extracellular	Intracellular and/or extracellular	Extracellular
Species specificity	Nonspecific	Occasionally species specific	Species specific
Metabolism/degradation	Activation or inactivation by metabolism	Degradation to nucleic acids and metabolism	*In vivo* degradation to amino acids
Predictability of toxicity	Unpredictable	On-target effects, predictable; off-target effects, unpredictable	Predictable
Toxicological mode of action	Off-target effects, (toxicity related to metabolites and nonspecific toxicity independent of mechanism of action)	Exaggerated pharmacology and off-target effects (target dependent/target independent)	Exaggerated pharmacology (known mechanism of action)

Nucleic Acid Ther. 2021 Apr;31(2):114-125. PMID: 33470890

Hybridization to the target sequence (exaggerated pharmacology)

- Pharmacologically relevant species
- Surrogate (generally, one animal species)

Toxicity

(OTEs) Unintended effects

Hybridization-dependent (bind to non-target sequence)

- *In silico* analysis
- *In vitro* tests (e.g., qPCR, microarray, RNAseq) using human cells
- Biological information of the intended gene

Hybridization/Sequence-independent (chemical modification, formulation, etc.)

- *Conventional toxicity studies*
- *Rodents and non-rodents*

Type of Study	New Chemical Entities (Generally)	*ONTs (Additional consideration)
POC	• *In vitro* and/*or in vivo* studies	
Safety Pharmacology	• *In vitro* hERG • Core battery (CNS, CVS, RS) • Additional: as needed	• *In vitro* hERG: generally not warranted
Single-Dose (Acute) Toxicity	• Separate studies are rarely needed	

Type of Study	New Chemical Entities (Generally)	*ONTs (Additional consideration)
Repeated-dose toxicity	• At least 2 mammalian species (rodents and non-rodents) required • Intended clinical route • Tox study duration ≥ clinical trial duration	• On-target & Off-target • On-target: - **Generally, at least one of the two species** (if only one pharmacologically relevant species) - **Surrogates** (if no appropriate pharmacologically relevant species)

Table 1 Recommended Duration of Repeated-Dose Toxicity Studies to Support the Conduct of Clinical Trials

Maximum Duration of Clinical Trial	Recommended Minimum Duration of Repeated-Dose Toxicity Studies to Support Clinical Trials	
	Rodents	Non-rodents
Up to 2 weeks	2 weeks[a]	2 weeks[a]
Between 2 weeks and 6 months	Same as clinical trial[b]	Same as clinical trial[b]
> 6 months	6 months[b, c]	9 months [b, c, d]

Table 2 Recommended Duration of Repeated-Dose Toxicity Studies to Support Marketing

Duration of Indicated Treatment	Rodent	Non-rodent
Up to 2 weeks	1 month	1 month
>2 weeks to 1 month	3 months	3 months
>1 month to 3 months	6 months	6 months
>3 months	6 months[c]	9 months [c,d]

Type of Study	New Chemical Entities (Generally)	*ONTs (Additional consideration)
Genotoxicity	• Standard battery • Typically 2 *in vitro* and 1 *in vivo* studies	• Consisting of only native nucleic acids: generally not necessary
DART	• FEED, PPND studies: typically in rodents • EFD studies: typically one rodent and one non-rodent species	• **Species-specific surrogate molecule** (if the clinical candidate is not pharmacologically active in the routine species)
Carcinogenicity	• If need, typically two species of rodents	
Local tolerance	• Separate studies are rarely needed	

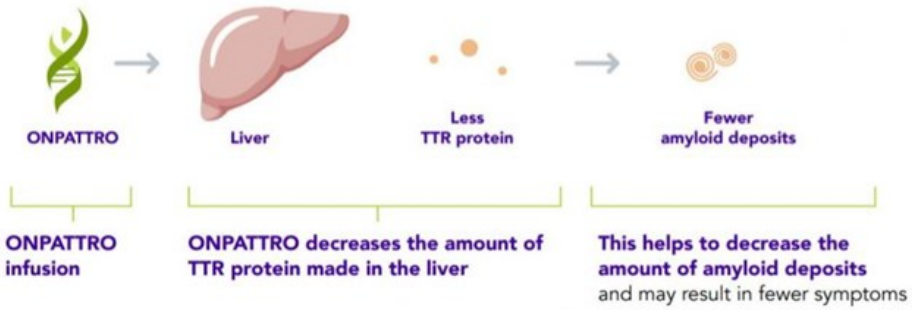
Type of Study	New Chemical Entities (Generally)	*ONTs (Additional consideration)
Immunotoxicity	• Separate studies are rarely needed	
Photosafety	• If there are particular photosafety concerns	• Chemically modified nucleic acids
Toxicokinetics	• Required	
Others	• Case-by-case	

CNS = central nervous system; CVS= cardiovascular system; DART = reproductive and developmental toxicity; EFD = Embryo-Fetal Development; FEED = Fertility and Early Embryonic Development; FIH = first in human; ONT= oligonucleotide therapeutics; PPND = Pre- and Postnatal Development; POC= proof of concept; RS = respiratory system

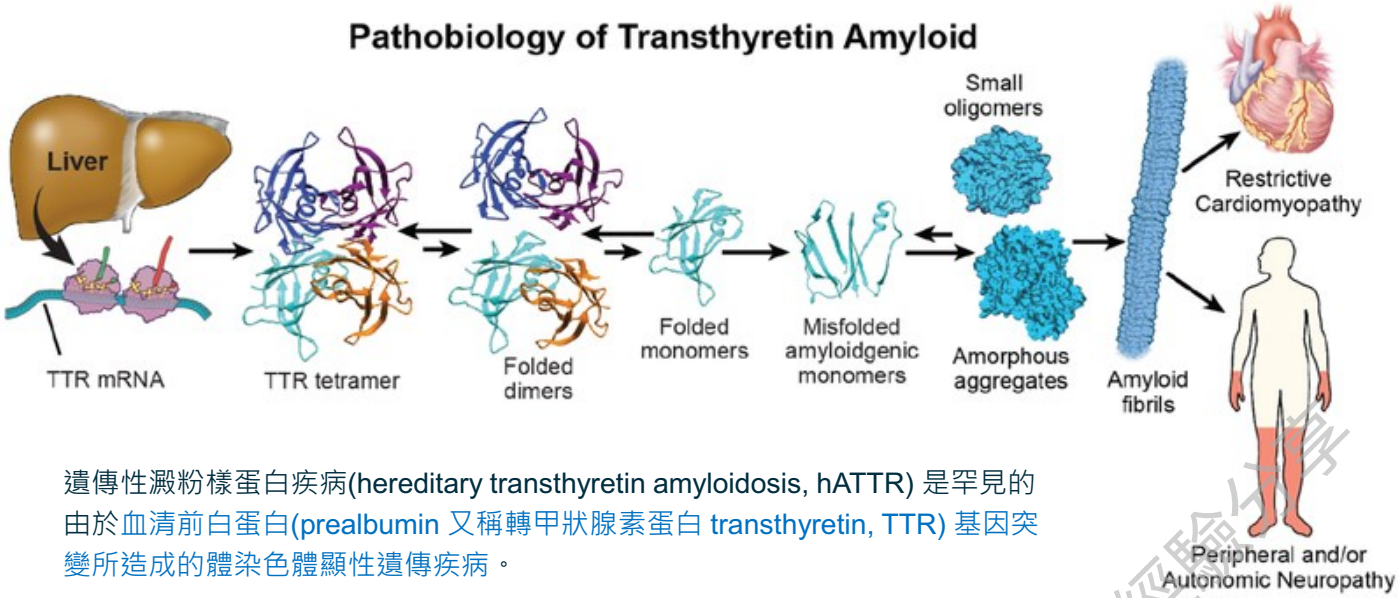
**Active ingredients include antisense oligonucleotides, siRNAs, or miRNAs, binding to a specific nucleotide sequence, and causing a biological reaction without synthesizing a new protein.*

CASE STUDY

onpattro™
(patisiran) lipid complex injection



Pathobiology of Transthyretin Amyloid

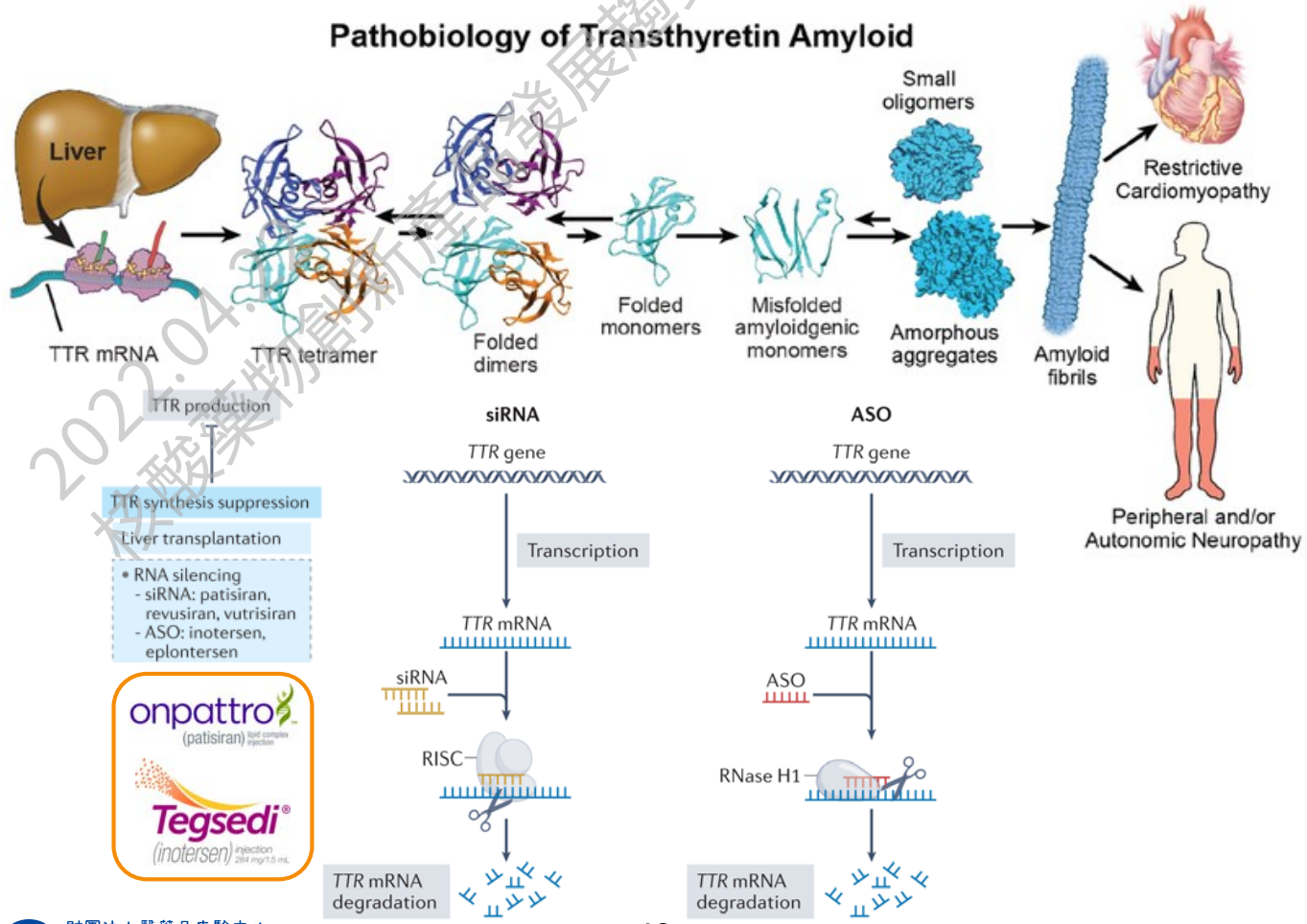


遺傳性澱粉樣蛋白疾病(hereditary transthyretin amyloidosis, hATTR) 是罕見的由於血清前白蛋白(prealbumin 又稱轉甲狀腺素蛋白 transthyretin, TTR) 基因突變所造成的體染色體顯性遺傳疾病。

TTR TETRAMERS	TTR MONOMERS	MISFOLDED TTR	AMYLOID DEPOSITS
TTR is primarily synthesized in the liver and is secreted as a tetramer composed of identical monomers.	In hATTR amyloidosis, the tetramer becomes destabilized, dissociating into monomers.	TTR monomers misfold and aggregate into amyloid fibrils.	Amyloid fibrils are deposited at multiple sites in the body, including the nerves, heart, and GI tract, causing damage that leads to clinical symptoms.

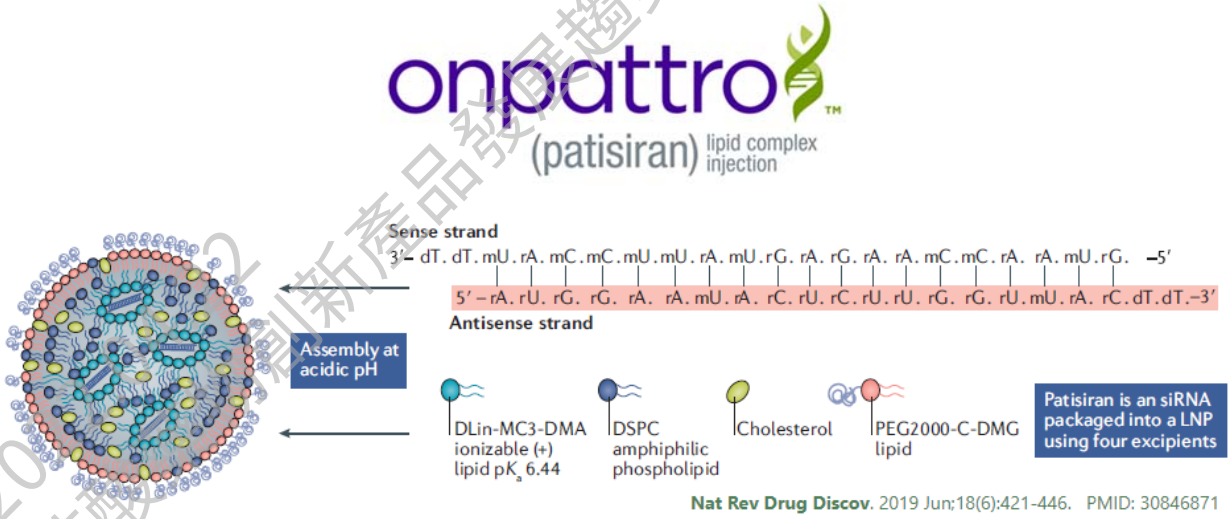
[PMID: 31171094]

Pathobiology of Transthyretin Amyloid

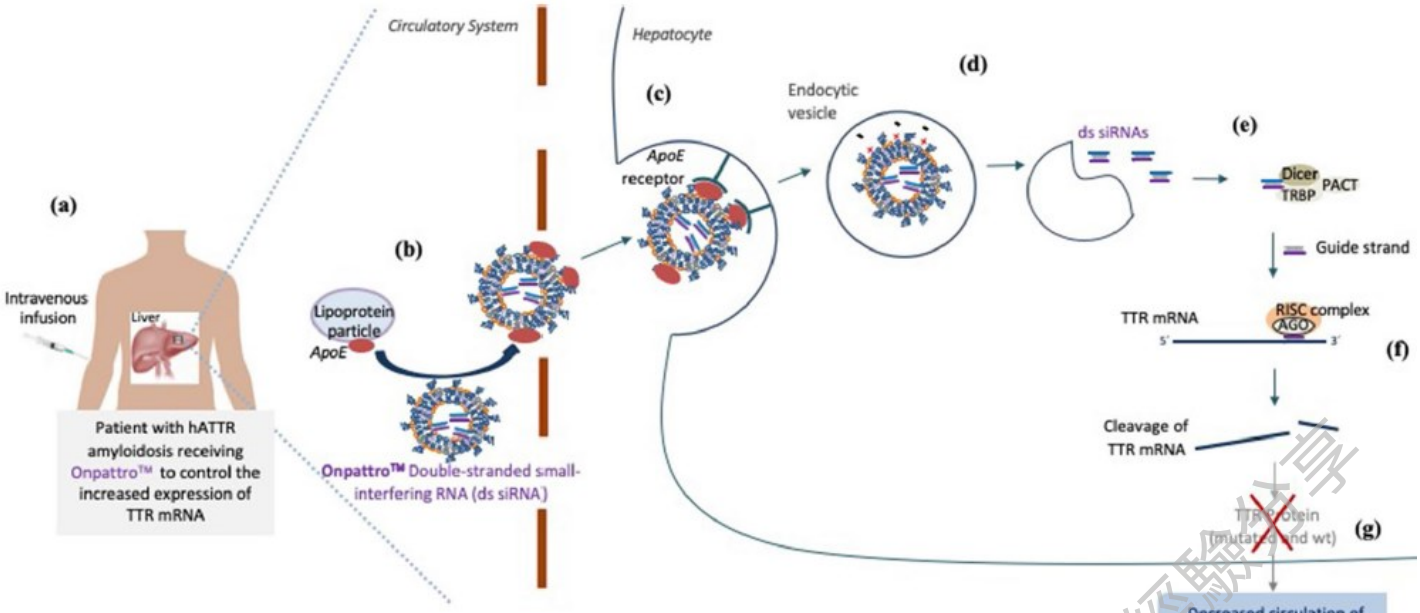


[PMID: 31171094; PMID: 35322226]

- **詠葆玖靜脈輸注濃縮液 2毫克/毫升**
- **衛部罕藥輸字第000063號**
- **適應症:** 適用於治療成人TTR (transthyretin)家族性澱粉樣多發性神經病變(Familial Amyloidotic polyneuropathy)。神經病變的疾病嚴重度限於第一、二期的病人。
- **用法用量:** 每公斤體重300微公克(300 μg/kg)，每3週靜脈輸注一次。



- The active substance, **patisiran**, is a **chemically-synthesized**, **double-stranded oligonucleotide**.
- The lipid excipients associate with the siRNA, **protect** it from immediate **degradation** in the circulatory system, and aid in its **delivery to the target site** in the liver.
- The drug product is a homogenous solution of nucleic acid/lipid nanoparticles with an average size of approximately 60-100 nm.
- DLin-MC3-DMA and PEG_{2000}-C-DMG are novel excipients.



作用機制:

Onpattro含有patisiran，一種雙鏈小分子干擾核糖核酸(**siRNA**)，可專一性針對所有突變型和野生型*TTR* mRNA的3'非轉譯區中的基因保守序列。Patisiran被製成**脂質奈米微粒**以將siRNA運送至**肝細胞**，肝細胞是循環中TTR蛋白的主要來源。藉由稱為RNA干擾(RNAi)的自然過程，patisiran**引起肝臟中TTR mRNA的催化降解，而使血清TTR蛋白質減少**。

[PMID: 31701435]

Key Nonclinical Studies with Onpattro (patisiran)

Pharmacology Studies

Type of Study	Study Description
Primary PD (proof-of-concept)	• *In vitro* studies - Patisiran is **active in human and nonhuman primate** but not in rodent - Reduces wild-type and mutant TTR alleles with similar potency - Potential off-target activity (bioinformatic analysis), further evaluated *in vitro* (cell-based) • *In vivo* studies: ↓ liver tissue TTR mRNA and serum TTR protein in mice and cynomolgus monkeys - Naïve cynomolgus monkeys: patisiran-LNP; a single dose; Q3W or Q4W over 24w +12w recovery - Mouse disease model (the V30M/Hsf-1 KO mouse model): 3mg/kg (only one dose was tested), bi-weekly, 6 doses ALN-TTR01 (LNP with same siRNA as patisiran-LNP)

Key Nonclinical Studies with Onpattro (patisiran)

Type of Study	Study Description
Secondary PD	• No stand-alone study • Evaluated potential drug-related effects in the toxicity study in monkeys: **TTR protein associated molecule levels** (i.e. Retinol binding protein, vitamin A and thyroxine concentrations); treatment impact on cytokine levels
Safety Pharmacology	• *In-vitro* hERG: only for LNP (with patisiran exchanged for an insect luciferase siRNA with no target in the human genome) • *In vivo*: CNS, cardiovascular, and respiratory parameters were assessed in cynomolgus monkey (single IV infusion; Patisiran-LNP)

Key Nonclinical Studies with Onpattro (patisiran)

Toxicity Studies

Type of Study	Study Description
Single dose	• Cynomolgus monkey, IV (up tp 100 mg/kg), unformulated siRNA
Repeat dose (Pivotal IV toxicity studies)	• **SD Rat** (3 GLP studies) - **4-week** (Q4W) study (+60-day recovery): patisiran-LNP; less relevant to clinical use because of the less frequent dosing, compared to that proposed for humans (Q3W) - **6-week** (Q2W) study (+60-day recovery): patisiran-LNP; a separate group received AF-011-1955 (non-pharmacologically active siRNA against insect luciferase, in same LNP formulation; 3 mg/kg only) - **26-week** (Q2W) study (+12-week recovery): *this study is not considered an adequate assessment of the chronic effects of patisiran-LNP

*50% of the animals had detectable ADA directed against PEG_{2000}-C-DMG; the lack of detectable patisiran in plasma at the Day 183 sampling time

Key Nonclinical Studies with Onpattro (patisiran)

Type of Study	Study Description
Repeat dose (Pivotal IV toxicity studies) (contd.)	• **Cynomolgus monkey** (2 GLP studies) - **6-week** (Q2W) study (+60-day recovery): patisiran-LNP; a separate group received AF-011-1955 (non-pharmacologically active siRNA against insect luciferase, in same LNP formulation; 3 mg/kg only) - **39-week** (Q3W) study (+13-week recovery): Patisiran-LNP
Genotoxicity	• Standard battery of genotoxicity assays (Patisiran-LNP) - *In vitro* Ames assay (bacterial reverse mutation assay) - *In vitro* chromosomal aberration assay in HPBL (human blood peripheral lymphocytes) - *In vivo* micronucleus assay in CD-1 mouse

Key Nonclinical Studies with Onpattro (patisiran)

Type of Study	Study Description
Carcinogenicity	• *A 2-year carcinogenicity study of patisiran-LNP in rats: not conducted • 26-week (Q2W) IV study in Tg.rasH2 mouse: patisiran-LNP
DART (Full battery)	Full battery <**rat-specific surrogate formulation AF-011-18534** > • Fertility and early embryonic development (FEED) in male Sprague-Dawley rat: Patisiran-LNP; AF-011-18534 (highest dose) • Embryo fetal development (EFD): - Combined fertility and early embryonic development and embryofetal development in female Sprague-Dawley rat: Patisiran-LNP; AF-011-18534 (highest dose) - New Zealand White rabbit: Patisiran-LNP • Pre- and postnatal development (PPND) in Sprague-Dawley rat: Patisiran-LNP; AF-011-18534 (highest dose)

*Immunogenicity (development of ADA) that was observed, substantial decrease in systemic exposure to patisiran, with no detectable levels near the end of the dosing period (Day 185) in the 26-week chronic rat study

Key Nonclinical Studies with Onpattro (patisiran)

Type of Study	Study Description
Others	• Immunotoxicity/ Immunogenicity: evaluated in the repeat-dose toxicity studies in rat and monkey • Immunostimulation: evaluated by measuring serum cytokines and complement in vitro, CD-1 mice and monkey • Phototoxicity: patisiran was typical for nucleic acids with peak absorbance at 260 nm that extends into the lower UV-B range (molar extinction coefficient=approximately 42,500 L mol-1 cm-1 at 290 nm); None of the lipid components of patisiran-LNP absorb light within the range of natural sunlight (290-700 nm); no dedicated phototoxicity studies are considered necessary. • Excipients (Novel lipids): genotoxicity • Bridging comparability studies: in monkeys

USFDA and/or EMA assessment report

Outline

法規參考

- 藥品查驗登記審查準則(110年09月14日修正;衛授食字第1101407694號)
- 藥品臨床試驗計畫-技術性文件指引 (104年11月02日)
- 藥品非臨床試驗安全性規範 (第五版) (103年07月07日)
- Evaluation of the quality, safety and efficacy of RNA-based prophylactic vaccines for infectious diseases: regulatory considerations (WHO, 7 December 2021) (The scope is confined to vaccines to prevent infectious diseases)
- Guidelines on the Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted Vaccines, In: WHO Expert Committee on Biological Standardization. Sixty-Fourth Report. Geneva, World Health Organization, 2014, Annex 2
- Guidelines on nonclinical evaluation of vaccines. In: WHO Expert Committee on Biological Standardization. Fifty-fourth Report. Geneva, World Health Organization, 2005, Annex 1

Basic Elements of Preclinical Evaluation and Testing of Vaccines

Safety
- Systemic Reactions/tox
- Local Tolerance
- ...

Pharmacological Properties
- **Functional Parameters**
  - Mode of action
  - Immunogenicity
  - Efficacy
  - Protection
  - Pharmacokinetics and Biodistribution

- Standard assays
- Clear requirements
- GLP compliance

- Highly product-specific
- GLP not required
- "Case-by-case"
- Based on sound science

→ Relevant Animal model is key!*

*"... a relevant animal model is one in which the test material is pharmacologically active..."

The more complex/novel the product the more complex and comprehensive testing scheme

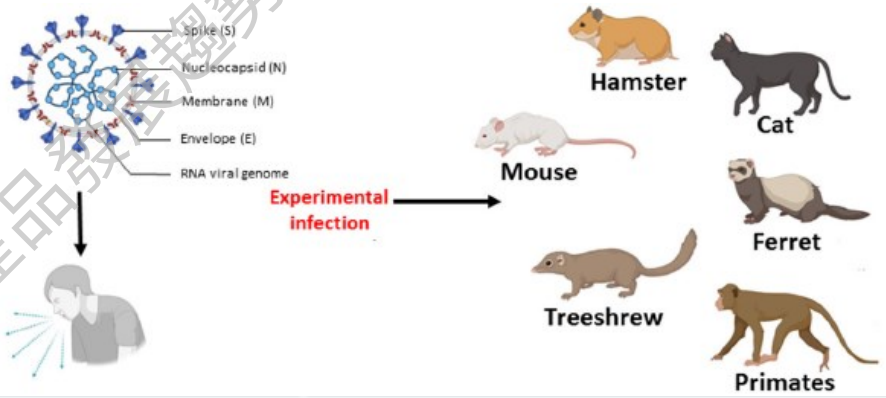
Primary Pharmacodynamics Studies

■ Proof-of-Concept

- **Assessment of immunogenicity**
- **Challenge-protection** studies in a relevant animal model, if an appropriate animal model is available

[redacted] GLP-compliance is not essential for such studies, but **studies shall be "of high quality and reliability"!!!**

Animal Models Currently being used in COVID-19 Research



Animal models	
Animal species	Key points
Mice — Wild type mice	SARS-CoV-2 cannot invade cells through mouse Ace2.
Mice — Human ACE2 transgenic mice	After SARS-CoV-2 infection, the mice show weight loss, virus replication in the lungs, and interstitial pneumonia.
Syrian hamster	After SARS-CoV-2 infection, the hamsters show rapid breathing, weight loss, and diffuse alveolar damage with extensive apoptosis.
Ferrets	After SARS-CoV-2 infection, acute bronchiolitis was observed in the lungs.
Cats	After SARS-CoV-2 infection, intra-alveolar edema and congestion in the interalveolar septa were observed. Abnormal arrangement of the epithelium with loss of cilia and lymphocytic infiltration into the lamina propria were also observed.
Cynomolgus macaques	SARS-CoV-2 can infect both type I and type II pneumocytes. After SARS-CoV-2 infection, pulmonary consolidation, pneumonia, and edema fluid in alveolar lumina were observed.
Rhesus macaques	Infected macaques had high viral loads in the upper and lower respiratory tract, humoral and cellular immune responses, and pathologic evidence of viral pneumonia. The therapeutic effects of adenovirus-vectored vaccine, DNA vaccine candidates expressing S protein, and remdesivir treatment could be evaluated.

[PMID: 33363234; PMID: 32553545]

Hamster

Animal model	Clinical symptoms	Histopathological features	Viral titer	Advantages	Disadvantages
- Syrian Golden hamster	- Progressive weight loss - Rapid breathing - Lethargy, hunched back, and ruffled furs	- Focal inflammation in the lung - Diffuse alveolar destruction - Pulmonary edema, and alveolar hemorrhage - Mononuclear inflammatory cell infiltration - Lung consolidation	- High level of virus titer in the nasal turbinates, trachea, and lungs at 2–7 dpi	- Easy to handle - High susceptibility to infection due to high binding affinity of ACE2 protein to the Spike of SARS-CoV-2 - Develop severe pneumonia similar to human patients - Infected hamsters develop immunity against reinfection	
- Chinese hamster	- Has a similar course of disease as in Syrian hamster - Milder and prolonged pneumonia than in Syrian hamster	- Has a similar histopathological picture of the disease as in Syrian hamster	- The same as Syrian hamser	- Highly susceptible to SARS-CoV-2 infection - More suitable than Syrian hamster due to its smaller size	

Primates

Animal model	Clinical symptoms	Histopathological features	Viral titer	Advantages	Disadvantages
Cynomolgus macaques	- Asymptomatic except serous nasal discharge in an aged animal	- Focal pulmonary consolidation in young and aged animals - Edema in alveolar and bronchiolar lumina - Thickened alveolar walls - Hyaline membrane formation - Hyperplasia of type II pneumocyte, and mononuclear infiltration	- Viral replication in both upper and lower respiratory tracts. - Viral replication peaks at the early stages of infection. - Higher level of viral RNA expression and prolonged virus shedding in aged animals compared to young animals	- Effective virus transmission to other animals - Development of lung disease - Early peak of virus resembles asymptomatic patients	- Slower reproduction rate - Limited clinical signs developed - High cost - Difficult handling - Ethical reasons
Rhesus macaques	- Asymptomatic or show mild and transient symptoms, such as, reduced appetite, weight loss, elevated body temperature, rapid respiration, hunched posture, dehydration, pale appearance, and occasional coughing	- Lung consolidation, edema, hemorrhage - Thickened alveolar walls, inflammatory infiltration - Mild to moderate interstitial pneumonia - Old animals shows diffuse severe interstitial pneumonia	- Viral RNA is in pharynx, trachea, bronchi, and lungs - High level of virus shedding from the nose and throat	- ACE2 receptors are 100% identical to those of humans - The moderate and transient disease resemble that of human cases - Similar virus shedding pattern to that of human	- Slower reproduction rate - Moderate clinical signs developed - High cost - Difficult handling [-] Ethical reasons

Non-clinical Studies on Safety Aspects

[redacted] Basic Objectives

- Risk mitigation before entering into clinical trial phase
- Detect any toxicities to be monitored in following clinical trials and identify a safe human dose
- If relevant, protection study shall include testing/monitoring for any indications of enhancement of disease due to vaccination

[redacted] **GLP-compliance of pivotal non-clinical safety studies is required!!!**

Basic Consideration - potential source of toxicity

- Inherent toxicity of vaccine product?
- Toxicity due to impurities or contaminations?
- Toxicity due interactions of vaccine components, such as adjuvants?
- Toxicity due to side-effects induced by immune response?

[PMID: 34068715]

Safety Pharmacology

- Detection of undesirable pharmacological effects on vital functions (e.g., CNS, CVS, respiratory, body temperature, renal function)
- If feasible, inclusion of safety pharmacology endpoints in general toxicology studies
- Before entering into FIH

Single-dose Toxicity and Local Tolerance

- Stand-alone: not generally required
- Preferably be integrated into repeated-dose toxicity studies if they are adequately designed
- Before entering into FIH

Repeat-dose Toxicity

- Species:
  - Generally, one relevant species
  - Should be able to elicit an immune response to the vaccine antigen
- Route: intended in human clinical trials
- Number of doses: should be equal to or more than the number of doses proposed in humans (N+1, generally)
- Dose: highest absolute dose intended to be used in humans (if feasible)

Repeat-dose Toxicity (contd.)

- Groups: generally, should include a negative controls group(s), treatment group(s), recovery group(s); if appropriate, active controls (formulation without antigen)
- End-points:
  - Similar to conventional repeated-dose toxicity studies
  - Immune end points and local tolerance needs special focus
- Before entering into FIH

Genotoxicity and Carcinogenicity

- Genotoxicity:
  - Normally not needed for the final vaccine formulation
  - A standard battery of genotoxicity studies is generally recommended for particular vaccine components (e.g., novel lipids or any novel excipients)
  - If needed
    - *in vitro* tests for mutations and chromosomal damage should be done prior to first human exposure
    - full battery tests parallel to clinical trials
- Carcinogenicity: generally not needed

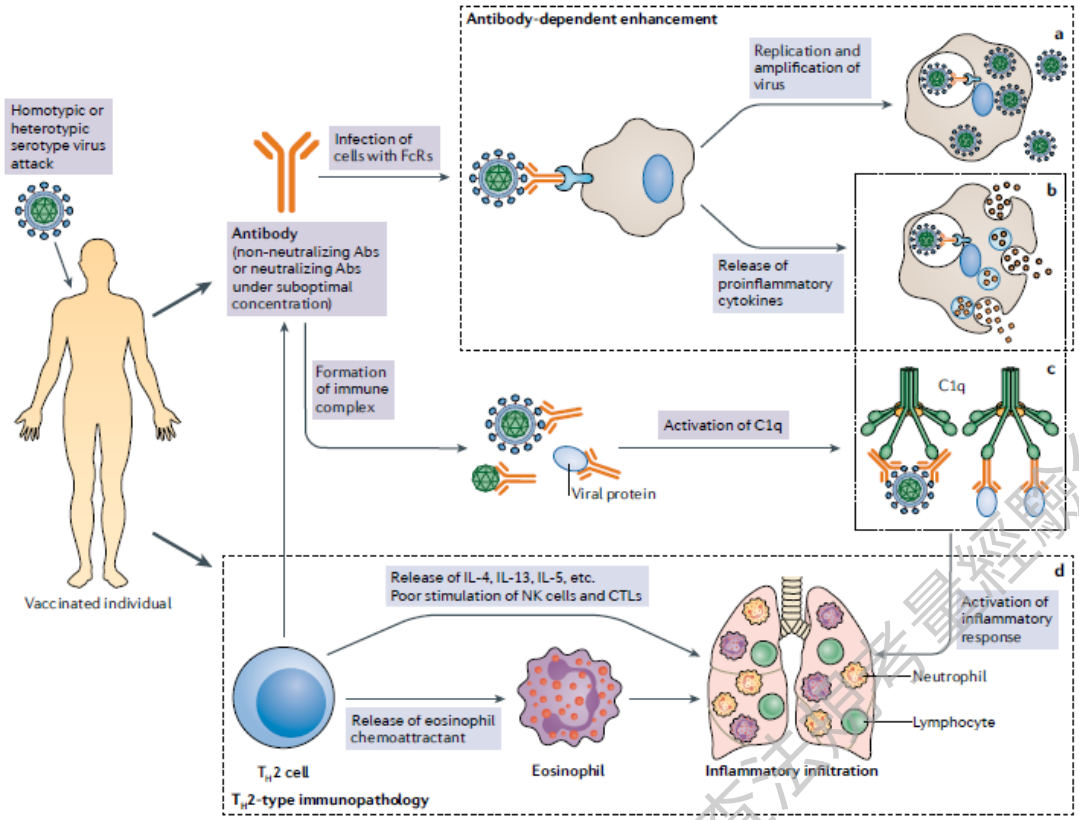
Developmental and Reproductive Toxicology

- Need depends on target population (e.g., pregnant women or women of childbearing potential)
- If need
  - Fertility can be assessed as part of repeated-dose toxicity studies by through standard histopathological examination on reproductive organs
  - In general, focus on prenatal and postnatal developmental (study endpoints covering the period from Stages C through F of ICH S5(3))
  - Species: should demonstrate an immune response
  - Route: should mimic the clinical route of administration
  - Dose: ideally, the maximal human dose should be administered to the test animal (typically full human, single dose level)

Biodistribution and Persistence

- To assess whether the mRNA vaccine distributes away from the tissue into which it was administered, into which tissues it distributes, and how long it persists

Vaccine-associated Disease Enhancement (VADE)



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Outline

Conclusion

- Nucleic acid-based drugs (i.g., ONTs) diversity: unique mechanisms of action with diverse toxicology profiles/concerns
- Case-by case!

財團法人醫藥品查驗中心
Center for Drug Evaluation, Taiwan

https://www.cde.org.tw/consultation_services/assistance_overview?id=6

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