Nonclinical Safety Evaluation of ADC Drugs

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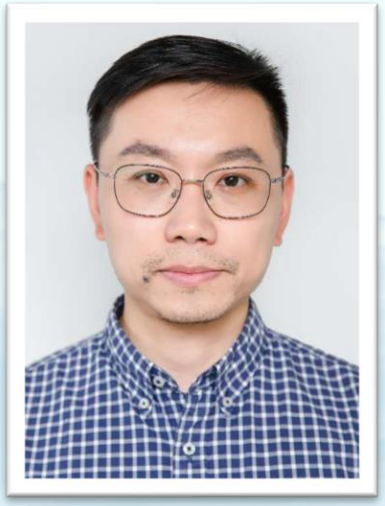
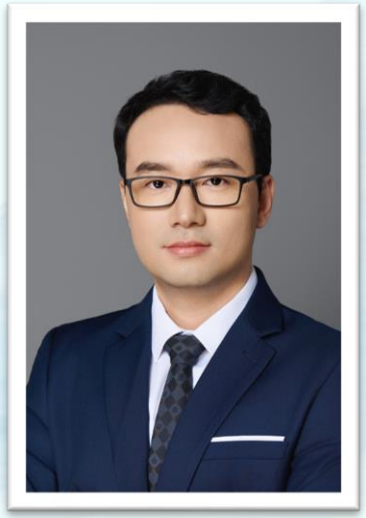
Presenters

Sue McPherson
Executive Director
Safety Assessment



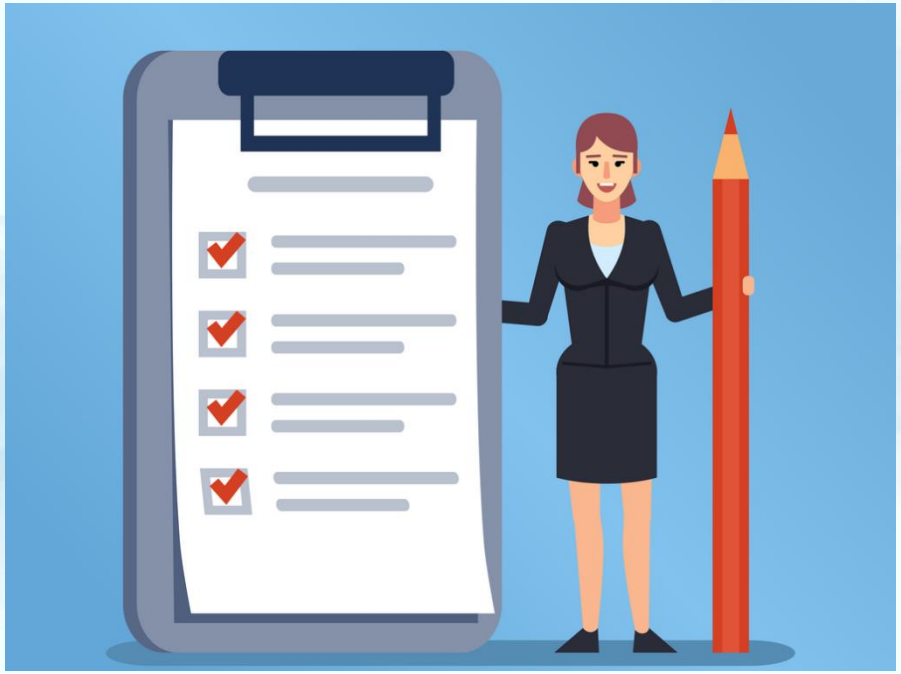
Leo Pan
Senior Director
Toxicology



Jinpeng Li
Director, Preclinical
Large Molecule
Bioanalysis

AGENDA

- History of Antibody Drug Conjugates (ADCs)
- Background for ADCs
- Nonclinical Safety Evaluations of ADCs
- Toxicity of ADCs
- Bioanalysis of ADCs
- ADC Case Review



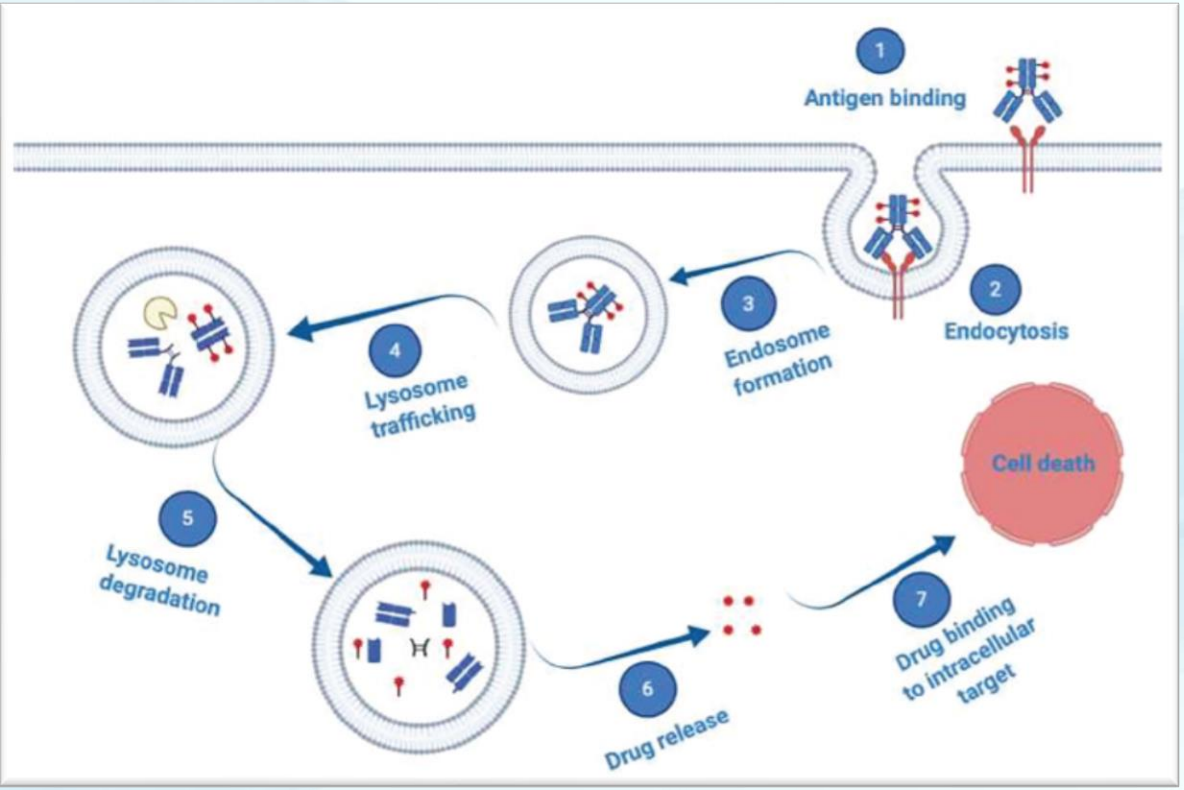
History of Antibody Drug Conjugates (ADC)

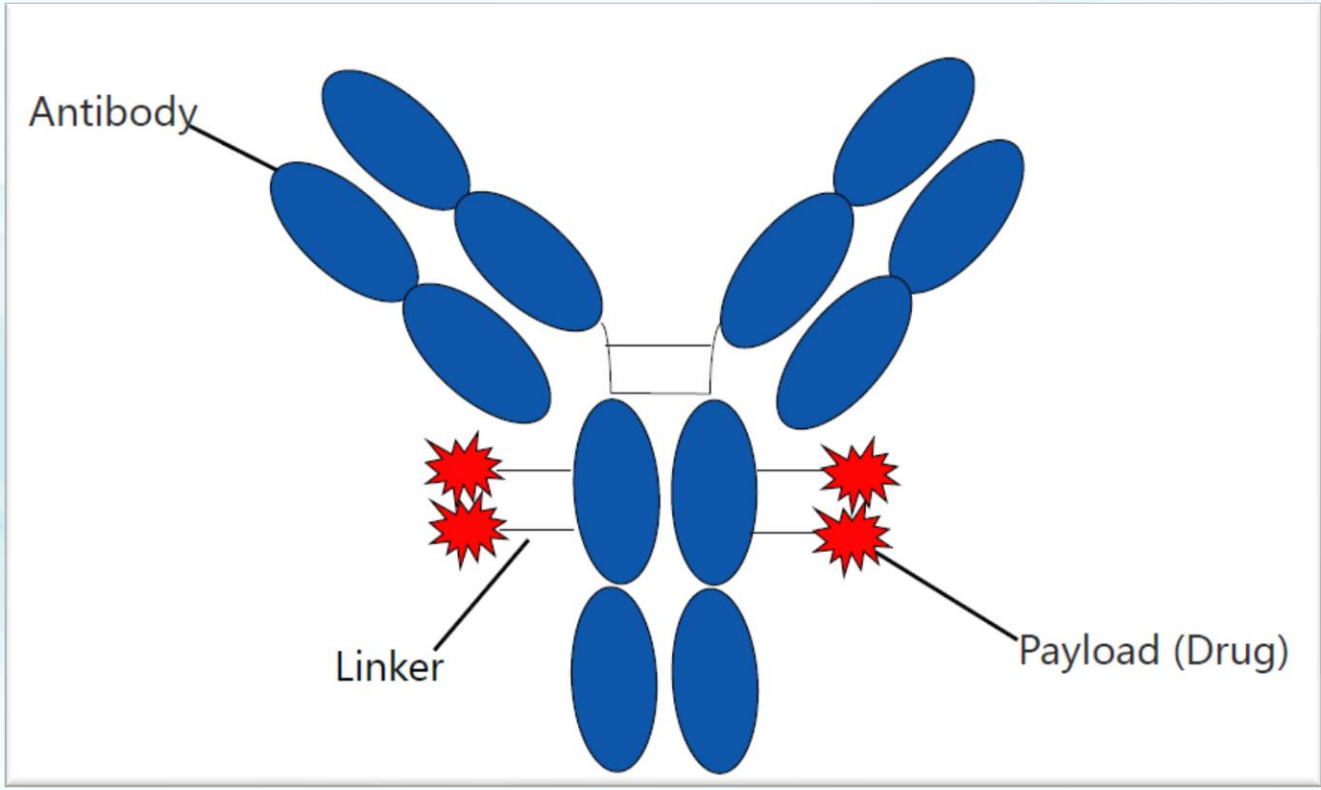
History of ADC

- Antibody Drug Conjugates (ADCs) embody a simple, but elegant, vision for cancer therapy—the delivery of a potent cytotoxic agent to tumor cells that are covalently coupled to tumor-specific antibodies The linkage between the antibody and the toxin should only release the toxin when the ADC binds and/or internalizes into the cancer, so-called smart chemo.
- In 1913, German physicist and scientist Paul Ehrlich pioneered the concept of targeted delivery of toxic agents against microbes or tumor cells.
- After nearly forty years of research, ADC has achieved promising results in clinical trials. The first successful clinical trial of ADC was in 1983, which used an anti-carcinoembryonic antigen antibody-vindesine conjugate.
- A whole chain of factors influences the overall success of ADC development starting with the selection of the right target, ie its only expressed in cancer cells.

Background of Antibody Drug Conjugates (ADC)

Magic Bullet-Components and Mechanism of Action





Biteghe FAN, et al. Advances in epidermal growth factor receptor specific immunotherapy: lessons to be learned from armed antibodies. Oncotarget. 2020

Pharmacokinetic Properties of ADCs

	Small Molecule	mAb	ADC
Administration	Variable	SC or IV high	IV high
Distribution	High	Limited (vascular), good to highly Vascularized tissues	Limited (vascular), good to highly vascularized tissues
Metabolism	Phase I and II	Proteolysis (FcR-mediated and unspecific) and Target Mediated Drug Disposition	Proteolysis and Target Mediated Drug Disposition (mAb and full ADC) Phase I and II (free payload)
Excretion	Renal and/or biliary	Very limited biliary	Limited biliary
Tumor accumulation	Low	High	High
Half-life	Short (h)	Long (days, recycling through FcR) dependent on immunogenicity	Long (days, recycling through FcR) dependent on immunogenicity
Drug Drug Interaction	Variable (perpetrator and/or victim)	low	Dependent on the payload (mainly as victim)

Pretto F, FitzGerald RE. In vivo safety testing of Antibody Drug Conjugates. Regul Toxicol Pharmacol. 2021

Antigen and Antibody

Antigen

- Tumor-specific
- Homogeneous expression
- High levels of expression
- Rapid internalization
- Minimal ectodomain shedding

Antibody

- High affinity for the target antigen
- Rapid internalization
- Favorable PK properties
- Minimal immunogenicity

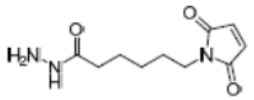
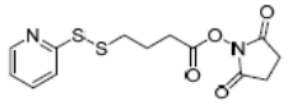
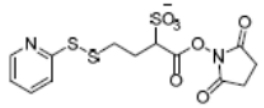
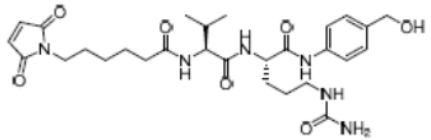
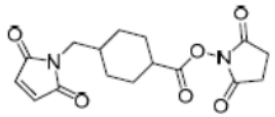
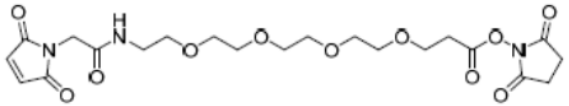
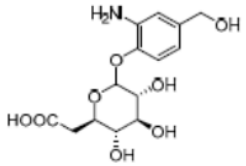
Payload-Classification and Requirements

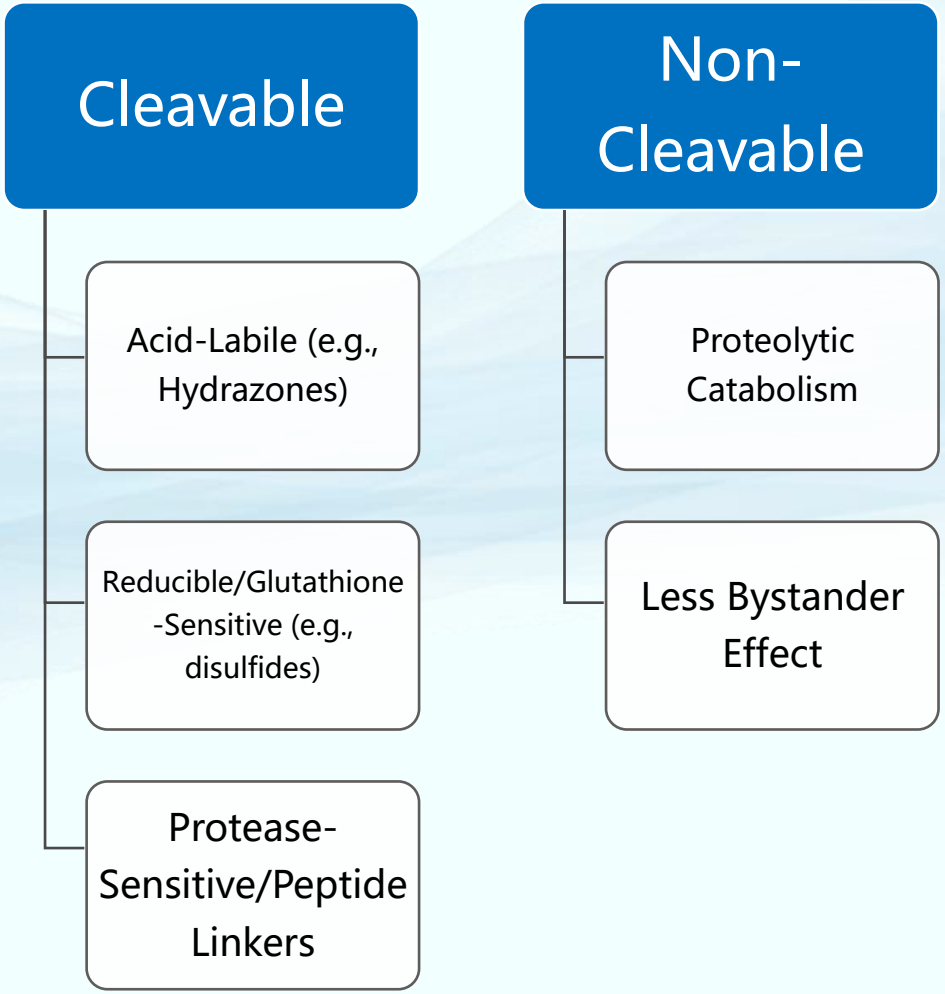
- High Cytotoxic
- Modifiable Functional Groups
- High Stability
- Bystander Effect

Target	MOA	Payload
Microtubule	Microtubule Disrupting Agents	Auristatins (MMAE, MMAF) Maytansinoids (DM1, DM4)
DNA	DNA Alkylators DNA Intercalators	Calicheamicins Pyrrolobenzodiazepines (PBDs) Duocarmycin Doxorubicin
	Topoisomerase Inhibitors	Camptothecin (SN38, Deruxtecan DXd)

Linker

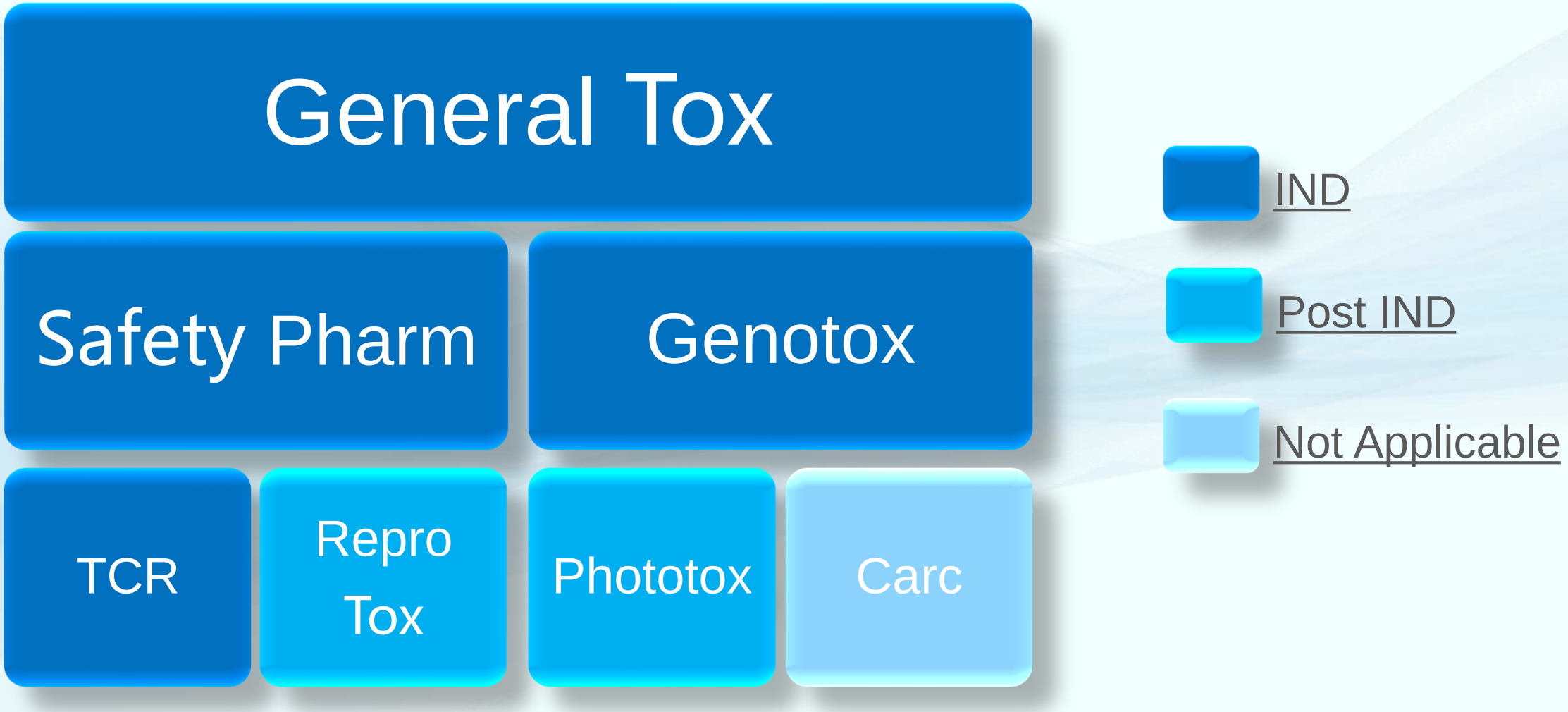
Table 1. Summary of commonly used linkers for the development of ADCs.

Abbreviation	Chemical Structure	Linker Type
MHH		Chemical labile (acid labile) linker
DSDM		Disulfide-containing reducible linker
Sulfo-SPDB		Disulfide-containing reducible linker
MC-VC-PABC		Enzymatically cleavable linker
SMCC		Non-cleavable bifunctional linker
Mal-PEG-NHS		Non-cleavable spacer linker
GBC		Enzyme-labile ß-Glucuronide linker



Evaluation

Nonclinical Safety Evaluations



Nonclinical Safety Evaluations Guidelines

- ICH M3 (R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. June 2009
- ICH S6 (R1): Preclinical Safety Evaluation of Biotechnology Derived Pharmaceuticals. June 2011
- ICH S7A. Guideline on Safety Pharmacology Studies for Human Pharmaceuticals. November 2000
- ICH S8 Immunotoxicity Studies for Human Pharmaceuticals. September 2005
- ICH S9 Nonclinical Evaluation for Anticancer Pharmaceuticals. October 2009
- ICH S9 Nonclinical Evaluation for Anticancer Pharmaceuticals Questions and Answers June 2016
- NMPA Technical guidelines for non-clinical studies of ADCs (draft for public comment, July 2022)

General Toxicology

What to Test

- Full ADC
- Unconjugated Antibody
- Payload
- Payload with Linker
- Linker

Species Selection

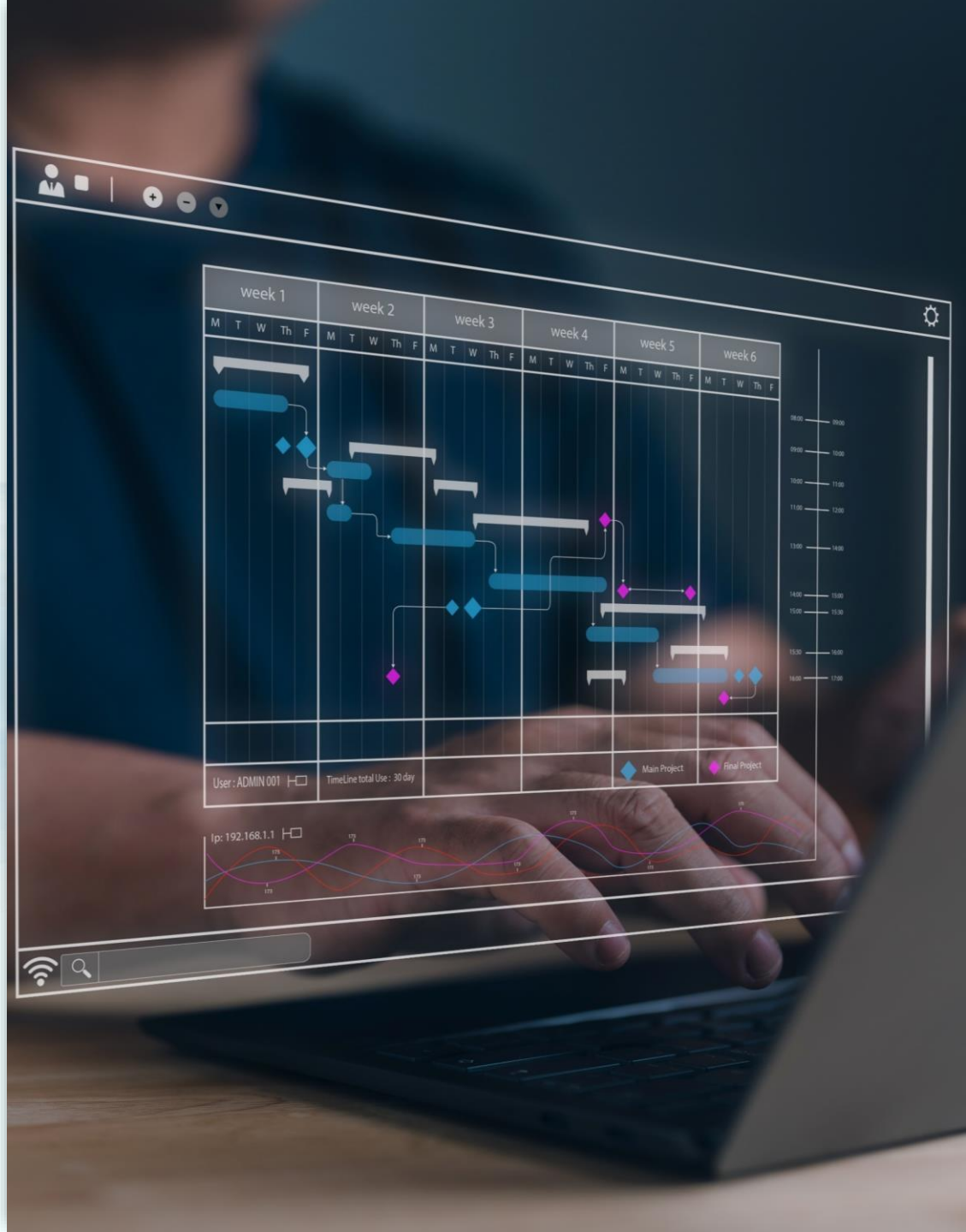
- Relevant species-same general principles as an unconjugated antibody
- Sequence homology, target binding affinities, receptor/ligand occupancy
- ADC in relevant species
- Novel payload in one or two species

General Toxicology Study Design

- Test Article and Species
  - ADC: one or two relevant species, mostly NHP
  - Payload: mostly in rats, single or short-term repeated dose

- Study Duration
  - IND: At least **two doses** of the ADC should be administered to support initial clinical trials of once every 3 or 4 weeks.
  - BLA: Up to **13 or 26 weeks**
  - Recovery phase included in as least one repeated dose study

- Dosing Interval
  - QW to Q3W



General Toxicology Study Design

Repeated Dose Toxicity Study in NHP (IND)

Groups	Dosing Phase	Recovery Phase
Vehicle Control	3/3	2/2
Low Dose ADC	3/3	2/2
Mid Dose ADC	3/3	2/2
High Dose ADC	3/3	2/2

Dosing:

- Q3W*2 to 4

Standard Endpoints:

- Clinical Signs
- Local Tolerance
- Body Weight
- Food Consumption
- Ophthalmology
- Clinical Pathology
- Full Tissue Histopathology

Incorporated Safety Pharmacology:

- Cardiovascular
- Respiratory
- Neurological Assessments

Immunotoxicity:

- Serum Cytokine
- Lymphocyte Phenotyping

Toxicokinetics:

- ADC
- Antibody
- Payload

Immunogenicity

GLP Compliance:

- GLP

Safety Pharmacology and Genotox

Safety Pharmacology

- *In Vitro*: hERG for novel payload, inhibition on Human ether-a-go-go related gene (hERG) potassium (K +) channels.
- *In Vivo*: CV, respiration, CNS (all or partially incorporated into tox studies) for ADC

Genotox for Payload

- Bacterial mutation (Ames) test
- *In Vitro* chromosome damage test (chromosome aberration, mouse lymphoma
- *In Vivo* micronucleus

Repro Tox, TCR, and Phototox

Repro Tox for ADC

- Embryofetal toxicology study (developmental tox, Seg II) in one species (usually rat)
- Fertility and early embryonic development and pre- and postnatal toxicology not needed
- Seg II can be waived if evidence suggests potential developmental toxicity due to MOA

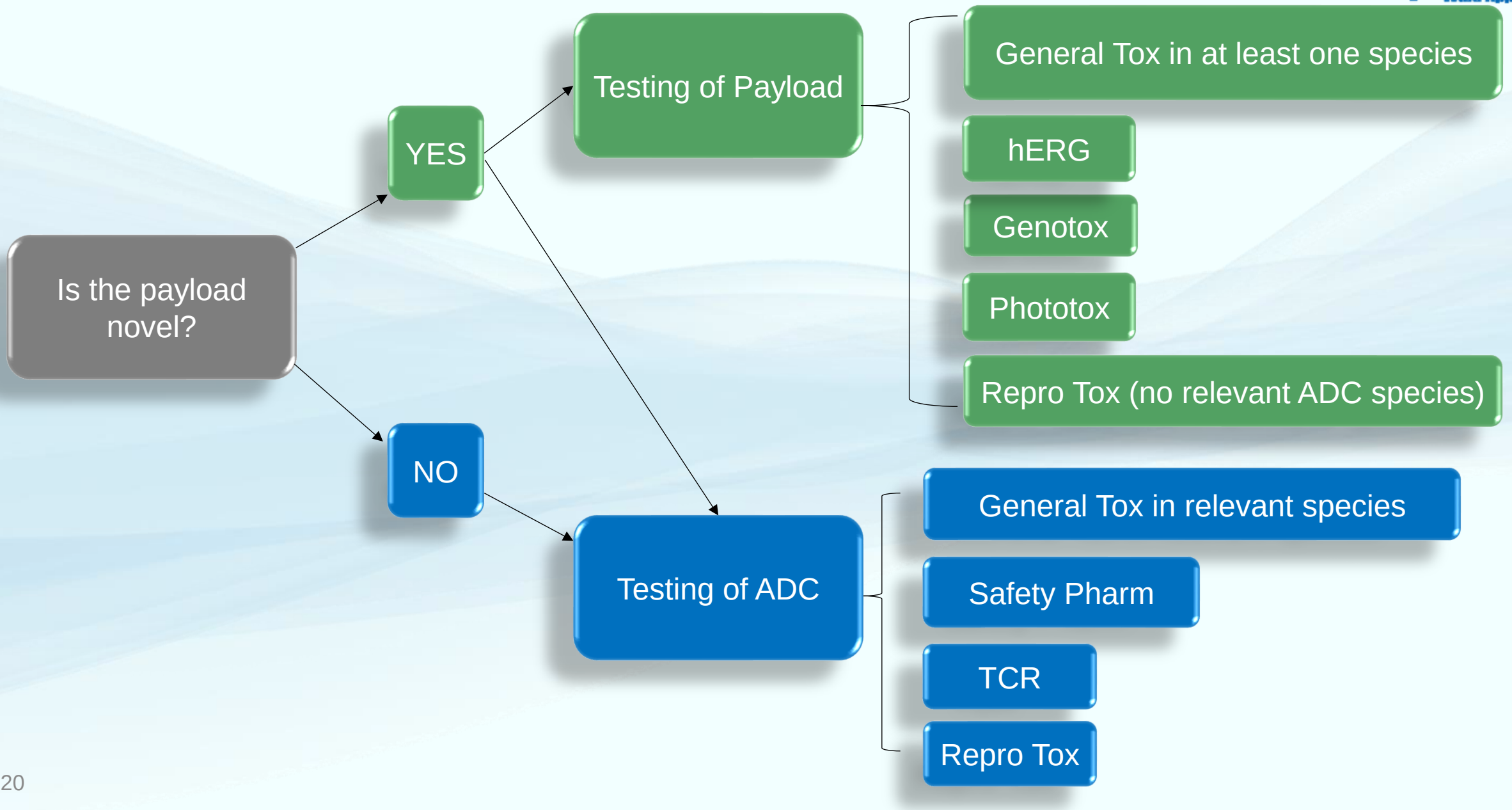
Tissue Cross Reaction (TCR) for ADC

- Limited value for species selection
- Provide information on potential unexpected binding
- Although not mandatory, TCR in at least one species (human) was completed for all approved ADCs

Phototox for Payload

- Photo absorption at 290-700 nm
- *In Vitro* 3T3 NRU
- *In Vivo* phototox in rats or guinea pigs

Nonclinical Safety Evaluation Strategy



Immunotoxicity and Biomarkers

Immunophenotyping (Flow Cytometry-Based Analysis)

Subpopulation	Species	
	NHP	Rodents
T cell	CD3,CD4,CD8,	CD3,CD4,CD8
Activated T cell	CD25,CD69,Ki67	CD25,CD69,Ki68
Treg	CD25, Foxp3	CD25, Foxp3
B cell	CD19,CD20,	CD45R
NK cell	CD16, CD56	CD335
Monocyte	CD14,CD16	CD11b
Neutrophils	CD11c,CD49d	CD11b,Ly-6B.2
Dendritic Cell	CD1c, CD123,CD83, MHC II	CD11c,MHC II
Macrophages	CD11b,CD68,CD163	F4/80,CD68
Intracellar staining	STAT3,STAT6,Bcl-6, PI/Annexin V	STAT3,STAT6,Bcl-6, PI/Annexin V

Note: In-house methods have been established for variety of species

Biomarkers

(Potentially relevant to ADC toxicity)

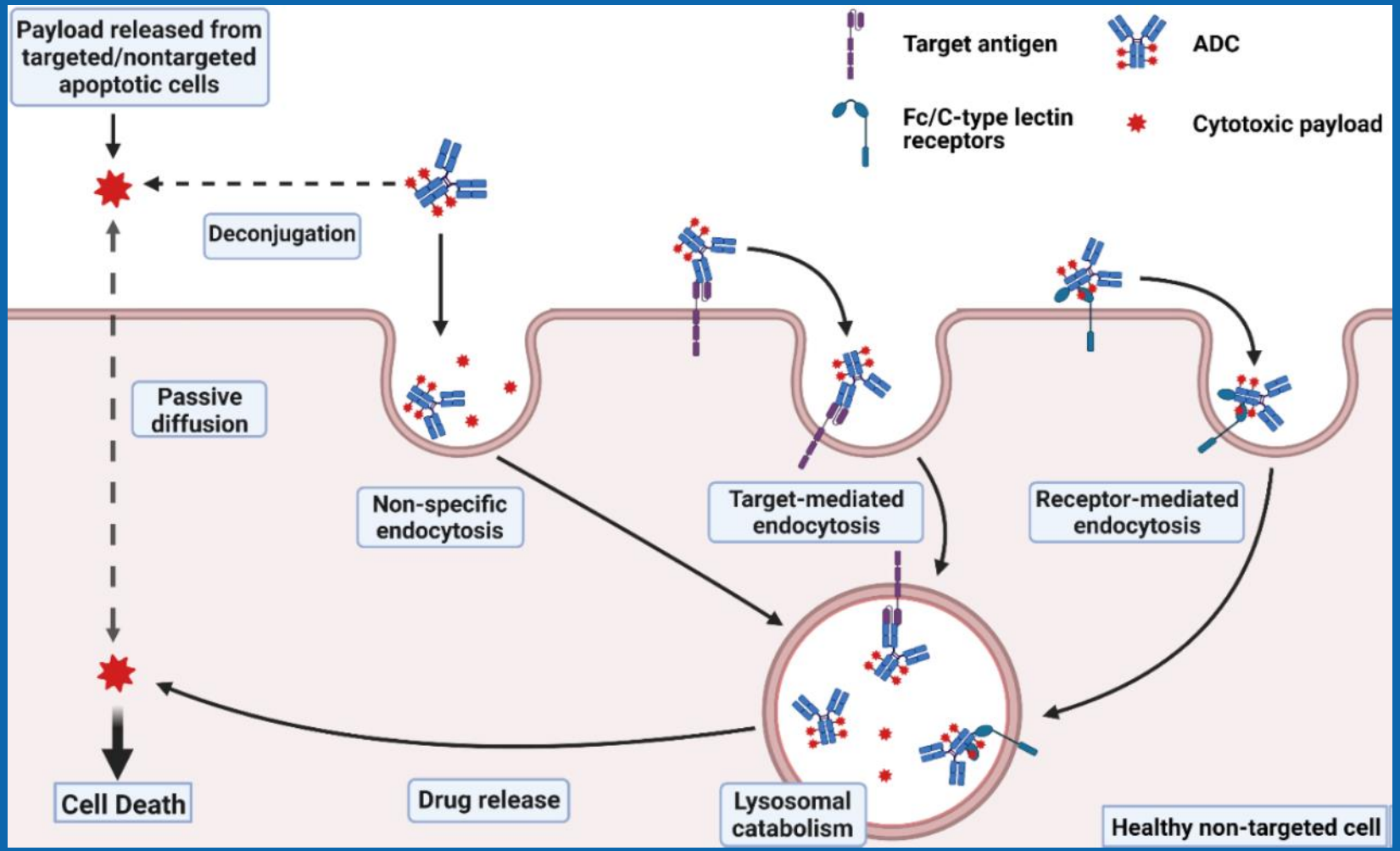
Nervous System	Liver Injury
M-CSF/Clusterin	Total Protein/Albumin/Prealbumin/
NfL	ALP/ALT/AST/GGT/LDH
DHA/EPA	Adiponectin/αGST/CICP
Thymidine&2-Deoxyuridine	TGF-β1/TIMP-1/IL-12P40/FGF-19
Dopamine	PIIINP-123C and COL5A3-1317N
MMP-9/(p)Tau	PINP-122C, COL3-610N
α-synuclein, Nfl, Aβ40/42	PVCP-1230N

Multiplex Cytokines

Panels	Species
U-PLEX Chemokine Combo 1 NHP (eotaxin, eotaxin-3, IL-8, IP-10, MCP-1, MCP-4, MDC, MIP-1α,MIP-1β,TARC)	NHP
U-PLEX Proinflam Combo 1 NHP (IFN-γ,IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12/ IL-23p40, TNF-α)	NHP
IL-2,IL-5,IL-6,IL-10,IL-13,TNF-α,IFN-r and IL-4	NHP
IL-2、IL-4、IL-5、IL-6、TNF-α and IFN-γ	NHP
IL-2, TNF-a, IFN-r	Rat
IFN-γ,IL-4, IL-5, IL-6, TNF-α	Rat
CRP, TNF-α; IL-2; IL-6; IL-8, IL-10; and IFN-γ	Mice
IL-2, IL-6, IL-13, TNF-α and INF-γ	Mice
GM-CSF, IFN-γ, IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, MCP-1, IP-10, KC-like and TNF-a	Dog
GM-CSF, IFN-γ, IL-2, IL-6, KC, IL-10, IL-18, MCP-1, IP-10, KC-like and TNF-a	Rat

Toxicity of ADC

Toxicity of ADCs-Mechanism



Premature release of payload

Off-Target: Receptor Mediated Uptake

FcγRs

Mannose receptor

Off-Site On-Target: Antigen Expression in Normal Cells

Toxicity of ADCs-Clinical Data

	Approved/Late-Stage ADCs	Linker Type	Key Toxicities
	Tubulin inhibitors		
MMAE	Brentuximab Vedotin, Polatuzumab Vedotin, Enfortumab Vedotin, Tisotumab Vedotin, Disitamab Vedotin	Cleavable	Neutropenia, peripheral neuropathy, anemia, skin toxicity
MMAF	Belantamab Mafodotin	Non-cleavable	Thrombocytopenia, ocular toxicity, hepatic toxicity
DM1	Trastuzumab Emtansine	Non-cleavable	Thrombocytopenia, hepatic toxicity
DM4	Mirvetuximab Soravtansine	Cleavable	Neutropenia, anemia, peripheral neuropathy, ocular toxicity
	DNA-crosslinkers/ DNA-alkylators		
Calicheamicin	Gemtuzumab Ozogamicin, Inotuzumab Ozogamicin	Cleavable	Neutropenia, thrombocytopenia, hepatic toxicity
PBD	Loncastuximab Tesirine	Cleavable	Neutropenia, thrombocytopenia, anemia, serosal effusion, nephron toxicity, skin toxicity
Duocarmycin	Trastuzumab Duocarmazine	Cleavable	Neutropenia, thrombocytopenia, serosal effusion
	Topoisomerase inhibitors		
SN-38	Sacituzumab Govitecan	Cleavable	Neutropenia, gastrointestinal toxicity
Deruxtecan	Trastuzumab Deruxtecan	Cleavable	Neutropenia, gastrointestinal toxicity

Toan D Nguyen et al. Mechanisms of ADC Toxicity and Strategies to Increase ADC Tolerability. Cancers (Basel). 2023. .

Toxicity of ADCs-Nonclinical vs Clinical

ADC	Target	Linker-Drug	Rat	Target Binding	Cynomolgus Monkey	Target Binding	Human SARs [2]
Adcetris (brentux-imab vedotin)	CD30	vc-MMAE	Hematopoietic system, liver, male reproductive organs	no	Hematopoietic system	yes	Peripheral neuropathy, hematologic toxicities, hepatotoxicity
Polivy (po-latuzumab vedotin)	CD79b	vc-MMAE	Hematopoietic system, liver, male reproductive organs	no	Hematopoietic system	yes	Peripheral neuropathy, myelosuppression, hepatotoxicity
Padcev (enfortumab vedotin)	Nectin-4	vc-MMAE	Hematopoietic system, liver, reproductive organs, skin, eye [3]	yes	Hematopoietic system, liver, GI tract, skin, eye [3]	yes	Peripheral neuropathy, ocular disorders, skin reactions
Blenrep (belantamab mafodotin)	BCMA	mc-MMAF	Hematopoietic system, liver, kidney, lung, reproductive organs, eye [4]	no	Hematopoietic system, liver, kidney, lung, reproductive organs	yes	Ocular toxicity, thrombocytopenia

J. Edward Fisher, Jr.. Considerations for the Nonclinical Safety Evaluation of Antibody–Drug Conjugates. Antibodies (Basel). 2021 .

Strategies to Reduce Toxicities

Conjugation Technology

- Site-specific conjugation
- Linker modification, i.e. PEG spacer
- Self-immolative linker
- More potent payload

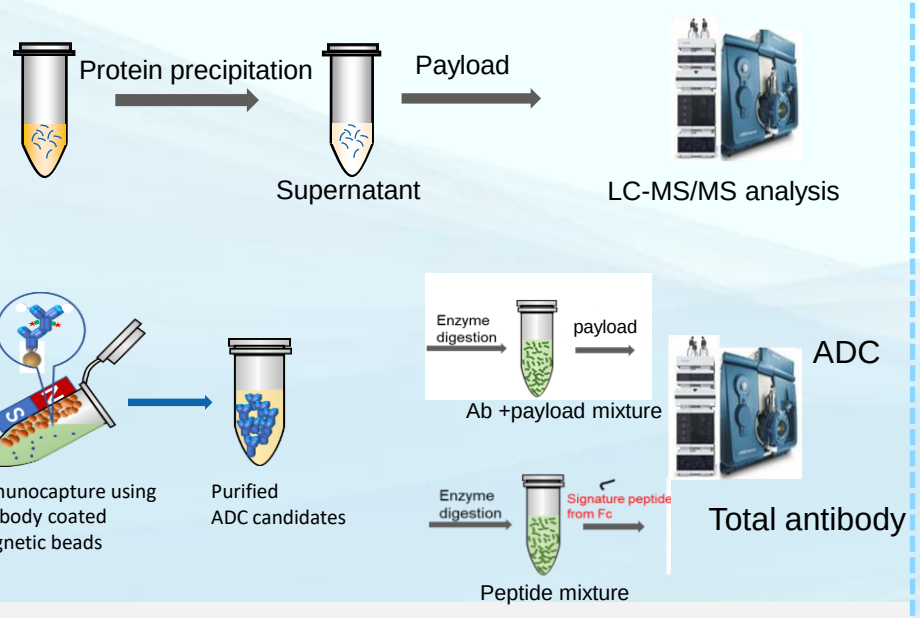
Antibody Modification and Others

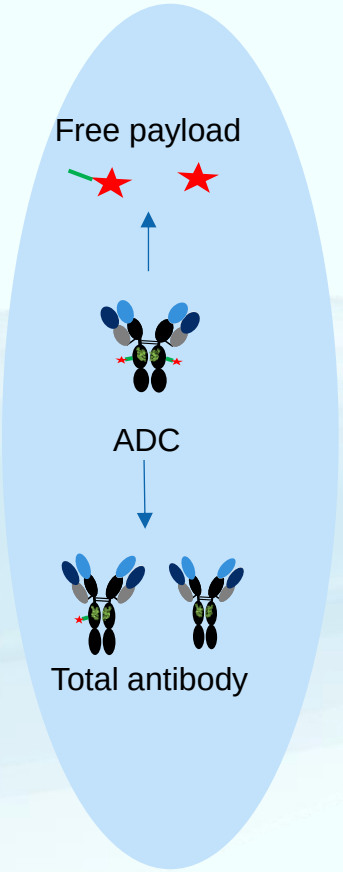
- Probody-Peptide masking
- Biospecific antibody
- Fractionated dosing
- Co-administration of payload binding antibody fragments

Bioanalysis

Nonclinical Bioanalysis Strategy: TK and DAR Profiling

Mass Spectrometry

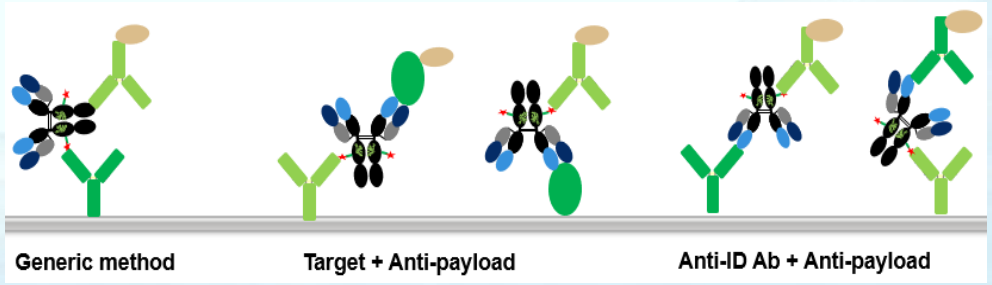




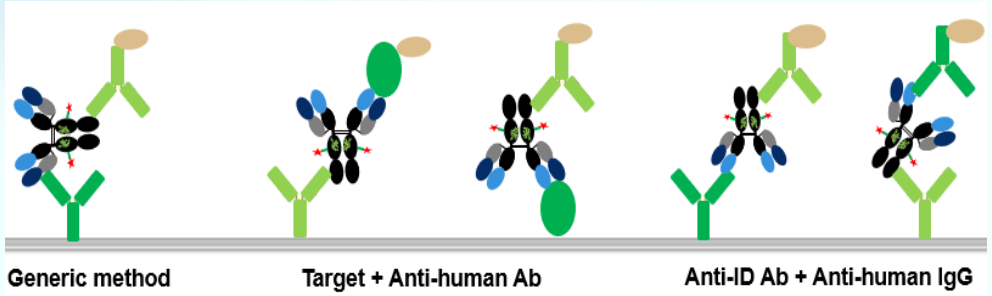
Heterogeneous Analytes for ADCs

Ligand Binding Assay

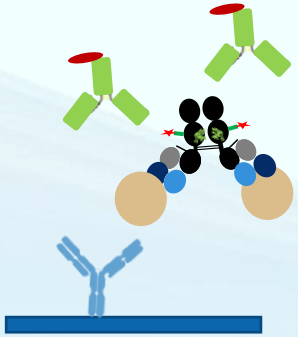
ADC

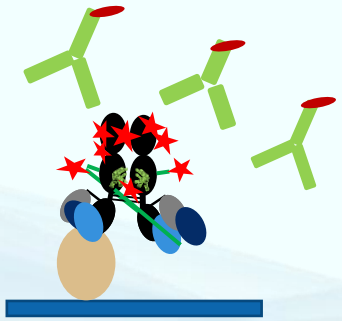


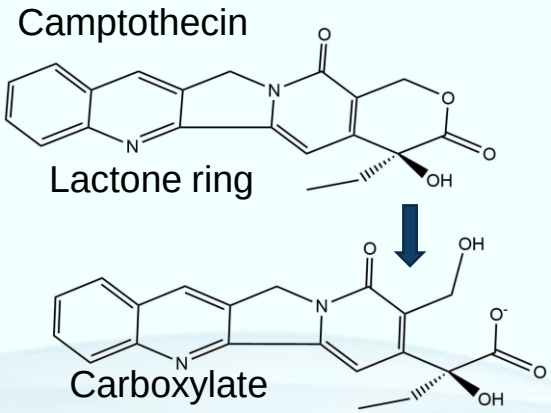
Total antibody

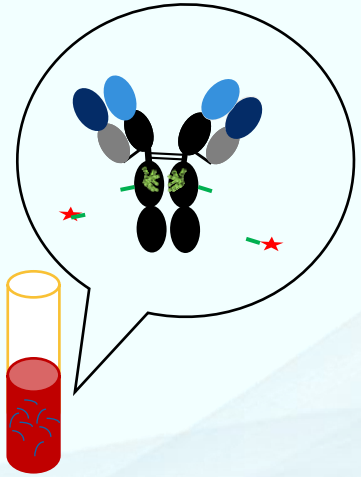


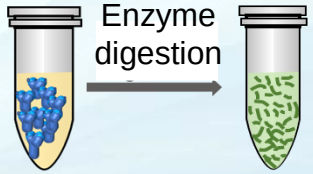
Challenges & Solutions









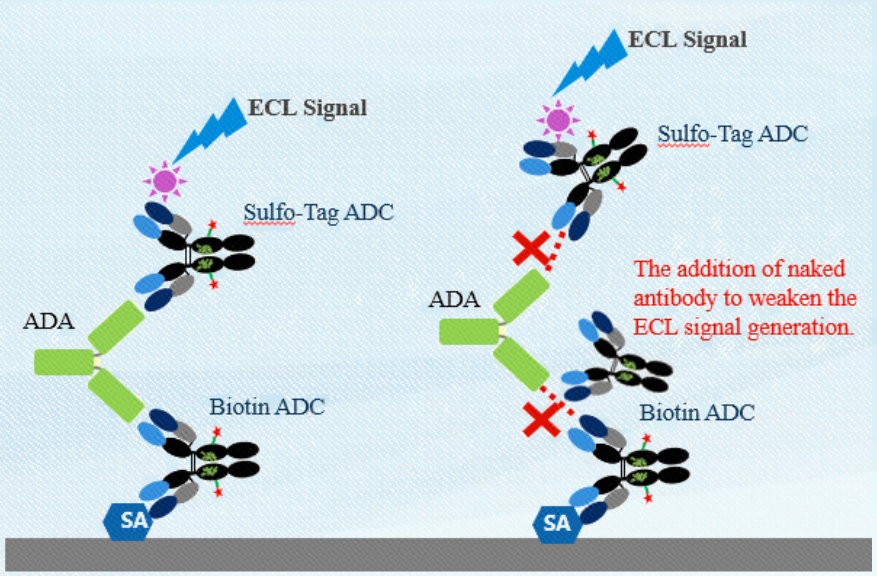


Detection	Total/ADC	Total	ADC	Free payload	Free payload	Total/ADC
Issue	Soluble target interference	Payload steric block	Structural change of camptothecin-derivative payload (e.g. Dxd, exatecan)		Free payload release & linker cleavage after sample collection	Low recovery after enzyme digestion
Solution	Target depletion, Non-competitive reagent	DAR insensitive reagents	Adjust pH		Stabilizers	Optimize digestion condition
platform	**LBA**			**LC-MS**		

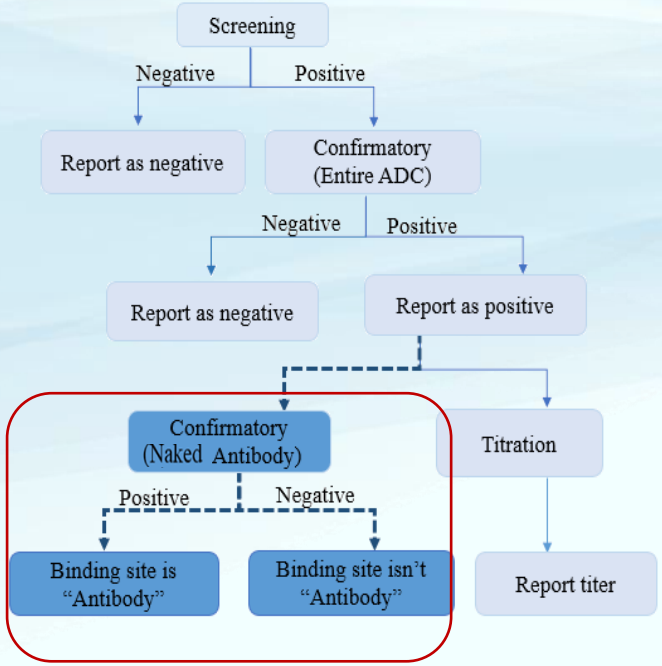
Immunogenicity Evaluation

ADA Assay

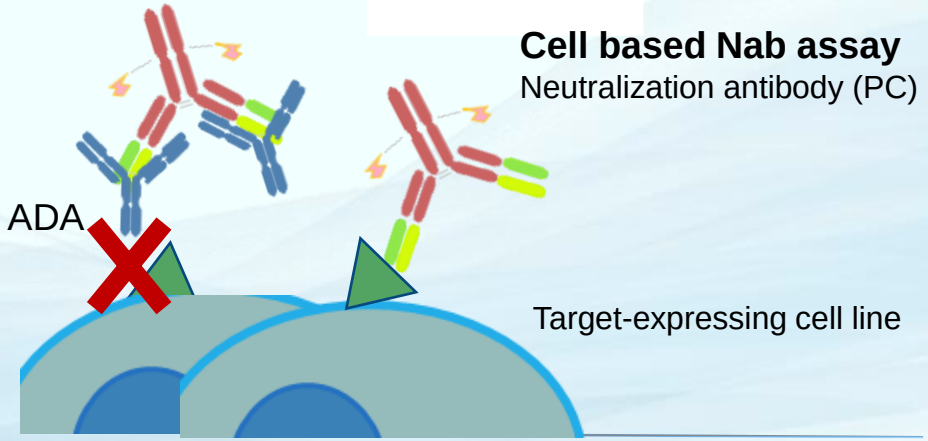
Domain specific analysis within one method (Bridging assay)



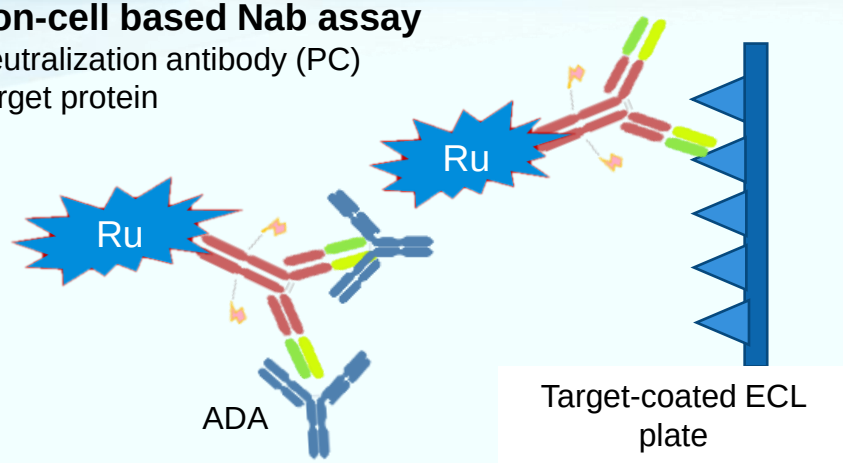
Tiered strategy for ADA against ADC



Neutralization Assay



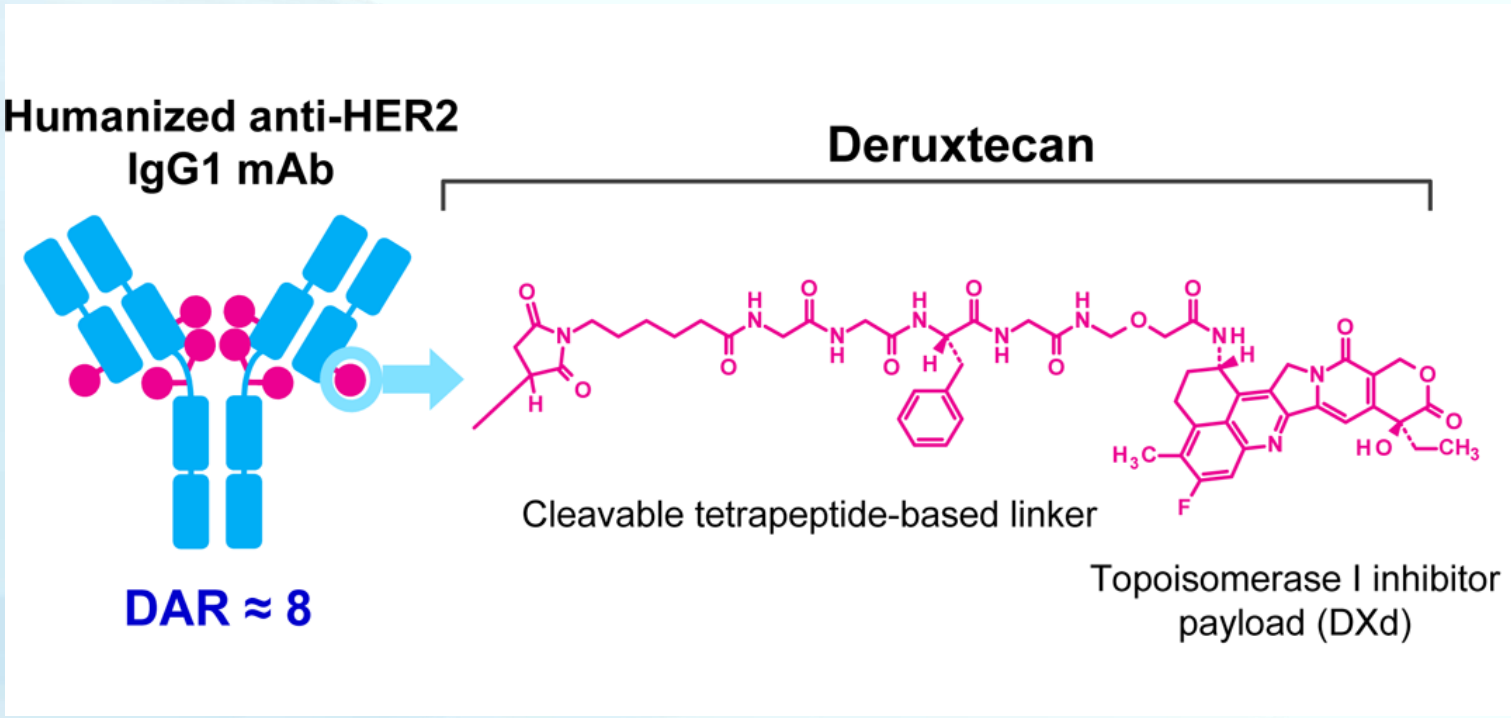
Non-cell based Nab assay
Neutralization antibody (PC)
Target protein



Critical reagent including positive control and naked antibody.

Case Study

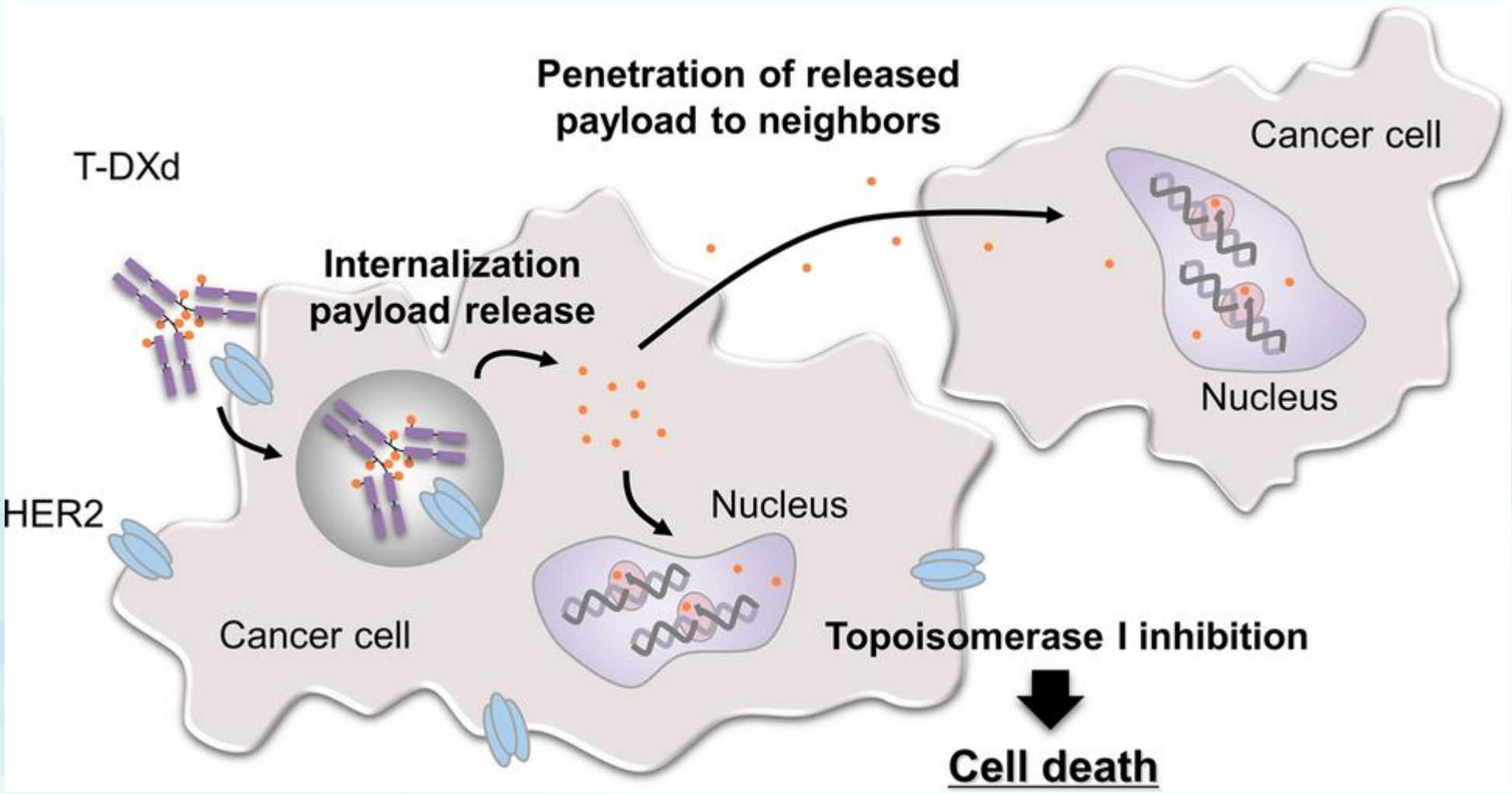
ADC Case Review-Trastuzumab Deruxtecan (Enhertu)



- Payload MOA: Topoisomerase I inhibitor
- High potency of payload
- High drug-to-antibody ratio (DAR) ≈ 8
- Stable linker-payload
- Payload with short systemic half-life
- Tumor-selective cleavable linker
- Bystander antitumor effect

Takashi Nakada.. Discovery research and translation science of trastuzumab deruxtecan, from non-clinical study to clinical trial. Translat Regulat Sci. 2021

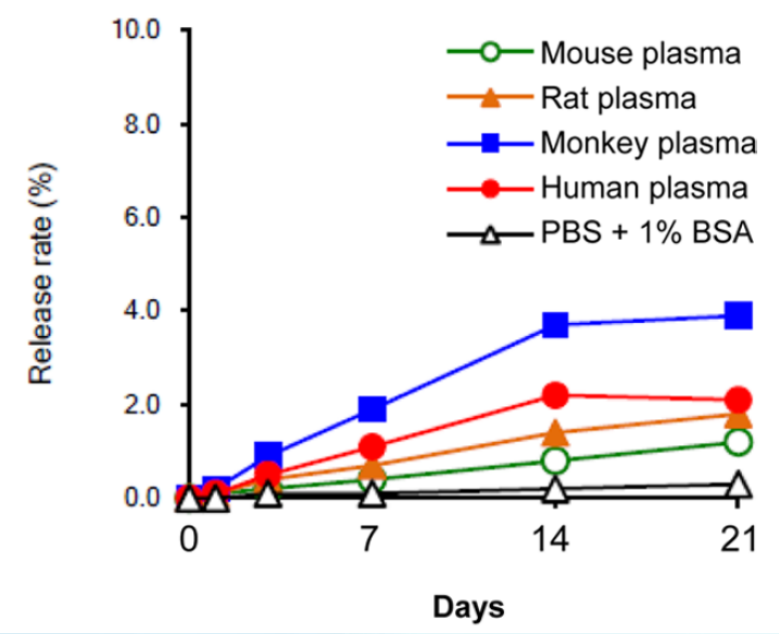
ADC Case Review-Trastuzumab Deruxtecan (Enhertu)



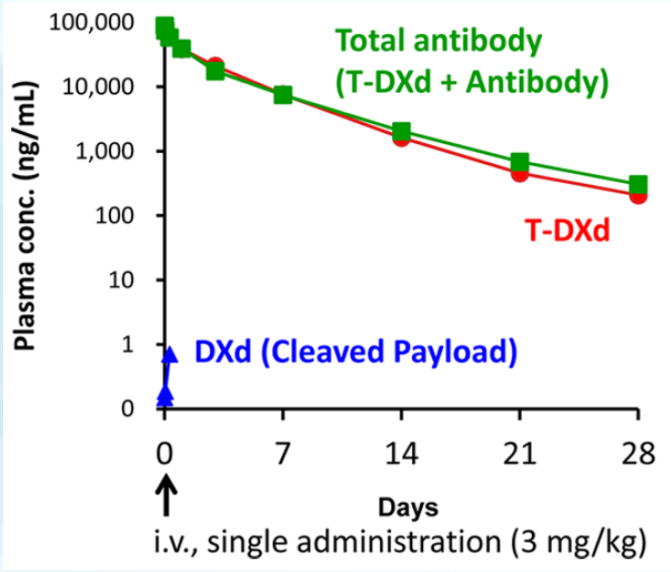
Kohei Shitara et a. Discovery and development of trastuzumab deruxtecan and safety management for patients with HER2-positive gastric cancer. Gastric Cancer, 2021.

ADC Case Review-Trastuzumab Deruxtecan (Enhertu)

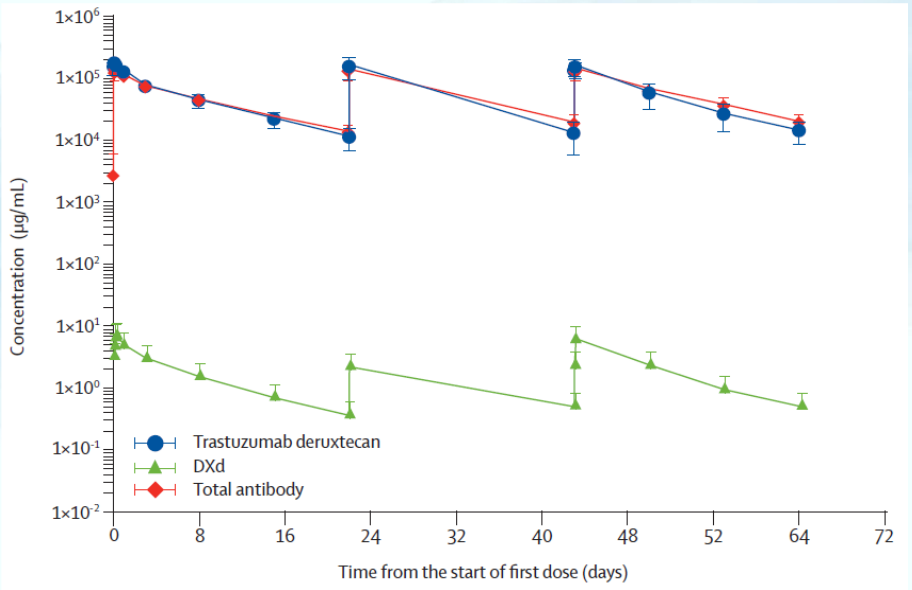
In Vitro Plasma Stability



In Vivo PK in NHP



Clinical PK



Takashi Nakada.. Discovery research and translation science of trastuzumab deruxtecan, from non-clinical study to clinical trial. Translat Regulat Sci. 2021

Toshihiko Doi et al. Safety, pharmacokinetics, and antitumour activity of trastuzumab deruxtecan (DS-8201), a HER2-targeting antibody–drug conjugate, in patients with advanced breast and gastric or gastro-oesophageal tumours: a phase 1 dose-escalation study. Lancet Oncol. 2017

ADC Case Review-Trastuzumab Deruxtecan (Enhertu)

Nonclinical Safety Evaluations of Enhertu

Type of Studies	ADC	Payload	Antibody
Safety Pharmacology	Telemetry CV with FOB and Res in monkeys	hERG	NA
Genetic Toxicology	NA	AMES, Chromosomal Aberration Assay, In Vivo Micronucleus Assay	NA
General Toxicology	Q3W*3, rec 6W, rat and monkey Q3W*5, rec 3M, monkey	QW*5, rec 4W, rat and monkey	Q2W*3, rat and monkey
Phototoxicity	NA	*In Vitro* 3T3 NRU *In Vivo* Phototoxicity in Rats	NA
TCR	Human and Monkey	NA	Not performed
Repro TOX	Not performed	Not performed	Not performed

ADC Case Review-Trastuzumab Deruxtecan (Enhertu)

Nonclinical Toxicity of Enhertu

Species	ADC	Payload	Antibody
Rat	Decreased WBC and Retics, and platelet Bone marrow,kidney, skin, intestines, testis and epididymides, lymph nodes and thymus, teeth	Decreased RBC, retics and WBC Bone marrow, intestines, lymph nodes and thymus	None
Monkey	Diarrhea, skin abnormalities Decreased RBC and retics Increased ALT, AST, CK, UREA, Bone marrow, kidney, skin, intestines, testis, lung	Vomiting,diarrhea Decreased RBC, retics, lymphocytes Increased ALT, AST, Bone marrow, heart, liver, intestines, testis, lymph nodes, spleen and thymus, corneal	None

Approved ADCs

ADC Drug	Trade name	Disease Indication	Payload/Payload Class	Payload Action	Target	mAb	Linker	DAR	Approval Year
Enfortumab vedotin	Padcev	Urothelial cancer	MMAE/auristatin	microtubule inhibitor	Nectin4	IgG1	valine-citrulline	3.8	2019
Polatuzumab vedotin-piiq	Polivy	Large B-cell lymphoma	MMAE/auristatin	microtubule inhibitor	CD79	IgG1	valine-citrulline	3.5	2019
Moxetumomab pasudotox	Lumoxiti	Hairy cell leukemia	PE38 (Pseudotox)	inhibition of protein synthesis	CD22	IgG1	valine-citrulline	N/A	2018
Inotuzumab ozogamicin	Besponsa	B-cell precursor acute lymphoblastic leukemia	ozogamicin/calicheamicin	DNA cleavage	CD22	IgG4	pH-sensitive hydrazone	6	2017
Trastuzumab emtansine	Kadcyla	Breast cancer	DM1/maytansinoid	microtubule inhibitor	HER2	IgG1	non-cleavable/SMCC	3.5	2013
Brentuximab vedotin	Adcetris	Anaplastic large cell lymphoma	MMAE/auristatin	microtubule inhibitor	CD30	IgG1	valine-citrulline	4	2011
Gemtuzumab ozogamicin	Mylotarg	Acute myelogenous leukemia	ozogamicin/calicheamicin	DNA cleavage	CD33	IgG4	pH-sensitive hydrazone	2–3	2017; 2000

Approved ADCs

ADC Drug	Trade name	Disease Indication	Payload/Payload Class	Payload Action	Target	mAb	Linker	DAR	Approval Year
Mirvetuximab soravtansine	ELAHERE	Ovarian Cancer	DM4/Maytansinoid	microtubule inhibitor	FRα	IgG1	disulfide	3.4	2022
Tisotumab vedotin-tftv	Tivdak	Cervical cancer	MMAE/auristatin	microtubule inhibitor	Tissue factor	IgG1	valine-citrulline	4	2021
Loncastuximab tesirine-lpyl	Zynlonta	Large B-cell lymphoma	SG3199/PBD dimer	DNA cleavage	CD19	IgG1	valine-citrulline	2.3	2021
Cetuximab Sarotalocan Sodium	Akalux	Head and neck cancer	IRDye700DX/phthalocyanine derivative.	photosensitizer	EGFR	IgG1	NA		2021
Belantamab mafodotin-blmf	Blenrep	Multiple myeloma	MMAF/auristatin	microtubule inhibitor	BCMA	IgG1	noncleavable maleimidocaproyl (mc)	4	2020
Sacituzumab govitecan	Trodelvy	Breast cancer	SN-38/camptothecin	TOP1 inhibitor	TROP2	IgG1	pH-sensitive hydrazone	7.6	2020
Trastuzumab deruxtecan	Enhertu	Breast cancer	DXd/camptothecin	TOP1 inhibitor	HER2	IgG1	maleimide tetrapeptide	8	2019

Questions

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Thank You