



Journal of Advanced Pharmaceutical Sciences and Natural Products (JAPSNP)

ANTIBODY-DRUG CONJUGATES IN SOLID TUMOURS: LINKER AND PAYLOAD ENGINEERING, BYSTANDER EFFECT, RESISTANCE MECHANISMS AND MANAGEMENT OF INTERSTITIAL LUNG DISEASE

Dr. Shivaraj D R^{*1}

Ms. Kusuma D S²

Ms. Yashaswini Raj²

¹Assistant Professor, Department of Pharmacy Practice, Akshaya Institute of Pharmacy, Tumkur 572106, Karnataka, India. Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru.

²Third year Pharm D, Department of Pharmacy Practice, Akshaya Institute of Pharmacy, Tumkur 572106, Karnataka, India. Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru.

Corresponding author: Dr. Shivaraj D R, Assistant Professor, Department of Pharmacy Practice, Akshaya Institute of Pharmacy, Tumkur 572106, Karnataka, India.

Email: Shivaraj.dr27@gmail.com.

ORCID: 0000-0002-9284-4360

ABSTRACT

Antibody-drug conjugates have moved from a niche technology to a standard of care across breast, gastric, urothelial, lung and ovarian cancer, yet the three agents used most widely in solid tumours embody incompatible design philosophies. Trastuzumab emtansine maximises linker stability and carries about 3.5 drugs per antibody; trastuzumab deruxtecan carries roughly eight on a tetrapeptide that loses 2.1% of payload over 21 days in plasma; sacituzumab govitecan carries 7.6 on a bond deliberately built to hydrolyse. This review argues that one physical property, whether the released payload can cross a

membrane and leave the cell that captured it, organises four literatures that are usually treated separately. The charge state of the released catabolite separates conjugates that kill antigen-negative neighbours from those that do not, and that distinction underwrites the extension of trastuzumab deruxtecan to HER2-low and HER2-ultralow breast cancer, where hazard ratios for progression of 0.50 and 0.63 were obtained without any change to the antibody. Drug-to-antibody ratio is shown not to be independently limiting once conjugate hydrophobicity is controlled. Resistance is moving away from the antigen and towards the payload: HER2 is lost or reduced in roughly two-thirds of tumours rebiopsied after trastuzumab deruxtecan, but efflux transporter upregulation, SLC46A3 loss, TOP1 mutation and SLFN11 loss now account for resistance in models where antigen expression is intact, which argues for switching payload class rather than target at progression. Interstitial lung disease is examined as the clinical expression of the same permeability. Adjudicated drug-related pneumonitis affected 15.4% of 1150 patients across nine trastuzumab deruxtecan studies, with 2.2% fatal and a median onset of 5.4 months, and the available mechanistic evidence points to target-independent uptake by alveolar macrophages rather than on-target HER2 binding. The consensus management algorithm is set out in full, together with the finding that no dataset yet demonstrates that its corticosteroid regimens alter outcome.

Keywords: Immunoconjugates; Antineoplastic Agents; Immunological; Drug Resistance; Neoplasm; Lung Diseases; Interstitial; Receptor; ErbB-2; Trastuzumab; Camptothecin.



1. Introduction

Three antibody-drug conjugates in routine use against solid tumours share an architecture and almost nothing else. Trastuzumab emtansine carries about 3.5 maytansinoid molecules per antibody through a non-reducible thioether linker that releases nothing until the antibody itself is degraded ¹. Trastuzumab deruxtecan carries roughly eight copies of a topoisomerase I inhibitor on a tetrapeptide linker so stable that only 2.1% of payload is lost after 21 days in human plasma ². Sacituzumab govitecan carries 7.6 copies of SN-38 on a carbonate bond chosen to hydrolyse, with an intact-conjugate mean residence time of 15.4 hours against roughly 300 hours for the carrier antibody ³. One design maximises stability, one maximises loading, one deliberately leaks.

These are not variations on a theme. They are opposing answers to the same question, and the answer chosen determines which tumours respond, how the tumour escapes, and which organ is injured.

The antibody receives most of the attention in discussions of ADC design, and it is the least discriminating component. Trastuzumab is the targeting moiety of both emtansine and deruxtecan conjugates, yet the two behave as different drugs: in DESTINY-Breast03 the deruxtecan conjugate extended median progression-free survival to 28.8 months against 6.8 months for emtansine, a hazard ratio of 0.33 ⁴. Nothing about the antigen changed. The linker and the payload changed.

This review argues that a single physical property organises four otherwise separate literatures. That property is whether the released payload can leave the cell it was delivered to. A payload that diffuses out produces the bystander effect that makes low-antigen tumours treatable; it shifts resistance away from the antigen and towards the payload and its efflux pumps; and it plausibly contributes to injury in tissues the antibody was never aimed at. Interstitial lung disease, which has become the defining toxicity of the deruxtecan class, is examined here at the length its clinical consequences demand, including the management algorithm now in use and the evidence that algorithm does and does not rest on.

2. Linker and payload engineering

2.1 The linker is the control point

A linker has to survive days in plasma and then release its cargo within minutes in a lysosome, and the chemistries that achieve this fall into two families whose consequences extend far beyond release kinetics.

Dipeptide linkers came first. Valine-citrulline and phenylalanine-lysine sequences flanked by a self-immolative spacer are hydrolysed by lysosomal cysteine proteases, and Dubowchik and colleagues showed that the phenylalanine-lysine version released doxorubicin some 30-fold faster than the valine-citrulline analogue in the presence of cathepsin B while both remained intact in human plasma ⁵. Replacing acid-labile hydrazones with these dipeptides raised



therapeutic indices as much as 60-fold in auristatin conjugates ⁶. The naming has outlived the biology, though. Deleting cathepsin B outright leaves the efficacy of valine-citrulline MMAE conjugates unchanged, because other cysteine cathepsins cleave the same bond, which also means that loss of a single protease is an improbable route to clinical resistance ⁷.

Stability then became the currency. The maleimidocaproyl-GGFG tetrapeptide of deruxtecan releases 2.1% of its payload over 21 days in human plasma; ado-trastuzumab emtansine releases 18.4% in four days ². That difference is what permits a drug-to-antibody ratio of eight without the systemic toxicity such loading would otherwise carry.

Maximal stability is nonetheless the wrong target, a point the disulfide series makes cleanly. Kellogg and colleagues varied steric hindrance at the carbons flanking the disulfide bond across a maytansinoid conjugate series and found that the construct with intermediate, not maximal, stability gave the best in vivo activity ⁸. Sacituzumab govitecan takes the same lesson further and pairs a moderately toxic payload with a bond designed to hydrolyse, yet at least 95% of serum SN-38 remains antibody-bound at any interval, and tumour delivery exceeds that achieved by irinotecan by a wide margin ³. Beta-glucuronide linkers occupy the opposite extreme, with an extrapolated plasma half-life of 81 days in the rat ⁹.

Non-cleavable linkers release nothing at all until the antibody is proteolysed, and what emerges is a fragment of the antibody still attached to the

drug. For the thioether-linked emtansine conjugate that fragment is lysine-N-epsilon-MCC-DM1, a charged species with an IC₅₀ of 0.1 micromolar. The corresponding catabolite of a disulfide-linked DM4 conjugate, S-methyl-DM4, is neutral and has an IC₅₀ of 2 picomolar, roughly fifty thousand times more potent, and unlike the charged adduct it crosses membranes ¹⁰. Lysosomal degradation is required either way; the linker decides only what the lysosome hands back.

That single contrast, drawn in Figure 1, is the origin of most of what follows in this review.

2.2 Payload classes and the potency illusion

Four payload chemistries carry almost all clinical ADCs. Microtubule inhibitors comprise the auristatins and the maytansinoids. Topoisomerase I inhibitors comprise SN-38 and the exatecan derivative deruxtecan, which is about ten times more potent than SN-38 in that assay ². DNA-damaging agents comprise the enediyne calicheamicin ¹¹ and the pyrrolobenzodiazepine dimers, of which SG3199 has a mean GI₅₀ of 151.5 picomolar and forms interstrand cross-links that persist beyond 36 hours, with a plasma half-life as short as eight minutes when given free ¹². Amanitins, which inhibit RNA polymerase II, represent the main attempt to add a genuinely new mechanism ¹³.

Potency is the variable most often assumed to drive success, and the clinical record contradicts the assumption. SN-38 is an unremarkable cytotoxin roughly a thousand-fold less potent



than a pyrrolobenzodiazepine dimer, and sacituzumab govitecan is among the most successful ADCs in triple-negative breast cancer¹⁴. Pyrrolobenzodiazepine conjugates, meanwhile, have repeatedly failed on tolerability. What distinguishes the successful payloads is a combination of moderate potency, membrane permeability and a therapeutic window wide enough to survive the fraction of dose that inevitably reaches normal tissue.

2.3 Drug-to-antibody ratio and the conjugation problem

Hamblett and colleagues purified an anti-CD30 auristatin conjugate into discrete species bearing two, four and eight drugs and produced the result that shaped a decade of design. Potency in vitro ranked E8 above E4 above E2. In vivo, at equal antibody dose, E4 matched E8 despite carrying half the drug. Clearance rose steeply with loading, the eight-drug species clearing three times faster than the four-drug and five times faster than the two-drug conjugate, and maximum tolerated dose moved the other way, so that reducing loading raised the therapeutic index¹⁵.

The field read this as a ceiling on DAR. It was a ceiling on hydrophobicity. Lyon and colleagues showed that the accelerated clearance tracks the hydrophobic character that conjugation confers rather than the drug count itself, and that hydrophilic drug-linkers restore the pharmacokinetics of homogeneous high-load conjugates¹⁶. Once that constraint was separated from DAR, loading of eight became achievable

by two different routes: deruxtecan pairs a hydrophilic, hyperstable tetrapeptide with a moderately potent payload², while sacituzumab govitecan pairs a hydrolysable bond with a payload mild enough that early release is tolerable³.

Heterogeneity remains the unfinished problem. Conventional conjugation to interchain cysteines yields a mixture spanning zero to eight drugs per antibody, and the species within that mixture differ in clearance and tolerability. Engineered-cysteine THIOMAB conjugates carry a defined load and were tolerated at higher doses in rats and cynomolgus monkeys while matching conventional conjugate efficacy¹⁷. Chemoenzymatic remodelling of the conserved Asn-297 glycan achieves homogeneity on native antibodies of any IgG isotype without protein engineering¹⁸. Neither approach yet dominates the approved agents, which remain heterogeneous mixtures.

3. The bystander effect

Released payload that crosses membranes kills the cell it was delivered to and then kills its neighbours, whether or not those neighbours express the antigen. The experiments that established this are specific and worth stating precisely, because the term is now applied loosely.

Ogitani and colleagues co-cultured HER2-positive KPL-4 cells with HER2-negative MDA-MB-468 cells and showed that the deruxtecan conjugate killed both populations while ado-trastuzumab emtansine and a deliberately low-



permeability control conjugate killed only the antigen-positive cells. The same separation held in mice bearing a mixed inoculum of HER2-positive and HER2-negative cells¹⁹. Kovtun and colleagues had earlier drawn the same line by linker chemistry, showing that disulfide-linked maytansinoid conjugates killed antigen-negative cells in co-culture while thioether-linked DM1 conjugates did not²⁰, and the catabolite chemistry explains why¹⁰.

One control in the Ogitani study deserves more attention than it usually receives. When the antigen-negative tumour was implanted on the opposite flank rather than mixed with the antigen-positive cells, the deruxtecan conjugate had no effect on it¹⁹. Bystander killing is a local phenomenon over a diffusion distance, not a systemic release of free drug. Any claim that the bystander effect explains a distant toxicity has to account for that result.

Internalisation may not even be a precondition. Proteases released by tumour cells and tumour-associated macrophages can cleave susceptible linkers extracellularly, which would allow payload liberation without antigen-mediated uptake²¹.

Why this matters in solid tumours specifically comes down to delivery. Antibody distribution within a tumour is heterogeneous and leaves large regions untargeted, a consequence of transport opposed by systemic and antigen-mediated clearance²², with the pattern set by dose, affinity, antigen copy number and molecular size²³. A payload confined to the cell that internalised the conjugate can only kill the

fraction of cells the antibody reached. A diffusible payload converts partial delivery into wider killing.

The clinical consequence is the HER2-low indication. DESTINY-Breast04 randomised 557 patients whose tumours scored 1+ by immunohistochemistry, or 2+ with negative in situ hybridisation, and reported median progression-free survival of 9.9 against 5.1 months and median overall survival of 23.4 against 16.8 months, hazard ratios of 0.50 and 0.64²⁴. DESTINY-Breast06 extended benefit to tumours scored 0 with faint membrane staining, with a hazard ratio for progression of 0.63 across 866 patients²⁵. A category that was previously HER2-negative became a treatable population without any change to the antibody.

The account is incomplete, and the DAISY trial shows where. Response rates fell with HER2 expression as expected, from 70.6% in overexpressing tumours to 37.5% in HER2-low disease, but reached 29.7% in tumours with no detectable HER2 at all²⁶. Antigen density predicts benefit only loosely, and a mechanism other than antigen-directed delivery is operating in the lowest stratum. Sacituzumab govitecan makes a related point from the other direction: ASCENT and TROPiCS-02 both enrolled without any TROP2 expression threshold and both were positive, with hazard ratios for survival of 0.51 and 0.79^{14,27}. Where a threshold is still applied, as for folate receptor alpha in platinum-resistant ovarian cancer, it is applied stringently, requiring at least 75% of cells staining at 2+ or above²⁸.



The property has a cost that is discussed far less than the benefit. A payload engineered to leave the cell it entered will also leave cells in normal tissue that took up conjugate by any route. Section 5 examines the clearest instance.

4. Mechanisms of resistance

Resistance can arise at any step between antigen binding and cell death, and the step at which it arises determines what should be given next. Figure 2 maps the documented mechanisms onto that cascade.

Antigen loss is the most clinically consequential and the best documented. Among 41 patients rebiopsied after trastuzumab deruxtecan, 34 had measurable HER2 at baseline, and of those 11 had lost HER2 entirely while a further 10 had a reduced score, so roughly two-thirds showed some antigen decline²⁹. A paired-biopsy series reported major decreases in 49% of cases, complete loss in just over half of those, together with *ERBB2* mutations at the trastuzumab-binding interface³⁰. The equivalent has been seen for TROP2, where a *TACSTD2* T256R mutation emerged in a patient progressing on sacituzumab govitecan³¹. Rebiopsy before the next HER2-directed line is therefore not an academic exercise.

Trafficking failures divert the conjugate without touching the antigen. Emtansine-resistant gastric cells route the conjugate into caveolin-1-positive vesicles rather than lysosomes, and an auristatin payload on an enzymatically cleavable linker overcomes that resistance³².

Lysosomal failures are more varied. Resistant HER2-positive clones with entirely normal HER2 expression accumulate conjugate in lysosomes that have raised pH and reduced proteolytic capacity³³. A separate and more specific mechanism affects non-cleavable linkers alone: the lysosomal transporter SLC46A3 is required to export maytansinoid and pyrrolobenzodiazepine catabolites into the cytosol, and its loss confers both innate and acquired resistance, with sensitivity restored when the transporter is re-introduced^{34,35}. Acquired emtansine resistance in one breast line combined SLC46A3 loss with PTEN deficiency³⁶.

Payload-level resistance is where the field is now converging. A systematic screen of 27 ADC payloads identified MMAE, DM1, DM4 and calicheamicin as P-glycoprotein substrates, with every pyrrolobenzodiazepine tested a substrate of P-glycoprotein, ABCG2 and MRP1³⁷. In deruxtecan-resistant gastric and lung models, HER2 expression was preserved while ABCG2 and ABCB1 were markedly upregulated, the cells were cross-resistant to free deruxtecan and to SN-38, and silencing the transporters restored sensitivity³⁸. Target-level and downstream mechanisms accompany this: a *TOP1* E418K mutation arose in a subclone of a patient progressing on sacituzumab govitecan, in parallel with the antigen mutation in a different subclone³¹, and deruxtecan resistance in HER2-mutant lung cancer was mediated by loss of SLFN11 with gain of ABCC1 while HER2 signalling and



tyrosine kinase inhibitor sensitivity were retained³⁹.

That convergence carries a practical implication. If resistance sits in the payload and its transporters rather than in the antigen, then switching to a second ADC against the same or a different antigen but carrying the same payload class should fail, while switching payload class should not. The clinical data are thin and retrospective. Among 85 patients given sacituzumab govitecan and trastuzumab deruxtecan in sequence, real-world progression-free survival was shorter on the second ADC in 75.2%, though a minority derived prolonged benefit, and PTEN loss was associated with de novo resistance to the deruxtecan conjugate but not to the govitecan conjugate⁴⁰. Sequencing remains reasonable; cross-resistance is real but incomplete; and no prospective trial has yet tested payload-class switching against target switching.

5. Interstitial lung disease

5.1 How common, how severe, and how soon

The reference dataset is an adjudicated pooled analysis of nine trastuzumab deruxtecan monotherapy studies covering 1150 patients. Independently adjudicated drug-related interstitial lung disease or pneumonitis occurred in 15.4%, with grade 1 in 4.2%, grade 2 in 7.7%, grade 3 in 1.2%, grade 4 in 0.1% and death in 2.2%, so that grades 1 and 2 accounted for just over three-quarters of affected patients⁴¹. Median time to onset was 5.4 months and 87.0%

of first events fell within the first year. Fatal events came earlier, at a median of 3.2 months⁴¹.

Incidence varies by tumour type more than by anything else measured: 20.6% in breast cancer, 15.3% in lung, 8.5% in gastric and 8.4% in colorectal disease⁴¹. An independent systematic review of 1193 patients found a lower overall figure of 11.40%, with the highest rates in uterine carcinosarcoma and non-small-cell lung cancer and a median time to recovery of 34 to 57 days⁴². Whether the difference between 15.4% and 11.4% reflects population or adjudication method is not resolved.

Rates in later trials are lower. DESTINY-Breast03 reported 10.5% with no grade 4 or 5 events, which the pooled analysis attributes to amended monitoring guidance alongside a less heavily pretreated population, an attribution that is plausible but uncontrolled⁴¹. DESTINY-Breast04 reported 12.1% including 0.8% grade 5²⁴ and DESTINY-Breast06 11.3%²⁵. Pooled label data separate the two dose levels: 12.9% adjudicated with 1.0% fatal at 5.4 mg/kg, against 15.4% with 1.6% fatal at 6.4 mg/kg⁴³.

The signal is not confined to one molecule. Datopotamab deruxtecan carries adjudicated drug-related ILD of 3.6% with 0.2% fatal in its pooled breast population⁴⁴, and patritumab deruxtecan reported a comparable order of magnitude⁴⁵. Enfortumab vedotin, an auristatin conjugate, causes pneumonitis in 3.3% as monotherapy with no fatal cases, rising to 9.4% with three deaths when combined with pembrolizumab⁴⁶. Pulmonary toxicity is therefore a property of several ADCs, most



pronounced in the deruxtecan class, and aggravated by checkpoint blockade.

permeability, but the gradient is also confounded by dose, schedule and indication.

5.2 What causes it

Only one non-clinical study carries most of the mechanistic weight, and it is informative precisely because its results are negative in the places that matter. Cynomolgus monkeys given trastuzumab deruxtecan developed interstitial pneumonitis at doses of 30 mg/kg and above, dose- and frequency-dependently, with diffuse lymphocytic infiltrates and mild fibrosis resembling human drug-induced lung injury ⁴⁷. Animals given exatecan alone developed no pulmonary toxicity despite higher systemic payload exposure, which excludes a simple free-payload effect. HER2 expression in monkey lung was confined to bronchial epithelium while the lesions were alveolar, a spatial mismatch that argues against a purely on-target mechanism. Immunohistochemistry localised the conjugate predominantly to alveolar macrophages ⁴⁷.

The best-supported account is therefore target-independent uptake of intact conjugate by alveolar macrophages, followed by local payload release in alveolar tissue. Whether the membrane-permeable payload then injures neighbouring pneumocytes by the same diffusion that produces bystander killing in tumours is a reasonable inference and is not directly demonstrated by any study retrieved for this review. The inference fits the class gradient, since ILD is commonest with the deruxtecan conjugates whose payload is designed for

5.3 Who is at risk, and how to watch for it

Stepwise multivariable analysis of the pooled cohort identified seven independent risk factors: enrolment in Japan, a dose above 6.4 mg/kg every three weeks, moderate or severe renal impairment, baseline oxygen saturation below 95%, more than four years since initial diagnosis, pre-existing lung comorbidity, and age under 65 ⁴¹. Two qualifications matter. The renal association held when creatinine clearance was estimated by Cockcroft-Gault and disappeared in a sensitivity analysis using CKD-EPI, so a risk factor that changes with the estimating equation should be treated as provisional ⁴¹. And the model found no significant difference between 5.4 and 6.4 mg/kg, even though unadjusted label rates differ between those doses ^{41,43}. The regional signal has been reproduced at trial level, with 22.2% incidence among 36 Japanese patients in DESTINY-Breast03 against 10.5% overall, though that subgroup is small enough that the estimate is imprecise ⁴⁸.

Radiologically, organising pneumonia is the dominant pattern at 52 to 72% of cases, followed by acute interstitial pneumonia or diffuse alveolar damage at 9 to 15%, hypersensitivity pneumonitis at 3 to 17%, and non-specific interstitial pneumonia in about 3% ⁴⁹. The distinction is prognostically useful, since organising pneumonia responds better to corticosteroids than the other patterns ⁴⁹.



Monitoring rests on computed tomography rather than physiology. A baseline chest CT should precede the first dose; trials scanned every six weeks, real-world practice generally every nine to twelve weeks timed to response assessment, and every six to nine weeks in higher-risk patients⁴⁹. Baseline pulmonary function tests are not routinely recommended, and spirometry should be deferred during an acute episode^{48,50}. Because symptoms are non-specific and the diagnosis is one of exclusion, infection has to be actively excluded before attribution⁵¹.

5.4 Management

Management is set out in Table 2 and rests on two published multidisciplinary guidance documents that agree with each other and with the product labels^{43,48,50}.

Asymptomatic, radiologically detected disease is grade 1. Treatment is interrupted until radiological resolution, corticosteroids at 0.5 mg/kg/day of prednisolone or equivalent are optional, and imaging is repeated at one to two weeks. Rechallenge is permitted: if the event resolves within 28 days the previous dose is resumed, and beyond 28 days the dose is reduced by one level, from 5.4 to 4.4 mg/kg every three weeks^{43,50}.

Any symptomatic event is grade 2 or above, and at that point trastuzumab deruxtecan is permanently discontinued. Prednisolone is started at 1 mg/kg/day for at least 14 days; if there is no improvement within five days the route is changed to intravenous and escalation to 2 mg/kg/day is considered; the taper then runs

over at least four weeks. Hospitalisation is not routinely required at grade 2⁵⁰.

Grade 3 and grade 4 disease share a regimen. Intravenous methylprednisolone at 500 to 1000 mg/day for three days is followed by prednisolone at 1 mg/kg/day or more for at least 14 days or until resolution, then tapered over at least four weeks. Hospitalisation is recommended at grade 3 and required at grade 4. If there is no improvement by five days at grade 3, or three to five days at grade 4, infliximab, intravenous immunoglobulin or mycophenolate mofetil may be considered⁵⁰. Prophylaxis against *Pneumocystis jirovecii* is advised while high-dose corticosteroids continue⁴⁸.

Two features of this algorithm deserve scrutiny. The first is that it is consensus rather than outcome-validated. Corticosteroids were given within 90 days of onset to 80.2% of patients with grade 2 to 4 events and 84.6% of those with fatal events, but the pooled analysis states that it could not determine how many received guideline-concordant treatment and reports no resolution rate attributable to steroids⁴¹. No dataset in this literature establishes that the recommended doses change outcome. The second is that permanent discontinuation at grade 2 is more conservative than the rule applied to other ADCs: enfortumab vedotin permits resumption after a grade 2 event and uses 1 to 2 mg/kg/day of prednisone⁴⁶. Given that grade 2 events constitute the largest single category and that most patients recover, a rule that permanently removes an effective drug from a patient whose pneumonitis fully resolved



is defensible on safety grounds and has never been tested against the alternative.

6. Discussion

The four themes reviewed here are usually treated separately, and they resolve into one design trade-off. Payload that can leave the cell it was delivered to buys efficacy in tumours the antibody only partly reaches, and it charges for that efficacy in normal tissue and in the mechanisms by which tumours escape.

Several things can now be asserted. Linker chemistry, not payload identity alone, determines whether an ADC kills antigen-negative neighbours, and the catabolite charge state is the proximate reason^{10,20}. Drug-to-antibody ratio is not independently limiting once conjugate hydrophobicity is controlled, which overturns the operative reading of the foundational loading study^{15,16}. Resistance is shifting from the antigen to the payload and its efflux transporters, with HER2 expression preserved in several deruxtecan-resistant models^{38,39}. Trastuzumab deruxtecan pneumonitis is common, mostly low-grade, concentrated in the first year, and fatal in one to two per cent of treated patients⁴¹.

The weaknesses in the evidence are specific rather than general. Mechanistic understanding of ADC lung injury rests substantially on a single cynomolgus study⁴⁷. The steroid regimens in universal clinical use have never been shown to alter outcome, because the only adjudicated dataset large enough to test the question did not capture treatment adequacy⁴¹. Cross-resistance between ADCs is supported by 85 retrospective

patients⁴⁰. Risk stratification for ILD depends on a multivariable model in which the renal signal does not survive a change of creatinine-estimating equation⁴¹. And the bystander account of ADC efficacy fails to explain a 29.7% response rate in tumours with no detectable HER2²⁶, which means some part of the mechanism is still unidentified.

Four studies would resolve most of this. Paired biopsy at progression should be mandatory in ADC trials rather than optional, because antigen loss in roughly a third of rebiopsied patients directly determines whether the next agent can work²⁹. An adjudicated ADC-pneumonitis registry recording imaging pattern, corticosteroid dose, timing and outcome would answer the steroid question that the pooled analysis explicitly could not. A randomised comparison of rechallenge against permanent discontinuation after fully resolved grade 2 pneumonitis would test the most consequential rule in the current algorithm. And a trial randomising patients progressing on one ADC to a second agent with the same payload class or a different one would convert the payload-resistance hypothesis into practice.

7. Conclusion

The component of an antibody-drug conjugate that determines its clinical behaviour is not the antibody. Linker chemistry sets whether the payload stays in the cell that captured it, and that single property propagates into the breadth of the treatable population, the mechanism by which tumours become resistant, and the profile of the



toxicity that limits use. Interstitial lung disease is the price currently paid for permeable payloads in the deruxtecan class, and it is manageable within an algorithm that is coherent, widely adopted, and still unvalidated against outcome.

Declarations

Funding. None

Ethics. This review synthesises published literature and required no ethics approval and no participant consent.

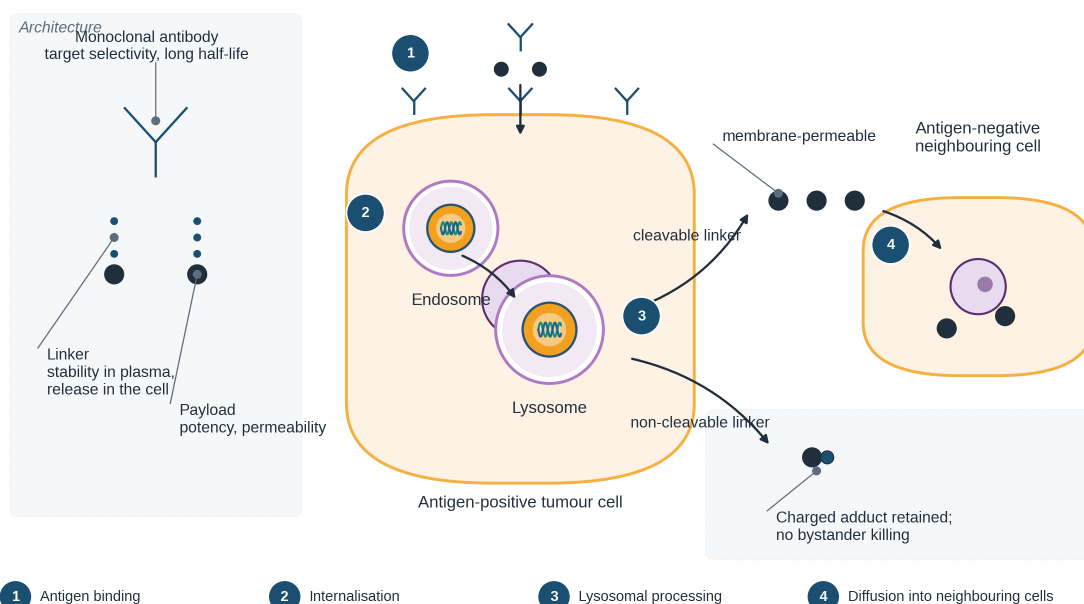


Figure 1. Linker chemistry, not the antibody, determines whether an antibody-drug conjugate kills antigen-negative neighbouring cells. The conjugate binds its antigen (1), is internalised (2) and is processed through the endosomal-lysosomal pathway (3), where the linker determines what is released.

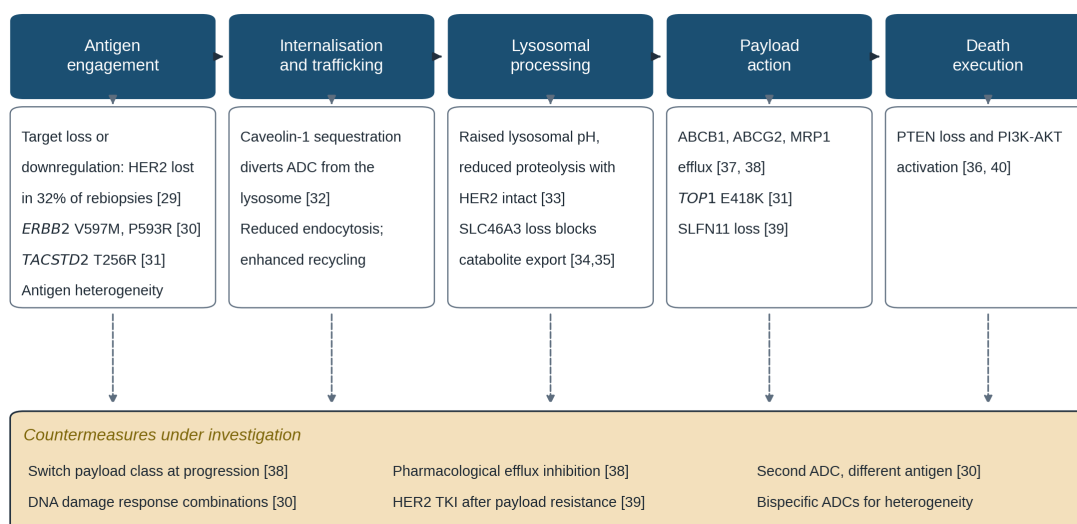


Figure 2. Documented mechanisms of resistance to antibody-drug conjugates, mapped onto the step of the mechanism at which each acts.



Table 1. Linker chemistry as the determinant of released species and bystander capability. Each row gives the stability datum reported by the cited source, the species the linker hands back after intracellular processing, and whether killing of antigen-negative neighbouring cells has been demonstrated experimentally for that chemistry. Bystander capability is recorded as established only where co-culture or mixed-xenograft evidence exists; it is not inferred from payload class alone.

Linker class	Representative conjugate	Release trigger	Reported stability	Released species	Bystander killing
Non-cleavable thioether (MCC)	Trastuzumab emtansine ¹	Proteolysis of the antibody itself ¹⁰	18.4% of payload released at 4 days in human plasma ²	Lysine- <i>N</i> - ϵ -MCC-DM1; charged; IC ₅₀ 0.1 μ M ¹⁰	No ^{19,20}
Cleavable dipeptide (valine-citrulline)	Auristatin conjugates ⁶	Lysosomal cysteine cathepsins; cathepsin B itself is dispensable ⁷	Therapeutic index up to 60-fold above the hydrazone equivalent ⁶	MMAE or MMAF, depending on construct ⁶	Not established
Cleavable tetrapeptide (GGFG)	Trastuzumab deruxtecan ²	Lysosomal proteases ²	2.1% of payload released at 21 days in human plasma ²	Deruxtecan; neutral and membrane-permeable; ~10-fold more potent than SN-38 ²	Yes ¹⁹
Cleavable disulfide	Maytansinoid DM4 conjugates ⁸	Intracellular reduction, tuned by steric hindrance ⁸	Intermediate, not maximal, stability gave the best in vivo activity ⁸	<i>S</i> -methyl-DM4; neutral; IC ₅₀ 2 pM ¹⁰	Yes ²⁰
Acid-labile hydrazone	Gemtuzumab ozogamicin ¹¹	Endosomal and lysosomal pH ¹¹	Less stable in plasma than dipeptide linkers ⁶	Calicheamicin ¹¹	Not established
Hydrolysable carbonate	Sacituzumab govitecan ³	Spontaneous hydrolysis, by design ³	SN-38 release half-life 17.5 h in mouse serum; $\geq$ 95% of serum SN-38 remains antibody-bound ³	SN-38; membrane-permeable ³	Yes ³
β -Glucuronide	Auristatin conjugates ⁹	β -Glucuronidase ⁹	Extrapolated rat plasma half-life 81 days ⁹	Payload-dependent ⁹	Not established



Table 2. Grading and management of trastuzumab deruxtecan-related interstitial lung disease. The regimen is reproduced from two multidisciplinary guidance documents that agree with each other and with the product label^{43,48,50}. Corticosteroid doses are prednisolone or equivalent unless methylprednisolone is specified.

	Grade 1 (asymptomatic, imaging only)	Grade 2 (symptomatic)	Grade 3-4 (severe or life- threatening)
Trastuzumab deruxtecan	Interrupt until radiological resolution to grade 0	Permanently discontinue	Permanently discontinue
Corticosteroid	Optional; 0.5 mg/kg/day	1 mg/kg/day for ≥ 14 days; switch to intravenous and consider 2 mg/kg/day if no improvement by day 5	Methylprednisolone 500- 1000 mg/day intravenously for 3 days, then ≥ 1 mg/kg/day for ≥ 14 days or until resolution
Taper	Over 4 weeks	Over ≥ 4 weeks	Over ≥ 4 weeks
Setting	Outpatient	Hospitalisation not routinely required	Recommended at grade 3; required at grade 4
Imaging and review	Repeat CT at 1-2 weeks	Review every 3-5 days	Review every 3-5 days (grade 3); re-image as clinically indicated (grade 4)
If steroid- refractory	Not applicable	Escalate dose and route by day 5	No improvement by day 5 (grade 3) or days 3-5 (grade 4): consider infliximab, intravenous immunoglobulin or mycophenolate mofetil
Rechallenge	Permitted after resolution to grade 0: same dose if ≤ 28 days, reduce 5.4 to 4.4 mg/kg every 3 weeks if > 28 days	Not permitted	Not permitted

References

1. Genentech Inc. KADCYLA (ado-trastuzumab emtansine) for injection: US prescribing information. Description and clinical pharmacology sections.
2. Nakada T, Sugihara K, Jikoh T, Abe Y, Agatsuma T. The latest research and development into the antibody-drug conjugate, [fam-] trastuzumab deruxtecan (DS-8201a), for HER2 cancer therapy. Chem Pharm Bull (Tokyo). 2019;67(3):173-85. doi:10.1248/cpb.c18-00744
3. Cardillo TM, Govindan SV, Sharkey RM, Trisal P, Arrojo R, Liu D, et al. Sacituzumab govitecan (IMMU-132), an anti-Trop-2/SN-38 antibody-drug conjugate: characterization and efficacy in pancreatic, gastric, and other cancers. Bioconj Chem. 2015;26(5):919-31. doi:10.1021/acs.bioconjchem.5b00223
4. Hurvitz SA, Hegg R, Chung WP, Im SA, Jacot W, Ganju V, et al. Trastuzumab deruxtecan versus trastuzumab emtansine in patients with HER2-positive metastatic breast cancer: updated results from DESTINY-Breast03, a randomised, open-



- label, phase 3 trial. *Lancet*. 2023;401(10371):105-17. doi:10.1016/S0140-6736(22)02420-5
5. Dubowchik GM, Firestone RA, Padilla L, Willner D, Hofstead SJ, Mosure K, et al. Cathepsin B-labile dipeptide linkers for lysosomal release of doxorubicin from internalizing immunoconjugates. *Bioconjug Chem*. 2002;13(4):855-69. doi:10.1021/bc025536j
6. Doronina SO, Toki BE, Torgov MY, Mendelsohn BA, Cervený CG, Chace DF, et al. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. *Nat Biotechnol*. 2003;21(7):778-84. doi:10.1038/nbt832
7. Caculitan NG, Dela Cruz Chuh J, Ma Y, Zhang D, Kozak KR, Liu Y, et al. Cathepsin B is dispensable for cellular processing of cathepsin B-cleavable antibody-drug conjugates. *Cancer Res*. 2017;77(24):7027-37. doi:10.1158/0008-5472.CAN-17-2391
8. Kellogg BA, Garrett L, Kovtun Y, Lai KC, Leece B, Miller M, et al. Disulfide-linked antibody-maytansinoid conjugates: optimization of in vivo activity by varying the steric hindrance at carbon atoms adjacent to the disulfide linkage. *Bioconjug Chem*. 2011;22(4):717-27. doi:10.1021/bc100480a
9. Jeffrey SC, Andreyka JB, Bernhardt SX, Kissler KM, Kline T, Lenox JS, et al. Development and properties of beta-glucuronide linkers for monoclonal antibody-drug conjugates. *Bioconjug Chem*. 2006;17(3):831-40. doi:10.1021/bc0600214
10. Erickson HK, Park PU, Widdison WC, Kovtun YV, Garrett LM, Hoffman K, et al. Antibody-maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing. *Cancer Res*. 2006;66(8):4426-33. PMID:16618769
11. Hamann PR, Hinman LM, Hollander I, Beyer CF, Lindh D, Holcomb R, et al. Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia. *Bioconjug Chem*. 2002;13(1):47-58. doi:10.1021/bc010021y
12. Hartley JA, Flynn MJ, Bingham JP, Corbett S, Reinert H, Tiberghien A, et al. Pre-clinical pharmacology and mechanism of action of SG3199, the pyrrolobenzodiazepine (PBD) dimer warhead component of antibody-drug conjugate (ADC) payload tesirine. *Sci Rep*. 2018;8(1):10479. doi:10.1038/s41598-018-28533-4
13. Pahl A, Lutz C, Hechler T. Amanitins and their development as a payload for antibody-drug conjugates. *Drug Discov Today Technol*. 2018;30:85-9. doi:10.1016/j.ddtec.2018.08.005
14. Bardia A, Rugo HS, Tolaney SM, Loirat D, Punie K, Oliveira M, et al. Final results from the randomized phase III ASCENT clinical trial in metastatic triple-negative breast cancer and association of outcomes by



- human epidermal growth factor receptor 2 and trophoblast cell surface antigen 2 expression. *J Clin Oncol.* 2024;42(15):1738-44. doi:10.1200/JCO.23.01409
15. Hamblett KJ, Senter PD, Chace DF, Sun MMC, Lenox J, Cervený CG, et al. Effects of drug loading on the antitumor activity of a monoclonal antibody drug conjugate. *Clin Cancer Res.* 2004;10(20):7063-70. doi:10.1158/1078-0432.CCR-04-0789
16. Lyon RP, Bovee TD, Doronina SO, Burke PJ, Hunter JH, Neff-LaFord HD, et al. Reducing hydrophobicity of homogeneous antibody-drug conjugates improves pharmacokinetics and therapeutic index. *Nat Biotechnol.* 2015;33(7):733-5. doi:10.1038/nbt.3212
17. Junutula JR, Raab H, Clark S, Bhakta S, Leipold DD, Weir S, et al. Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index. *Nat Biotechnol.* 2008;26(8):925-32. doi:10.1038/nbt.1480
18. van Geel R, Wijdeven MA, Heesbeen R, Verkade JMM, Wasieleski AA, van Berkel SS, et al. Chemoenzymatic conjugation of toxic payloads to the globally conserved N-glycan of native mAbs provides homogeneous and highly efficacious antibody-drug conjugates. *Bioconjug Chem.* 2015;26(11):2233-42. doi:10.1021/acs.bioconjchem.5b00224
19. Ogitani Y, Hagihara K, Oitate M, Naito H, Agatsuma T. Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity. *Cancer Sci.* 2016;107(7):1039-46. doi:10.1111/cas.12966
20. Kovtun YV, Audette CA, Ye Y, Xie H, Ruberti MF, Phinney SJ, et al. Antibody-drug conjugates designed to eradicate tumors with homogeneous and heterogeneous expression of the target antigen. *Cancer Res.* 2006;66(6):3214-21. doi:10.1158/0008-5472.CAN-05-3973
21. Staudacher AH, Brown MP. Antibody drug conjugates and bystander killing: is antigen-dependent internalisation required? *Br J Cancer.* 2017;117(12):1736-42. doi:10.1038/bjc.2017.367
22. Thurber GM, Schmidt MM, Wittrup KD. Antibody tumor penetration: transport opposed by systemic and antigen-mediated clearance. *Adv Drug Deliv Rev.* 2008;60(12):1421-34. doi:10.1016/j.addr.2008.04.012
23. Thurber GM, Schmidt MM, Wittrup KD. Factors determining antibody distribution in tumors. *Trends Pharmacol Sci.* 2008;29(2):57-61. doi:10.1016/j.tips.2007.11.004
24. Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. *N Engl J Med.* 2022;387(1):9-20. doi:10.1056/NEJMoa2203690



25. Bardia A, Hu X, Dent R, Yonemori K, Barrios CH, O'Shaughnessy JA, et al. Trastuzumab deruxtecan after endocrine therapy in metastatic breast cancer. *N Engl J Med*. 2024. doi:10.1056/NEJMoa2407086
26. Mosele F, Deluche E, Lusque A, Le Bescond L, Filleron T, Pradat Y, et al. Trastuzumab deruxtecan in metastatic breast cancer with variable HER2 expression: the phase 2 DAISY trial. *Nat Med*. 2023;29(8):2110-20. doi:10.1038/s41591-023-02478-2
27. Rugo HS, Bardia A, Marme F, Cortes J, Schmid P, Loirat D, et al. Overall survival with sacituzumab govitecan in hormone receptor-positive and human epidermal growth factor receptor 2-negative metastatic breast cancer (TROPiCS-02): a randomised, open-label, multicentre, phase 3 trial. *Lancet*. 2023;402(10411):1423-33. doi:10.1016/S0140-6736(23)01245-X
28. Moore KN, Angelergues A, Konecny GE, Banerjee S, Pignata S, Colombo N, et al. Mirvetuximab soravtansine in FRalpha-positive, platinum-resistant ovarian cancer. *N Engl J Med*. 2023;389(23):2162-74. doi:10.1056/NEJMoa2309169
29. Gouda MA, Gonugunta A, Dumbrava EE, Yap TA, Rodon J, Piha-Paul SA, et al. Human epidermal growth factor receptor 2 loss following treatment with trastuzumab deruxtecan in patients with metastatic breast cancer. *Clin Cancer Res*. 2025;31(7):1268-74. doi:10.1158/1078-0432.CCR-24-3468
30. Chen W, Gupta A, Mai N, Nag S, Lau JS, Singh S, et al. Trastuzumab deruxtecan resistance via loss of HER2 expression and binding. *Cancer Discov*. 2026;16(2):235-49. doi:10.1158/2159-8290.CD-25-0647
31. Coates JT, Sun S, Leshchiner I, Thimmiah N, Martin EE, McLoughlin D, et al. Parallel genomic alterations of antigen and payload targets mediate polyclonal acquired clinical resistance to sacituzumab govitecan in triple-negative breast cancer. *Cancer Discov*. 2021;11(10):2436-45. doi:10.1158/2159-8290.CD-21-0702
32. Sung M, Tan X, Lu B, Golas J, Hosselet C, Wang F, et al. Caveolae-mediated endocytosis as a novel mechanism of resistance to trastuzumab emtansine (T-DM1). *Mol Cancer Ther*. 2018;17(1):243-53. doi:10.1158/1535-7163.MCT-17-0403
33. Rios-Luci C, Garcia-Alonso S, Diaz-Rodriguez E, Nadal-Serrano M, Arribas J, Ocana A, et al. Resistance to the antibody-drug conjugate T-DM1 is based in a reduction in lysosomal proteolytic activity. *Cancer Res*. 2017;77(17):4639-51. doi:10.1158/0008-5472.CAN-16-3127
34. Hamblett KJ, Jacob AP, Gurgel JL, Tometsko ME, Rock BM, Patel SK, et al. SLC46A3 is required to transport catabolites of noncleavable antibody maytansine conjugates from the lysosome to the cytoplasm. *Cancer Res*. 2015;75(24):5329-40. doi:10.1158/0008-5472.CAN-15-1610



35. Kinneer K, Meekin J, Tiberghien AC, Tai YT, Phipps S, Kiefer CM, et al. SLC46A3 as a potential predictive biomarker for antibody-drug conjugates bearing noncleavable linked maytansinoid and pyrrolobenzodiazepine warheads. *Clin Cancer Res.* 2018;24(24):6570-82. doi:10.1158/1078-0432.CCR-18-1300
36. Li G, Guo J, Shen BQ, Yadav DB, Sliwkowski MX, Crocker LM, et al. Mechanisms of acquired resistance to trastuzumab emtansine in breast cancer cells. *Mol Cancer Ther.* 2018;17(7):1441-53. doi:10.1158/1535-7163.MCT-17-0296
37. Roth JS, Robey RW, Gottesman MM, et al. Identification of antibody-drug conjugate payloads that are substrates of ATP-binding cassette drug efflux transporters. *Cancer Drug Resist.* 2026;9:2. doi:10.20517/cdr.2025.151
38. Murase Y, et al. Therapeutic strategies to overcome payload resistance of trastuzumab deruxtecan in HER2-positive cancers. *Cancer Sci.* 2026;117(4):996-1009. doi:10.1111/cas.70319
39. Nilsson MB, et al. Loss of payload sensitivity and other mechanisms of resistance to T-DXd in HER2-mutant NSCLC: implications for subsequent responsiveness to HER2 TKIs. *J Thorac Oncol.* 2026;21(6):103555. doi:10.1016/j.jtho.2026.01.007
40. Mai N, Klar Lieberman MM, Ferraro E, Bromberg M, Chen Y, Razavi P, et al. Clinical outcomes of sequential antibody-drug conjugate therapy in metastatic breast cancer. *JCO Precis Oncol.* 2025;9:e2400898. doi:10.1200/PO-24-00898
41. Powell CA, Modi S, Iwata H, Takahashi S, Smit EF, Siena S, et al. Pooled analysis of drug-related interstitial lung disease and/or pneumonitis in nine trastuzumab deruxtecan monotherapy studies. *ESMO Open.* 2022;7(4):100554. doi:10.1016/j.esmoop.2022.100554
42. Abuhelwa Z, Alloghbi A, Alqahtani A, Nagasaka M. Trastuzumab deruxtecan-induced interstitial lung disease/pneumonitis in ERBB2-positive advanced solid malignancies: a systematic review. *Drugs.* 2022;82(9):979-87. doi:10.1007/s40265-022-01736-w
43. Daiichi Sankyo Inc. ENHERTU (fam-trastuzumab deruxtecan-nxki) for injection: US prescribing information. Warnings and precautions: interstitial lung disease and pneumonitis.
44. Daiichi Sankyo Europe GmbH. DATROWAY (datopotamab deruxtecan) summary of product characteristics. Special warnings and precautions for use: interstitial lung disease.
45. Yu HA, Goto Y, Hayashi H, Felip E, Yang JCH, Reck M, et al. HERTHENA-Lung01, a phase II trial of patritumab deruxtecan (HER3-DXd) in epidermal growth factor receptor-mutated non-small-cell lung cancer after epidermal growth factor receptor



- tyrosine kinase inhibitor therapy and platinum-based chemotherapy. *J Clin Oncol.* 2023;41(35):5363-75.
doi:10.1200/JCO.23.01476
46. Astellas Pharma Europe BV. PADCEV (enfortumab vedotin) summary of product characteristics. Special warnings and precautions for use: pneumonitis and interstitial lung disease.
47. Kumagai K, Aida T, Tsuchiya Y, Kishino Y, Kai K, Mori K. Interstitial pneumonitis related to trastuzumab deruxtecan, a human epidermal growth factor receptor 2-targeting Ab-drug conjugate, in monkeys. *Cancer Sci.* 2020;111(12):4636-45.
doi:10.1111/cas.14686
48. Chiu JWY, Lee SC, Ho JCM, Park YH, Chao TC, Kim SB, et al. Clinical guidance on the monitoring and management of trastuzumab deruxtecan-related adverse events: insights from an Asia-Pacific multidisciplinary panel. *Drug Saf.* 2023;46(10):927-49. doi:10.1007/s40264-023-01328-x
49. Cereser L, Della Rossa S, Sparascio F, Gerratana L, Minisini AM, Puglisi F, et al. Imaging of trastuzumab deruxtecan-related interstitial lung disease. *Eur J Radiol.* 2025;194:112499.
doi:10.1016/j.ejrad.2025.112499
50. Wekking D, Porcu M, Pellegrino B, Lai E, Mura G, Denaro N, et al. Multidisciplinary clinical guidelines in proactive monitoring, early diagnosis, and effective management of trastuzumab deruxtecan (T-DXd)-induced interstitial lung disease (ILD) in breast cancer patients. *ESMO Open.* 2023;8(6):102043.
doi:10.1016/j.esmoop.2023.102043
51. Swain SM, Nishino M, Lancaster LH, Li BT, Nicholson AG, Bartholmai BJ, et al. Multidisciplinary clinical guidance on trastuzumab deruxtecan (T-DXd)-related interstitial lung disease/pneumonitis: focus on proactive monitoring, diagnosis, and management. *Cancer Treat Rev.* 2022;106:102378.
doi:10.1016/j.ctrv.2022.102378