ADCのバイオアナリシス

パドセブを例に

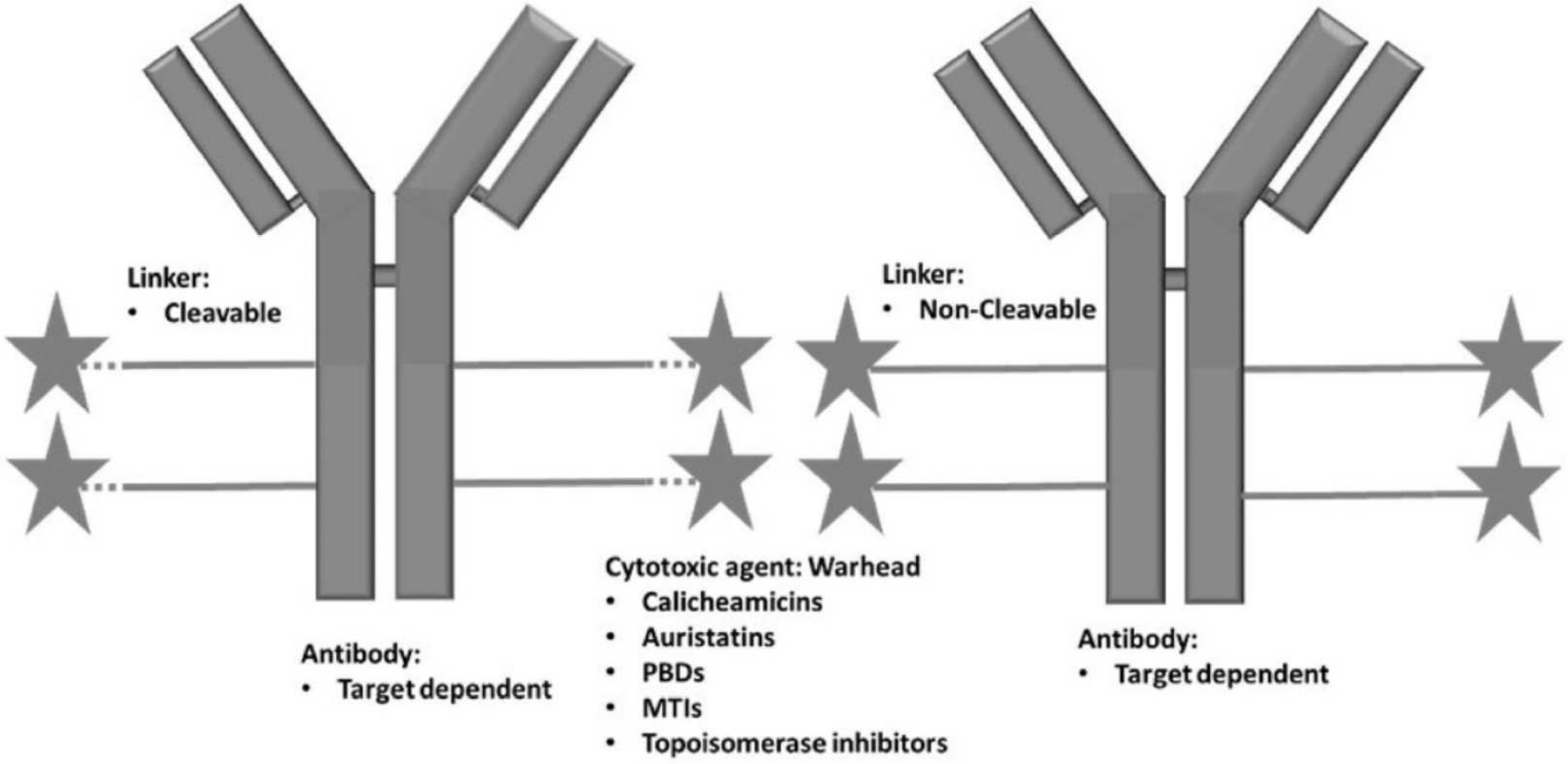
永多正憲
アステラス製薬株式会社　非臨床バイオメディカルサイエンス
March 03, 2023

Funding Statement: This research was funded by Astellas.

目次

ADC

ADC



K Beaumont, et al, Xenobiotica, 52:8, 770-785, 2022

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近年FDAに承認されたADC薬剤(2000-2018)

ADC Drug	Trade name	Maker	Disease Indication	Payload/Payload Class	Payload Action	Target	mAb	Linker	DAR	Approval Year
Moxetumomab pasudotox	Lumoxiti	Astrazeneca	Relapsed or refractory hairy cell leukemia (HCL)	PE38 (Pseudotox)	/	CD22	IgG1	Cleavable	N/A	2018
Inotuzumab ozogamicin	Besponsa	Pfizer/Wyeth	relapsed or refractory CD22-positive B-cell precursor acute lymphoblastic leukemia	ozogamicin /calicheamicin	DNA cleavage	CD22	IgG4	acid cleavable	6	2017
Trastuzumab emtansine	Kadcyla	Genentech, Roche	HER2-positive metastatic breast cancer (mBC)	DM1/maytansinoid	microtubule inhibitor	HER2	IgG1	non-cleavable	3.5	2013
Brentuximab vedotin	Adcetris	Seagen Genetics, Millennium/Takeda	relapsed HL and relapsed sALCL	MMAE/auristatin	microtubule inhibitor	CD30	IgG1	enzyme cleavable	4	2011
Gemtuzumab ozogamicin	Mylotarg	Pfizer/Wyeth	relapsed acute myelogenous leukemia (AML)	ozogamicin /calicheamicin	DNA cleavage	CD33	IgG4	acid cleavable	2–3	2017; 2000

https://axispharm.com/antibody-drug-conjugatesadcs-list-approved-by-fda2000-2022/ より改変

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近年FDAに承認されたADC薬剤(2019-2022)

ADC Drug	Trade name	Maker	Disease Indication	Payload/Payload Class	Payload Action	Target	mAb	Linker	DAR	Approval Year
Mirvetuximab soravtansine	ELAHERE	Immuno Gen	Platinum-Resistant Ovarian Cancer	Maytansinoid DM4	Folate receptor alpha	FRα	IgG1	/	N/A	2022
Tisotumab vedotin-tftv	Tivdak	Seagen Inc	Recurrent or metastatic cervical cancer	MMAE/auristatin	microtubule inhibitor	Tissue factor	IgG1	enzyme cleavable	4	2021
Loncastuximab tesirine-lpyl	Zynlonta	ADC Therapeutics	Large B-cell lymphoma	SG3199/PBD dimer	DNA cleavage	CD19	IgG1	enzyme cleavable	SG3199/PBD dimer	2021
Belantamab mafodotin-blmf	Blenrep	GlaxoSmithKline (GSK)	Relapsed or refractory multiple myeloma	MMAF/auristatin	microtubule inhibitor	BCMA	IgG1	non-cleavable	4	2020, withdrawn on 22 Nov. 2022
Sacituzumab govitecan	Trodelvy	Immunomedics	mTNBC	SN-38/camptothecin	TOP1 inhibitor	TROP2	IgG1	acid cleavable	7.6	2020
Trastuzumab deruxtecan	Enhertu	AstraZeneca/Daiichi Sankyo	Unresectable or metastatic HER2-positive breast cancer	DXd/camptothecin	TOP1 inhibitor	HER2	IgG1	enzyme cleavable	8	2019
Enfortumab vedotin	Padcev	Astellas/Seagen Genetics	Locally advanced or metastatic urothelial cancer	MMAE/auristatin	microtubule inhibitor	Nectin4	IgG1	enzyme cleavable	3.8	2019
Polatuzumab vedotin-piiq	Polivy	Genentech, Roche	relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBCL)	MMAE/auristatin	microtubule inhibitor	CD79	IgG1	enzyme cleavable	3.5	2019

https://axispharm.com/antibody-drug-conjugatesadcs-list-approved-by-fda2000-2022/ より改変

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パドセブ

パドセブ(エンホルツマブ　ベドチン)

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＊2022年６月改訂（第３版）
2021年11月改訂

抗悪性腫瘍剤／抗Nectin-4抗体微小管阻害薬複合体
エンホルツマブ　ベドチン（遺伝子組換え）注

生物由来製品、劇薬、
処方箋医薬品
注意－医師等の処方箋により
使用すること

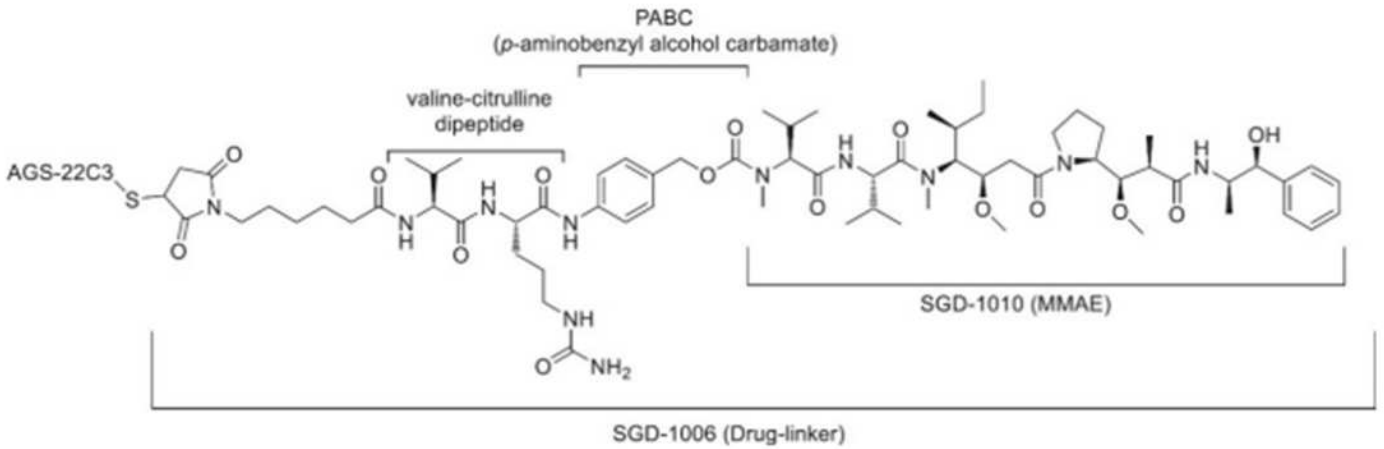
貯法：２～８℃で保存
＊有効期間：30箇月

パドセブ®点滴静注用30mg

PADCEV® for I.V. infusion 30mg

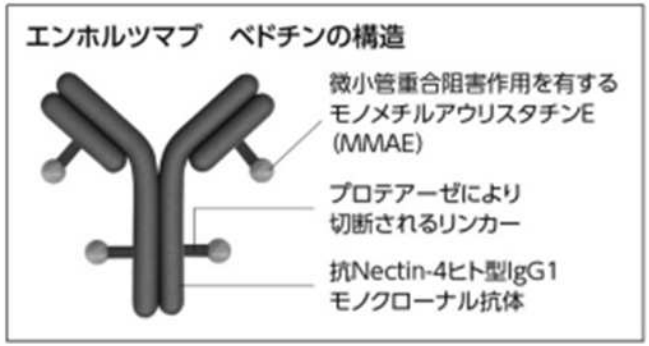
日本標準商品分類番号
８７４２９１

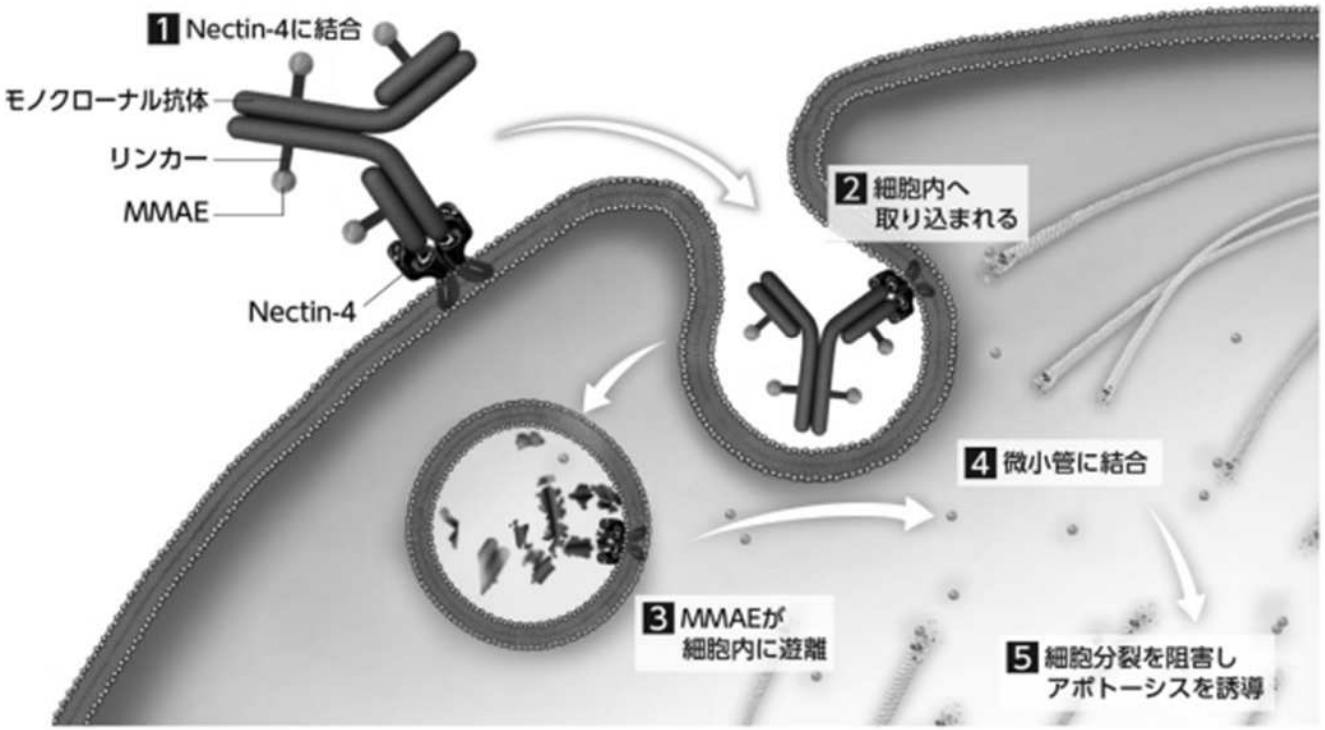
承認番号	30300AMX00454
販売開始	2021年11月



パドセブ(エンホルツマブ　ベドチン)

エンホルツマブ　ベドチンの作用機序[8~10, 12]





パドセブのバイオアナリシス

PK測定

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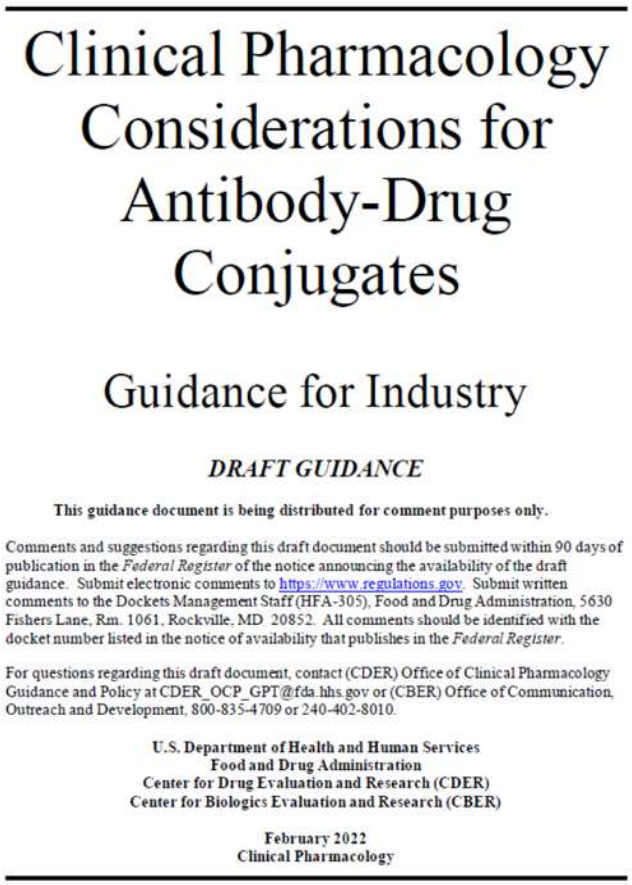
ADCのバイオアナリシス戦略
～FDA DRAFT GUIDANCE(2022)～



Clinical Pharmacology Considerations for Antibody-Drug Conjugates

Guidance for Industry

DRAFT GUIDANCE

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Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact (CDER) Office of Clinical Pharmacology Guidance and Policy at CDER_OCP_GPT@fda.hhs.gov or (CBER) Office of Communication, Outreach and Development, 800-835-4709 or 240-402-8010.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

February 2022
Clinical Pharmacology

III. CLINICAL PHARMACOLOGY CONSIDERATIONS

Given that ADCs are composed of an antibody, a chemical linker, and a payload, evaluating the clinical pharmacology of ADCs can be more complex than for small or large molecules alone.

A. Bioanalytical Approach

All bioanalytical methods should be validated and reported as outlined in the FDA's guidance entitled *Bioanalytical Method Validation* (May 2018). In general, beginning with first-in-human studies, the ADC, its constituent parts, and its pharmacologically active metabolites, if any, should be measured. Later in development, the ADC, its constituent parts, and its pharmacologically active metabolites that are quantifiable in systemic circulation should be measured to inform exposure-response analyses as described in section III.B Dose- and Exposure-Response. Any decisions to exclude measurements of constituent parts of the ADC or pharmacologically active metabolites in later development should take into consideration:

- **Constituent parts of the ADC** –includes the total antibody and the unconjugated payload

パドセブのバイオアナリシスもこれに準じて対応した(ADC、Total antibody、およびPayload)

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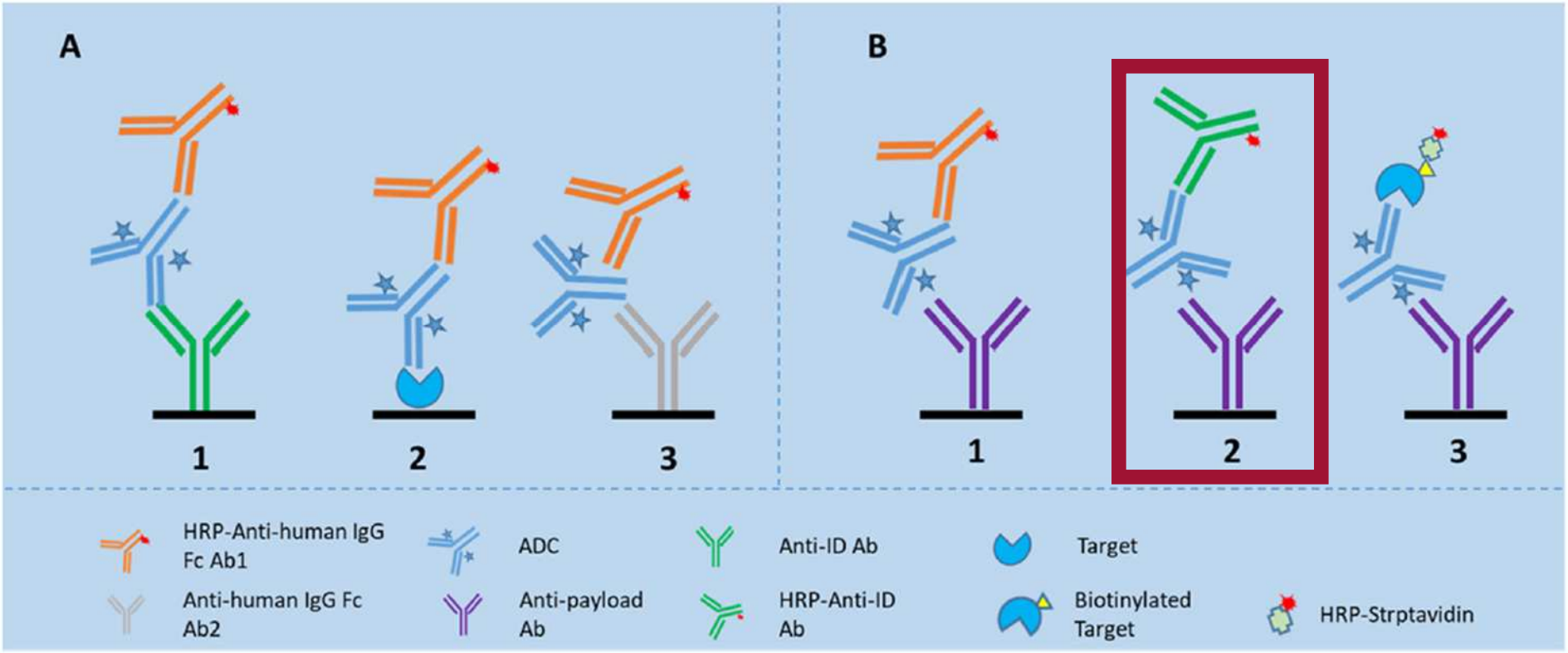
パドセブのバイオアナリシス

測定対象	測定法	必要とする重要試薬
ADC	LBA（ELISA）	抗payload抗体 抗イディオタイプ抗体
Total antibody	LBA（ELISA）	抗イディオタイプ抗体x2
Payload（MMAE）	LC-MS/MS	
ADA	LBA（ECLIA）	

パドセブのバイオアナリシス（ADC WITH LBA）
①抗PAYLOAD抗体



開発ステージやタイムラインに合わせた抗payload抗体の取得が必要

Q Qin, et al, Molecules, 27, 6299, 2022

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パドセブのバイオアナリシス（ADC WITH LBA）
②DAR0体含有率による補正

ADC標準物質を用いる検量線範囲
1-1000 ng/mLとすると
↓（DAR 0 = 5.6%で補正）
実際には
0.944-944 ng/mL

RESULTS OF ANALYSIS

Product:	AS2567465-00 (Enfortumab Vedotin) Reference Standard
Lot No.:	1002173G
Potency:	100 %
Expiry date:	January 08, 2024
Storage:	Store in a freezer (-80°C). Protect from light.
Appearance:	Colorless, slightly opalescent
pH:	6.0
Osmolality:	191 mOsm/Kg
Identity	
Peptide Mapping:	Conforms
Purity test	
(1) CE-SDS (Reduced):	98.7 %
(2) Size Exclusion Chromatography:	97.4 % Monomer 1.1 % HMWs 1.5 % LMWs
(3) icIEF:	40.6 % Acidic 57.1 % Main
(4) %DAR0:	5.6 %
(5) Free Drug Related Impurities:	SGD-1010 < 0.10 % (w/w) Any other single impurity < 0.15 % (w/w) Total Quantified Impurities (TQI) < 0.15 % (w/w)
Protein Concentration:	9.8 mg/mL
DAR:	3.9
Binding Potency:	100 %
Cytotoxicity:	100 %
Polysorbate 20:	0.02 % (w/v)

パドセブのバイオアナリシス（PAYLOAD: MMAE）

LC-MS/MSを用いる濃度測定法バリデーション試験における主な安定性評価項目

- Short term stability
- Long term stability
- Freeze/Thaw stability
- Whole blood stability（血漿）
- Autosampler stability
- Processed sample stability
- Stock/working solution stability

実サンプルを考慮し、ADC存在下での安定性を追加検討

＜注意点＞

・ADC製剤中のpayload含有量を考慮

・LC-MS/MS測定感度

・Cleavable/Non-cleavable linker

・ADC添加濃度（実サンプルを考慮）

パドセブのバイオアナリシス(PAYLOAD: MMAE)

ヒト血漿中-10 to -30℃安定性(90日間)

	添加濃度(pg/mL)	Mean(n=6)	RE (%)	CV(%)
LQC	30.0	29.8	99.3	3.5
HQC	2000	2020	101.0	1.9

ヒト血漿中-10 to -30℃安定性(90日間)＊50 μg/mL パドセブ存在下

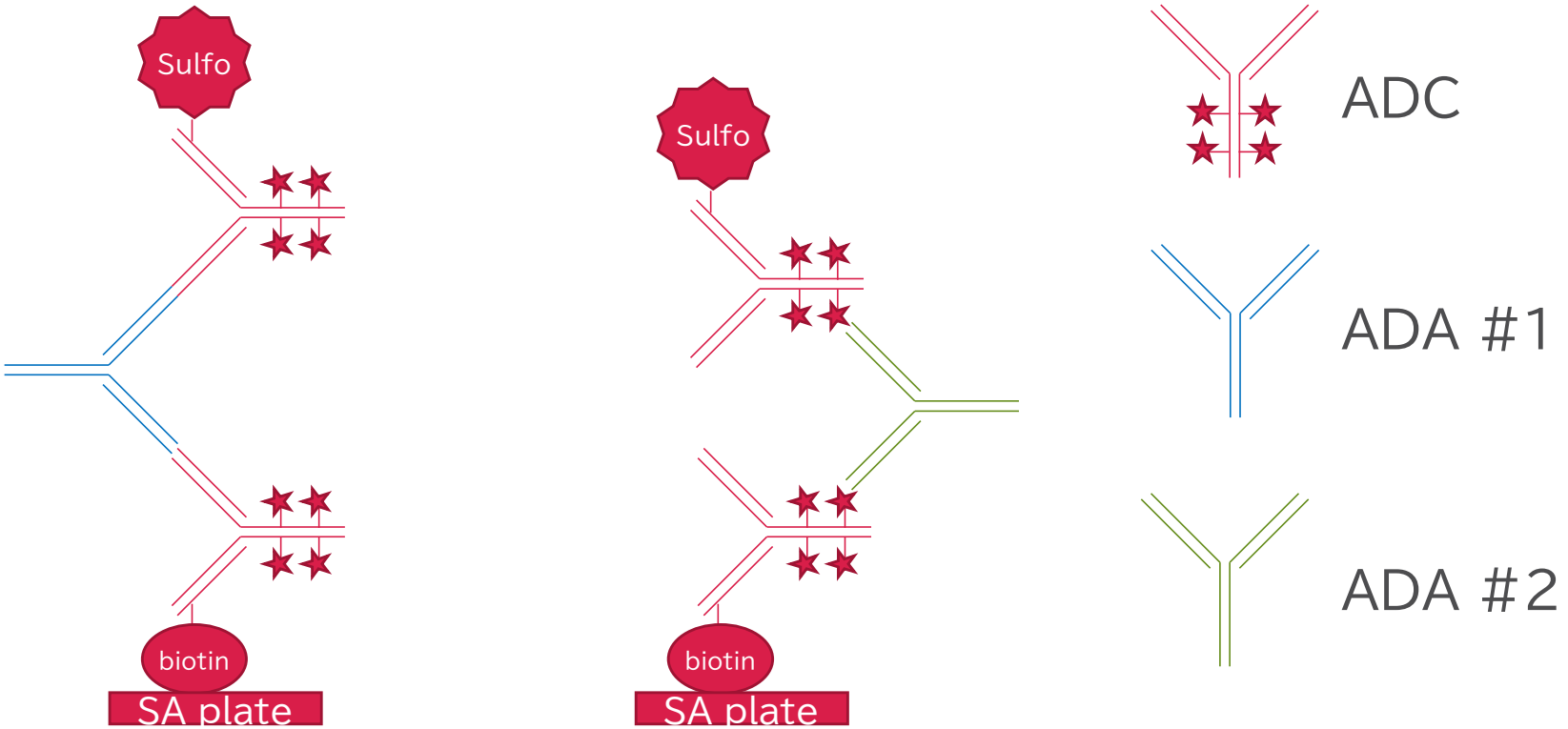
	MMAE添加濃度(pg/mL)	MMAE濃度(理論値、pg/mL)	Mean(n=6)	RE(%)	CV(%)
LQC	30.0	54.8	56.0	102.2	3.2
HQC	2000	2060	2070	100.5	1.0

特に低濃度で影響を受ける可能性あり

パドセブのバイオアナリシス

ADA測定

ADCのADA測定に関するバイオアナリシス戦略



シグナルだけではADCのどの部位に対するADAが産生されたか判別できない

ADCのADA測定に関するバイオアナリシス戦略 ～FDA GUIDANCE（2019）～

Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2019
Pharmaceutical Quality/CMC

3. Domain Specificity

Some proteins possess multiple domains that function in different ways to mediate clinical efficacy. An immune response to one domain may inhibit a specific function while leaving others intact. FDA recommends that sponsors direct initial screening and confirmatory tests against the whole therapeutic protein product. For multi-domain therapeutic protein products, the sponsor may need to investigate whether the ADA binds to specific clinically relevant domains in the protein. For example, to adequately understand the risk of ADA to subjects for therapeutic protein products with modifications such as pegylation, sponsors should develop assays to determine the specificity of ADA for the protein component as well as the modification to the therapeutic protein product (Gorovits et al. 2014).

The domain specificity is generally assessed in ADA samples confirmed positive using the whole molecule. Examination of immune responses to therapeutic protein products with multiple functional domains such as bispecific antibodies may require development of multiple assays to measure immune responses to different domains of the molecules (see section IV.L.4).

4. Conjugated Proteins

Antibody-drug conjugates (ADCs) are antibodies conjugated with small molecule drugs, so they represent a classic hapten-carrier molecule. Therefore, the immunogenicity assays should measure the responses to all components of the ADC therapeutic protein product, including the antibody, linker-drug, and new epitopes that may result from conjugation. When ADCs need to be labeled for immunogenicity assays, the conjugation should consider the potential for increased hydrophobicity of the labeled molecules because they may cause aggregation. The stability and solubility of these capture reagents should be adequately characterized (see section IV.A.3).

Guidance for Industry: Immunogenicity Testing of Therapeutic Protein Products - Developing and Validating Assays for Anti-Drug Antibody Detection, 2019

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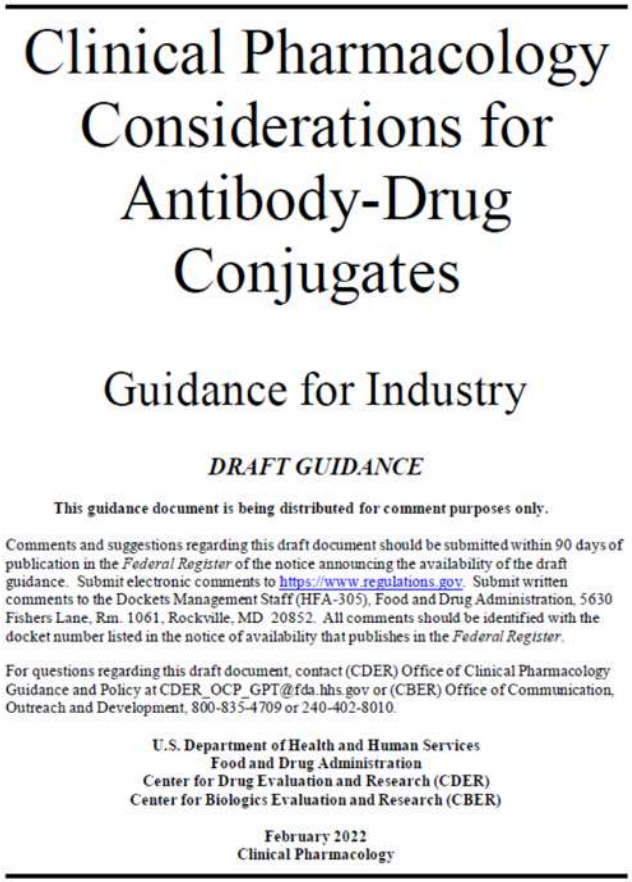
ADCのADA測定に関するバイオアナリシス戦略 ～FDA DRAFT GUIDANCE（2022）～



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February 2022
Clinical Pharmacology

353 **E. Immunogenicity**
354
355 An immune response to an ADC can be generated to any constituent part of the ADC, including
356 the antibody, the payload, or epitopes created by the conjugation linker. Given that ADCs
357 generally have a relatively narrow therapeutic window, it is important to evaluate
358 immunogenicity to ADCs and the potential impact on PK, safety and efficacy. A multitiered
359 immunogenicity assessment should be conducted as outlined in the FDA guidances
360 *Immunogenicity Assessment for Therapeutic Protein Products* (August 2014) and
361 *Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays*
362 *for Anti-Drug Antibody Detection* (January 2019), including a confirmatory assessment detecting
363 anti-drug antibodies (ADAs) against the ADC. Additionally, it could be appropriate to develop
364 multiple assays to measure the immune responses to the constituent parts of the ADC, such as
365 additional epitopes or domains resulting from the conjugation of the constituent parts.

ADA陽性となる場合、寄与するADCのドメインを明らかにする必要がある

Guidance for Industry: Clinical Pharmacology Considerations for Antibody-Drug Conjugates, 2022

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パドセブのバイオアナリシス（ADA）

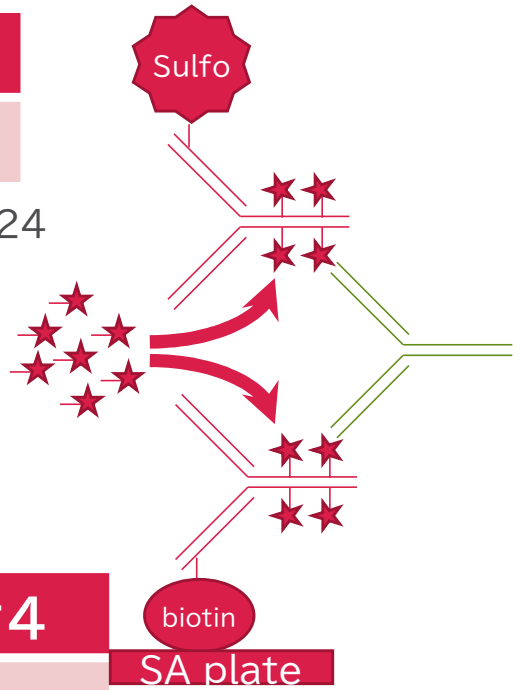
ドナーのブランク血清にポジコンADA（抗payload）を添加

	ドナー#1	ドナー#2	ドナー#3	ドナー#4
シグナル	19548	19075	19714	21757

カットオフポイント：219.24

Payload-linker添加

	ドナー#1	ドナー#2	ドナー#3	ドナー#4
シグナル	211	199	182.5	214
% inhibition	98.9	99	99.1	99.0



Payload-linkerへの特異性を確認

まとめと今後の展望

まとめ

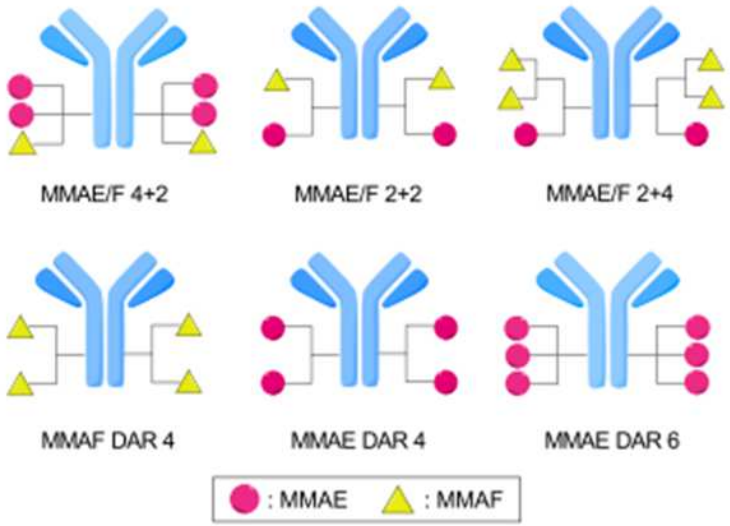
パドセブのバイオアナリシスを考えるうえで考慮したポイント

測定対象	測定法	ポイント
ADC	LBA （ELISA）	・抗payload抗体の調製 ・DAR0値による補正
Total antibody	LBA （ELISA）	・抗イディオタイプ抗体x2
Payload （MMAE）	LC-MS/MS	・ADC存在下での安定性の考察
ADA	LBA （ECLIA）	・Domain specificity

ADCのバイオアナリシスにおける今後の展望

・ADCの多様化

例：複数payload



・Intact MS
LBAでは確認できない、ADC構造特異的な情報（DARの経時的変化など）の収集が可能

→M&S予測性、MOAの理解、分子デザイン戦略などへのさらなる貢献が期待される

C.M Yamazaki, et al, Nature Communications, 12, 3528 (2021)

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謝辞

アステラス製薬株式会社　開発研究
非臨床バイオメディカルサイエンス
非臨床レギュラトリーサイエンス