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Beyond ADC target expression: Understanding ADC properties and pharmacology

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ADC Therapeutic Development
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Conflict of Interest

Raffaele Colombo

I have the following relevant financial relationships to disclose:

Employee of: Zymeworks Inc.
Stockholder in: Zymeworks Inc., AstraZeneca

In addition:

I will not discuss side effects or endorse any of the drugs mentioned in this presentation

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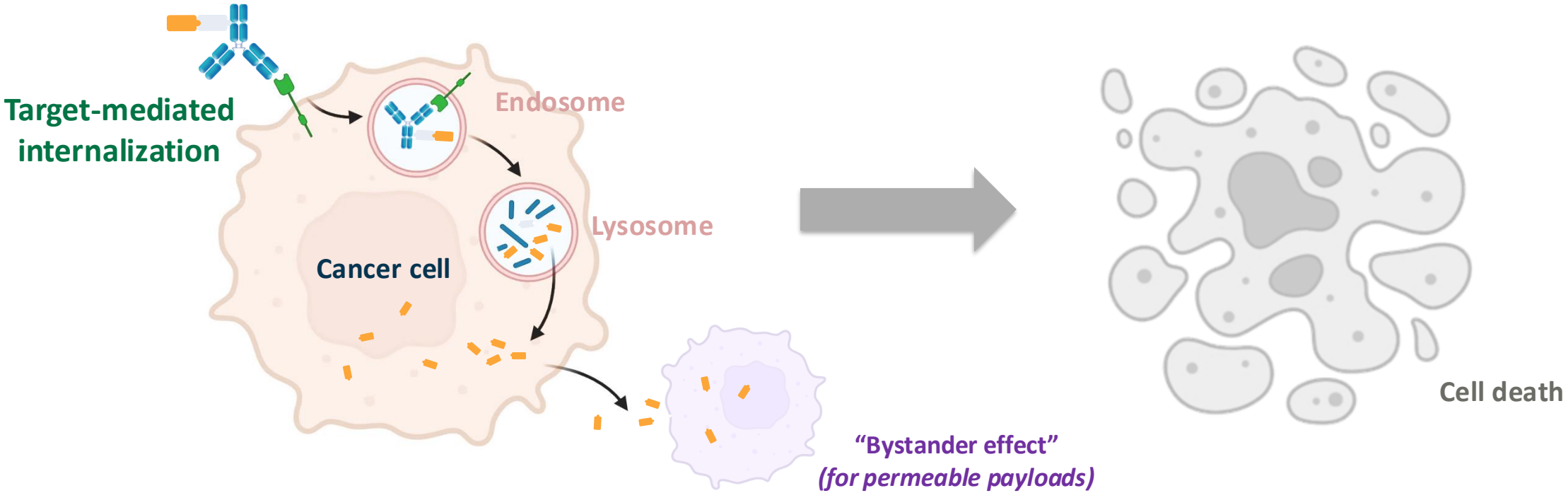
ADC target expression: an elusive biomarker?



- *Does target expression correlate with ADC response?*
- *Are there alternative mechanisms for ADCs over and above direct tumor targeting?*

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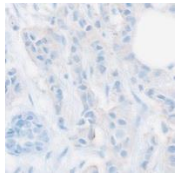
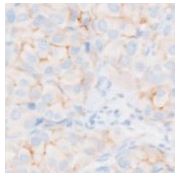
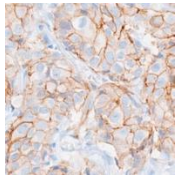
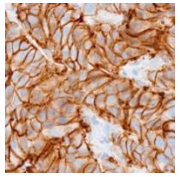
Different scores are used to represent target expression based on immunohistochemical (IHC) staining

IHC (0-3+)


IHC = 0

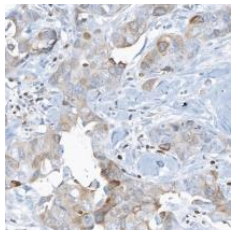

IHC = 1+

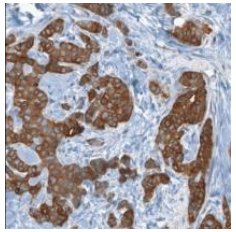

IHC = 2+

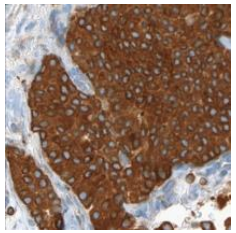

IHC = 3+

IHC 0 (no staining), IHC 1+ (weak staining), IHC 2+ (moderate staining), and IHC 3+ (strong staining)

H-score (0-300)

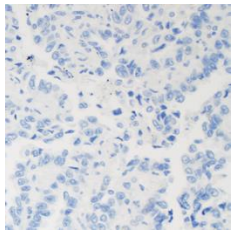

H-score = 50

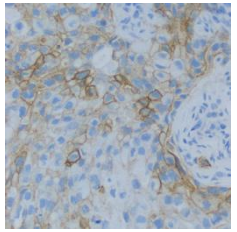

H-score = 150

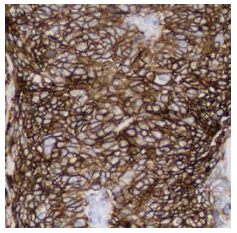

H-score = 270

H-score = (1 × percentage of weak staining) + (2 × percentage of moderate staining) + (3 × percentage of strong staining)

TPS (0-100)

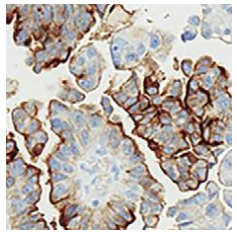

TPS = 3

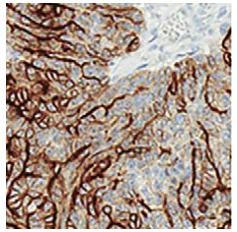

TPS = 40

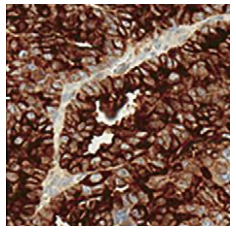

TPS = 100

Tumor proportion score (TPS) = percentage of viable tumor cells with partial or complete membrane staining at any intensity

PS2+ (0-100)


PS2+ = 30


PS2+ = 60


PS2+ = 95

PS2+ scoring = percentage of viable tumor cells with moderate [2+] or strong [3+] staining intensity

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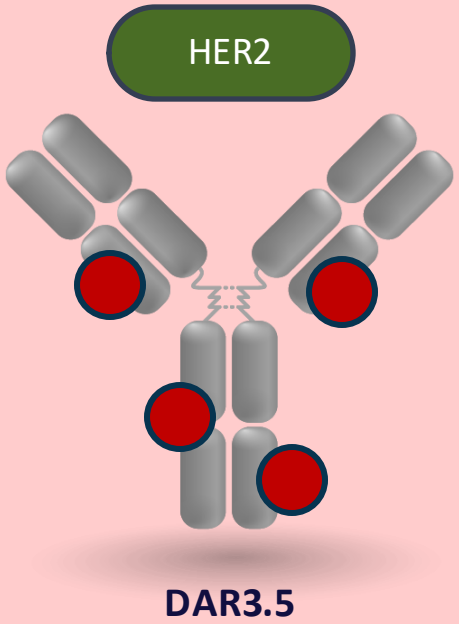
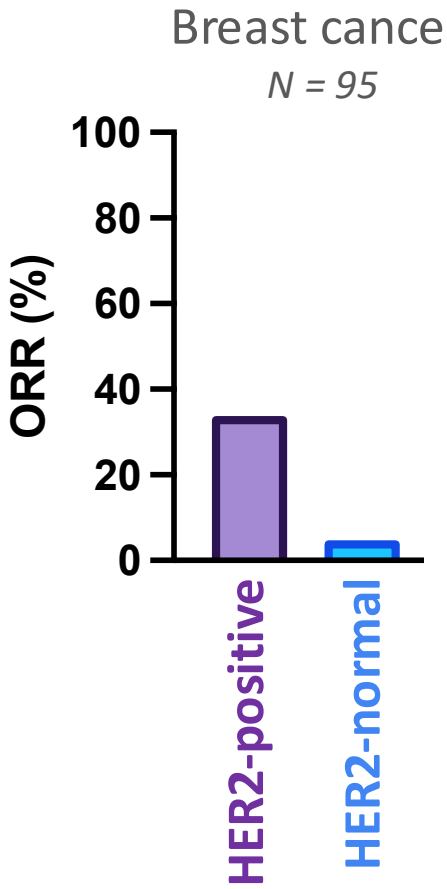
T-DM1 showed clear benefits in patients with HER2-positive breast cancer

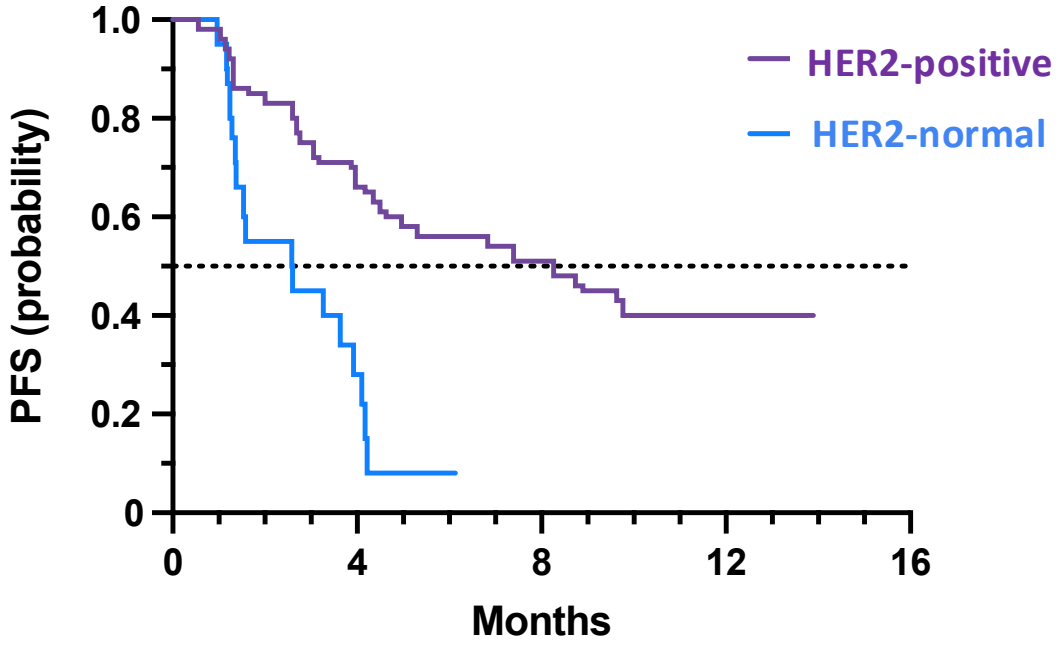
Trastuzumab emtansine (T-DM1)



DAR3.5

DM1 (maytansinoid)
Non-cleavable linker





HER2-positive = IHC 3+ or IHC 2+/FISH+

HER2-normal = IHC 2+/FISH- or IHC 1+ or IHC 0

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H. A. Burris III et al. *J. Clin. Oncol.* **2011**, *29*, 398–405. Similar trends reported by I. E. Krop et al. *J. Clin. Oncol.* **2012**, *30*, 3234-3241

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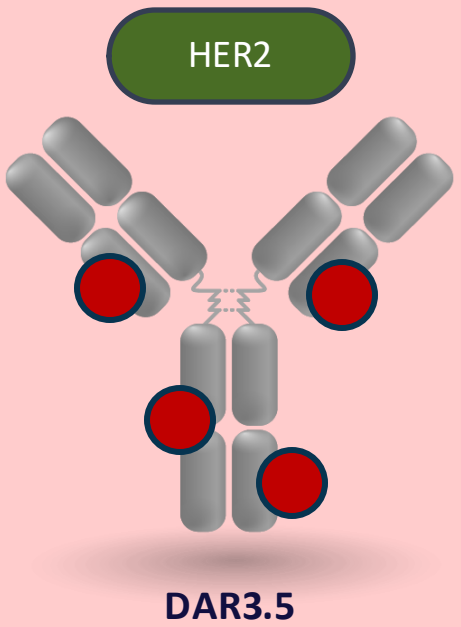
T-DM1 showed clear benefits in patients with HER2-positive breast cancer

Trastuzumab emtansine (T-DM1)

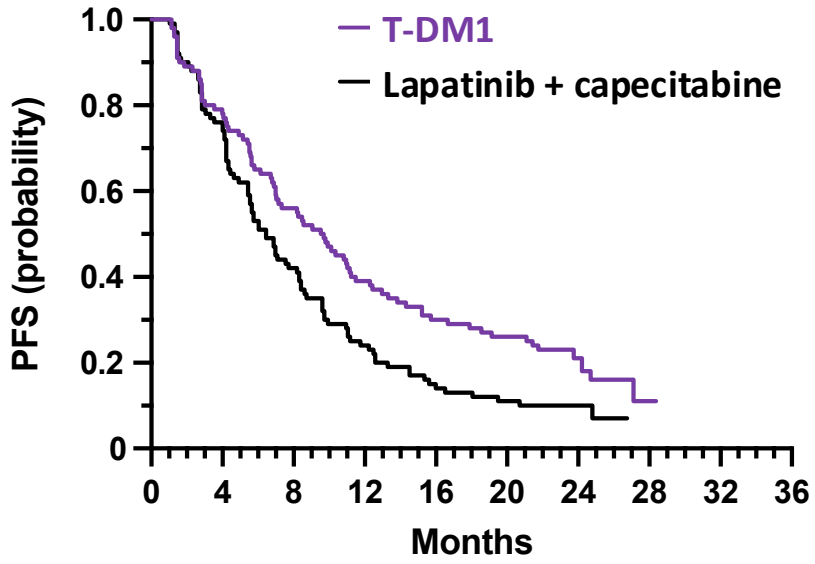


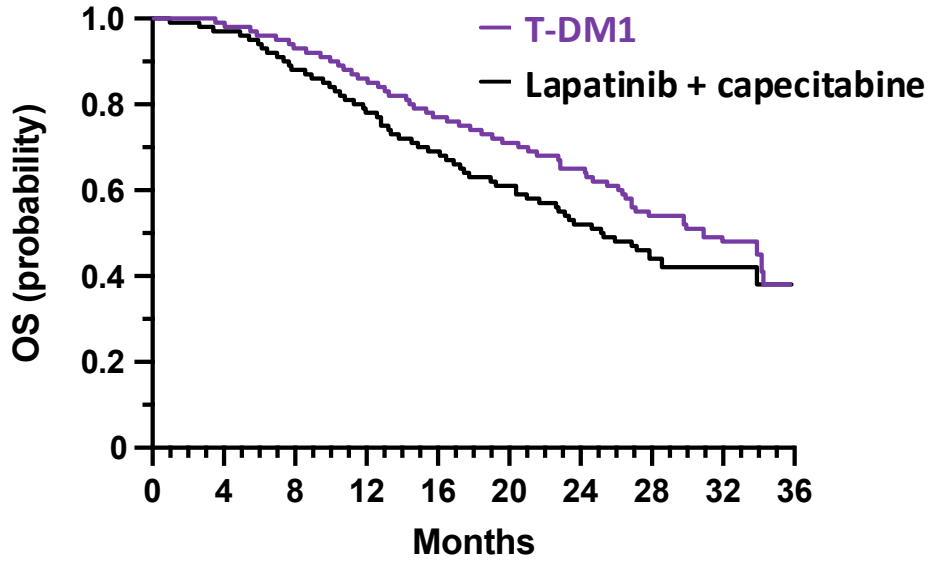
DAR3.5

DM1 (maytansinoid)
Non-cleavable linker

EMILIA trial

(HER2-positive breast cancer)
N = 991





… which led to the approval of T-DM1 for patients with HER2-positive breast cancer!

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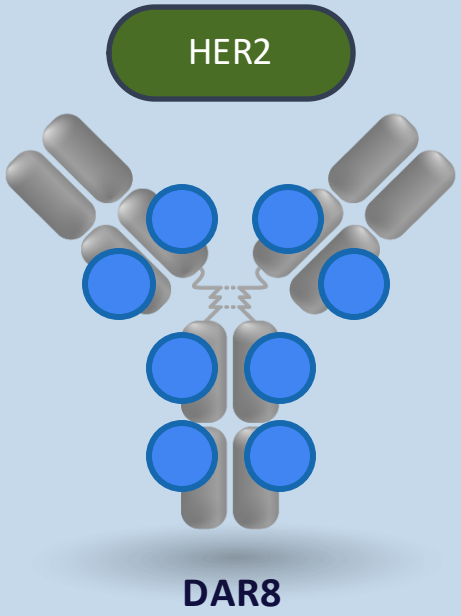
S. Verna et al. *N. Engl. J. Med*. **2012**, *367*, 1783-1791

T-DXd showed benefits across all HER2-expressions, but better efficacy in patients with HER2-high breast cancer

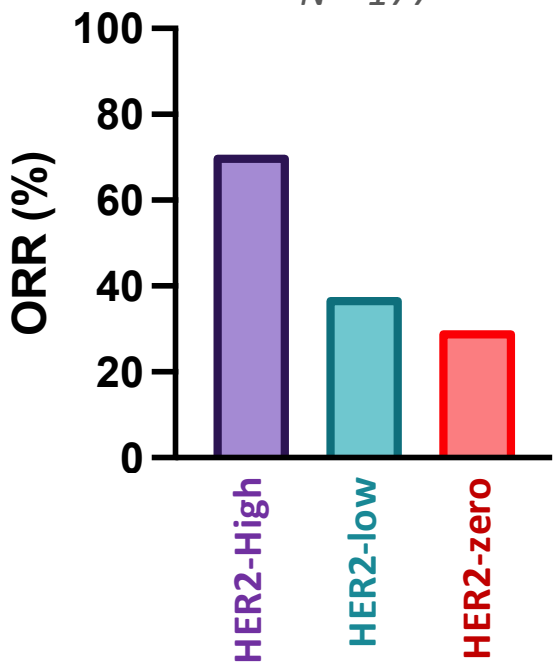
Trastuzumab deruxtecan (T-DXd)

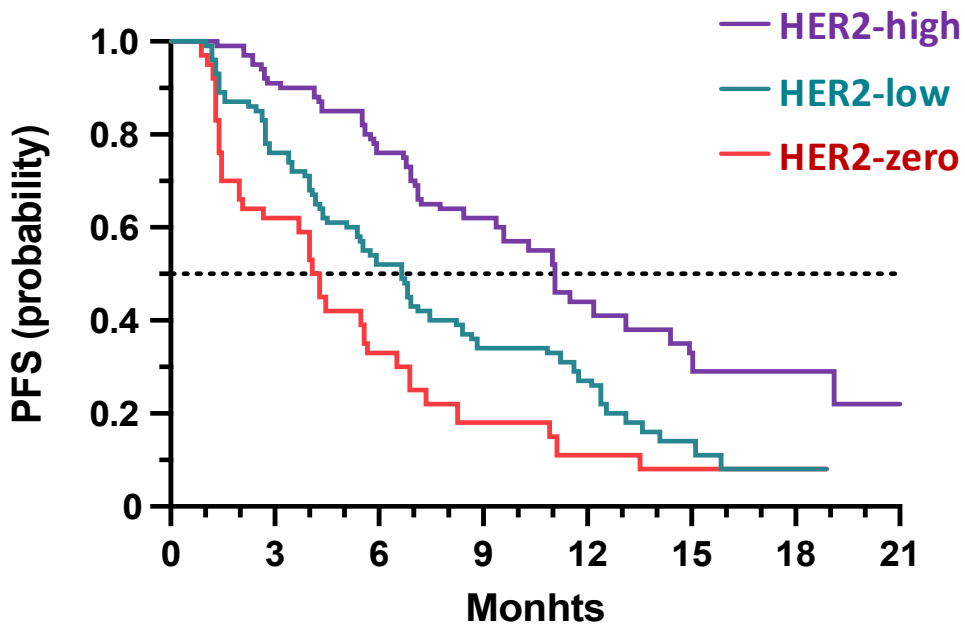


DXd (TOPO1i)
Cleavable linker (GGFG)

DAISY trial

N = 177





HER2-high = IHC 3+ or IHC 2+/FISH+

HER2-low = IHC 2+/FISH- or IHC 1+

HER2-zero = IHC 0

Similar trend observed for OS, with median OS of 31, 19, and 12 months for patients with HER2-high, HER2-low, and HER2-zero, respectively

DAISY: F. Mosele et al. *Nat. Med.* **2023**, *29*, 2110. HER2-high, *N* = 68; HER2-low, *N* = 72; HER2-zero, *N* = 37

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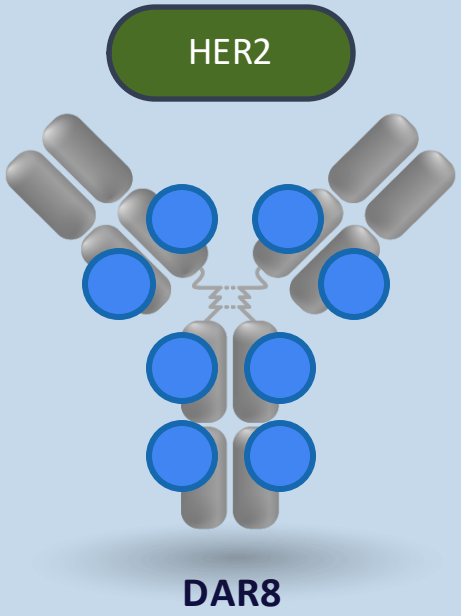
Benefits observed for T-DXd vs treatment of physician's choice in patients with HER2-high, low, and ultralow breast cancer

HER2 ultralow = IHC >0 and <1+

Trastuzumab deruxtecan (T-DXd)

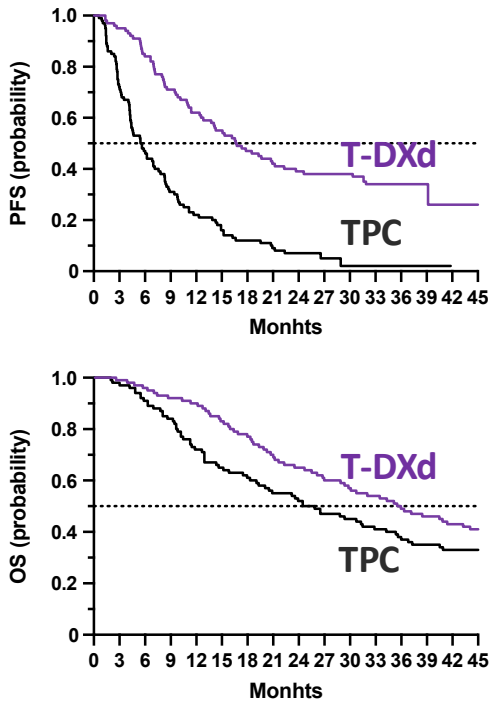


DAR8

DXd (TOPO1i)
Cleavable linker (GGFG)

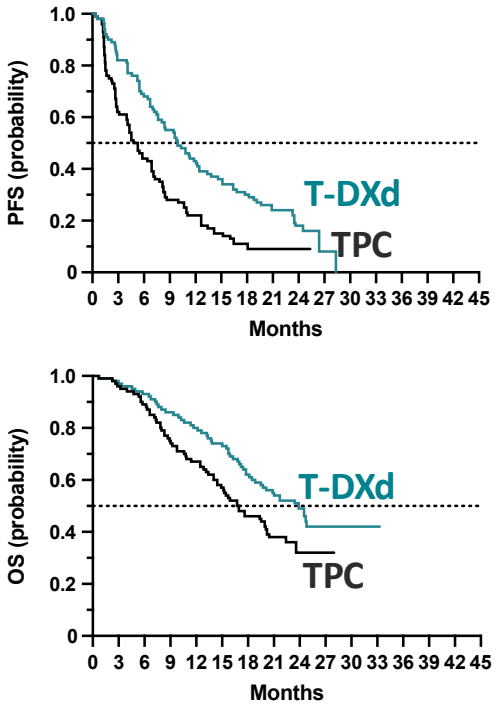
HER2-high

(DESTINY-Breast02)
N = 524



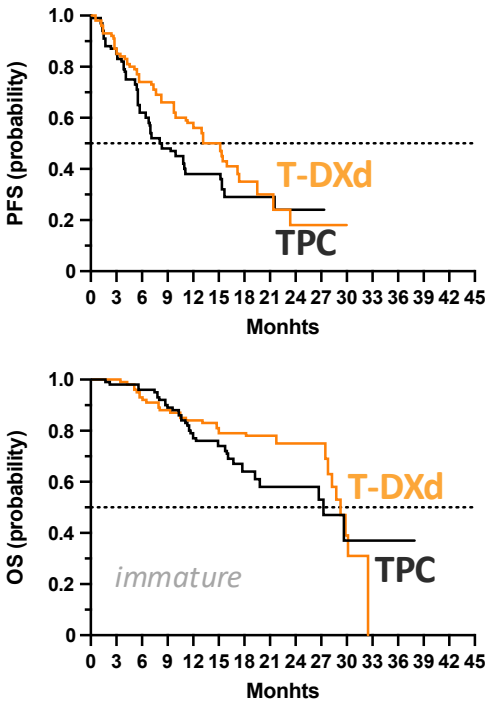
HER2-low

(DESTINY-Breast04)
N = 557



HER2-ultralow

(DESTINY-Breast06)
N = 152



TPC = treatment of physician's choice

DESTINY-Breast02: F. André et al. *Lancet*. **2023**, *401*, 1773; DESTINY-Breast04: S. Modi et al. *N. Engl. J. Med*. **2022**, *387*, 9;
DESTINY-Breast06: A. Badia et al. *N. Engl. J. Med*. **2024**. doi: 10.1056/NEJMoa2407086.

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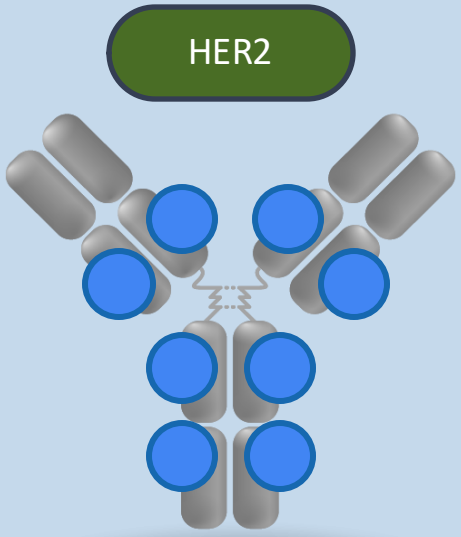
Benefits observed for T-DXd *vs* treatment of physician's choice in patients with HER2-high, low, and ultralow breast cancer

Trastuzumab deruxtecan (T-DXd)

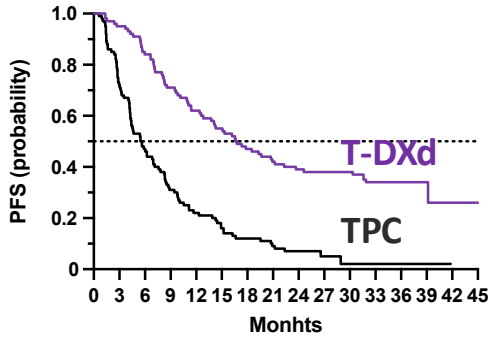


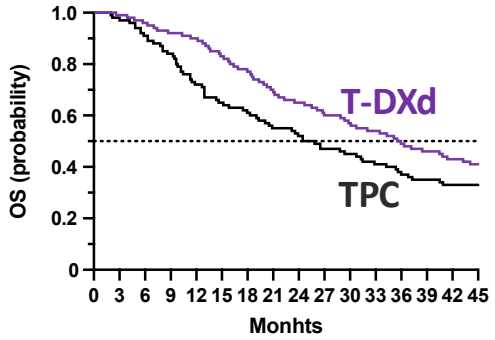
DAR8

DXd (TOPO1i)
Cleavable linker (GGFG)

HER2-high

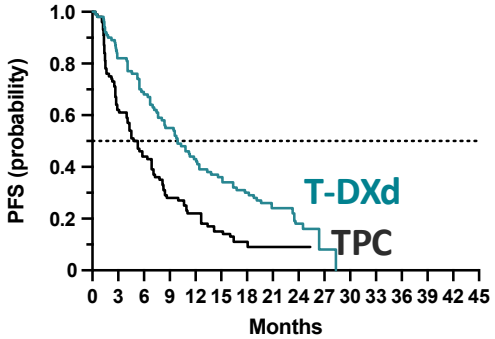
(DESTINY-Breast02)
N = 524

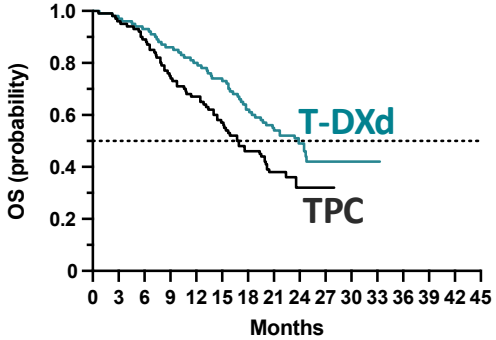




HER2-low

(DESTINY-Breast04)
N = 557





HER2-zero

(DESTINY-Breast15)

?

NCT05950945

TPC = treatment of physician's choice

DESTINY-Breast02: F. André et al. *Lancet*. **2023**, *401*, 1773; DESTINY-Breast04: S. Modi et al. *N. Engl. J. Med*. **2022**, *387*, 9;
DESTINY-Breast15: https://clinicaltrials.gov/study/NCT05950945

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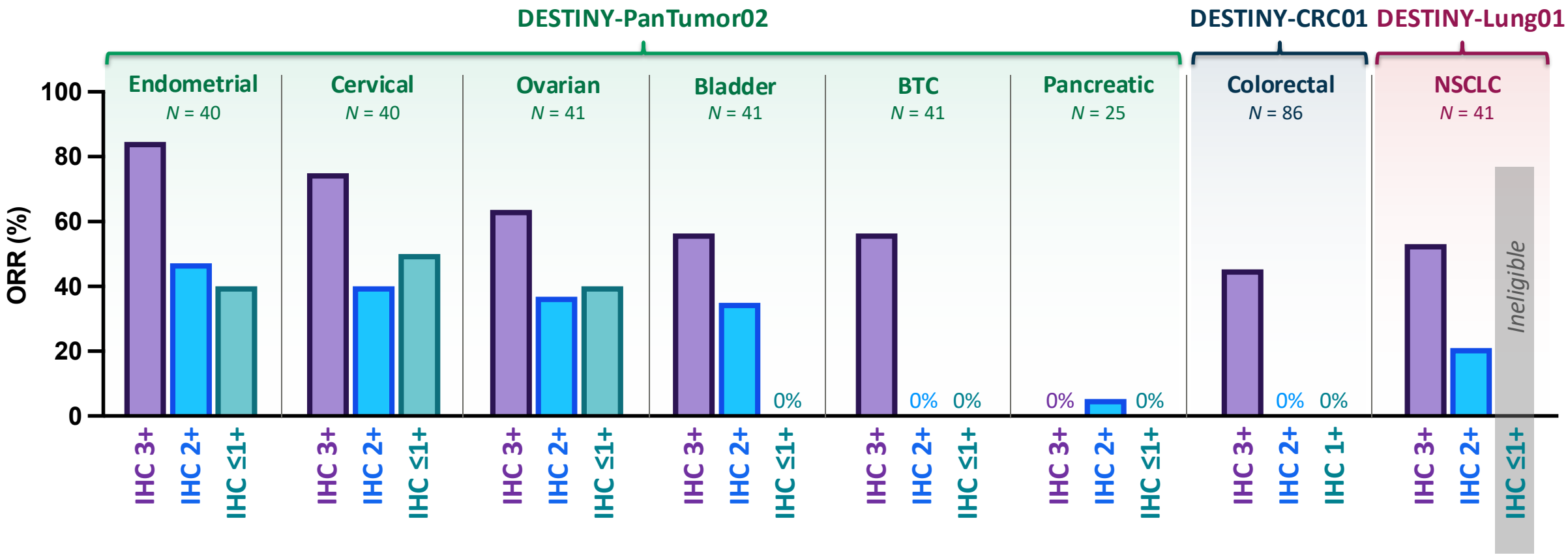
Additional trials reinforce the increased activity observed with T-DXd in HER2-high (IHC 3+) *vs* HER2-low/zero



….which led to the accelerated approval of T-DXd in "IHC 3+" solid tumors!

F. Meric-Bernstam et al. *J. Clin. Oncol*. **2024,** *42*, 47-58, T-DXd 5.4 mg/kg Q3W; T. Yoshino et al. *Nat. Commun.* **2023**, *14*, 3332, T-DXd 6.4 mg/kg Q3W; E. F. Smit et al. *Lancet Oncol.* **2024**, *25*, 439-454, T-DXd 5.4 mg/kg Q3W.

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Dissecting the contributions of ADC components

Antibody contribution

Can the antibody deliver more payload to high expressing cells?

Can the antibody contribute to efficacy itself?

Payload contribution

Can systemic payload exposure contribute to efficacy?

Are certain tumors more sensitive to certain payloads?

(linker stability for another time)

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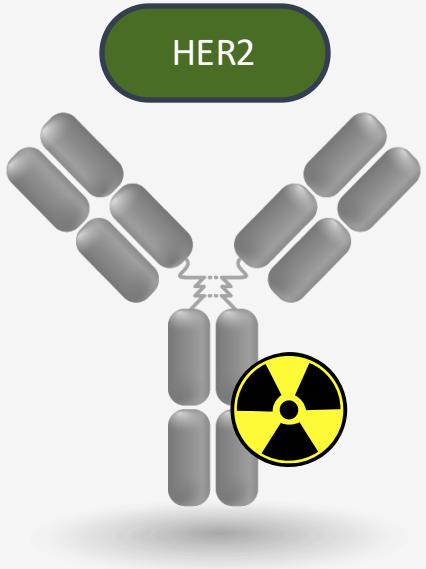
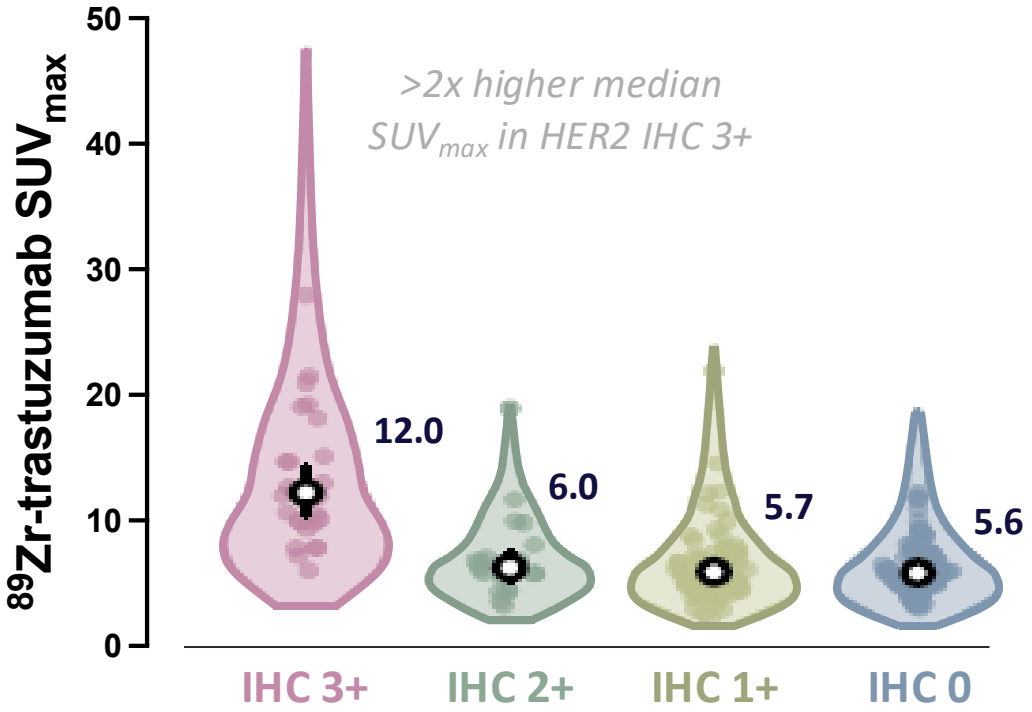
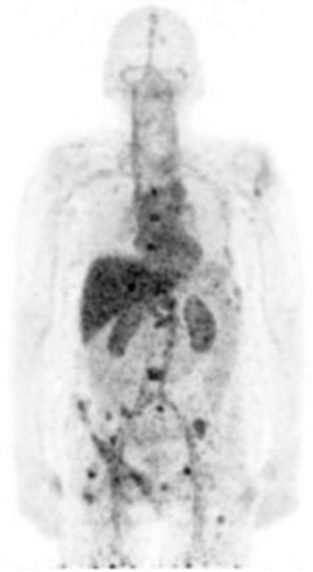
^{89}Zr-trastuzumab showed better uptake (SUV_{max}) in HER2-high (IHC 3+) lesions



^{89}Zr-trastuzumab

^{89}Zr-trastuzumab uptake (SUV_{max}) by IHC status





Evident ^{89}Zr-trastuzumab uptake in patients with HER2-low tumors, but higher uptake in HER2-high

$$\text{Standardized Uptake Value (SUV)} = \frac{Ac \cdot BW}{ID \cdot 2^{(-\Delta t / t_{1/2})}}$$

Ac = active concentration(Bq/mL)
ID = injected dose (Bq)
BW = body weight (g)
Δt = delay between injection time and scan time (s)
$t_{1/2}$ = radionuclide decay half-life (s)

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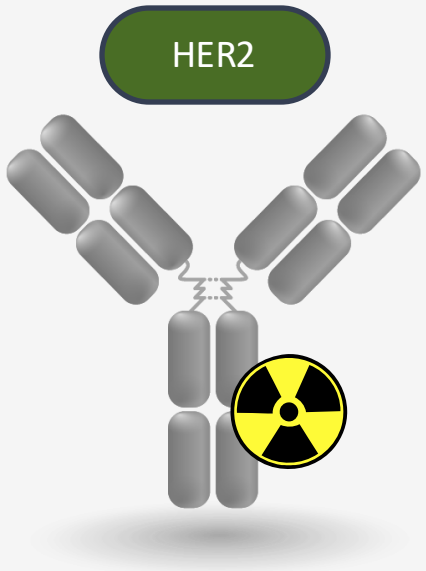
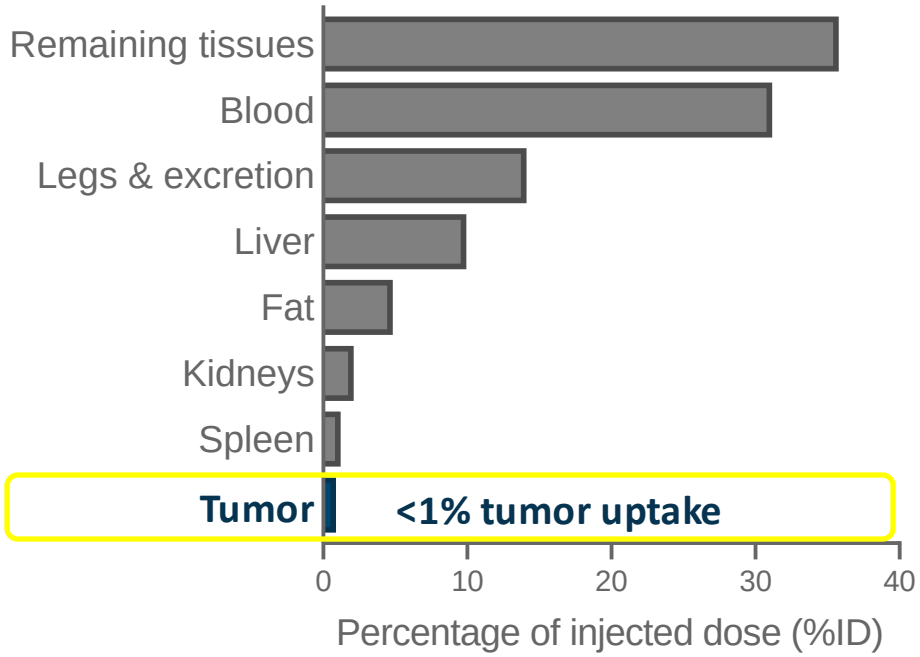
SUV_{max} from 5163 lesions in 189 patients, based on biopsied HER2 IHC status. B. Eisses et al. *J. Nucl. Med.* **2024**, *65*, 1540-1547

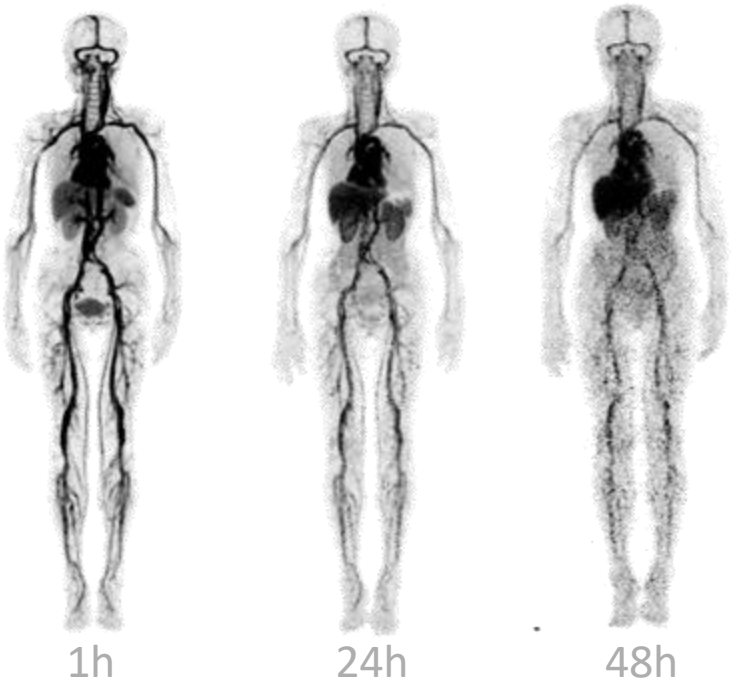
^{89}Zr-trastuzumab showed better uptake (SUV_{max}) in HER2-high (IHC 3+) lesions… but low absolute uptake



^{89}Zr-trastuzumab

Low absolute uptake (%ID) in tumor lesions





PET acquisition at 4 days after dosing

F. Bensch et al. *Theranostics* **2018**, *8*, 4295-4304

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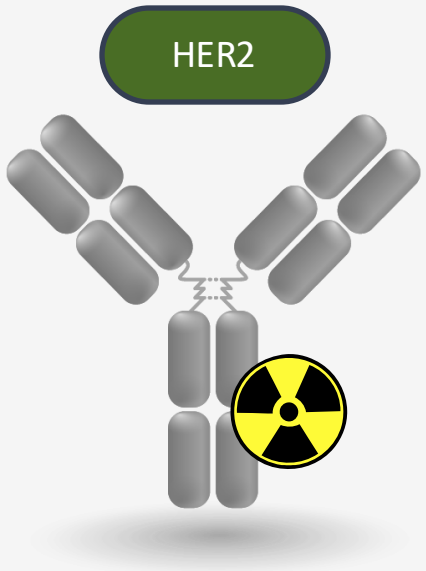
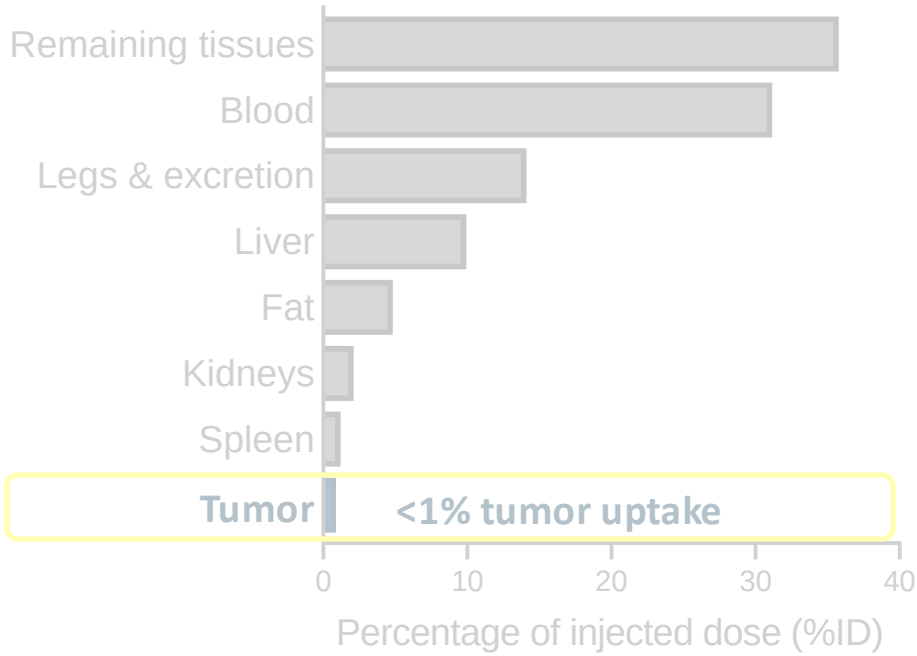
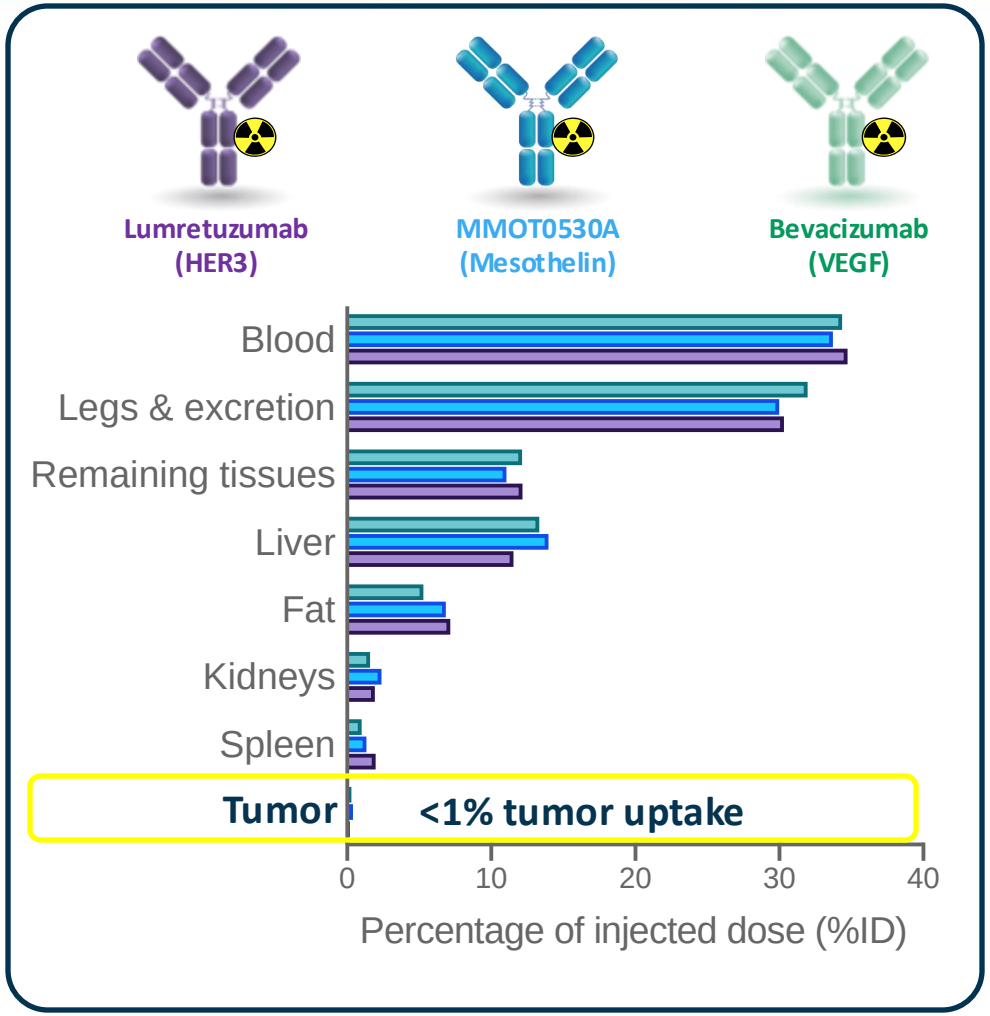


^{89}Zr-trastuzumab





PET acquisition at 4 days after dosing

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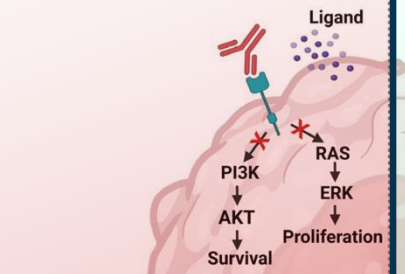
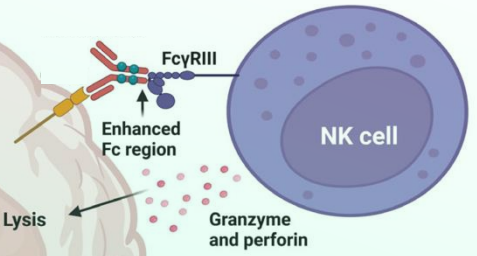
F. Bensch et al. *Theranostics* **2018**, *8*, 4295-4304

Antibody component can be more than just a carrier

Antibody can induce an antitumor effect via different mechanisms!

Blocking the downstream **signaling** pathways

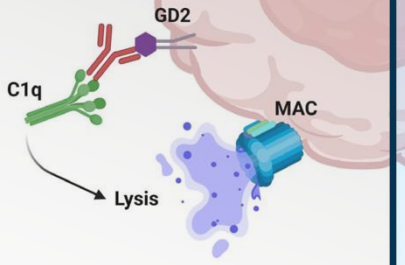


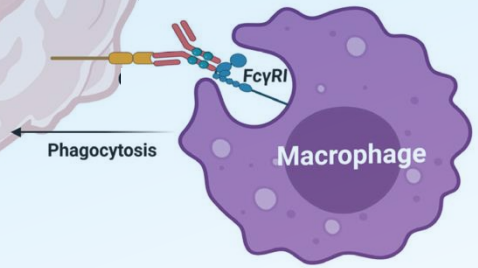


Antibody-dependent cellular cytotoxicity (**ADCC**)

Cancer cell

Complement-dependent cytotoxicity **(CDC)**





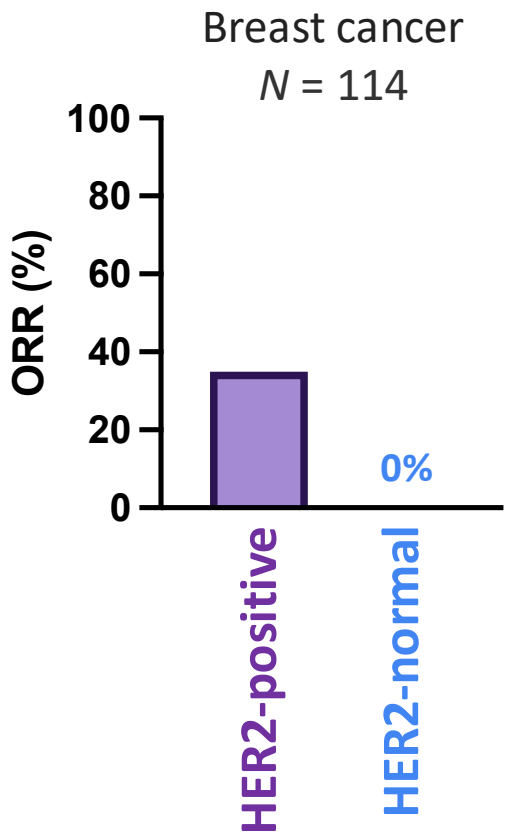
Antibody-dependent cellular phagocytosis **(ADCP)**

Figure adapted from: C. Rodríguez-Nava et al. *Biomedicines* **2023**, *11*, 1610

"Naked" trastuzumab showed benefits in HER2-positive *vs* HER2-normal, highlighting the antibody contribution

Trastuzumab ("naked")

Tras: 4 mg/kg QW



HER2-positive = IHC 3+ or IHC 2+/FISH+

HER2-normal = IHC 2+/FISH- or IHC ≤1+

C. L. Vogel et al. *J. Clin. Oncol.* **2002**, *20*, 719-726; A. D. Seidman et al. *J. Clin. Oncol.* **2001**, *19*, 2587-2595

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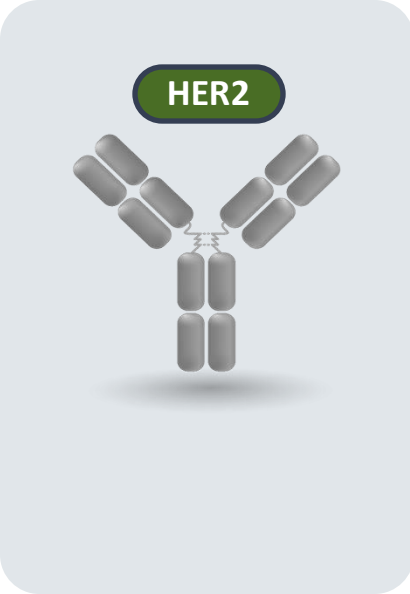
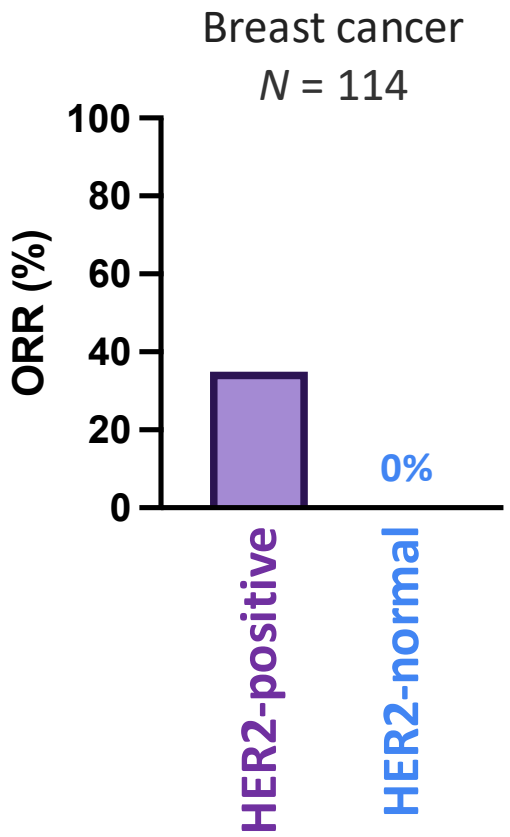
"Naked" trastuzumab showed benefits in HER2-positive *vs* HER2-normal, highlighting the antibody contribution

Trastuzumab ("naked")

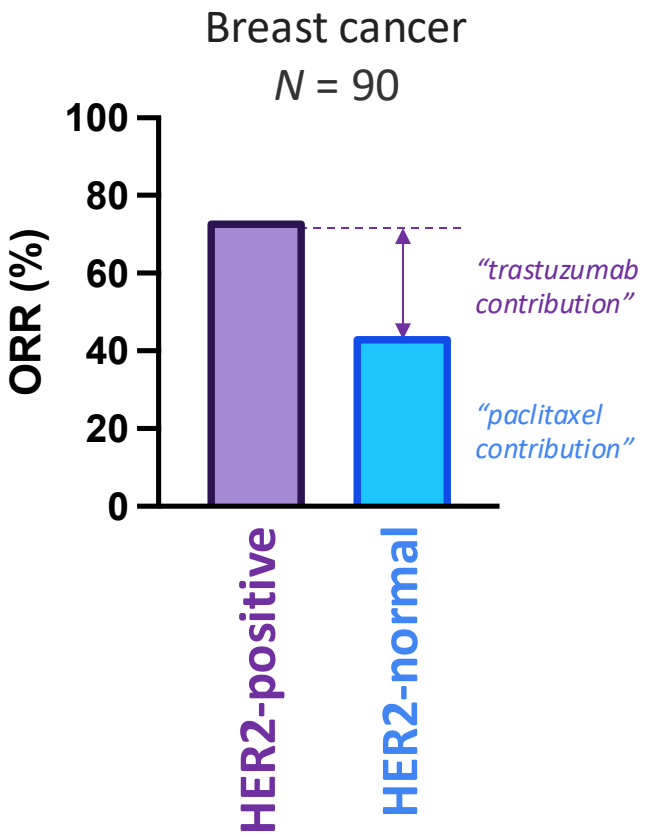


Tras: 4 mg/kg QW



Trastuzumab ("naked") + paclitaxel combination

Tras: 2 mg/kg QW
Paclitaxel: 90 mg/m² QW



HER2-positive = IHC 3+ or IHC 2+/FISH+

HER2-normal = IHC 2+/FISH- or IHC ≤1+

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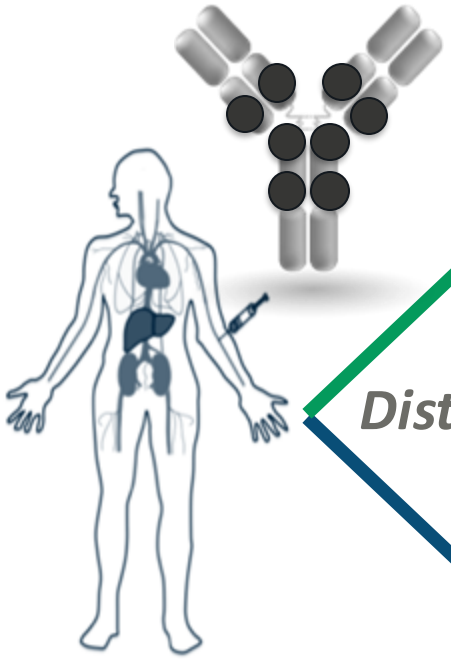
C. L. Vogel et al. *J. Clin. Oncol.* **2002**, *20*, 719-726; A. D. Seidman et al. *J. Clin. Oncol.* **2001**, *19*, 2587-2595

On-tumor and off-tumor ADC disposition generates free payload in circulation



On-tumor

On-target on-tumor

(typically <1%)

Distribution

Off-tumor

Off-target
(including on-target off-tumor)

(typically >99%)

Clearance

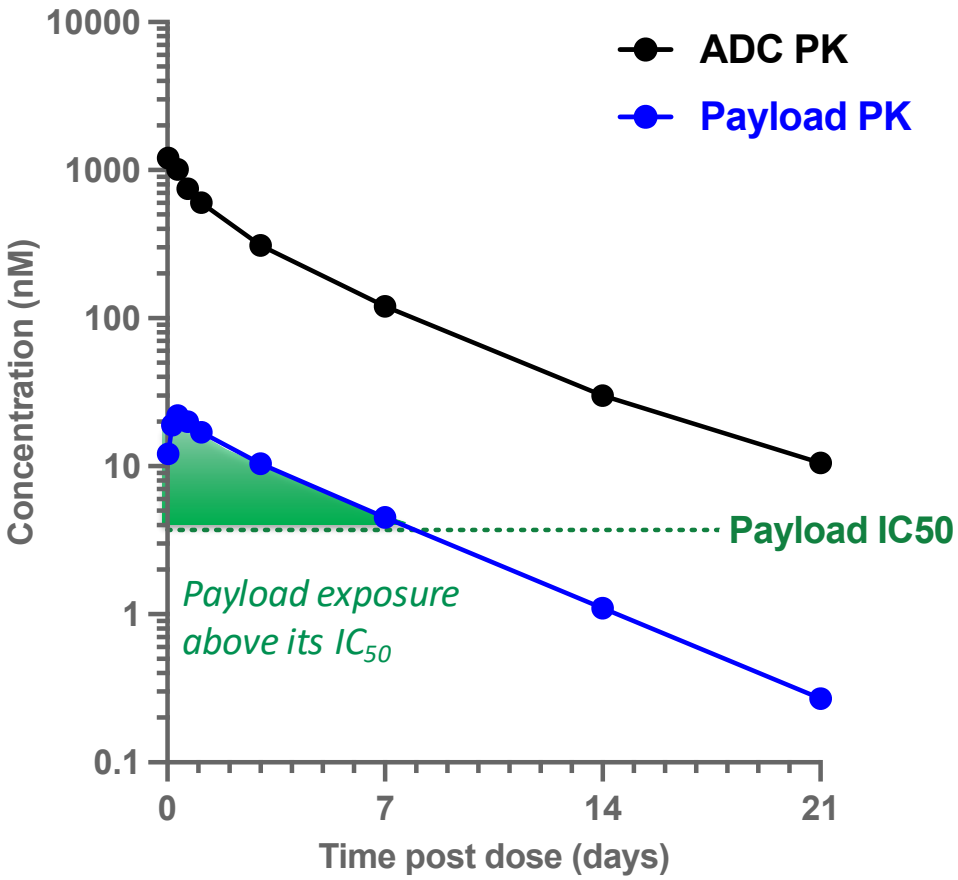
ADC metabolism

Payload in circulation

R. Colombo, P. Tarantino, J. R. Rich, P. M. LoRusso, E.G.E de Vries, *Cancer Discovery*, **2024**, *OnlineFirst*

Circulating payload concentrations achieve pharmacologically active levels in humans

PK data for T-DXd and DXd from DESTINY-Gastric01



For ADCs with bystander active (= permeable) payloads:

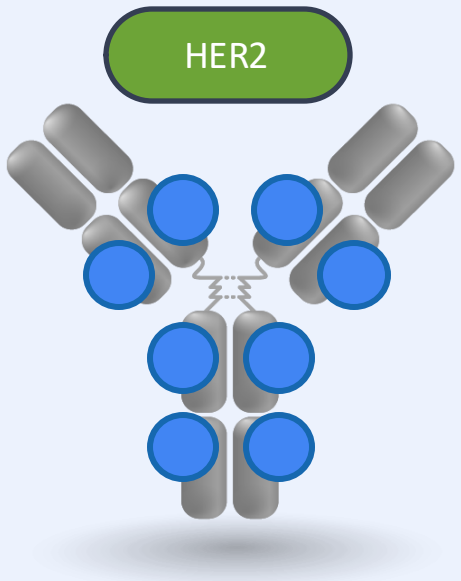
- in addition to ADC **direct target delivery**
- in addition to **bystander killing** (payload released in the TME or in heterogeneous tumor)

Systemic payload exposure likely contributes to efficacy observed in patients with low or even absent antigen expression

DESTINY-Gastric01: K. Shitara et al. *N. Engl. J. Med.* **2020**, *382*, 2419-2430

T-DXd showed better efficacy in patients with HER2-high, but benefits observed across all HER2-expressions

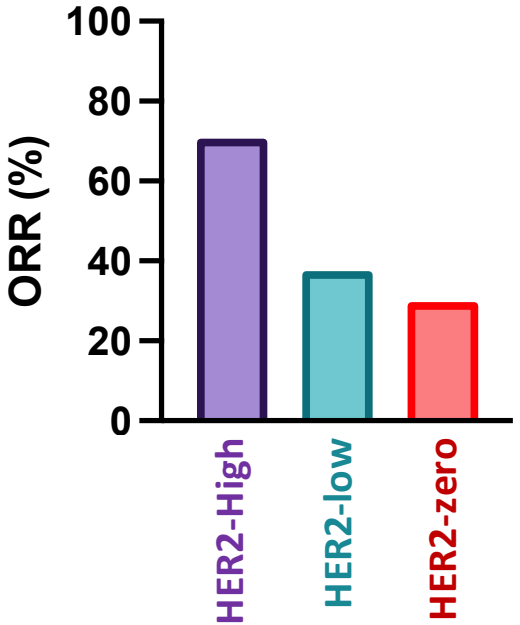
Trastuzumab deruxtecan (T-DXd)

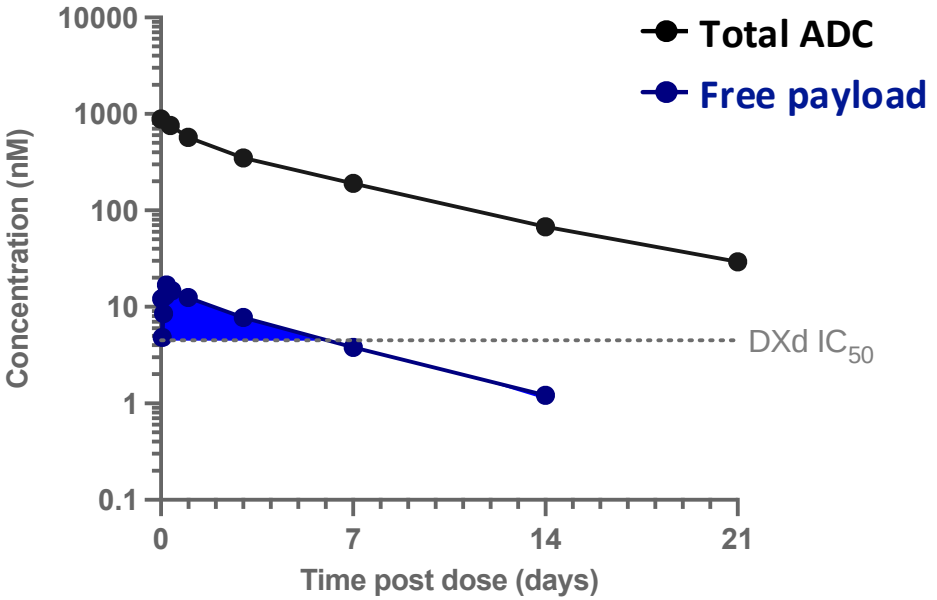


DAR8

DXd (TOPO1i)
Cleavable linker (GGFG)

Breast cancer
(DAISY study)
N = 177

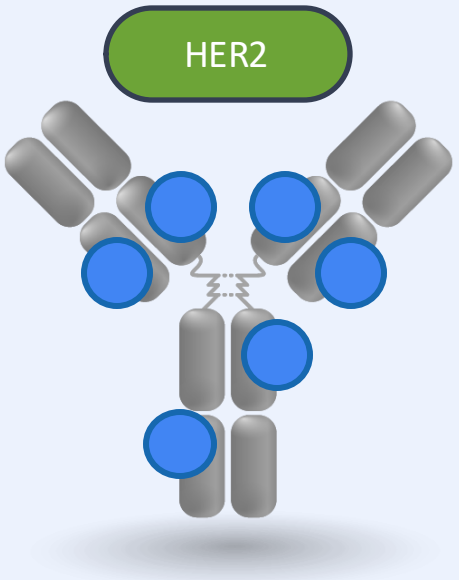




DAISY: F. Mosele et al. *Nat. Med*. **2023**, *29*, 2110. PK from Destiny-Breast01, O. Yin et al. presented at ASCPT **2019**

Trastuzumab rezetecan (T-DXh) showed trends similar to trastuzumab deruxtecan (T-DXd)

Trastuzumab rezetecan (SHR-A1811)

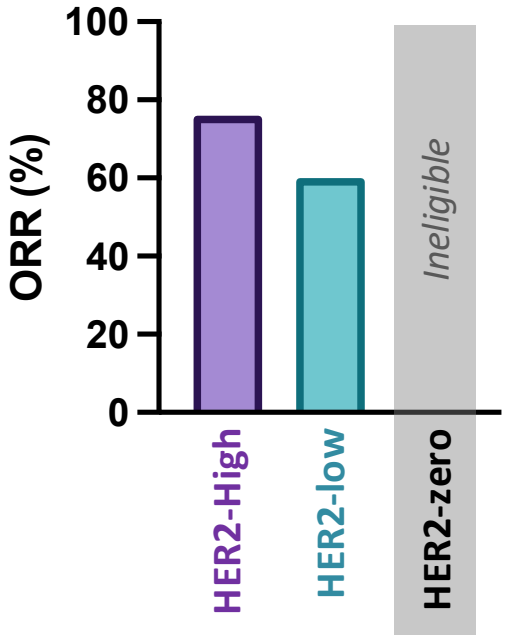


DAR5.7

DXh (TOPO1i)
Cleavable linker (GGFG)

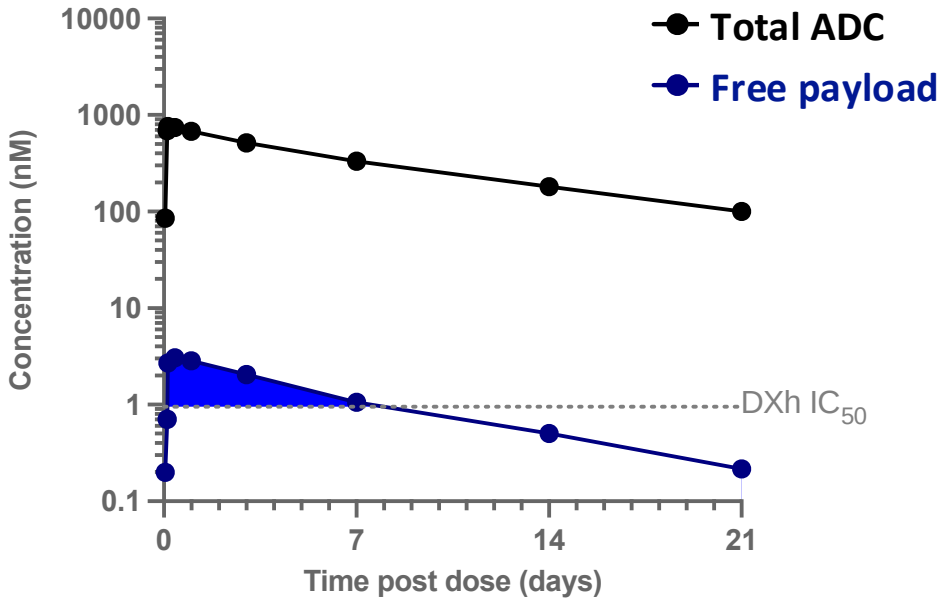
Breast cancer

N = 202



Doses 1-8 mg/kg Q3W (majority ≥4.8 mg/kg)

PK for 5.6 mg/kg



HER2-high, 114 patients; HER2-low, 88 patients.
H. Yao et al. *J. Clin. Oncol.* **2024**, *42*, 3453-3465. Similar trends observed in other solid tumors.

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Disitamab vedotin showed efficacy in patients with HER2-high and low breast cancer

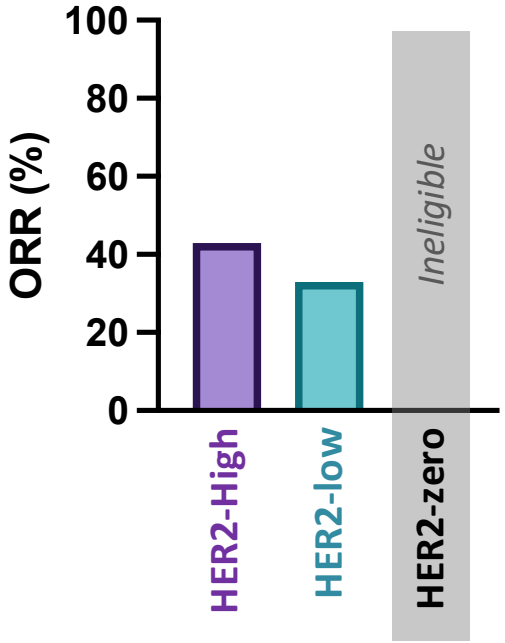
Disitamab vedotin (DV)

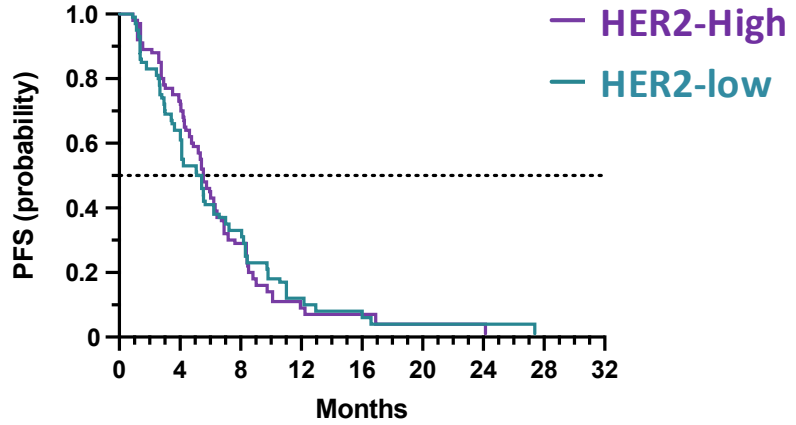
DAR4

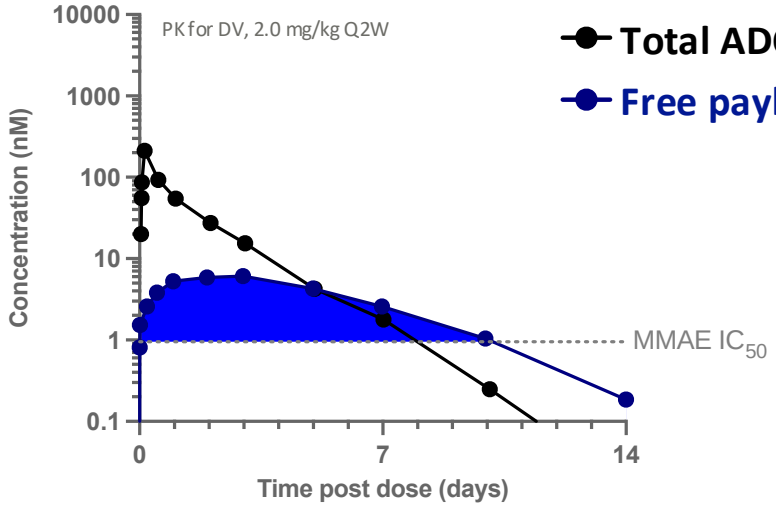
MMAE (auristatin)
Cleavable linker (Val-Cit)

Breast cancer

N = 136



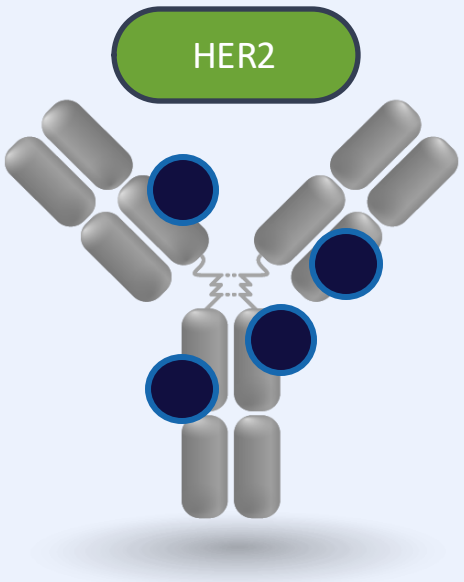




HER2-high (IHC 3+ or IHC 2+/ISH+), 70 patients. HER2-low (IHC 1+ or IHC 2+/ISH-), 66 patients.
Doses: 1.5-2.5 mg/kg Q2W. RP2D 2.0 mg/kg Q2W. J. Wang et al. *Cancer Commun*. **2024**, *44*, 833-851.

Trastuzumab vedotin showed efficacy in patients with HER2-high and low breast cancer

Trastuzumab vedotin (MRG002)

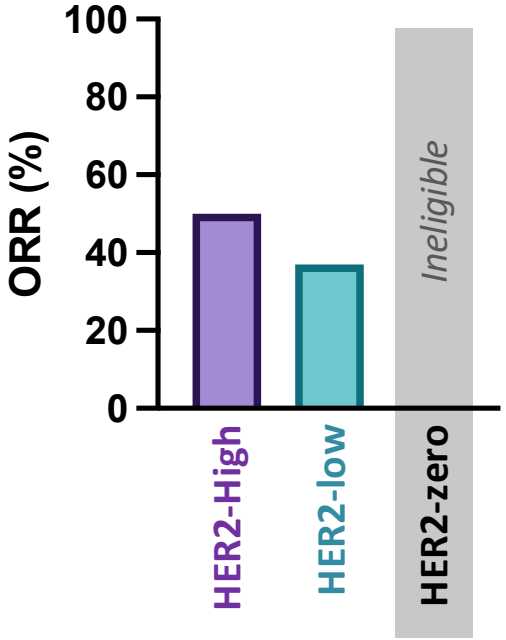


DAR4

MMAE (auristatin)
Cleavable linker (Val-Cit)

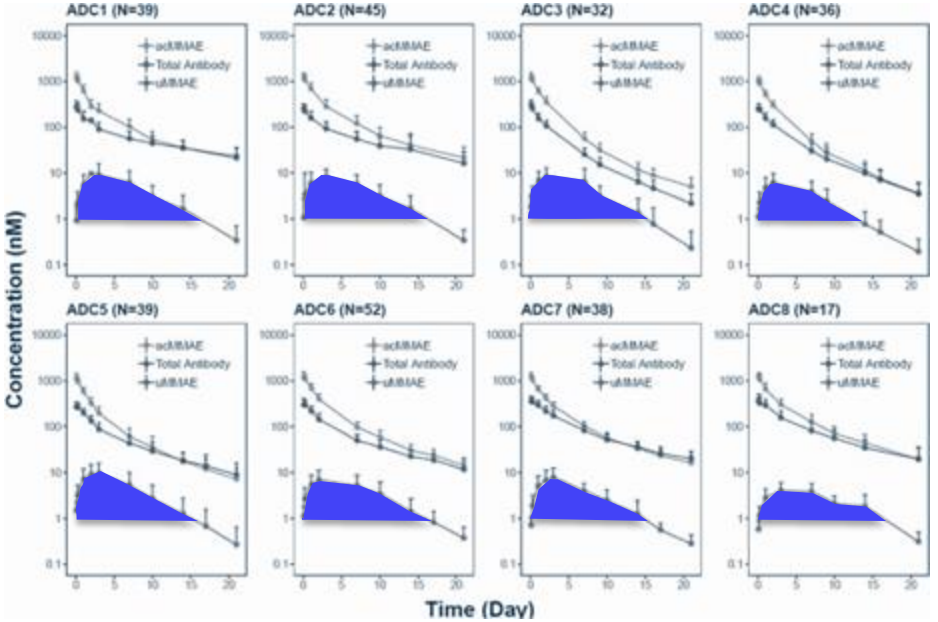
Breast cancer

N = 81



No PK reported (yet) for MRG002

… but stochastic DAR4 vedotin ADCs have similar payload PK across multiple targets and indications



HER2-high, 25 patients. Y. Guo et al. *Ann. Oncol.* **2021**, *32*, S480-S481; HER2-low, 56 patients: Z. Jiang et al. *J. Clin. Oncol.* **2022**, *40*, 1102-1102. MMAE ADC PK data adapted from: C. Li et al. *MAbs*. **2020**, *12*, 1699768.

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Antibody and payload contribution to efficacy

Antibody component

Can deliver more payload to high-expressing cells

If target is expressed high enough. But overall low absolute uptake (typically <1%)

Can inhibit intracellular signaling cascades

If the antibody is active as single agent and the ADC is dosed at a relevant antibody dose.

Can induce ADCC, ADCP, CDC

If Fc-mediated effector functions are preserved

(linker stability for another time)

Payload component

May contribute to efficacy via localized (TME release, bystander) and/or systemic exposure

If the payload and/or payload metabolite(s) are bystander active / permeable

May be more efficacious in tumors with higher sensitivity to its mechanism of action

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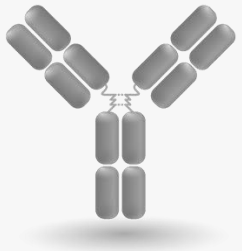
Same antibody but different "active drugs" lead to different clinical outcome

Naked antibody

	Trastuzumab
	
Antibody dose	6 mg/kg Q3W (2 mg/kg QW)
Clinical benefits	*Responses only in HER2-high*
Antibody contribution	🟢
Direct payload delivery	🔴
Chemo exposure	🔴

🟢 = *contribution*
🟡 = *limited contribution*
🔴 = *not a contribution*

*For T-DM1, the major active metabolite generated from ADC catabolism is Lys-MCC-DM1 (less permeable, non-bystander active)

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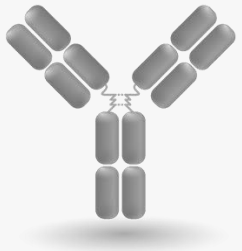
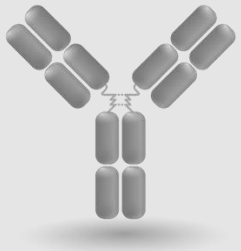
Same antibody but different “active drugs” lead to different clinical outcome

	Naked antibody	Naked antibody + permeable MTI combo
	Trastuzumab	**Trastuzumab + paclitaxel**
		
		+ Paclitaxel
Antibody dose	**6 mg/kg Q3W (2 mg/kg QW)**	**6 mg/kg Q3W (2 mg/kg QW)**
Clinical benefits	***Responses only in HER2-high***	***Responses in HER2-high, low, and zero; higher responses in HER2-high***
Antibody contribution	🟢	🟢
Direct payload delivery	🔴	🔴
Chemo exposure	🔴	🟢

🟢 = *contribution*
🟡 = *limited contribution*
🔴 = *not a contribution*

*For T-DM1, the major active metabolite generated from ADC catabolism is Lys-MCC-DM1 (less permeable, non-bystander active)

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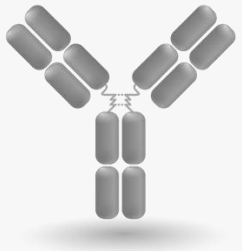
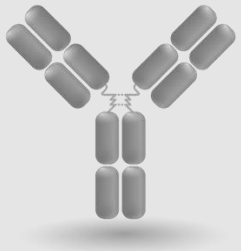
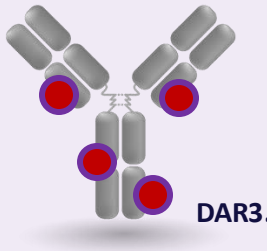
Same antibody but different "active drugs" lead to different clinical outcome

	Naked antibody	Naked antibody + permeable MTI combo	ADC releasing a less permeable payload*
	Trastuzumab	**Trastuzumab + paclitaxel**	**Trastuzumab emtansine (T-DM1)**
			
		+ **Paclitaxel**	DAR3.5
			DM1 (maytansinoid) Non-cleavable linker (MCC)
Antibody dose	**6 mg/kg Q3W (2 mg/kg QW)**	**6 mg/kg Q3W (2 mg/kg QW)**	**3.6 mg/kg Q3W**
Clinical benefits	***Responses only in HER2-high***	***Responses in HER2-high, low, and zero; higher responses in HER2-high***	***Responses mainly in HER2-high***
Antibody contribution	🟢	🟢	🟡
Direct payload delivery	🔴	🔴	🟢
Chemo exposure	🔴	🟢	🔴

🟢 = *contribution*
🟡 = *limited contribution*
🔴 = *not a contribution*

*For T-DM1, the major active metabolite generated from ADC catabolism is Lys-MCC-DM1 (less permeable, non-bystander active)

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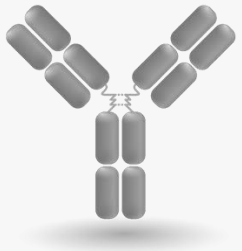
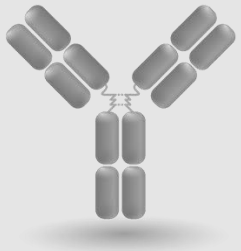
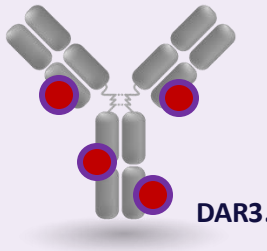
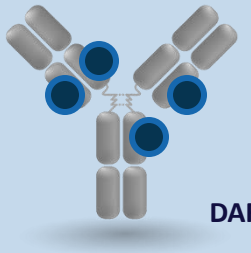
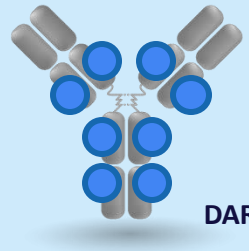
Same antibody but different "active drugs" lead to different clinical outcome

	Naked antibody	Naked antibody + permeable MTI combo	ADC releasing a less permeable payload*	ADC releasing a permeable MTI payload	ADC releasing a permeable topo1i payload
	Trastuzumab	**Trastuzumab + paclitaxel**	**Trastuzumab emtansine (T-DM1)**	**Trastuzumab vedotin (MRG002)**	**Trastuzumab deruxtecan (T-DXd)**
		 + ● Paclitaxel	 DAR3.5	 DAR4	 DAR8
			DM1 (maytansinoid) Non-cleavable linker (MCC)	MMAE (auristatin) Cleavable linker (Val-Cit)	DXd (camptothecin) Cleavable linker (GGFG)
Antibody dose	**6 mg/kg Q3W (2 mg/kg QW)**	**6 mg/kg Q3W (2 mg/kg QW)**	**3.6 mg/kg Q3W**	**2.6 mg/kg Q3W**	**5.4 mg/kg Q3W**
Clinical benefits	***Responses only in HER2-high***	***Responses in HER2-high, low, and zero; higher responses in HER2-high***	***Responses mainly in HER2-high***	***Responses in HER2-high, low, and zero; higher responses in HER2-high***	
Antibody contribution	🟢	🟢	🟡	🟡	🟢
Direct payload delivery	🔴	🔴	🟢	🟢	🟢
Chemo exposure	🔴	🟢	🔴	🟢	🟢

🟢 = *contribution*
🟡 = *limited contribution*
🔴 = *not a contribution*

*For T-DM1, the major active metabolite generated from ADC catabolism is Lys-MCC-DM1 (less permeable, non-bystander active)

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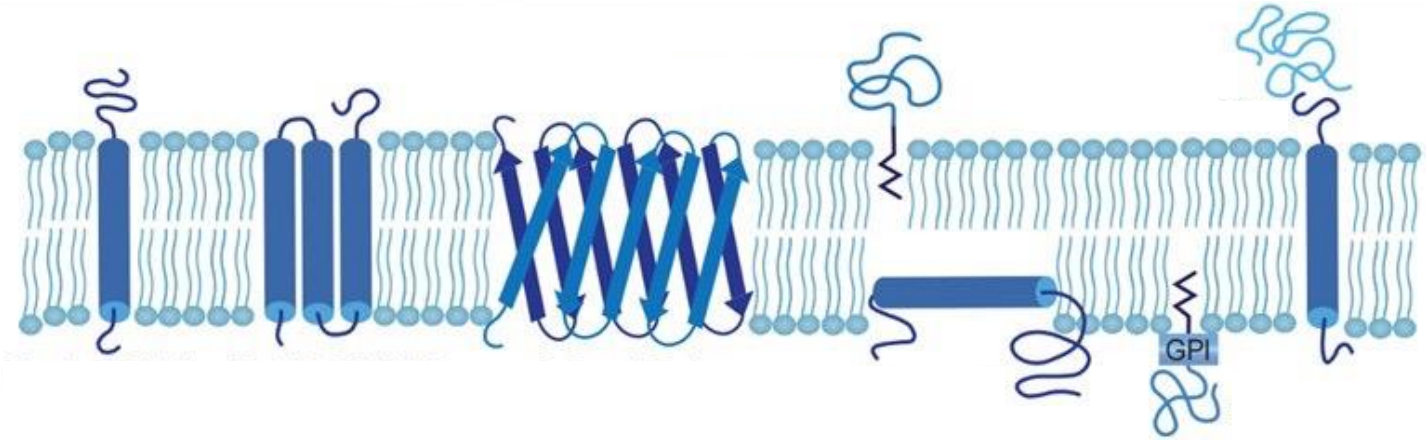
What about non-HER2 ADCs?

… is the target expression adequate to see a differentiation?

… is the antibody inhibiting relevant biology pathways?

… is the antibody just a carrier?

… is the efficacy mainly due to systemic payload exposure?



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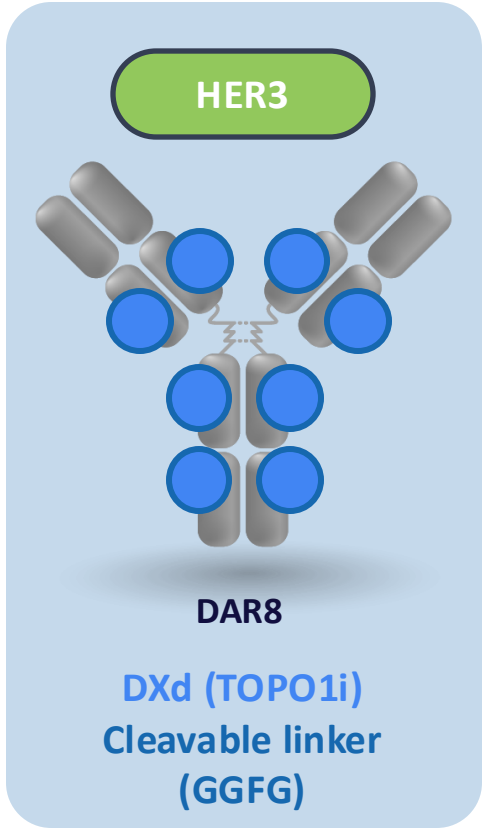
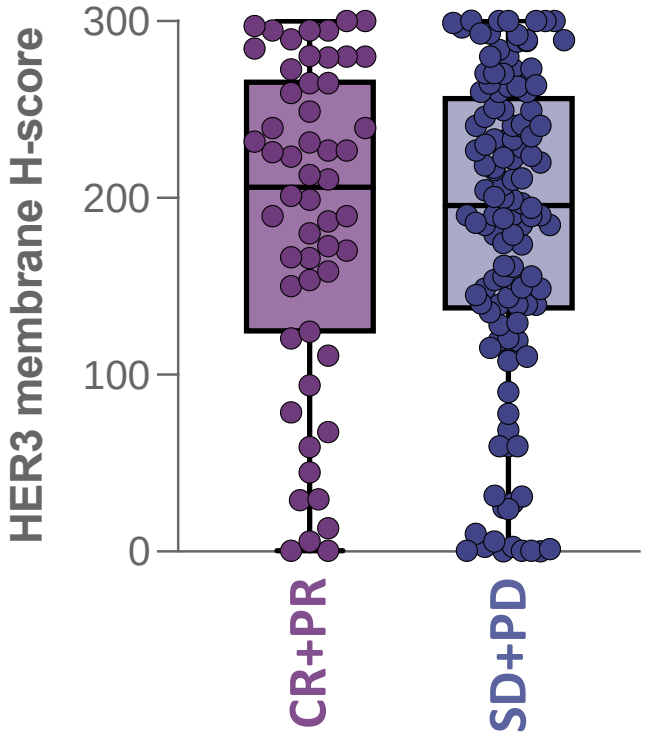
Benefits observed with HER3-DXd in patients across all HER3-expressions in different indications



Patritumab deruxtecan

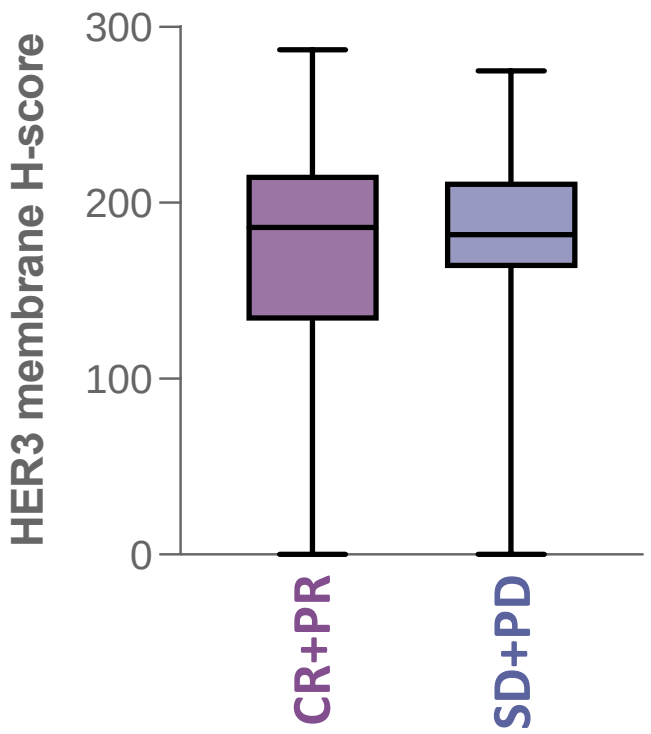
HERTHENA-Lung01 (Lung cancer)

N = 209



ICARUS-BREAST01 (Breast cancer)

N = 72



CR = complete response; **PR** = partial response; **SD** = stable disease; **PD** = progressive disease

HERTHENA-Lung01: H. A. Yu et al. *J. Clin. Oncol.* **2023**, *41*, 5363-5375; ICARUS-BREAST01: B. Pistilli et al. 340O, **ESMO24.**

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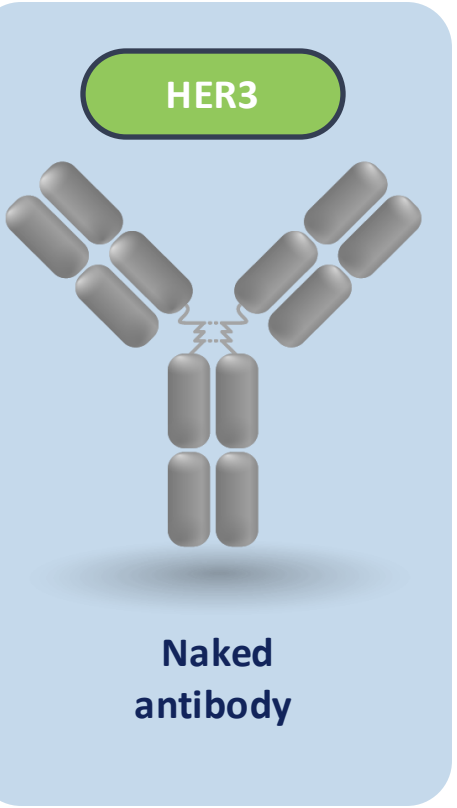
No benefits observed with HER3 naked antibodies as single agents or in combinations



Patritumab

	Dose	Monotherapy	+ erlotinib	+ cetuximab and platinum
Patritumab	**9 mg/kg Q3W**	**No objective responses**	**No improvements over erlotinib alone**	**No improvements over cetuximab + platinum doublet**

In advance solid tumors, including lung, breast, and head and neck cancers

P. M. LoRusso et al. *Clin. Cancer Res.* **2013**, *9*, 3078-3087; L. Paz-Arez et al. *J. Thorac. Oncol.* **2017**, *12*, S1214-S1215; M. D. Forster et al. *Eur. J. Cancer.* **2019**, *123*, 36-47; D. Meulendijks et al. *Clin. Cancer Res.* **2016**, *22*, 877-885; D. Meulendijks et al. *Clin. Cancer Res.* **2017**, *23*, 5406–5415; L. Gandullo-Sánchez, *J. Exp. Clin. Cancer Res.* **2022**, *41*, 310

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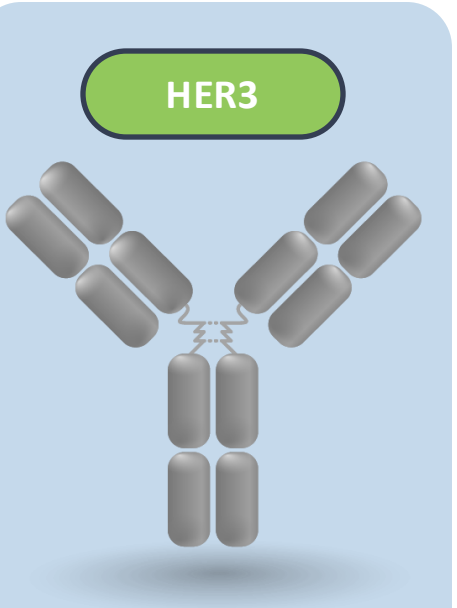
No benefits observed with HER3 naked antibodies as single agents or in combinations



Naked antibody

Patritumab
… and others

	Dose	Monotherapy	+ erlotinib	+ cetuximab and platinum
Patritumab	**9 mg/kg Q3W**	**No objective responses**	**No improvements over erlotinib alone**	**No improvements over cetuximab + platinum doublet**
Lumretuzumab	800 mg Q3W	No responses		
Barecetamab	20 mg/kg Q3W	No responses		
GSK2849330	30 mg/kg QW	3% ORR		
Seribantumab	20 mg/kg QW	No responses		
CDX-3379	12 mg/kg Q3W	4% ORR		
Elgemtumab	40 mg/kg QW	No responses		
AV-203	20 mg/kg Q2W	5% ORR		
REGN1400	20 mg/kg Q2W	No responses		

In advance solid tumors, including lung, breast, and head and neck cancers

P. M. LoRusso et al. *Clin. Cancer Res.* **2013**, *9*, 3078-3087; L. Paz-Arez et al. *J. Thorac. Oncol.* **2017**, *12*, S1214-S1215; M. D. Forster et al. *Eur. J. Cancer.* **2019**, *123*, 36-47; D. Meulendijks et al. *Clin. Cancer Res.* **2016**, *22*, 877-885; D. Meulendijks et al. *Clin. Cancer Res.* **2017**, *23*, 5406–5415; L. Gandullo-Sánchez, *J. Exp. Clin. Cancer Res.* **2022**, *41*, 310

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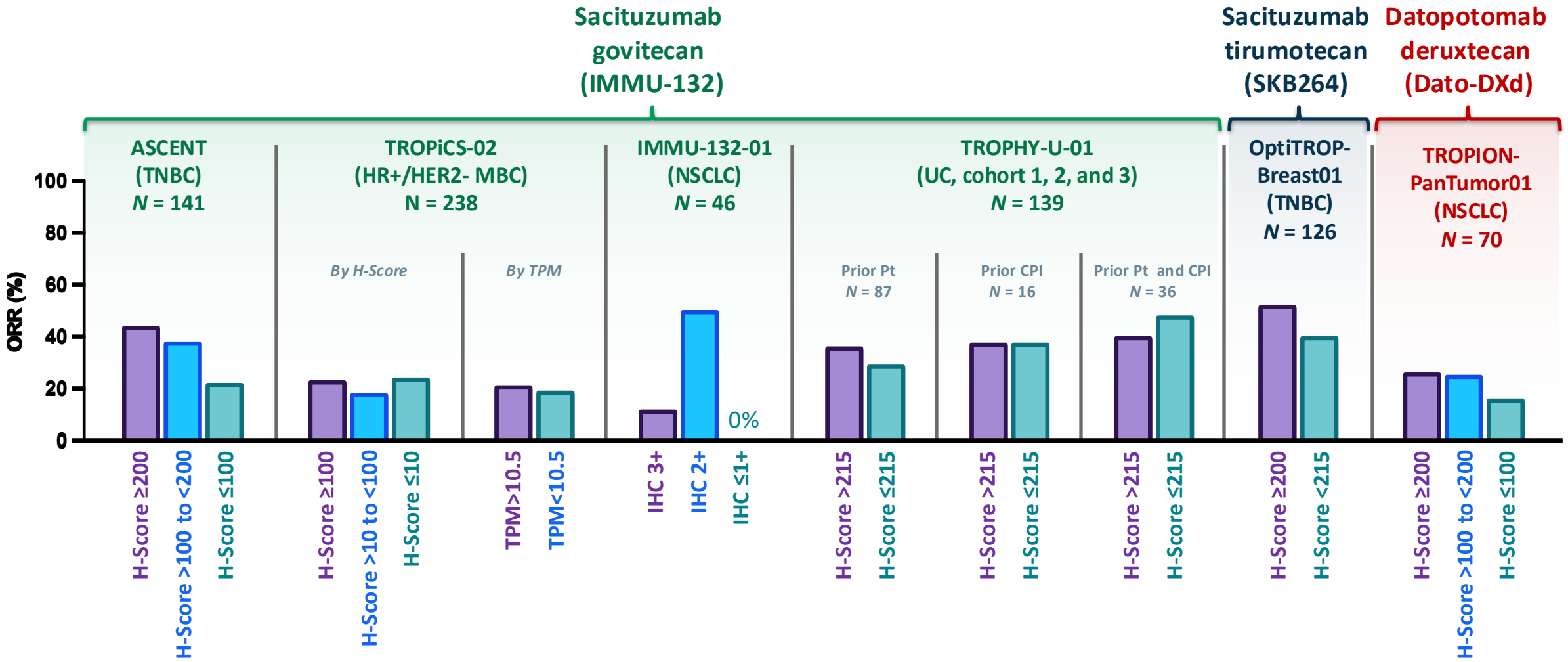
No clear relationship between TROP2 expression and responses with TROP2 ADCs



A. Bardia et al. *Ann. Oncol.*, **2021**, *32*, 1148-1156; H. Rugo et al. *Cancer. Res.* **2023**, 83(5_Supplement): GS1-11; A. Bardia et al. *J. Clin. Oncol.* **2023**, *41*, 1082-1082; R. S. Heist et al. *J. Clin. Oncol.* **2017**, *35*, 2790-2797; Y. Loriot et al. J. Clin. Oncol. 2023, 41, 4579-4579; U-01; Binghe Xu et al. *J. Clin. Oncol.* **2024**, *42*, 104-104; T. Shimizu et al. *J. Clin. Oncol.* **2023**, *41*, 4678-4687

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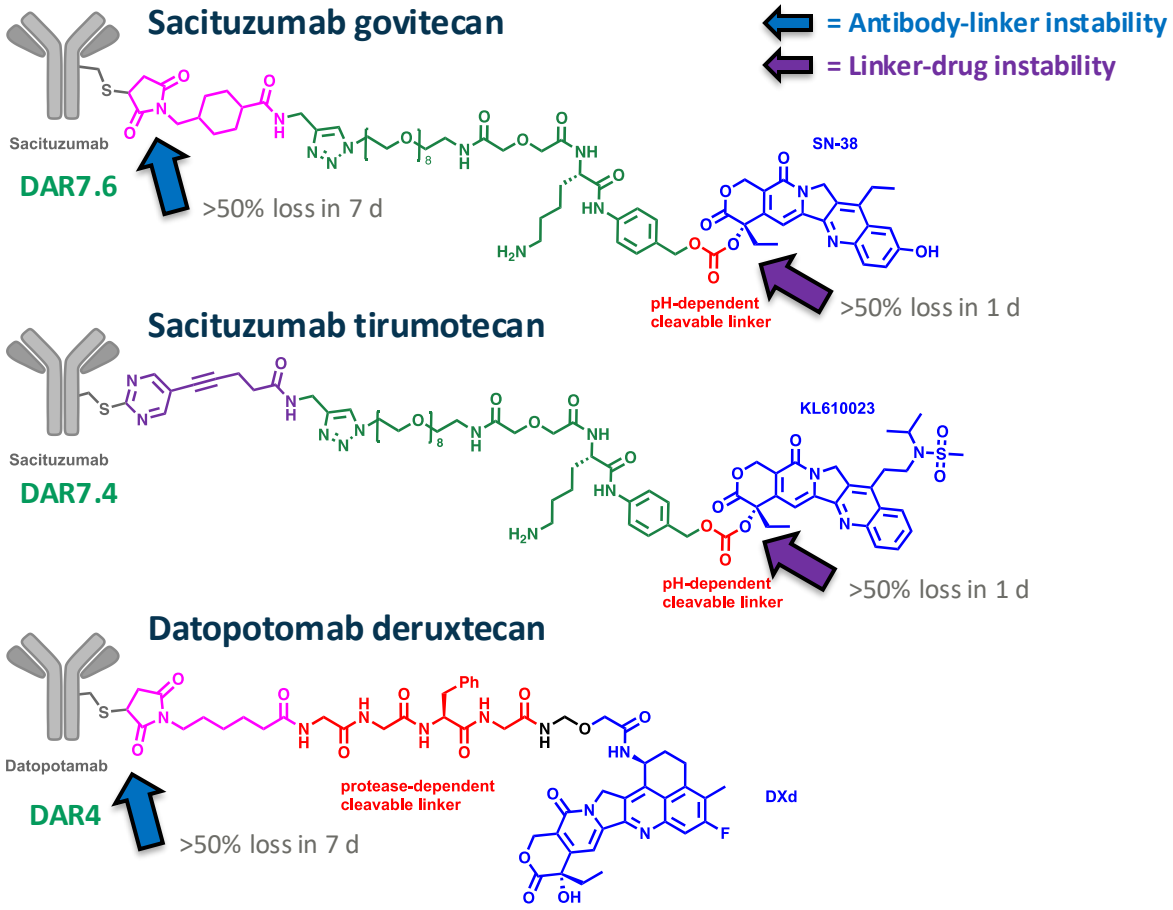
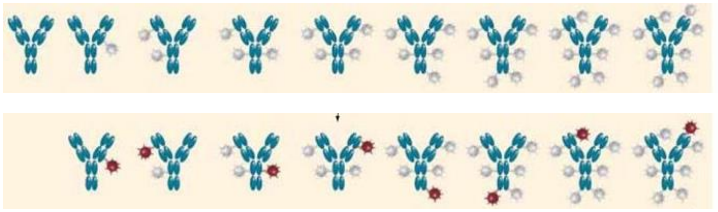
Comparisons for TROP2 ADCs are complicated by different linker stabilities





	Total antibody half-life	Total ADC half-life
Sacituzumab govitecan (SG)	6 days	0.7 days
Sacituzumab tirumotecan (sac-TMT)	Not reported	1.5 days
Datopotomab deruxtecan (Dato-DXd)	5.3 days	4.9 days

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761115Orig1s000 Multidiscipline Review; J. Rodon et al. *Ann. Oncol.*, **2021**, *32*, S585; A. Bardia et al. *J. Clin. Oncol.* **2024**, *42*, 2281

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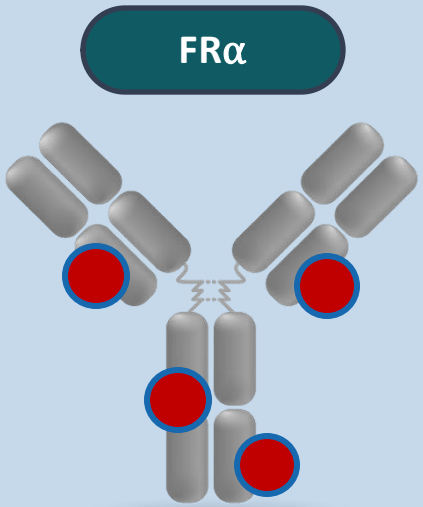
Mirvetuximab soravtansine didn't show a statistically significant improvement in OS using FRα "10X Scoring"

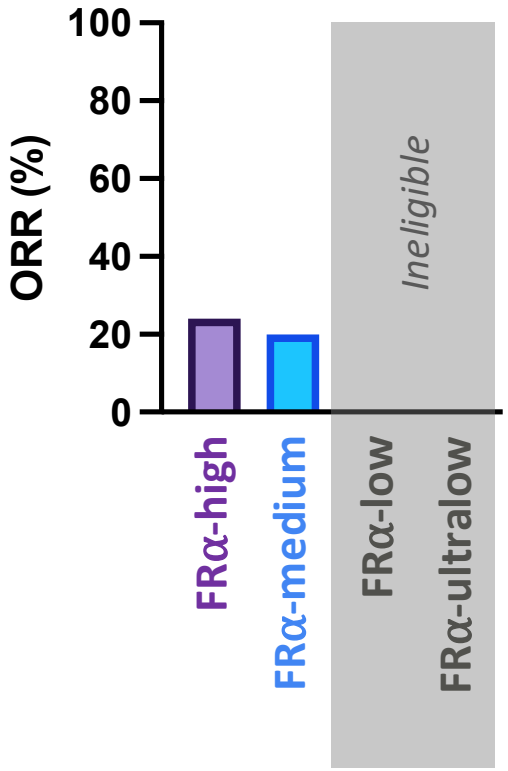


DM4 (maytansinoid)
Cleavable linker (disulfide)

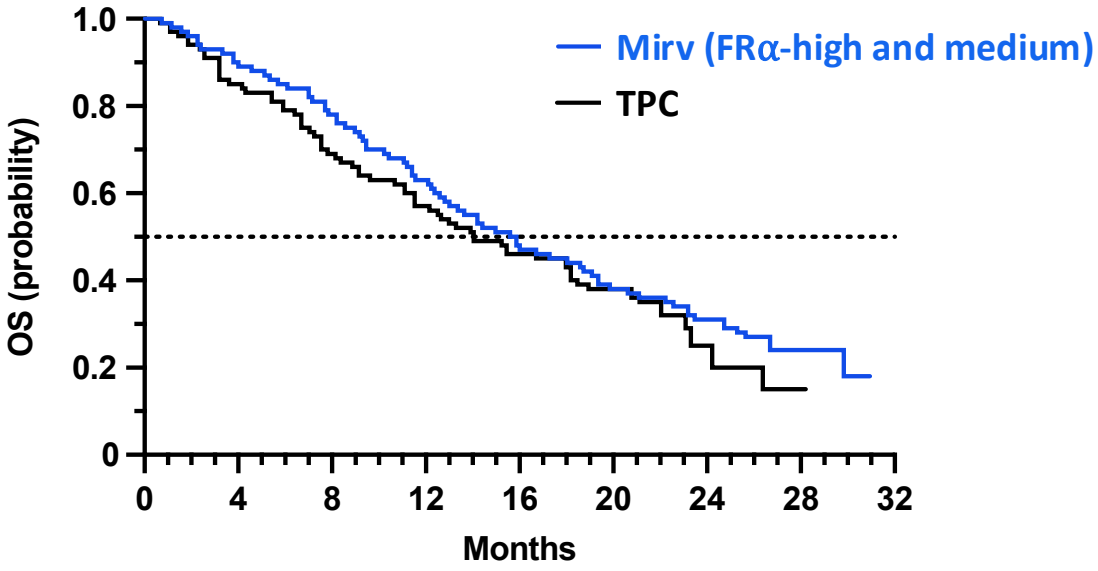
Mirvetuximab soravtansine (Mirv)

10X scoring

FORWARD I
(*N* = 248)



Ovarian Cancer
N = 366



10X Scoring: percentage of tumor cells with FRα membrane staining visible at 10X microscope objective

FRα-high = 10X scoring ≥75%

FRα-medium = 10X scoring ≥50% and <75%

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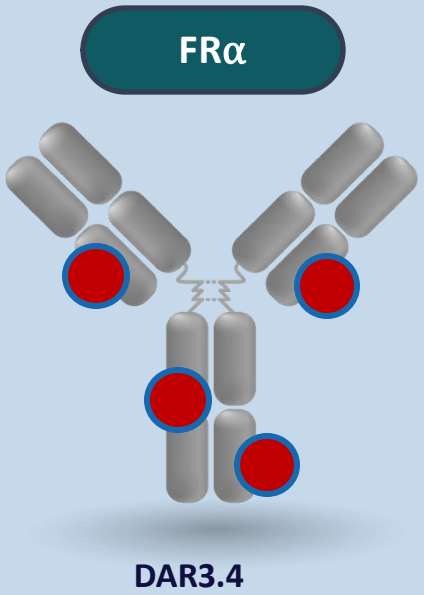
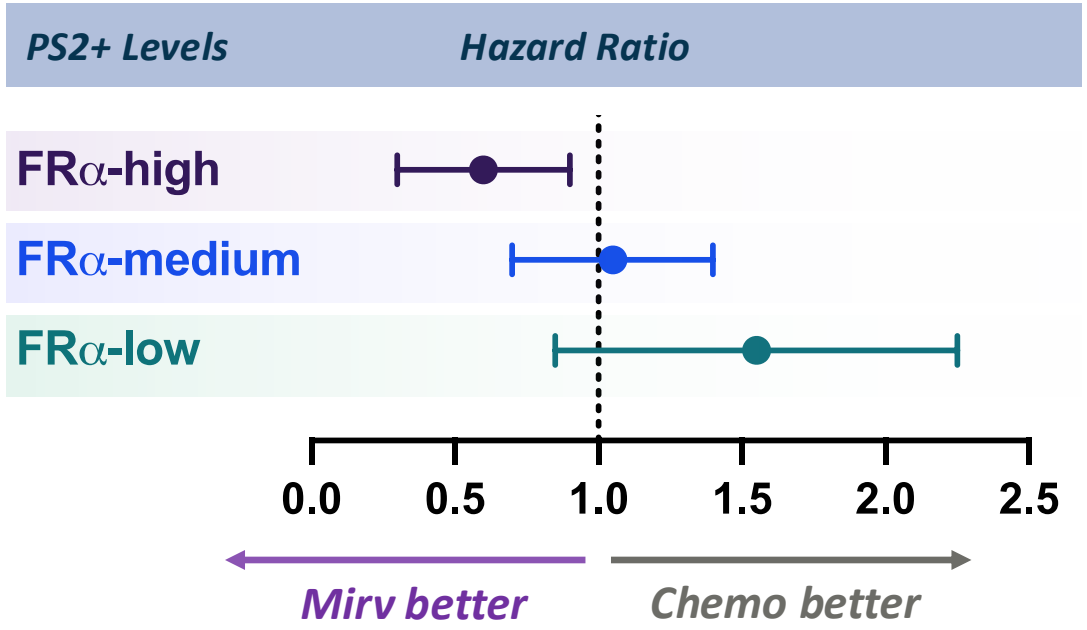
K. N. Moore et al. *Ann. Oncol.* **2021**, *32*, 757-765. TPC = Treatment of physician's choice

Mirvetuximab soravtansine showed better PFS in patients with FRα-high (using PS2+ Scoring)



Mirvetuximab soravtansine (Mirv)

FORWARD I (*N* = 248)
PFS Hazard Ratio Plot



PS2+ scoring: percentage of viable tumor cells with moderate [2+] or strong [3+] staining intensity:

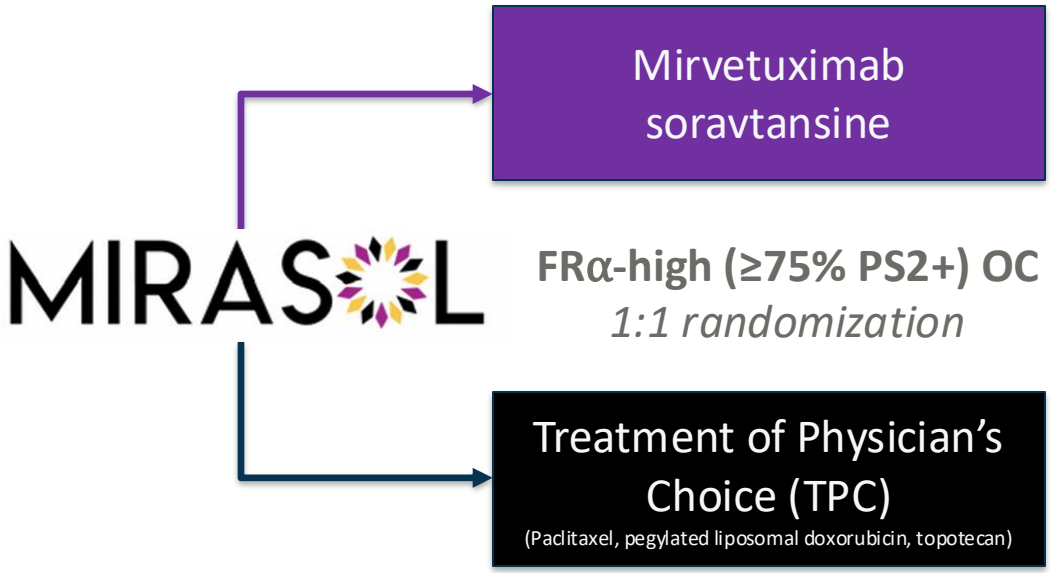
FRα-high = PS2+ ≥75%

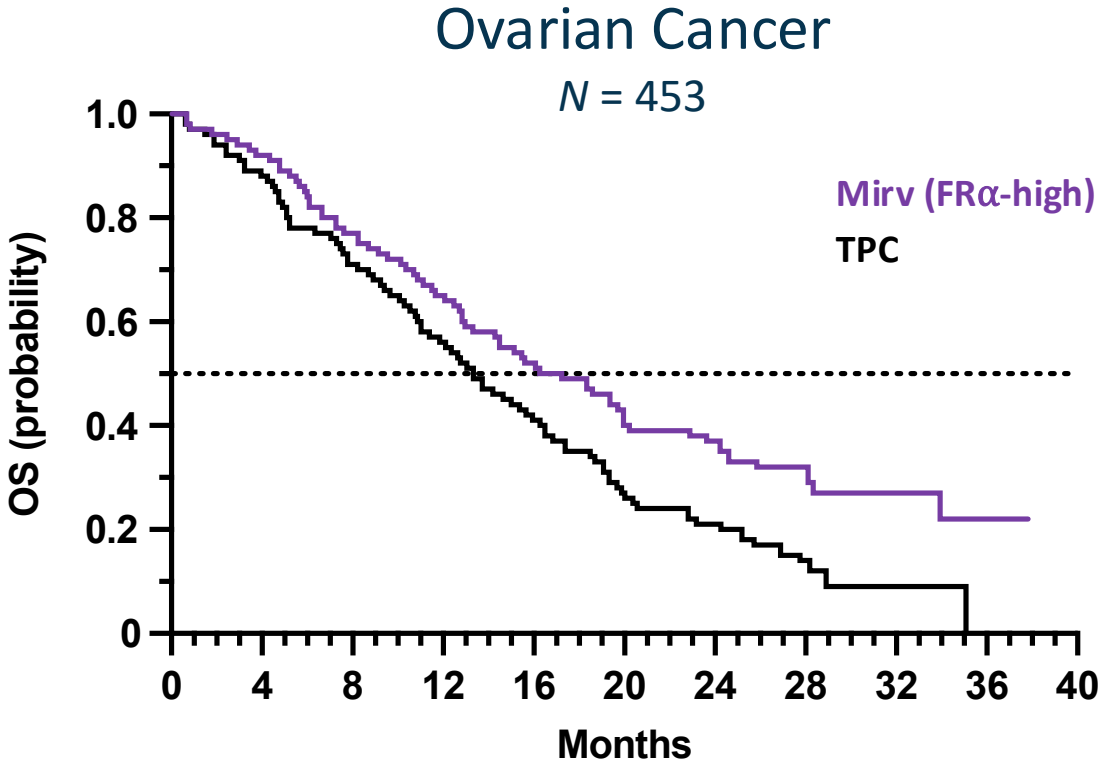
FRα-medium = PS2+ ≥50% and <75%

FRα-low = PS2+ ≥25% and <50%

K. N. Moore et al. *Ann. Oncol.* **2021**, *32*, 757-765

Mirvetuximab soravtansine showed better OS than TPC in patients with FRα-high (≥75% PS2+) ovarian cancer

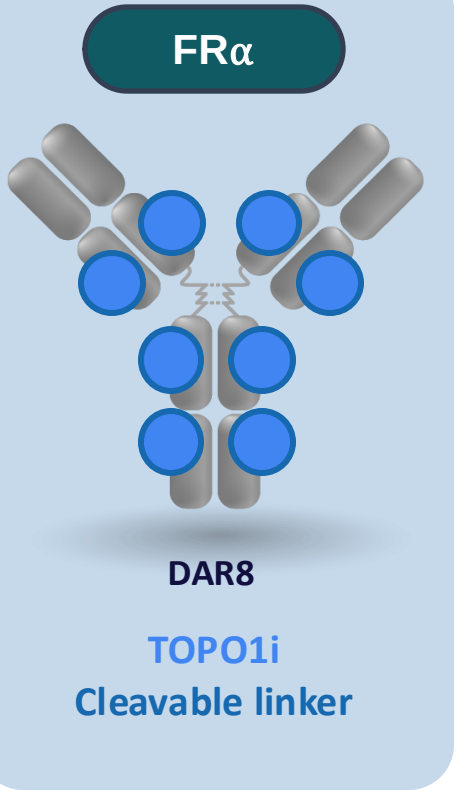




….which led to the approval of Mirv for patients with "FRα-high" (PS2+ ≥75%) ovarian cancer

K. N. Moore et al. *N. Engl. J. Med.* **2023**, *389*, 2162-2174; Updated OS from L. G. Coffman et al. 746P, presented at **ESMO24**

Emerging data for novel TOPO1i ADCs suggest responses across all FRα expressions



BAT8006: "Preliminary efficacy in all PROC patients regardless of FRα expression"

PRO1184: "Responses in patients with OC were observed regardless of FRα expression levels"

AZD5335: "Objective responses observed in patients with FRα-high and FRα-low"

data are immature and no PFS/OS reported

BAT8006: H Jia et al. Presented at **ASCO24**
PRO1184: E. K. Lee et al. Presented at **ESMO24**
AZD5335: R. Shapira-Frommer et al. Presented at **ESMO24**

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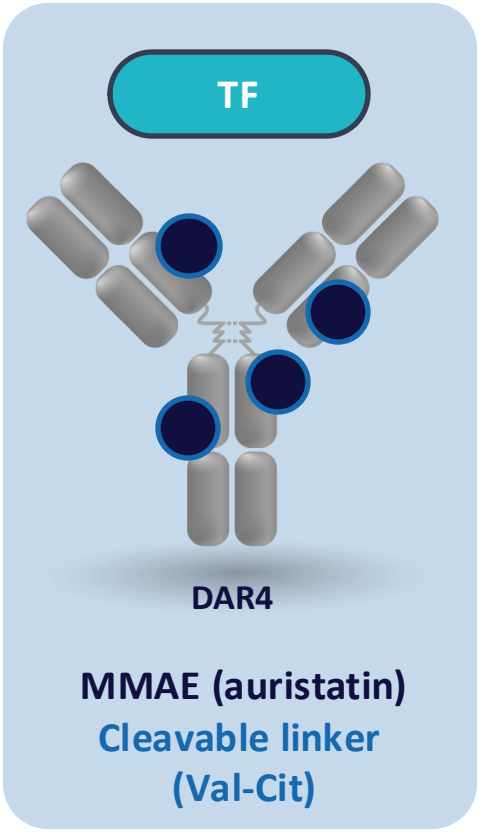
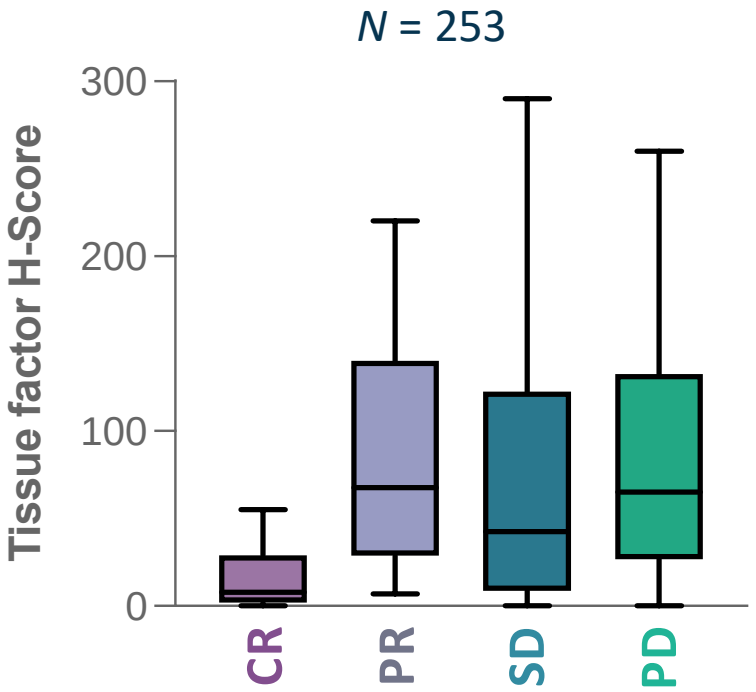
Tisotumab vedotin showed no association between TF expression and tumor response in cervical cancer



Tisotumab vedotin (TV)

InnovaTV 301 (cervical cancer)



CR = complete response; **PR** = partial response; **SD** = stable disease; **PD** = progressive disease.

MULTI-DISCIPLINE REVIEW 761208Orig1s000

*"Response to tisotumab vedotin was observed **irrespective of the level of membrane tissue factor expression***

Based on the data available, a companion diagnostic is not needed to select patients"

I. Vergote et al. *N. Engl. J. Med.* **2024**, *391*, 44-55. Similar trends observed in InnovaTV 201 and 204 trials, see: J. S. de Bono et al. *Lancet Oncol.* **2019**, *20*, 383-393; R. L. Coleman et al. *Lancet Oncol.* **2021**, *22*, 609-619;

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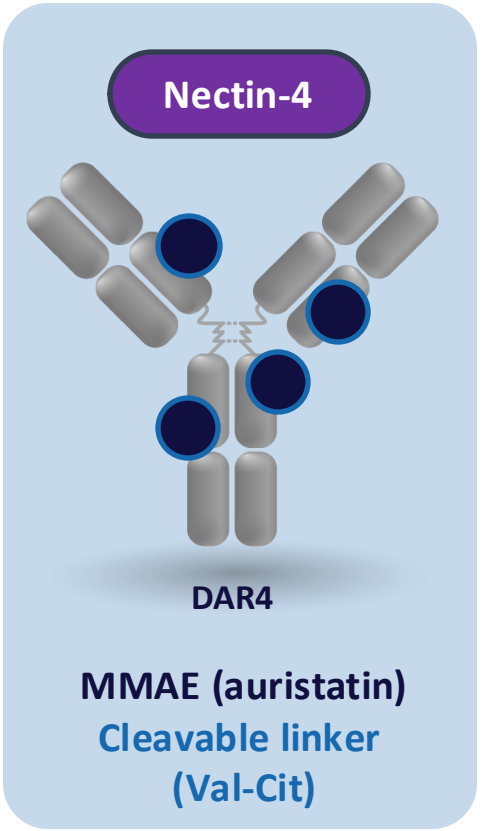
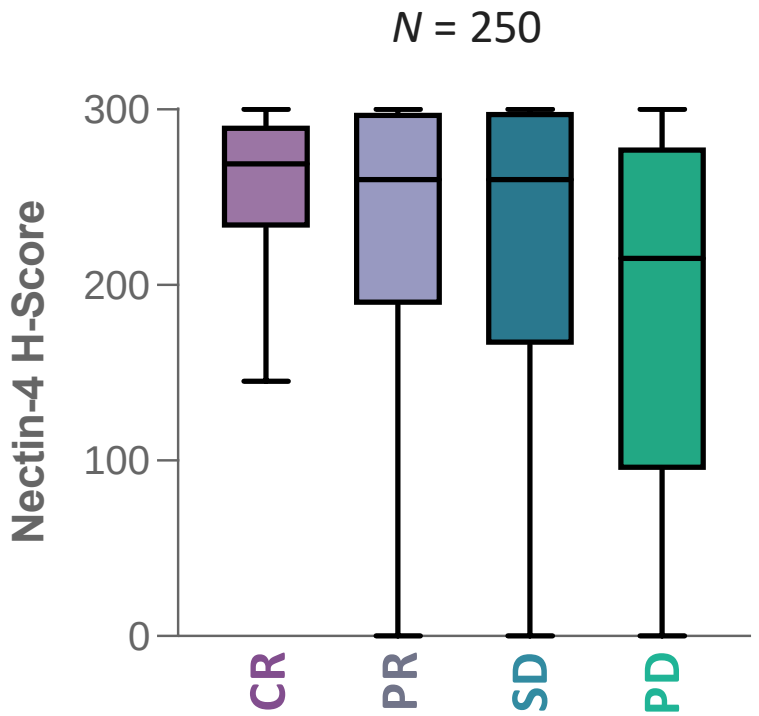
Enfortumab vedotin showed responses across all nectin-4 expression levels in urothelial carcinoma



Enfortumab vedotin (EV)

EV-301 (urothelial carcinoma)



CR = complete response; **PR** = partial response; **SD** = stable disease; **PD** = progressive disease.

MULTI-DISCIPLINE REVIEW
761137Orig1s000

*"There is likely **no lower H-score cutoff for nectin-4 expression** below which patients would not be expected to benefit from treatment with enfortumab vedotin"*

Patient selection for treatment with enfortumab vedotin based on Nectin-4 expression levels is not warranted."

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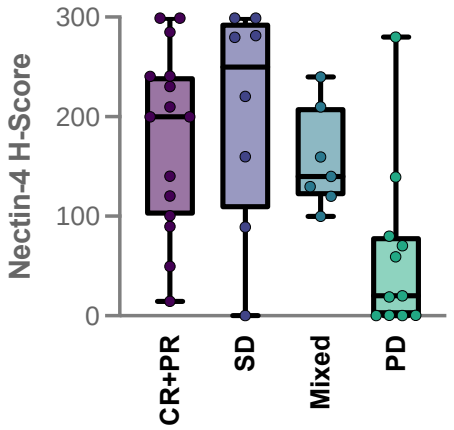
T. Powles et al. *N. Engl. J. Med.* **2021**, *384*, 1125-1135

Better efficacy in patients with nectin-4 high or amplified treated with enfortumab vedotin (retrospective study)

A different nectin-4 antibody was used for IHC, highlighting well-known IHC challenges, including sensitivity, specificity, and dynamic range

Responses based on nectin-4 H-Score

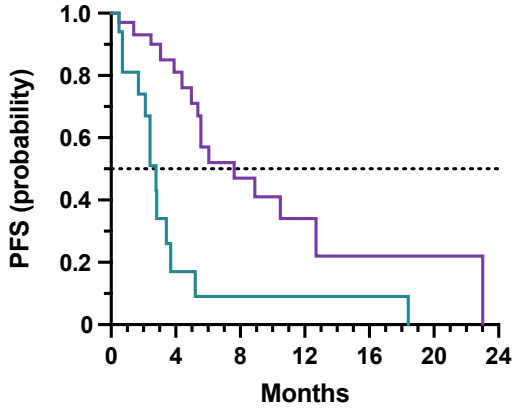
N = 47



CR = complete response; **PR** = partial response; **SD** = stable disease; **Mixed** = mixed responses; **PD** = progressive disease

Nectin-4 **H-score ≥100** *vs* **H-score <100**

N = 47



"The assumption of widespread nectin-4 expression in urothelial carcinoma requires re-evaluation"

N. Klümper et al. *Clin. Cancer Res.* **2023**, *29*, 1496–1505; N Klümper et al. *J. Clin. Oncol.* **2024**, *42*, 2446–2455.

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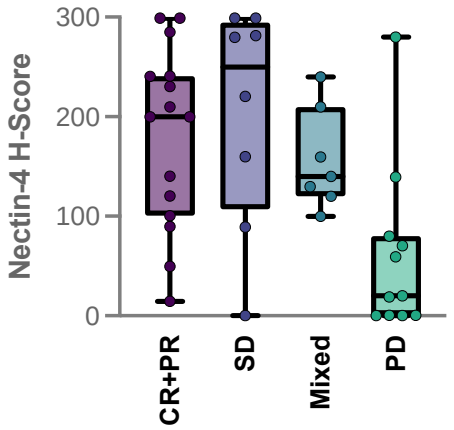
Responses based on nectin-4 H-Score

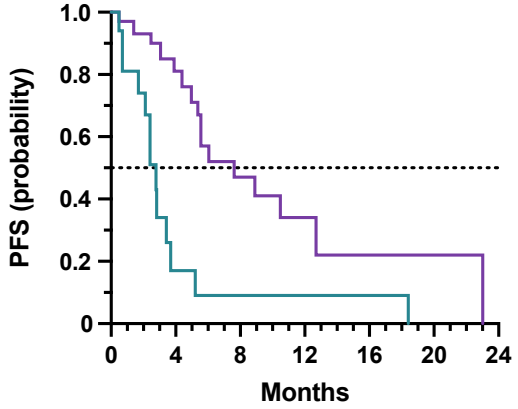
N = 47



CR = complete response; **PR** = partial response; **SD** = stable disease; **Mixed** = mixed responses; **PD** = progressive disease

Nectin-4 **H-score ≥100** *vs* **H-score <100**

N = 47

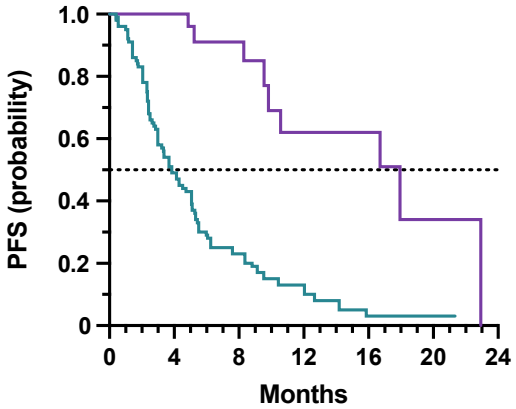


"The assumption of widespread nectin-4 expression in urothelial carcinoma requires re-evaluation"

Nectin-4 amplification was determined with a newly developed fluorescence in situ hybridization (FISH) assay.

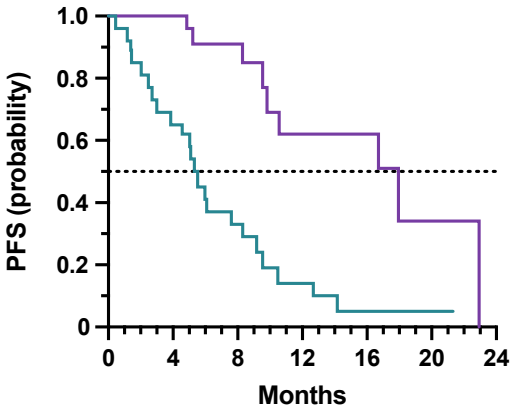
Nectin-4 **amplified** vs **non-amplified**

N = 108



Nectin-4 **amplified** *vs* **non-amplified with H-score ≥200**

N = 54



Similar trends observed for OS (not shown)

"Nectin-4 amplification could be a predictive biomarker for EV in mUC and other tumors"

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N. Klümper et al. *Clin. Cancer Res.* **2023**, *29*, 1496–1505; N Klümper et al. *J. Clin. Oncol.* **2024**, *42*, 2446–2455.

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Does target expression correlate with responses in solid tumors for approved ADCs?

	Payload	Bystander active	Target	Target/response correlation
Trastuzumab emtansine	DM1	No*	HER2	**Better responses in HER2-high**
Trastuzumab deruxtecan	DXd	Yes	HER2	**Better responses in HER2-high, but responses observed across all HER2-expression**
Mirvetuximab soravtansine	DM4	Yes#	FRα	**Better PFS and OS in FRα-high (≥75% PS2+)**
Sacituzumab govitecan	SN-38	Yes	TROP2	**No clear relationship**
Enfortumab vedotin	MMAE	Yes	Nectin-4	**Responses observed across all nectin-4 levels** **Emerging data suggest correlation**
Tisotumab vedotin	MMAE	Yes	TF	**No correlation**

With over 200 ADCs currently in clinical development, the relationship between target expression and treatment efficacy will likely be better understood in the near future!

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* For T-DM1, the major active metabolite generated from ADC catabolism is not cell permeable
\# Not all the metabolites of DM4 are cell permeable

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Does target expression correlate with responses in hematological malignancies?

	Payload	Bystander active	Target	Target/response correlation
Brentuximab vedotin	MMAE	Yes	CD30	**No relationship observed in several types of non-Hodgkin lymphomas**
Polatuzumab vedotin	MMAE	Yes	CD79b	**Responses observed across all target levels in DLBCL**
Loncastuximab tesirine	PBD	Yes	CD19	**Responses observed across all target levels in DLBCL**
Inotuzumab ozogamicin	Calicheamicin	Yes	CD22	**No statistically significant relationship in ALL**
Gemtuzumab ozogamicin	Calicheamicin	Yes	CD33	**Contradictory results in AML**
Belantamab mafodotin*	MMAF	No	BCMA	**No relationship observed in MM**

*Belantamab mafodotin has been withdrawn from the market but is currently under review by several regulatory authorities for its potential use in combination therapies

Gemtuzumab ozogamicin responses **inversely correlate** with **P-gp expression!**

BV: D. Jagadeesh et al. *The Oncologist*, **2022**, *27*, 864; **PV:** L. H. Sehn et al. *J. Clin. Oncol.* **2020**, *38*, 155; **Lonca-T:** M. Hamadani et al. *Blood* **2021**, *137*, 2634; **Blenrep:** https://www.ema.europa.eu/en/documents/assessment-report/blenrep-epar-public-assessment-report_en.pdf; **IO:** E. Pennesi et al. *Leukemia*. **2022**, *36*, 1516; **GO:** M. Molica et al. *Cancers* **2021**, *13*, 3214. DLBCL = Diffuse large B cell lymphoma; ALL = Acute lymphoblastic leukemia; AML = Acute myeloid leukemia; MM = multiple myeloma.

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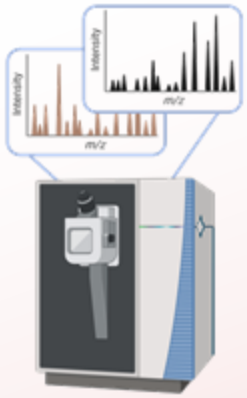
Where do we go next? Target(s) identification, quantification, and functionality

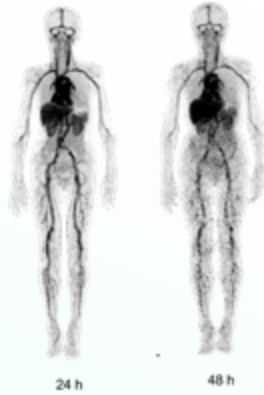


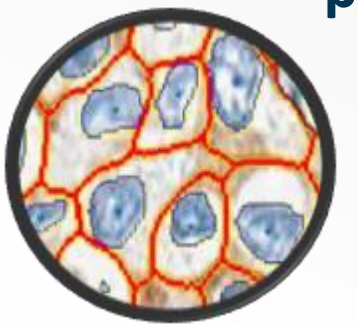
Proteomics

- Mass spectrometry (MS)
- Reverse Phase Protein Array (RPPA)

Companion diagnostic imaging tools

- Radioconjugates (*e.g.*, radiolabeled antibody)



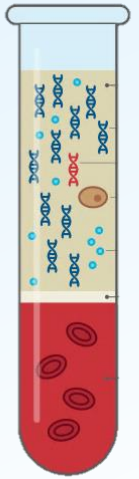


Digital and computational pathology

- Quantitative Continue Scoring (QCS)
- Normalized Membrane Ratio (NMR)
- Proximity models

Liquid biopsy

- *Cell-free DNA (cfDNA)*
- *Circulating tumor DNA (ctDNA)*
- *Circulating tumor cells (CTCs)*
- *Epigenomic signatures*



…among others!

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Key takeaways and final thoughts

1) ADC **target-mediated delivery is not the only way** for an ADC or its payload to get into a cell
2) Pharmacology of ADCs is more **complex with bystander** active payloads

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1) ADC **target-mediated delivery is not the only way** for an ADC or its payload to get into a cell
2) Pharmacology of ADCs is more **complex with bystander** active payloads
3) For many ADCs, **benefits are observed across all levels of target expression**
4) Patients with no target expression tended to be **excluded** from trials, based on the **classic view of ADCs**
5) Patients with low or no target expression may benefit from a certain ADC, but they might **benefit more** from another ADC with a more **optimal target or payload**

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Key takeaways and final thoughts

1) ADC **target-mediated delivery is not the only way** for an ADC or its payload to get into a cell
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5) Patients with low or no target expression may benefit from a certain ADC, but they might **benefit more** from another ADC with a more **optimal target or payload**
6) Biomarkers for **payload sensitivity or resistance** are likely important but lag far behind
7) We have **limited biomarkers for toxicities** (e.g. UGT1A1)
8) Many biomarker/expression **analyses are not standardized** across institutions/companies and are often retrospective

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Acknowledgments

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Zymeworks

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Jamie Rich
Senior Director, Technology

Stuart Barnscher
Senior Director, Preclinical Programs

Steve Seredick
Director, Portfolio Strategy

Paul Moore
CSO

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