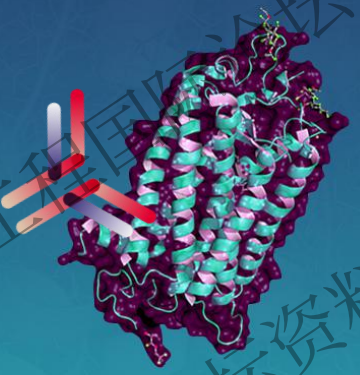
Comparability Study for GPCR Antibody--Getagozumab



Speaker: Kesuo Fan
Date: 2020.9

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Gmax Biopharm and Getagozumab (GMA301)

鸿运华宁药物研发现状

战略定位	• 以专有技术为依托，以产品创新为导向；专注于具有自主知识产权，针对重大疾病的一类新药的研究、开发、生产和销售。
技术平台	• 专注于治疗性G蛋白偶连受体(GPCR) 抗体制备、筛选、优化、和开发技术平台 • 独创的GPCR Bibody (双特异性分子) 构建和开发技术平台
治疗领域	• 心血管疾病：肺动脉高压（PAH），心衰等 • 代谢系统疾病：二型糖尿病，肥胖症，非酒精脂肪性肝炎（NASH）等 • 肿瘤(Bibody)：急性淋巴细胞白血病（ALL），卵巢癌，鼻咽癌，食管癌等
产品管线	• 2个临床二期在研新药（用于治疗二型糖尿病和肥胖症的GMA102和GMA105） • **1个临床一期在研新药（用于治疗肺动脉高压的GMA301）** • 4个进入临床前阶段的抗体和双体（Bibody）在研新药 • 6个处于不同的早期研究和开发阶段治疗性抗体和双体（Bibody）

Gmax GPCR mAb Platform

GPCRs: privileged families as drug targets

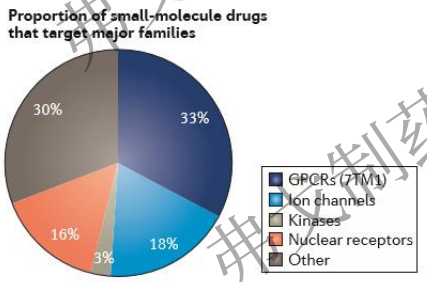
- *Well validated targets for over 35% approved therapeutics*
- *Huge market potential: Sales of drugs targeting GPCRs generated more than $120 billion in 2015*
- *Therapeutic areas covered: cardiovascular diseases and metabolic disorders*

Challenges to generate mAbs targeting GPCRs

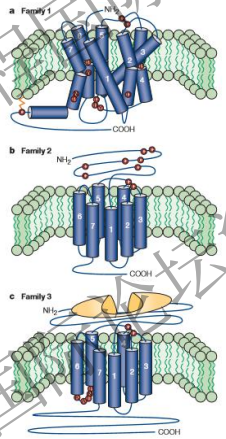
- *~ 80 GPCRs are appropriate mAb targets (C.J. Hutchings et al, /mAbs 2:6, 2012)*
- *Only 2 mAbs approved in world (MogamulizumAb/Poteligeo, Kyowa Hakko Kirin Co.)*
- *Technical challenges to develop mAbs Against GPCR s*

Gmax's unique and integrated mAb platform enables successful discovery and development of therapeutic mAbs against GPCRs

Innovation, Integration, Streamlining

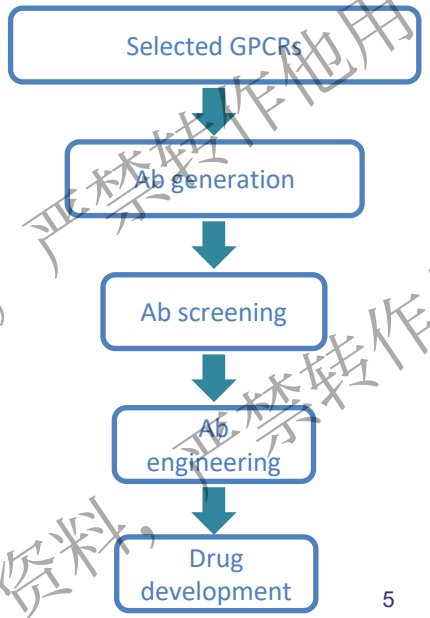


Nature Reviews | Drug Discovery | Dec. 2016

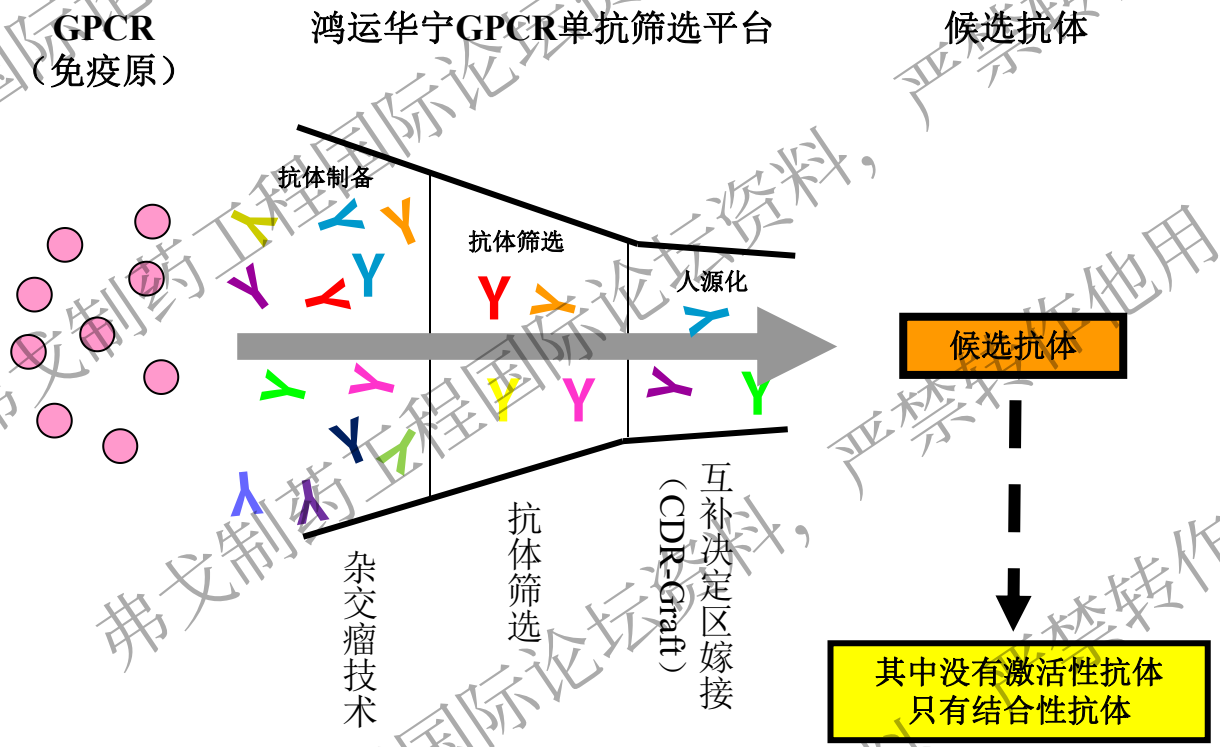


GPCR families and structures

Nature Reviews | Drug Discovery | Vol. 3 | July 2004



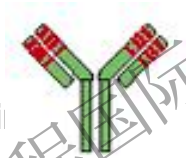
抗GPCR人源化单抗获得过程



GPCR antibody GMA301 (Getagozumab)

GMA301：

- 人源化单克隆抗体 (IgG4)
- 内皮素受体拮抗剂 (Endotheli receptor antagonist, ERA)



- 引起血管扩张

适应症：肺动脉高压，卵巢癌（Possible）

目前的ERA药物：

bosentan (Tracleer)
ambrisentan (Letairis/Volibris)
macitentan (Opsumit)

这些药物均为小分子类药物，其缺陷：短效，肝脏毒性。

- 专利状况： 原创一类新药，全球自主发明专利
- 市场前景： 该类药物有22亿美元销售额-2014；
  中国每年有100-200万新病人。
- 开发现状： 临床前研究；澳大利亚临床I期-完成；
  获FDA孤儿药认证。

GMA301是世界唯一的在研内皮素受体的抗体药物

靶向性强，药效好，半衰期长，没有肝脏毒性

GMA-301研究背景

肺动脉高压 (Pulmonary arterial hypertension, PAH)

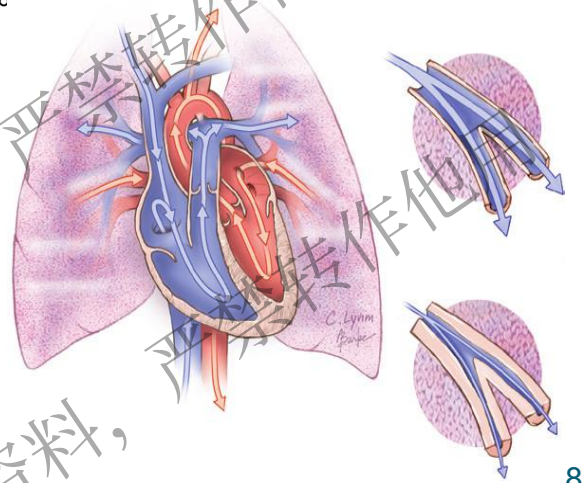
肺动脉高压是一种诊断难、治疗难的致命性疾病，被称为“心肺血管系统的癌症”。它是因为肺内或者与肺关联血管的不断束紧(vasoconstriction)，引起心脏对肺供血量不足，之后心脏对肺供血压力补偿性增加而引起的，其微观表现为肺小动脉内膜增厚，血管紧缩，重构，僵硬或者血栓造成的局部闭塞，进而血管对肺血液循环的阻力上升，最终导致右心室衰竭而死亡。病人不加治疗生存期仅为3年左右。

病因：

- 1）不明原因；
- 2）遗传性；
- 3）并发症（HIV，组织硬化症，小分子减肥药等）。

两个典型的症状：

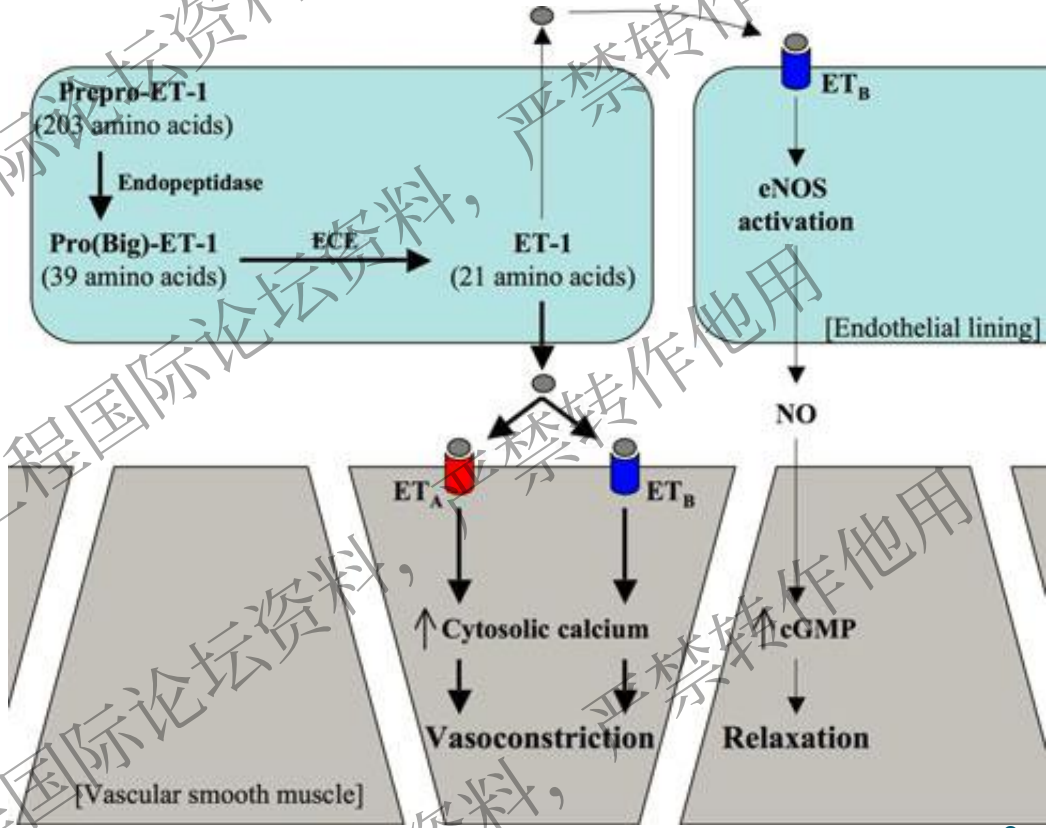
1. 肺动脉血压升高（25-30mmHg）
2. 血管壁组织增生，管壁增厚



内皮素(ET-1)在PAH中的作用

PAH发病过程中，体内内皮素(Endothelin-1,ET-1)水平升高。内皮素可以引起血管强而持久的收缩和血管壁增厚，从而引起血管压力上升。

ET-1 是通过与内皮素受体结合而发挥作用的。内皮素受体分为A和B两种[endothelin receptor subtype A (ET_A) and endothelin receptor subtype B (ET_B)]。内皮素受体是GPCR（G-Protein Coupled Receptor）的一种。



临床项目二：GMA301临床前研究

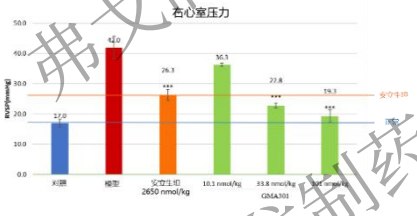
Getagozumab (吉他格株单抗)

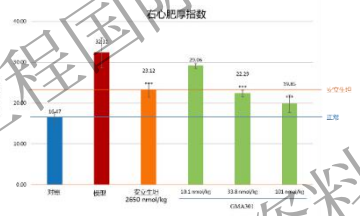
靶向ETa，用以治疗肺动脉高压（PAH）的单抗药

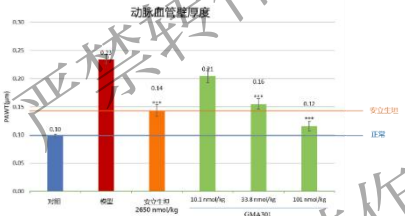
优势总结

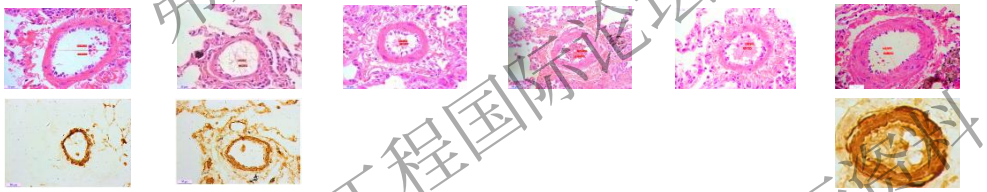
1. **疗效优：** 显著优于Ambrisentan
2. **靶向性强：** 仅阻断ETa (不阻断 ETb)，因此没有水肿的副作用
3. **安全性好：** 未观察到肝毒以及其他毒性反应
4. **半衰期长：** 有望做到每月给药一次

在MCT造模的食蟹猴PAH模型中，GMA301与Ambrisentan相比，可显著降低肺动脉压力、减少右心肥厚和肺动脉增生

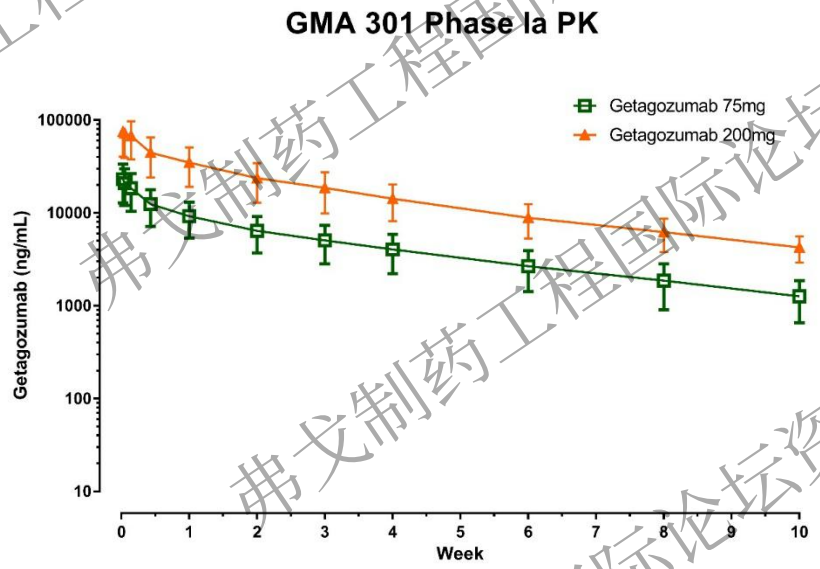








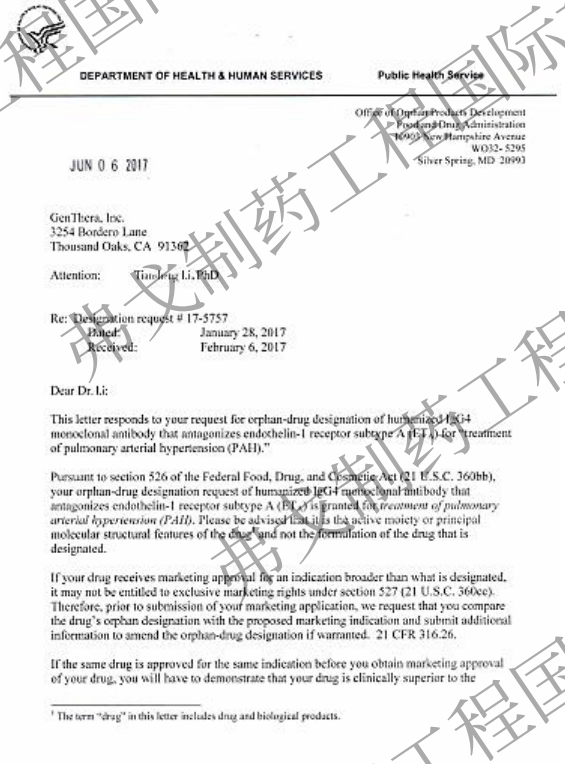
GMA301 Ia期临床药代数据（75mg、200mg剂量组）



PK参数	75 mg (N=6)	200 mg(N=6)
T1/2 (h)	498.4±107.1	565.3±60.8
Tmax (h)	4.0±0.0	8.0±8.0
Cmax (ng/mL)	26993.2±4959.0	89927.1±17341.0
AUC0-t (h*ng/mL)	8803269.8±1735715.6	31997574.4±5813593.7
AUC0-∞ (h*ng/mL)	9745076.6±2456720.3	35871252.9±6396907.3

GMA301 已获美国FDA孤儿药认证，入选国家重大新药专项

- GMA301在欧美是孤儿药，肺动脉高压发病人数每万人中小于5人。
- 在中国有100-200万病人，服用进口药每人每年的花费高达20万元, 市场规模巨大。



DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

Office of Orphan Products Development
Food and Drug Administration
10903 New Hampshire Avenue
WO32- 5295
Silver Spring, MD 20993

JUN 0 6 2017

GenThera, Inc.
3254 Bordero Lane
Thousand Oaks, CA 91362

Attention: Tiansheng Li, PhD

Re: Designation request # 17-5757
Dated: January 28, 2017
Received: February 6, 2017

Dear Dr. Li:

This letter responds to your request for orphan-drug designation of humanized IgG4 monoclonal antibody that antagonizes endothelin-1 receptor subtype A (ET-1) for "treatment of pulmonary arterial hypertension (PAH)."

Pursuant to section 526 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360bb), your orphan-drug designation request of humanized IgG4 monoclonal antibody that antagonizes endothelin-1 receptor subtype A (ET-1) is granted for *treatment of pulmonary arterial hypertension (PAH)*. Please be advised that it is the active moiety or principal molecular structural features of the drug[1] and not the formulation of the drug that is designated.

If your drug receives marketing approval for an indication broader than what is designated, it may not be entitled to exclusive marketing rights under section 527 (21 U.S.C. 360cc). Therefore, prior to submission of your marketing application, we request that you compare the drug's orphan designation with the proposed marketing indication and submit additional information to amend the orphan-drug designation if warranted. 21 CFR 316.26.

If the same drug is approved for the same indication before you obtain marketing approval of your drug, you will have to demonstrate that your drug is clinically superior to the

[1] The term "drug" in this letter includes drug and biological products.

GenThera, Inc. 2

already approved same drug in order to obtain orphan-drug exclusivity. Failure to demonstrate clinical superiority over the already approved same drug will result in your drug not receiving orphan-drug exclusivity. 21 CFR 316.34(c).

You must submit to the Office of Orphan Products Development a brief progress report of drug development within 14 months after this date and annually thereafter until marketing approval. 21 CFR 316.30.

Please notify this Office within 30 days of submitting a marketing application for the drug's designated use. Once your marketing application is approved, please contact Florence Moore, M.S., Ph.D., at 301-796-9226 or alternatively at 301-796-8660 to assess eligibility for orphan-drug exclusivity.

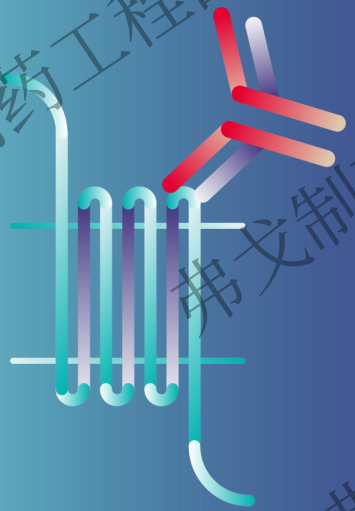
If you have questions regarding the development of your designated product, please feel free to contact Soumya Patel, PharmD, at 301-796-8678 or alternatively at 301-796-8660. Congratulations on obtaining your orphan-drug designation.

Sincerely,

for Gayatri R. Rao

Gayatri R. Rao, MD, JD
Director
Office of Orphan Products Development

十三五 国家重大新药创制专项



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Comparability Study
(可比性研究)

可比性研究是生物药开发中的关键步骤

- 目标：证明产品的质量、安全性和活性在工艺改变前后没有产生负面性的变化。(Products made using pre- and post-change processes are required to be comparable as demonstrated by comparability studies to qualify for continuous development and commercial supply.)

- 可比性研究的结果并不是确认产品完全一致，而是证明产品高度相似。(Not to confirm that the quality attributes of the pre-change and post-change product are identical, but highly similar quality attributes.)

- 以达到避免重做临床前或临床试验。(If a manufacturer can provide assurance of comparability through analytical studies alone, nonclinical or clinical studies with the post-change product are not warranted.)

工艺变更的主要原因：

- improving the manufacturing process
- increasing scale
- Manufacturing site change
- improving product stability
- complying with changes in regulatory requirements

During early development, it is common practice for pharmaceutical companies to focus on rapid advancement to first-in-human studies in order to achieve proof-of-concept while gaining knowledge to inform subsequent development decisions. Continued process optimization is therefore necessary to meet regulatory requirements toward late-stage development, and to have a robust process heading into commercial manufacturing

Comparability is phase related（可比性研究和开发阶段相关）：

The earliest time that the comparability exercise applies is between the nonclinical materials used for investigational new drug (IND) application-enabling studies and Phase 1 clinical material. During early phases of nonclinical and clinical studies, comparability testing is generally not as extensive as for an approved product.

For late stage comparability studies, comprehensive comparability studies, including a thorough evaluation of the product quality using data from routine lot release; extended characterization, including isolation and characterization of variants and impurities; process testing, stability and forced degradation, are performed.

if analytical comparability is not established, nonclinical and clinical studies will be required.

Phase-appropriate comparability

Phase-appropriate comparability refers to the strategy adopted to ensure that the comparability study is designed to meet phase specific requirements (see following Table), which vary in depth and scope for different phases of development.

Table 2. Scope of analytical comparability at different phases of development.

Phase of development	Scope of comparability	Acceptance Criteria
Nonclinical and Phase 1 clinical study	Release Characterization	Not necessary for pre-defined acceptance criteria
Between Phases 1, 2 and 3	Release Extended characterization (including peak isolation and characterization if new peaks or the same peak with increased intensity are seen) In-process (assays and controls) Stability, if appropriate Forced degradation, if appropriate, selected conditions	Pre-defined acceptance criteria based on limited experience and limited statistical analysis
After pivotal study	Release Extended characterization (Including peak isolation and characterization if new peaks are seen) In-process (assays and controls) Stability Forced degradation, including more conditions	Pre-defined acceptance criteria based on statistical analysis

Critical quality attributes，

风险评估 Risk-assessment

Risk is defined as "the combination of the probability of occurrence of harm and the severity of that harm. Risk assessment is composed of risk identification, risk analysis and risk evaluation. Risk assessment is an integral part of a comparability study. Risk assessment helps define the extent of comparability studies, driving the selection of lots, analytical methods, and

Table 3. Examples of proposed manufacturing changes and the associated risks.

Proposed changes	Potential risks to CQAs
Manufacturing site change	High risk
Drug substance scale change	Medium risk
Facility fit change	Medium risk, depending on the nature of change
Cell line change	High risk
Medium and feed change	High risk
Fermentation set point change	Medium to low risk, may have been covered during process characterization
Chromatography matrix change	High risk on clearance of residuals, adventitious agents, product-related substances/impurities and process-related impurities
Chromatography operation parameter change	Medium risk, may have been studied during process characterization
Raw material changes	Medium risk, potentially impacting extractable and leachable
Drug substance storage container and temperature	Medium risk, extractable, leachable, stability
Formulation change-new excipients	High risk, stability
Formulation change-same excipients at different concentrations	Low risk
Drug product storage temperature	Low risk, supported by development data
Drug product packing	Low risk, supported by development data
Drug product presentation	Medium to high risk, depending if raw material or device are changed

可比性研究方案 Comparability protocols

Table 7. Contents of a typical comparability protocol.

Sections	Contents
Process history and comparison	Brief process history Rational for process change Comparison of pre- and post-change process
Risk assessment	Leverage on development knowledge and scientific literature to predict which quality attributes are likely to be impacted and the potential impact on safety and efficacy Leverage knowledge of CQA for this risk assessment
Comparability strategy	Release Extended characterization In-process Stability, if needed Forced degradation, if needed Non clinical and clinical, if needed Provide justifications for the selected tests and studies
Lot selection	Number of lots Lot genealogy Representative lots of the pre- and post-change lots
Methods and studies	List of methods, studies and justification
Acceptance criteria	Quantitative and qualitative

可比性研究的分析方法

- Release testing methods
- Physicochemical characterization
- In-process testing methods
- Stability testing methods

The battery of tests for the comparability exercise should be carefully selected and optimized to maximize the potential for detecting relevant differences in the quality attributes of the product that might result from the proposed manufacturing process change.

The measurement of quality attributes in characterization studies does not necessarily entail the use of validated assays, but the assays should be scientifically sound and provide results that are reliable. Those methods used to measure quality attributes for batch release should be validated, as appropriate.

分析方法要全面

Although the pre- and post-change product appear highly similar, the analytical procedures used are not sufficient to discern relevant differences that can impact the safety and efficacy of the product. The manufacturer should consider employing additional testing (e.g., further characterization) or nonclinical and/or clinical studies to reach a definitive conclusion.

It is essential to apply more than one analytical procedure to evaluate the same quality attribute

可比性研究的分析与判断

Where the change results in the appearance of new impurities, the new impurities should be identified and characterized when possible.

Manufacturers should consider evaluating all relevant functional activities. Biological assay results can serve multiple purposes in the confirmation of product quality attributes that are useful for characterization and batch analysis, and, in some cases, could serve as a link to clinical activity. The manufacturer should consider the limitations of biological assays, such as high variability, that might prevent detection of differences that occur as a result of a manufacturing process change.

The manufacturer should confirm that the specifications after the process change are appropriate to ensure product quality. Results within the established acceptance criteria, but outside historical manufacturing control trends, might suggest product differences that warrant additional study or analysis.

可比性研究的几种结果

- The pre- and post-change product is highly similar.
- Although the pre- and post-change product appear highly similar, some differences have been identified in the comparison of quality attributes and a possible adverse impact on safety and efficacy profiles cannot be excluded. In such situations, the generation and analysis of additional data on quality attributes are unlikely to assist in determining whether pre- and post-change product are comparable. The manufacturer should consider performing nonclinical and/or clinical studies.
- Differences in the quality attributes are so significant that it is determined that the products are not highly similar and are therefore not comparable.

达到放行检验的标准是可比性研究的先决条件：
(Meeting the release specifications is a basic requirement for demonstrating product consistency with respect to product quality, safety and efficacy.)

Table 4. A typical list of batch release assays for mAb drug substance.

Attributes	Methods
Safety	Bioburden
Safety	Endotoxin
General	Appearance (color and clarity)
General	pH
General	Concentration
Identity	Peptide mapping (LC-UV)
Purity	SDS-PAGE/CE-SDS (non-Reducing and reducing)
Purity	SEC-HPLC
Potency	Antigen binding
Potency	Cell-based assay
Potency	Effector functions
Charge/identity	IEX-HPLC/IEF/cIEF/CZE
Glycosylation	N-glycan profiling by NP-HPLC of labeled glycans
Impurities	HCPs
Impurities	Host cell DNA
Impurities	Residual protein A

Table 8. Proposed acceptance criteria for mAb comparability assessment.

Category of testing	Specific assays	Acceptance criteria
Routine batch release	Peptide mapping	• Meeting release specification •Comparable peak profiles based on retention times and relative intensity •No new or missing peaks in the post-change lots
	SDS-PAGE/CE-SDS	•Meeting release specification •Percentage of main band/peak within the acceptance criteria based on statistical analysis •Same banding/peak pattern •No new species
	SEC-HPLC	•Meeting release specification •Percentage of main peak within the acceptance criteria based on statistical analysis •Same retention times of the aggregate, monomer and fragment peaks
	Charge (CEX, cIEF)	•Meeting release specification •Percentage of major peaks within the acceptance criteria based on statistical analysis •No new peaks in the post-change lots
	Oligosaccharides	•Meeting release specification •Percentage of major peaks within the acceptance criteria based on statistical analysis •No new peaks in the post-change lots
	Binding affinity	•Meeting release specification •Binding affinity within the acceptance criteria based on statistical analysis
	Cell based assay	•Meeting release specification · Potency within the acceptance criteria based on statistical analysis
Extended characterization	Molecular weight analysis by LC-MS	•Mass error within the instrument accuracy •The same species
	Peptide mapping with LC-MS detection	•Confirmation of the primary sequence •Percentages of post-translational modifications within the acceptance criteria
	Disulfide bonding pattern	•Confirmation of the correct disulfide bond linkage
	Free thiol	•Level of free cysteine within the acceptance criteria based on statistical analysis
	CD	•No substantial difference in the spectra and conformational fractions, if calculated
	AUC	•Percentage of main peak within the acceptance criteria based on statistical analysis •Aggregates, monomer, and fragments with comparable sedimentation velocity
Process comparison	Process controls	•Equal or better process control
	Product quality	•Equal or better impurities clearance •Equal or better product intermediate stability •Comparable product-related substance
Stability	Real time and accelerated	•Comparable or slower degradation rates •Same degradation pathways
Forced degradation	Various conditions	•Comparable degradation kinetics •Same degradation pathways

Comparability with in-process controls

The DS should be evaluated at the process step most appropriate to detect a change in the quality attributes

Adequacy of the in-process controls including critical control points and in-process testing: In-process controls for the post-change process should be confirmed, modified, or created, as appropriate, to maintain the quality of the product;

稳定性研究 Stability

Stability studies are used to demonstrate that the post-change material has a stability profile comparable to that of the pre-change material.

Stability studies have the potential to detect differences that cannot be detected by release and extended characterization assays

Stability studies include real time, accelerated, and forced degradation studies.

Table 6. Various forced degradation conditions and their effects on mAbs.

Forced degradation conditions	Quality attributes to evaluate
Thermal	Aggregations and chemical modifications such as oxidation, deamidation
Low pH	Aggregation and fragmentation
High pH	Aggregation, deamidation, degradation of disulfide bonds
Agitation	Aggregation
Freeze/thaw	Aggregation
Oxidation	Susceptible sites of oxidation, which may be altered if structure changes introduced
Deamidation	Susceptible sites of deamidation, which may be altered if structure changes introduced
Glycation	Susceptible sites of glycation, which may be altered if structure changes introduced
Photo	Tryptophan oxidation

稳定性研究的重要性

Any change with the potential to alter protein structure or purity and impurity profiles should be evaluated for its impact on stability.

For example, the presence of trace amounts of a protease might only be detected by product degradation that occurs over an extended time period; or, in some cases, divalent ions leached from the container closure system might change the stability profile because of the activation of trace proteases not detected in stability studies of the pre-change product.

Accelerated and stress stability studies are often useful tools to establish degradation profiles and provide a further direct comparison of pre-change and post-change product.

可比性研究报告 Comparability report:

A comparability report is generated and ultimately used for regulatory submission to obtain approval of the changed process.

The core data used for the comparability study come from routine batch release testing, extended characterization and process comparison in terms of process controls and in-process results. Depending on the phase of development, the nature of changes, and the outcome of the risk assessment, stability and forced degradation data may also be needed to establish comparability.

If there are differences, the report should primarily focus on the differences and justifications as to whether or not the differences will negatively impact product quality, and thus adversely impact product safety and efficacy.

In cases where comparability cannot be established based on quality data, nonclinical and clinical studies are required.

抗体分子常见修饰及在可比性研究中的应用

N-terminal modifications

The two most common N-terminal modifications of a mAb are the presence of pyroglutamate (pyroGlu) as the first amino acids of the mature light chain or the heavy chain and the presence of unprocessed leader sequences.

The presence of N-terminal pyroGlu or a leader sequence is not expected to affect the overall structure and function of recombinant mAbs.

C-terminal modification

Removal of C-terminal lysine (Lys) and C-terminal amidation are the two major C-terminal modifications.

From this body of knowledge, it can be concluded that neither C-terminal Lys nor amidation is expected to impact mAb structure, stability, function or safety.

N-linked glycosylation

N-glycosylation is one of the most sensitive indicators of manufacturing consistency, and therefore is of particular interest for comparability studies.

Sialic acid

Sialic acids on the conserved Fc-glycans of mAbs are present at levels rarely exceeding 5%. But it is important to closely monitor and control the level of NGNA and carefully evaluate the levels when comparing pre- and post-change lots during comparability studies.

Fucose

In contrast to other receptors, low core-fucosylation results in a dramatic improvement in antibody binding to FcgRIIIa89-92 and leads to higher ADCC activity. The correlation between low core-fucosylation and higher ADCC was found to translate into higher efficacy.

High mannose

Heightened concern around high mannose structures is related to studies indicating their effect on the PK properties of recombinant mAbs. Such studies have demonstrated that the presence of high mannose resulted in shorter in vivo half-life in animal models as well as in humans.

Aglycosylation

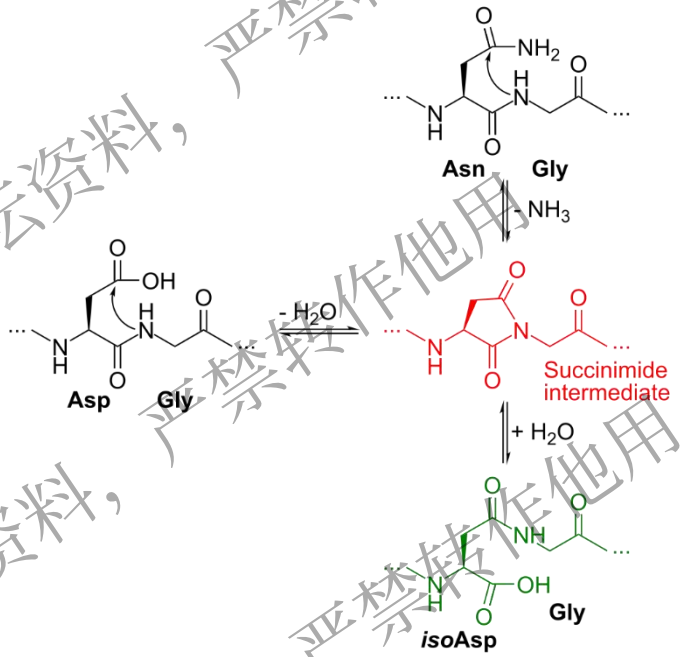
Aglycosylated IgG1 antibodies show substantial conformational differences, decreased stability and almost complete loss of the Fc effector-triggered biological functions such as ADCC and CDC.

Deamidation

Deamidation has been reported to occur in antibody complementarity-determining regions (CDRs), and resulted in decreased antigen binding affinity. However, deamidation has been reported more frequently in the constant domains of recombinant mAbs.

The effect of deamidation varies depending on the location of the Asn residue and the resulting products.

The presence of succinimide in the CDRs has been shown to decrease mAb antigen binding affinity and potency.



Oxidation

The most prevalent oxidation events of mAbs occur to methionine128–131 and tryptophan132–134 residues.

Modifications related to cysteine residues

Glycation

Glycation of lysine residues has not been shown to affect potency and PK.

Many of the modifications result in the generation of acidic species because they are either on the side chains of lysine or arginine residues or the light chain and heavy chain N-terminal primary amine groups. The reaction products are undesirable in all these cases and need to be evaluated as part of the comparability assessment.

Aggregation

Aggregates are one of the major impurities in mAb therapeutics, and are classified, by default, as a CQA.

The major concerns with aggregation are loss of efficacy, receptor activation through cross-linking and, most importantly, immunogenicity.

Coloration

Coloration of mAb drug substances is a common quality attribute, especially for high concentration solutions. Oxidation of tryptophan residues, the presence of advanced glycation end products (AGEs), and association of mAb with B vitamins, their degradation products or B-vitamin mediated reaction products have been identified as contributors to the coloration of mAb solutions. In addition, Formulation buffer excipients could have a substantial impact on mAb coloration.

Charge variants

From the perspectives of product quality and comparability, charge variants are important because they are the most commonly cited reason for heterogeneity.

Acidic variants are typically the sum of unrelated mAb variants containing various degrees of sialylation, Asn deamidation and glycation.

Basic species are mainly formed due to uncyclized N-terminal Gln, C-terminal lysine and C-terminal amidation.

If process changes result in differences in the formation of new product-related variants or impurities, the differences would likely be detected by a charge-based method. When differences in charge profile are observed, thorough characterization is required to understand their chemical nature, and their impacts on safety and potency.

Product-related impurities
Process related impurities

This distinction is very relevant to the comparability exercise because the expectations for a tighter control of product-related impurities will be notably higher than for the variants.

The safety risks associated with process-related impurities and contaminants call for particular attention to be paid to both when evaluating pre- and post-change materials for comparability.

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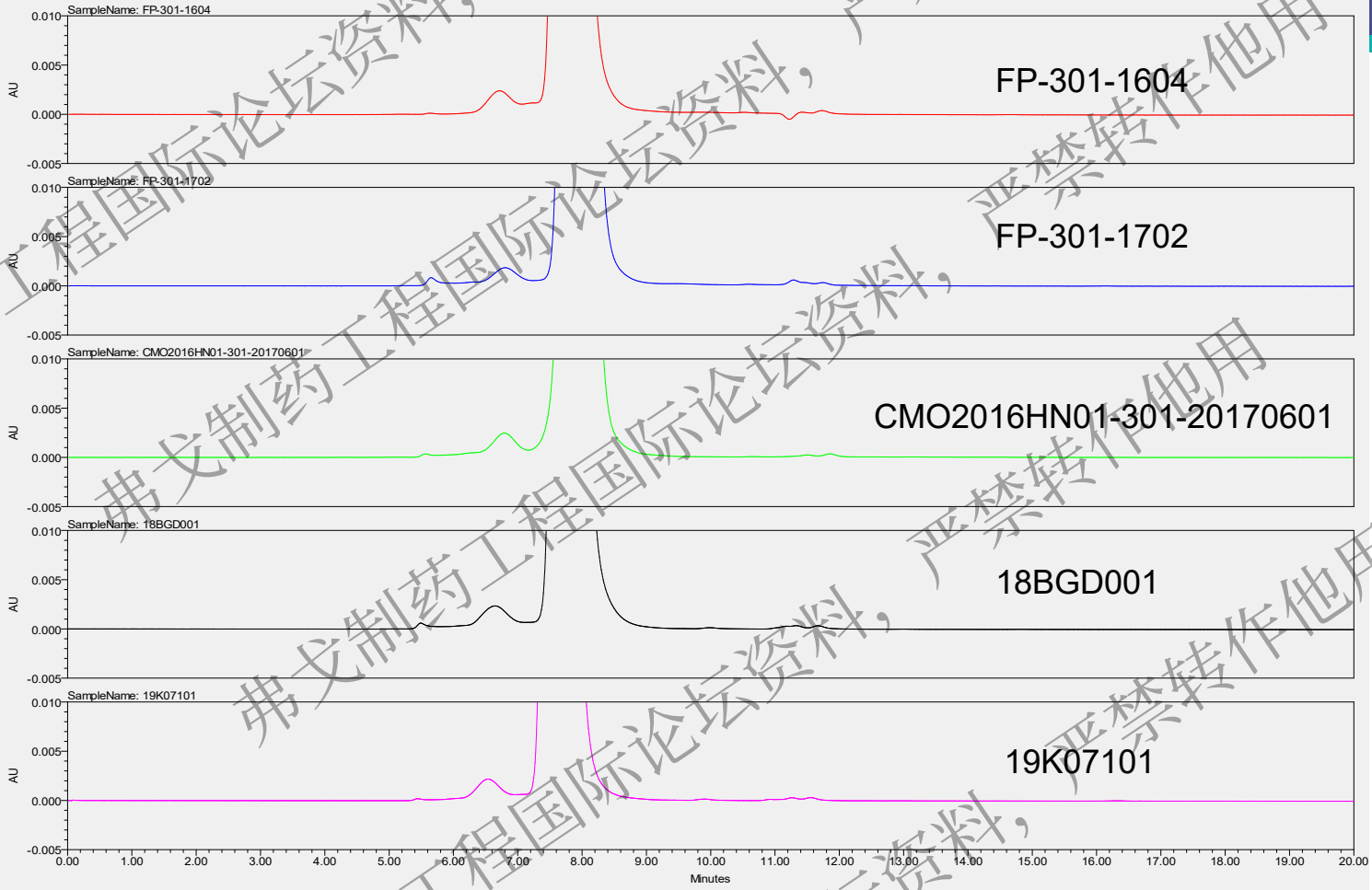
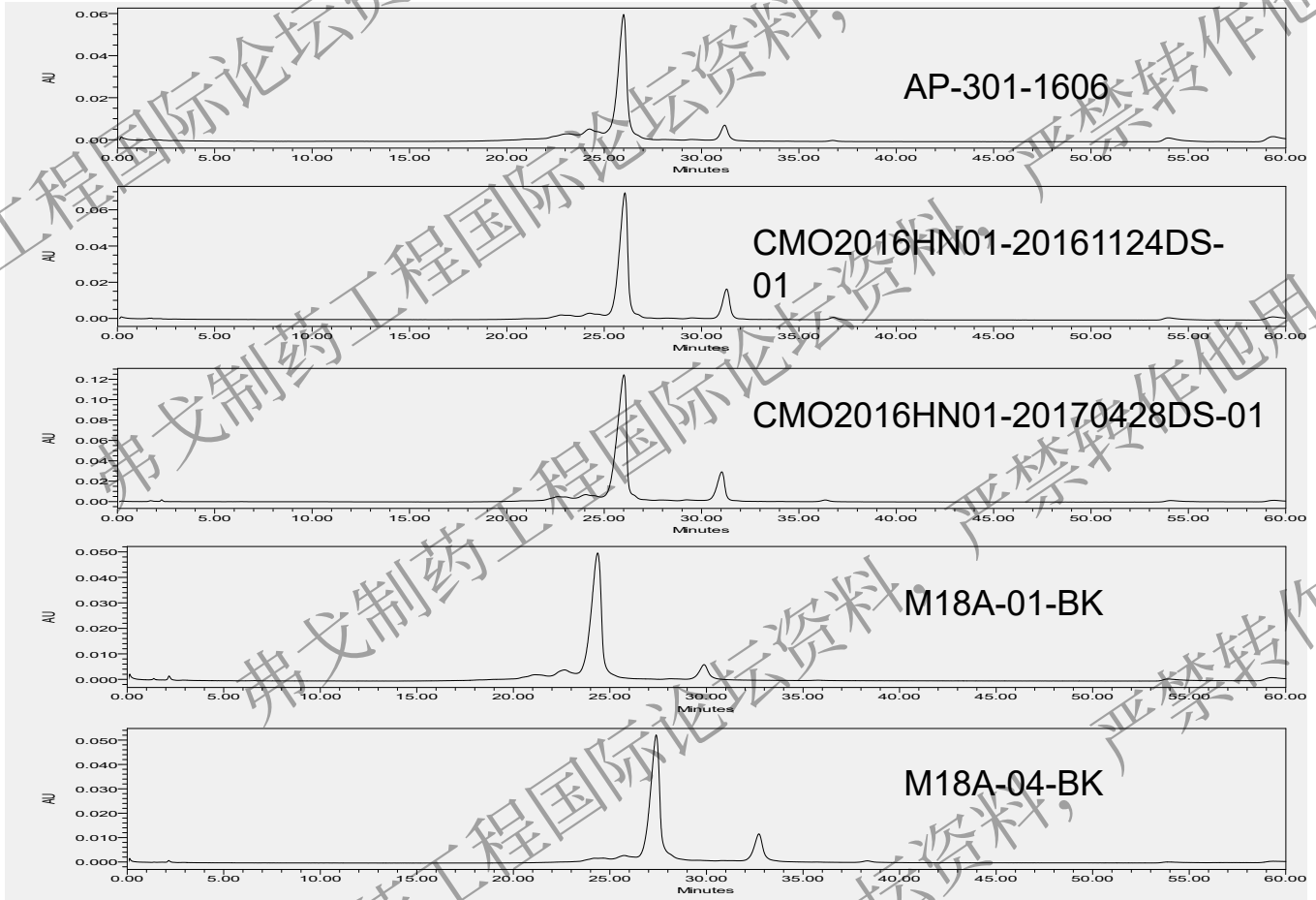
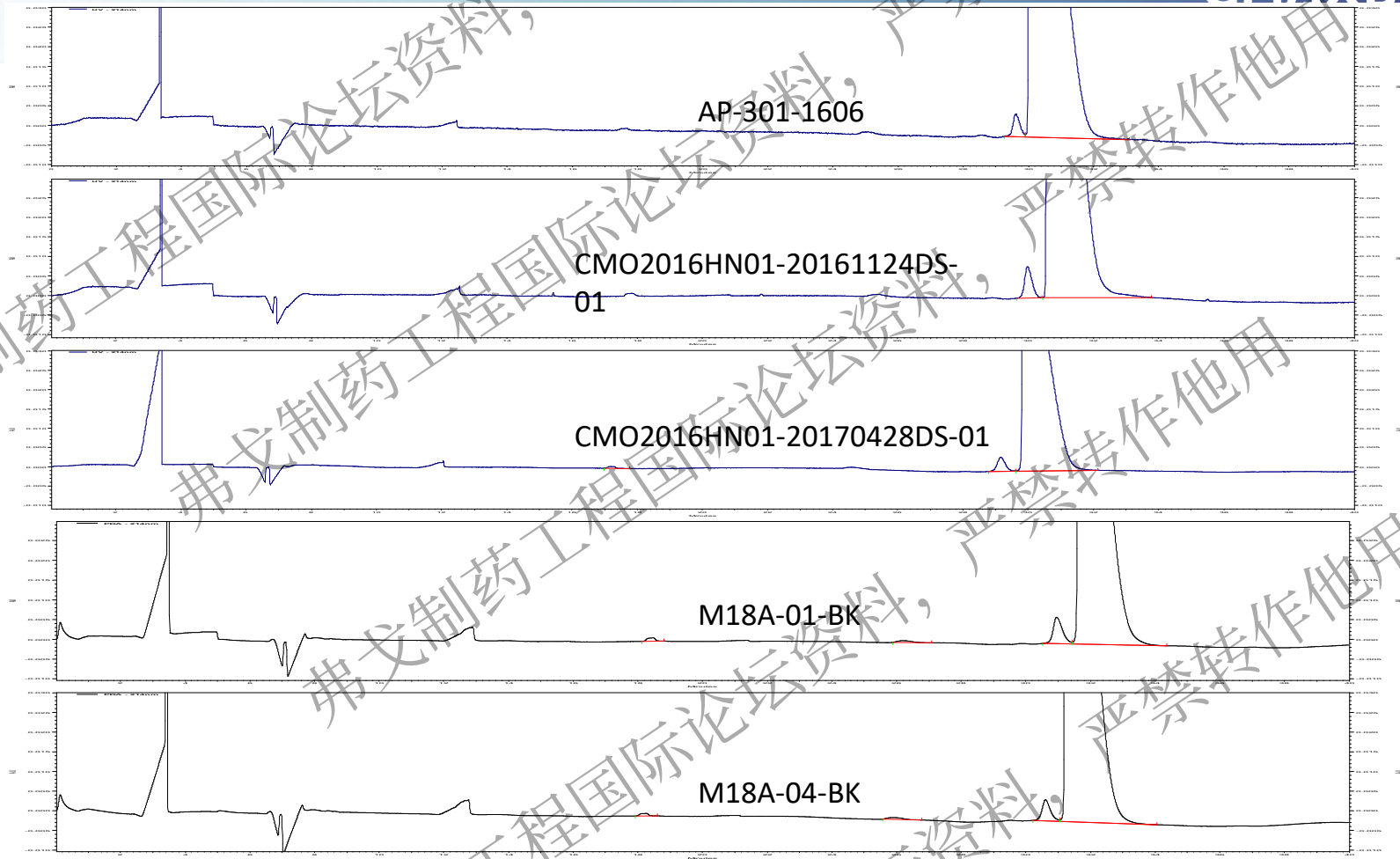
Case study
场地变更可比性

SEC-HPLC





非还原CE-SDS



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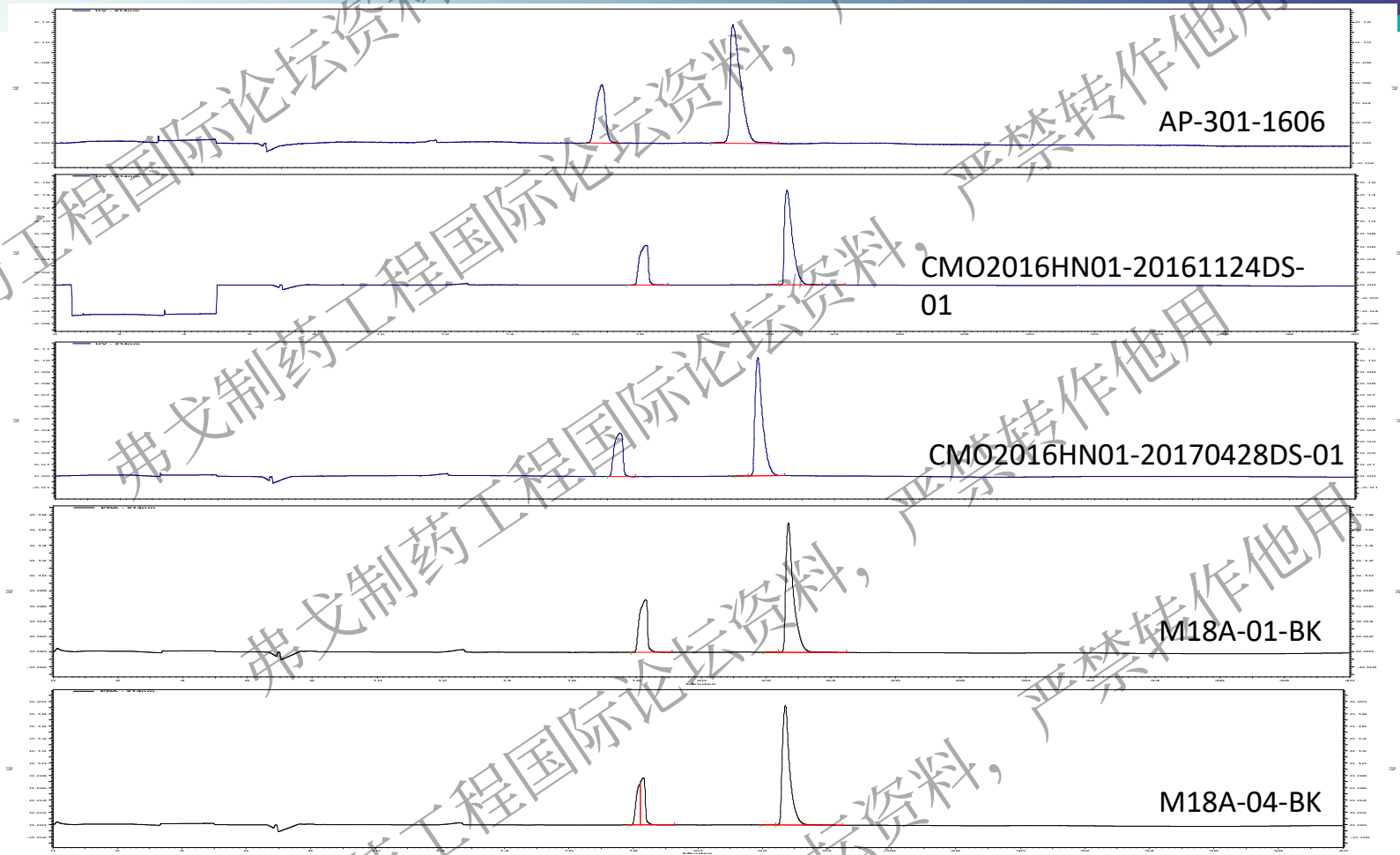
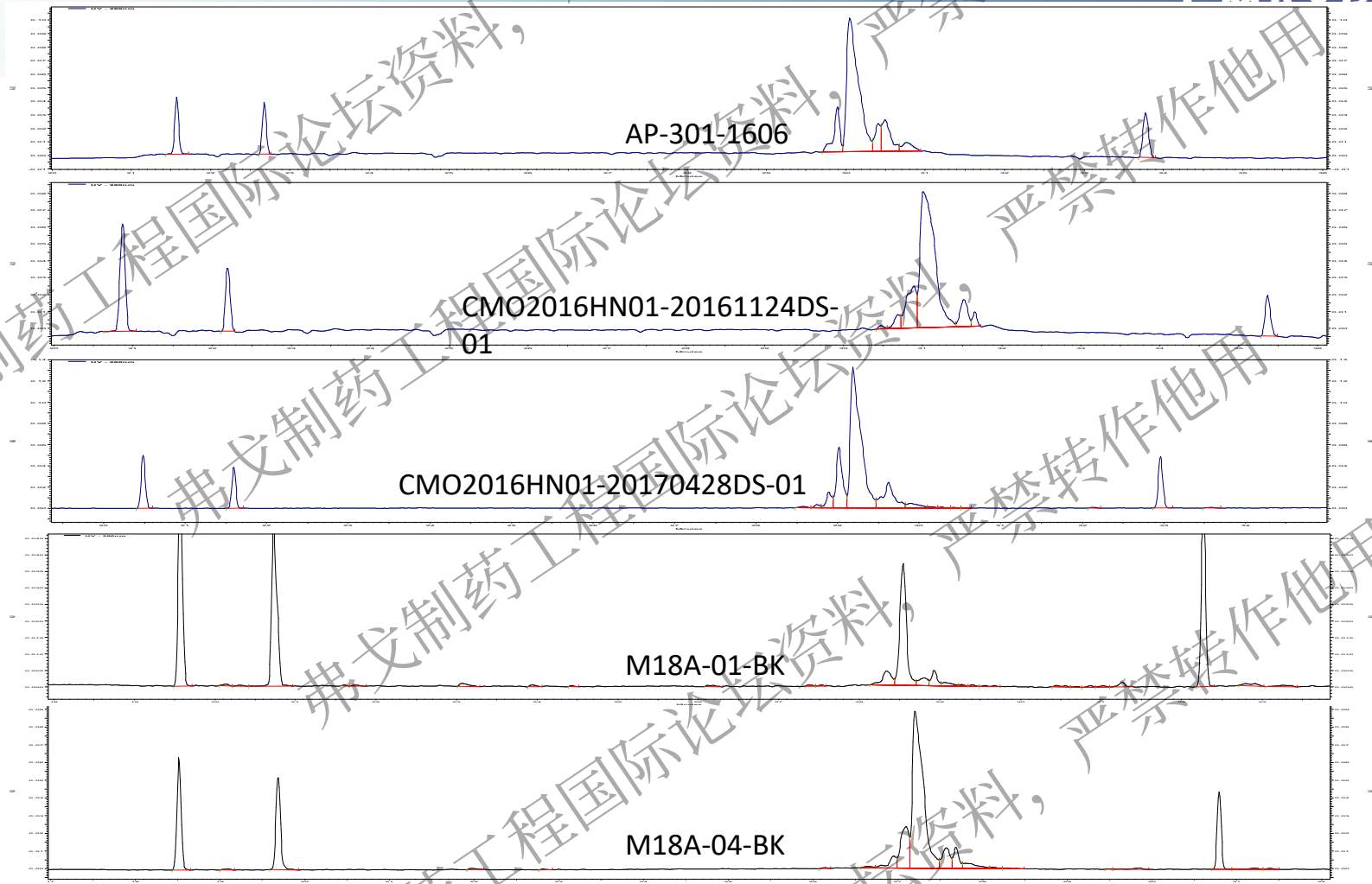
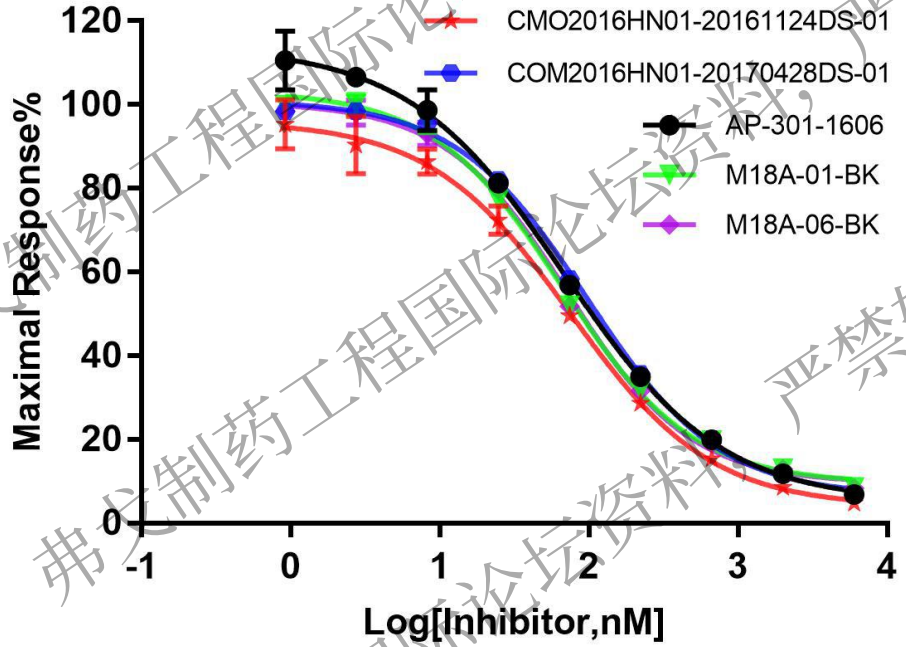


CIEF



GMA301原液相对结合活性



Contents

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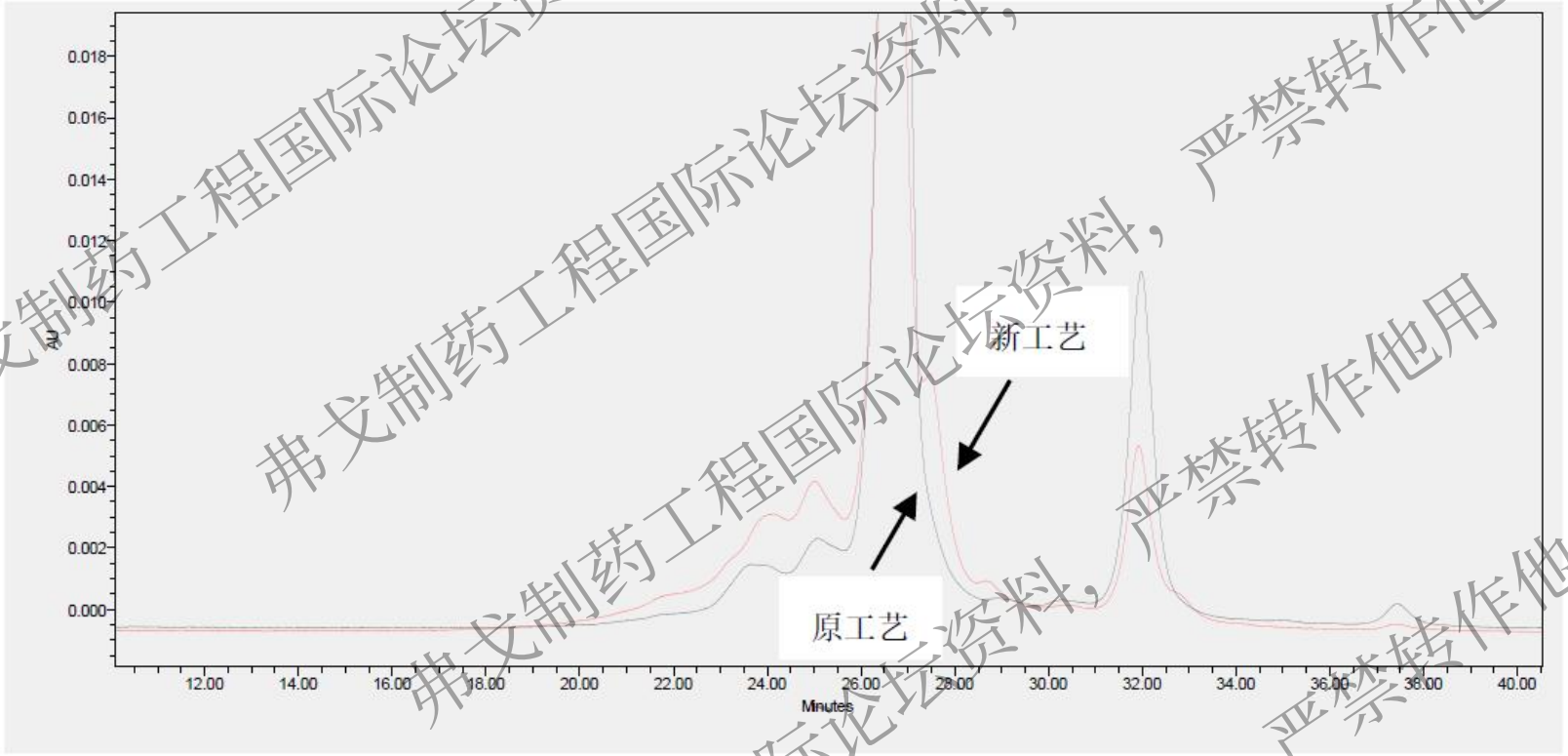
Case study
克隆变更可比性

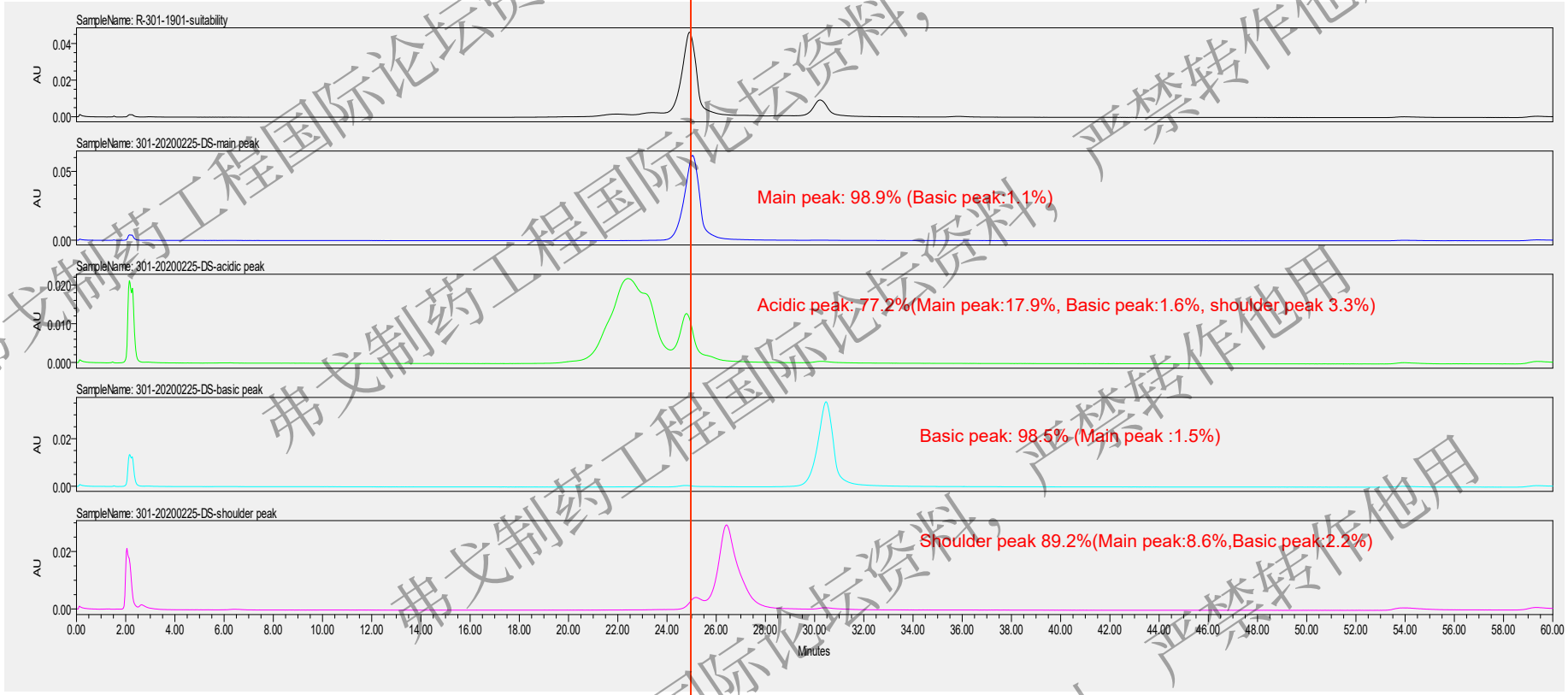
原液放行检测结果

Lot	Items	Results	Specifications
M18A-20-E-200L-1-BK (New clone)	HCP	0.0015%	≤ 0.0100%
	HCD	＜0.18 pg/mg	≤10 pg/mg protein
	Pro.A	0.00004%	≤ 0.0010%
	SEC	Monomer: 99.4% HMW: 0.6%	Monomer: ≥ 95.0% HMW: ≤5.0%
	Reduced CE	(LC+HC):99.6%	(LC+HC): ≥ 90.0%
	Non-reduced CE	97.4%	Monomer: ≥ 90.0%
	WCEX	Acidic variants: 24.8% Basic variants: 10.8% Main peak: 52.6% Shoulder peak：11.8%	Acidic variants: ≤ 30.0% Basic variants: ≤ 25.0% Main peak: ≥ 50.0%
	Peptide mapping	Conform	The profile should conform to working reference standard
	Relative binding activity	Not tested	50.0%~150.0%
	Biological activity	115.6%	50.0%~150.0%
	Polysorbate 80	Not tested	0.2~0.6 mg/ml

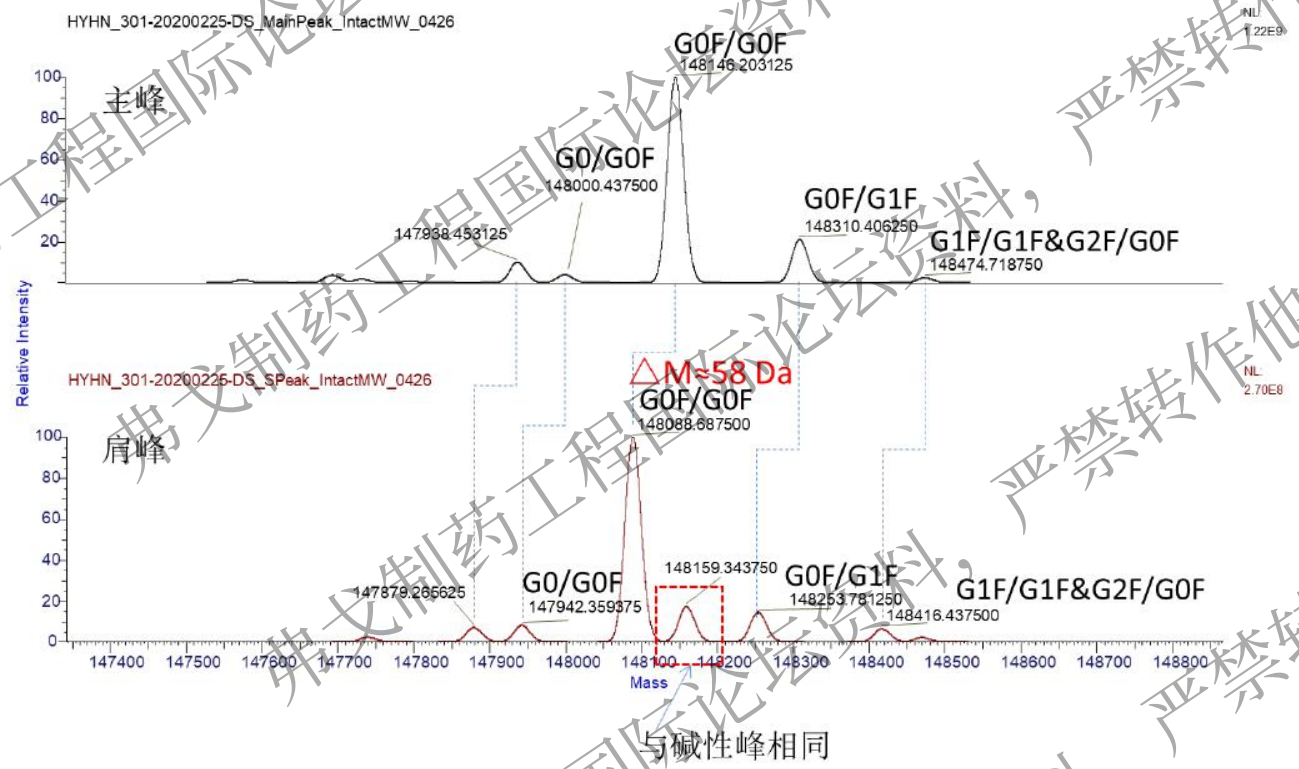
新旧克隆电荷异构体差异



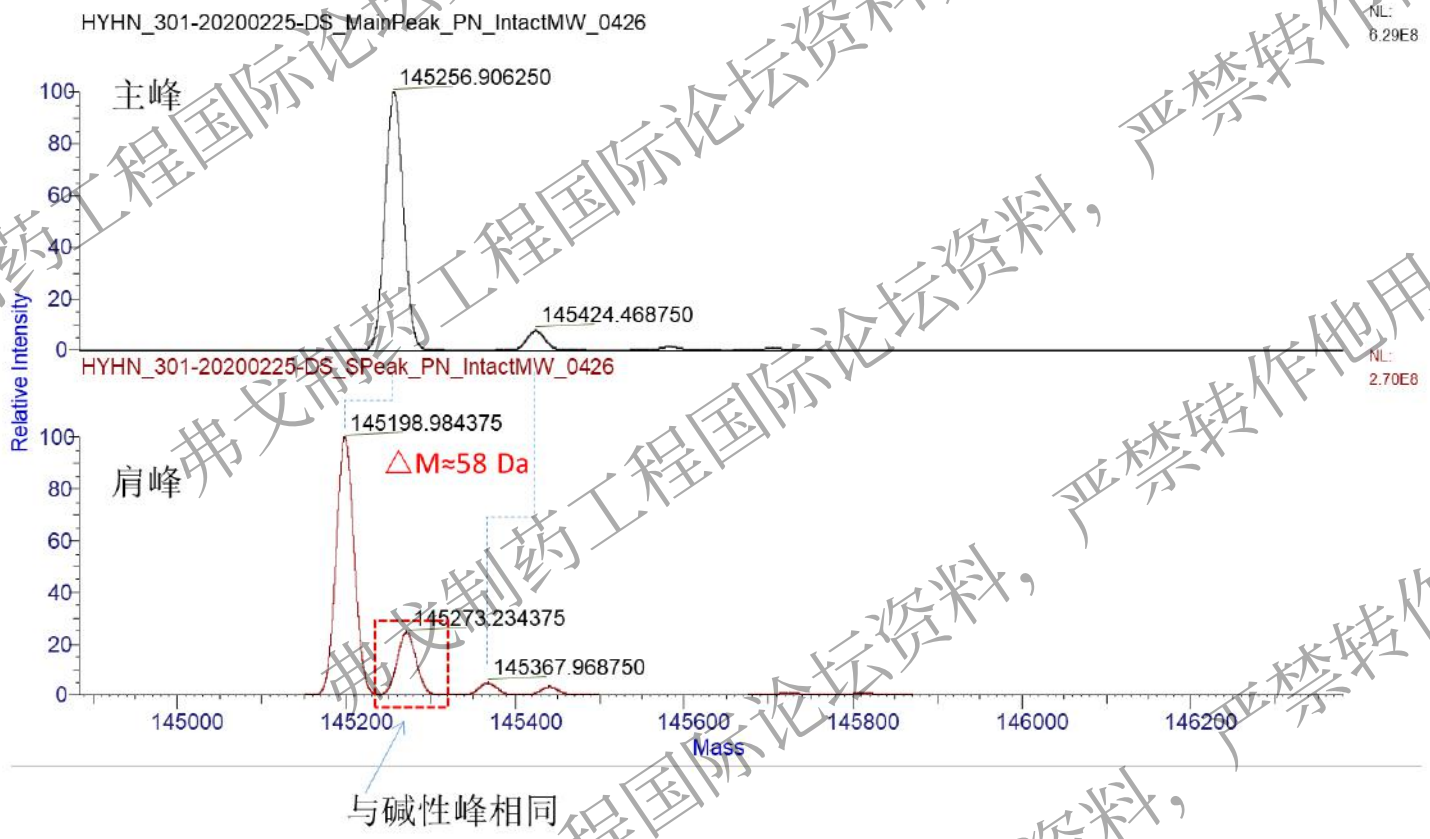
半制备HPLC电荷异构体制备



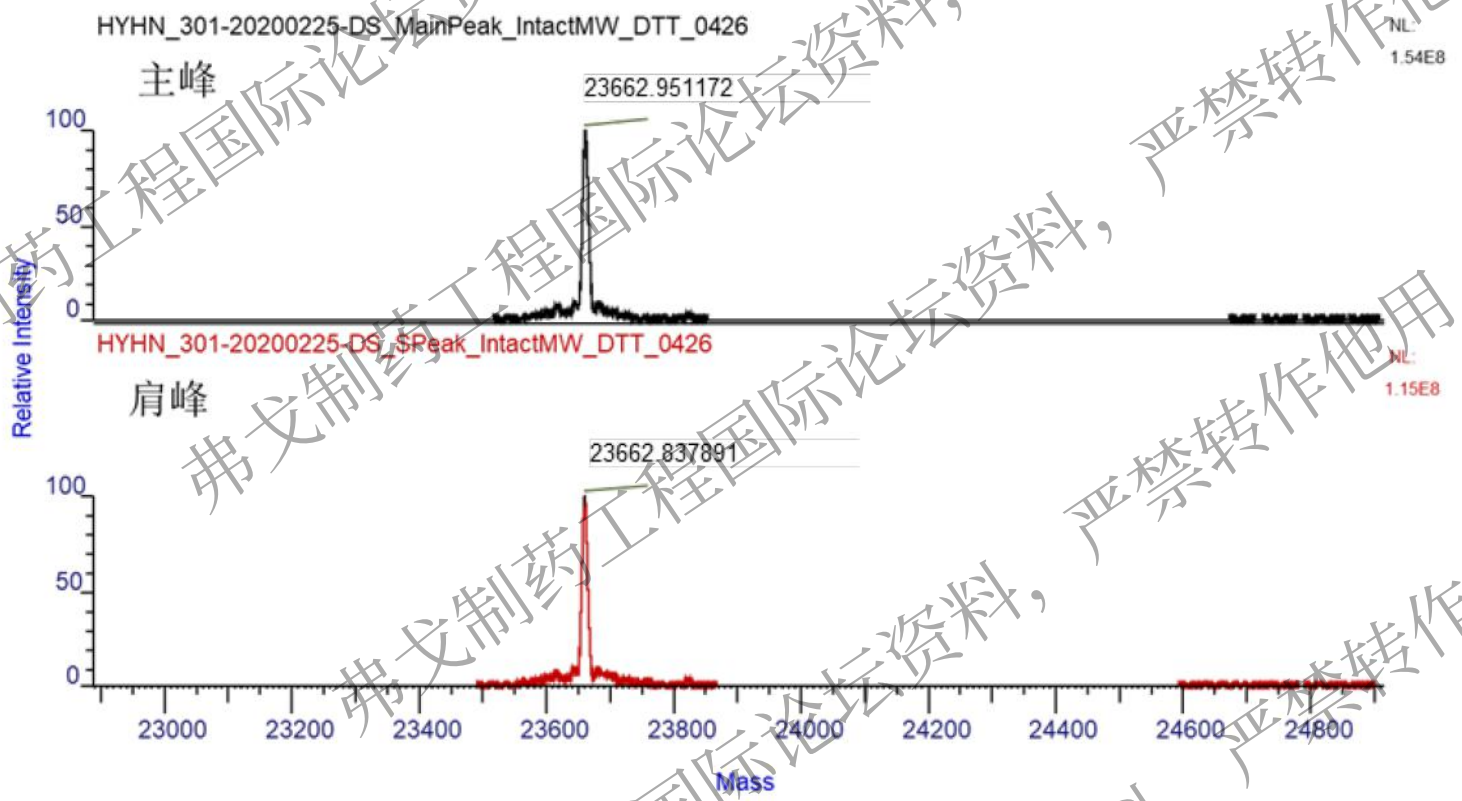
电荷异构体表征-完整分子量对



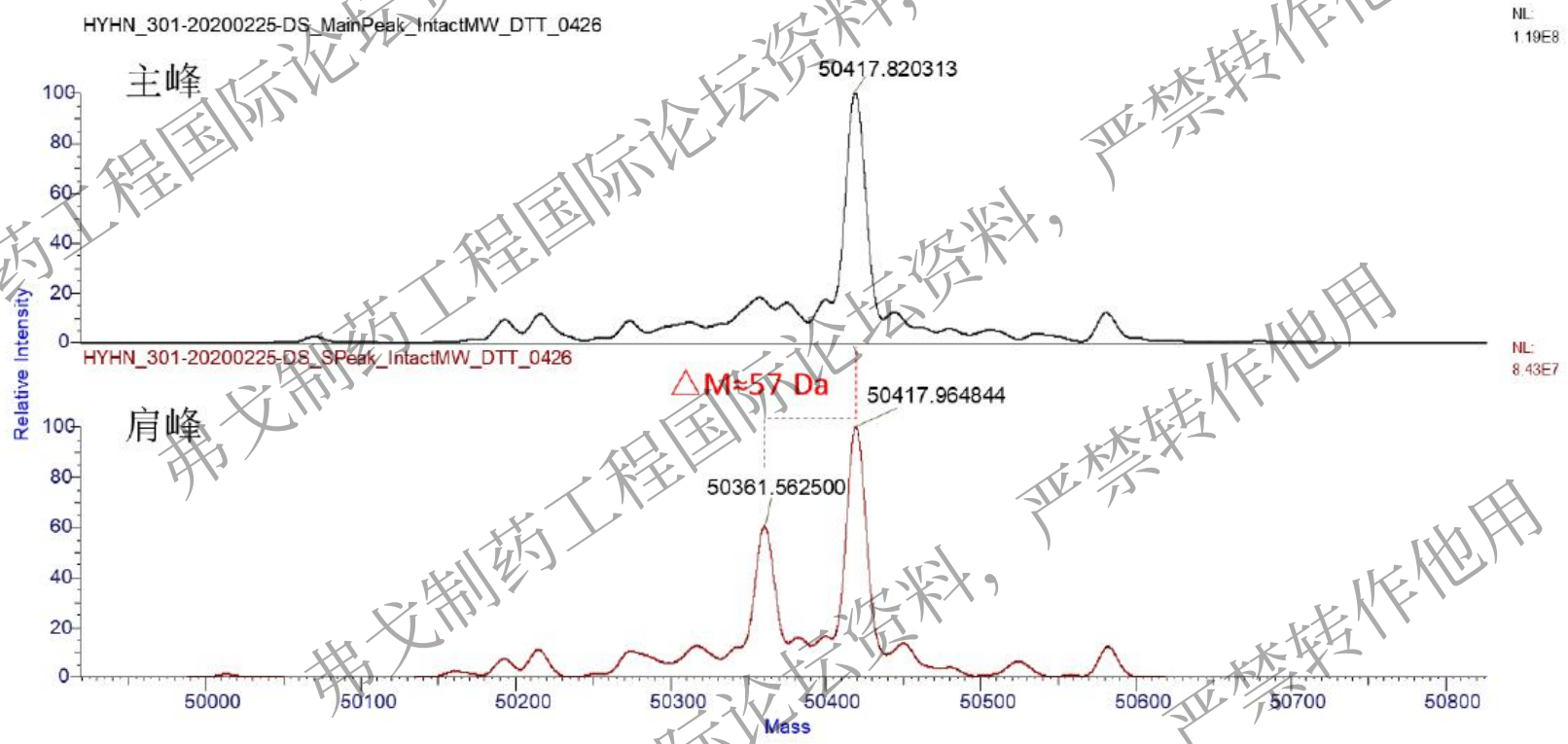
电荷异构体表征-去糖完整分子量对比



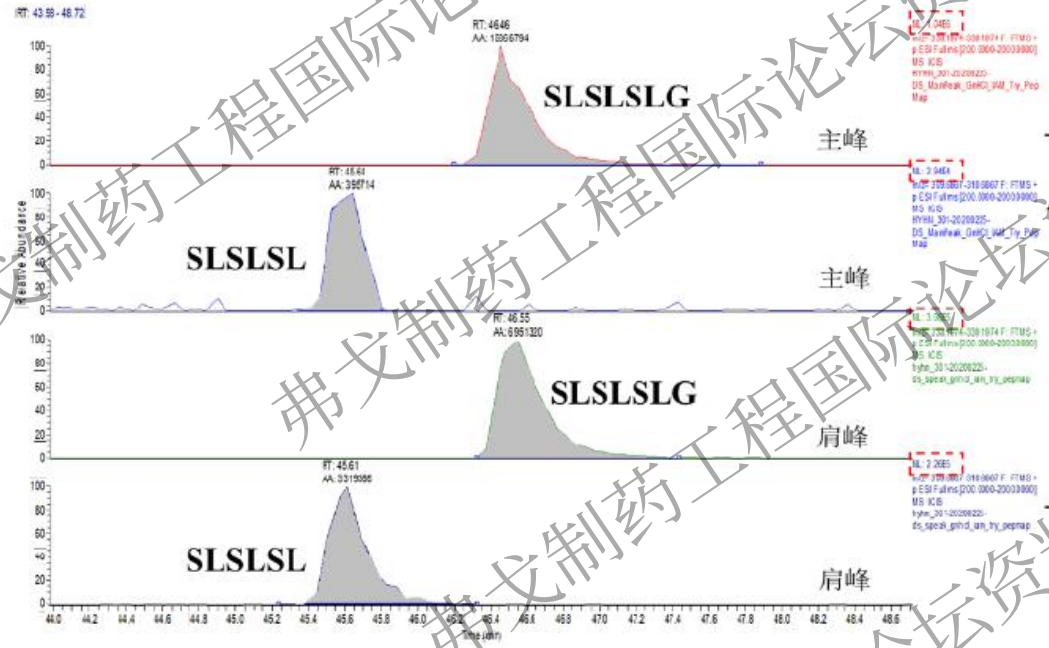
电荷异构体表征-还原轻链分子量对比



电荷异构体表征-还原重链分子量对比



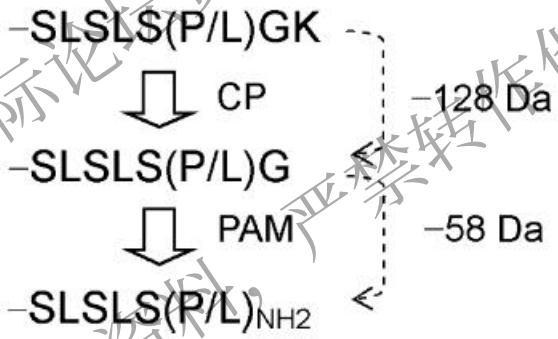
电荷异构体表征-提取离子流（XIC）对比



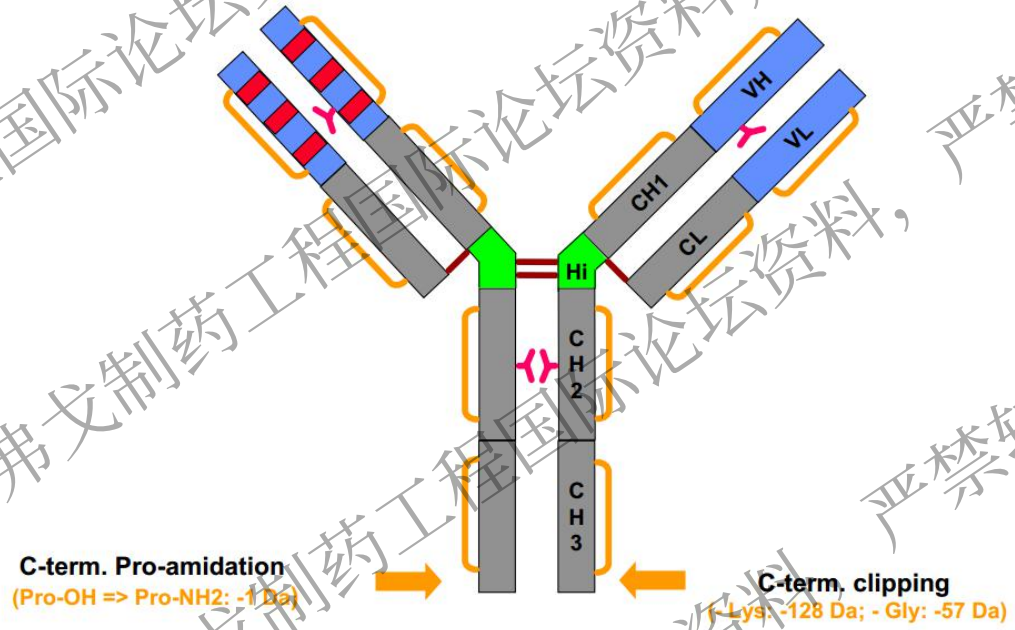
	肽段	肽段编号	峰面积
主峰	SLSLSLG	T38	1.59E+07
	SLSLSL	T38*	3.96E+05
肩峰	SLSLSLG	T38	6.95E+06
	SLSLSL	T38*	3.32E+06

碱性峰的确认

- 收集的电荷异质体酶解，LCMS分析，Biopharma Finder搜索，添加常见的修饰，额外添加末端的丢失Gly并发生amidation（DesG_Amidation，质量减少58Da）。
- Amidation修饰可以导致碱性峰。
- 根据三种酶解的结果，BP峰可能发生末端丢失Gly并同时在C末端有amidation，导致BP和MP相差59Da。



Scheme 1. Speculated process of the C-terminal processing of recombinant therapeutic monoclonal antibodies. C-terminal Lys on the heavy chains of mAbs that is encoded in the gene sequences is removed by intracellular CP(s) resulting in a 128 Da reduction in molecular mass. PAM cleaves C-terminal Gly and produces C-terminal Pro amide for IgG1, IgG2 and IgG3, or Leu amide for IgG4 leading to a 58 Da reduction in molecular mass [12]. CP stands for carboxypeptidase. PAM stands for peptidylglycine alpha-amidating monooxygenase.



脱去甘氨酸：-57Da

酰胺化：-1Da

总共-58Da分子量

Conclusions: LC-MS results showed that the post-shoulder-peak was caused by the glysine depletion and leucine amidation at the C-terminal. (Biopharma Finder_DesG_Aimdation, **△M≈58Da)**

Beck, A.; *et al*. Characterization of Therapeutic Antibodies and Related Products. *Analytical Chemistry*. 2013, 85(2): 715-736

电荷异构体表征-生物学活性对比

Sample	Lot of ref.	参比品活性IC50	对照活性IC50	Relative activity%	Mean value %	CV%
GMA301-20200225-DS-main Peak（主峰）	R-301-1901	25.29	27.44	92.2	117.9	22.9
		41.55	33.71	123.3		
		18.94	15.69	120.7		
		12.36	11.83	104.5		
		16.9	10.1	167.3		
		12.69	12.73	99.7		
GMA301-20200225-DS-acidicPeak（酸性峰）		25.29	24.51	103.2	103.4	12.2
		41.55	39.91	104.1		
		18.94	17.2	110.1		
		12.36	14.93	82.8		
		16.9	13.99	120.8		
		12.69	12.8	99.1		
GMA301-20200225-DS-basicPeak（碱性峰）		28.93	19.55	148.0	132.1	12.0
		28.93	22.16	130.6		
		25.06	20.8	120.5		
		20.16	13.01	155.0		
		14.92	12.16	122.7		
		23.17	19.97	116.0		
GMA301-20200225-DS-shoulder Peak（肩峰）		24.4	20.78	117.4	133.9	8.7
		24.4	18.75	130.1		
		16.88	12.41	136.0		
		20.16	13.22	152.5		
		14.92	10.76	138.7		
		23.17	18.01	128.7		
GMA301-20200225-DS（原液）		31.37	29	108.2	100.7	NA
		41.27	44.25	93.3		

电荷异构体表征-结合活性对比

样品名称	参比品批号	参比品IC50	样品IC50	相对结合活性%	平均值%	CV%
GMA301-20200225-DS-main Peak（主峰）	R-301-1901	80.94	90.27	89.7	86.1	4.0
		62.27	70.46	88.4		
		49.56	59.61	83.1		
		55.33	66.51	83.2		
GMA301-20200225-DS-acidicPeak（酸性峰）		80.94	90.47	89.5	86.3	4.0
		62.27	72.38	86.0		
		49.56	56.25	88.1		
		55.33	67.78	81.6		
GMA301-20200225-DS-basicPeak（碱性峰）		72.58	66.27	109.5	103.2	18.6
		36.02	41.54	86.7		
		46.84	52.69	88.9		
		93.53	73.34	127.5		
GMA301-20200225-DS-shoulder Peak（肩峰）		72.58	79.21	91.6	85.0	9.2
		36.02	44.03	81.8		
		46.84	62.12	75.4		
		93.53	102.7	91.1		

Thank You

GMAXBio