THERAPEUTIC ADVANCES in
Medical Oncology

Review

Innovative payloads for ADCs in cancer treatment: moving beyond the selective delivery of chemotherapy

Ther Adv Med Oncol

2025, Vol. 17: 1–16

DOI: 10.1177/17588359241309461

Davide Izzo, Liliana Ascione, Lorenzo Guidi, Renato Maria Marsicano, Chrysanthi Koukoutzeli, Dario Trapani and Giuseppe Curigliano

***Abstract*:** Antibody–drug conjugates (ADCs) have emerged as a transformative approach in cancer therapy by enhancing tumor targeting and minimizing systemic toxicity compared to traditional chemotherapy. Initially developed with chemotherapy agents as payloads, ADCs have now incorporated alternative payloads, such as immune-stimulating agents, natural toxins, and radionuclides, to improve therapeutic efficacy and specificity. A significant advancement in ADC technology is the integration of Proteolysis Targeting Chimeras (PROTACs), which enable the precise degradation of cellular targets involved in tumorigenesis. This strategy enhances the specificity and precision of cancer therapies, addressing key mechanisms in cancer cell survival. Moreover, incorporating radioactive isotopes into ADCs is an emerging strategy aimed at further improving therapeutic outcomes. By delivering localized radiation, this approach offers the potential to enhance the efficacy of treatment and expand the therapeutic arsenal. Despite these innovations, challenges remain, including dysregulated immune activation, severe adverse effects, and intrinsic immunogenicity of some agents. These emerging issues highlight the ongoing need for optimization in ADC therapy. This review summarizes the latest developments in ADC technology, focusing on novel payloads, PROTAC integration, and the potential for combining ADCs with other therapeutic modalities to refine cancer treatment and improve patient outcomes.

Plain language summary

New treatments for cancer: using antibody–drug conjugates to deliver more than just chemotherapy

Antibody-drug conjugates (ADCs) are a new type of cancer treatment that can target cancer cells more precisely, reducing side effects compared to traditional chemotherapy. ADCs were first combined with chemotherapy drugs, but now they also use other treatments like immune-boosting agents, natural toxins, and even radioactive substances to make the treatment more effective. These advances allow ADCs to deliver treatment directly to cancer cells, improving the chances of success. While there are still some challenges to overcome, such as managing side effects, researchers are working on making these therapies safer and more effective. ADCs offer a more targeted approach to cancer treatment, with the potential to improve outcomes and reduce harm to healthy cells.

Keywords: ADC, bacterial toxins, payloads, precision medicine, PROTAC, TLR

Received: 16 July 2024; revised manuscript accepted: 9 December 2024.

Correspondence to:
Giuseppe Curigliano
Division of New Drugs and Early Drug Development, European Institute of Oncology, IRCCS, Via Giuseppe Ripamonti 435, Milan 20141, Italy

Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy
Giuseppe.curigliano@ieo.it

Davide Izzo
Liliana Ascione
Lorenzo Guidi
Renato Maria Marsicano
Dario Trapani
Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

Division of New Drugs and Early Drug Development, European Institute of Oncology, IRCCS, Milan, Italy

Chrysanthi Koukoutzeli
Division of New Drugs and Early Drug Development, European Institute of Oncology, IRCCS, Milan, Italy

Introduction

The advent of antibody–drug conjugates (ADCs) has radically changed the landscape of cancer treatments, by adding sophisticated approaches to specifically target cancer cells with potent antineoplastic payloads. While broadly carrying an unacceptable safety profile when used as systemic therapeutics, payloads delivered through ADCs allow for more selective, tailored cancer delivery of drugs, while largely sparing normal tissues[1,2] yielding improved safety. With few exceptions, ADCs have been developed as conjugated to payloads that are chemotherapy compounds, exerting cytotoxic activities—mostly by targeting microtubules or disrupting DNA by inhibiting the topoisomerases enzymes. However, with ADCs' ability to precisely deliver virtually any payload, their potential for clinical application has expanded through new ADC technologies, carrying potent cytotoxins that can be of a different chemical or biological species than classic chemotherapy drugs. Conventional chemotherapies have shown inherent limitations, including their off-target toxicity and cross-resistance mechanisms with traditional chemocompounds. As such, there is an urgent need to identify novel payloads, to overcome and prevent treatment resistances, and ultimately result in improved patient outcomes. Specifically, immune-conjugates, innate immune system activators, bacterial toxins, and radioligands appear each to be offering distinct mechanisms of action and therapeutic potential, with promising results from preclinical and early clinical studies (Figure 1). In this review, we will describe the most prominent non-chemotherapy payloads under development, to portray an emerging landscape of ADCs exerting their clinical benefits through innovative mechanisms of action.

Immune-stimulating antibody conjugates

Immune conjugates represent an innovative approach in ADC design, intended to leverage the power of the immune system to enhance

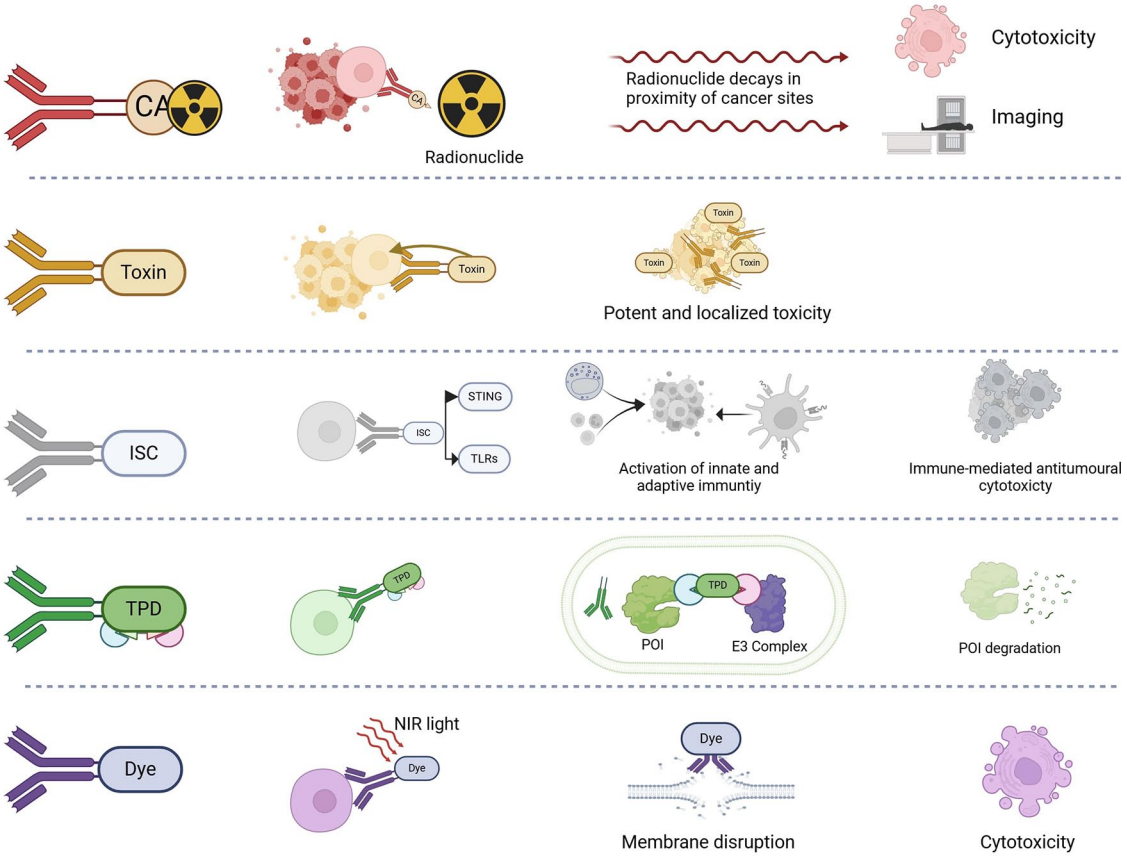

Figure 1. A concise visual representation of selected innovative ADCs and immune conjugates. The figure highlights their key components and mechanisms.
CA, chelating agent; ISC, immune-stimulating compound; NIR, near-infrared; POI, protein of interest; STING, stimulator of interferon genes; TLR, Toll-like receptors; TPD, targeted protein degrader.

anti-tumor activity. Immune-activating payloads can stimulate immunity by enhancing the specific, lymphocyte-mediated responses or the innate immune system. The introduction of immune checkpoint inhibitors (ICIs) in routine clinical practice has revolutionized cancer treatment. While widespread and well consolidated, ICIs' activity is usually impaired in so-called cold tumors (i.e., poorly immunogenic cancers); hence, new strategies to enhance immune response are needed and largely under investigation.[1] Approaches to enhance the immune response and turn "cold" tumors into "inflamed" immunogenic cancers include immuno-agonists small molecules: the Toll-like receptors (TLRs) agonists and the stimulator of interferon genes (STING) agonists. These agents engage with the pattern recognition receptors (PRRs), favoring the recognition of damage-associated molecular patterns (DAMPs) released by tumor cells.[2] The systemic administration of such immune stimulators has shown to yield a non-selective systemic immune response, causing immune-related adverse events that can be severe (e.g., cytokine release syndrome). As such, selectivity through ADC conjugation may better direct their activities within tumors, while sparing normal tissues. In an attempt to more selectively deliver such therapies, intra-tumoral administration of immune-stimulants has been investigated,[3] with limited efficacy. The targeted delivery of immune agonists provided by immune-stimulating antibody conjugates (ISACs), however, may represent a better alternative to overcome immune-activation-related toxicity. ISACs are structurally similar to traditional ADCs, comprising an antibody targeting a tumor antigen covalently linked to an immuno-agonist, representing the payload.[4] However, ISACs differ from traditional ADCs due to their mechanism of action. Once the antibody has bound to the target antigen, the complex antigen-ISAC is internalized by endocytosis mediated by the Fcγ receptor (FcγR) on immune cells, especially the antigen-presenting cells (APCs). This process locally reshapes the tumor immune microenvironment and activates the anti-tumor immune response.[5] Of note, being ISACs' uptake mediated by the FcγR, anti-drug antibodies (ADAs) could develop, whose role in impairing drug efficacy, pharmacokinetics, and patients' safety is still unknown. Hence, ADAs' detection may have an important role in this setting: while with traditional ADCs, a certain amount of ADAs is detected, their role is unclear (and not routinely searched for clinical practice).[6–8] The main PRR agonists used as payloads are TLR agonists and STING agonists.

Toll-like receptors. Innate immune system activators offer a promising strategy to bolster anti-tumor immunity. These agents can activate PRRs such as TLRs or STING pathways, initiating a cascade of immune responses that enhance the innate and adaptive immune responses against cancer cells. TLRs are transmembrane receptors expressed by both immune and non-immune cells that play a key role in innate immunity recognizing danger signals known as pathogen-associated molecular patterns, which are external (e.g., bacterial parts), and DAMPs, which come from the inside as remnants of dead cells. TLRs are located on the cell surface (i.e., TLR1, 2, 4, 5, 6, 10) and on the endosomes of APCs (i.e., TLR3, 7, 8, 9). Once activated, TLRs enhance antigen presentation by APCs, such as dendritic cells and macrophages, hence TLR agonists improve immune response, including anti-tumor response.[9] The integration of these activators into ADCs aims to exploit the body's natural defense mechanisms to achieve a more sustained and complete anti-tumor effect. Due to safety concerns, no TLR agonist has been approved for systemic administration or intratumoral injections. Immune conjugates have been designed to avoid toxicity issues related to the ISACs given systemically, with TLR7, TLR8, and TLR9 agonists as the main employed payloads.[10] Preclinical data in vitro[11] and in vivo[5] suggested a significant anti-tumor activity of ISACs with TLR agonist payloads. Specifically, the human epidermal growth factor receptor 2 (HER2)-directed and TLR8-conjugated SBT6050 (pertuzumab zuvotolimod) ISAC was evaluated in combination with an anti-PD1 (pembrolizumab or cemiplimab) in a phase I trial (NCT04460456), reporting an acceptable safety profile and no unexpected adverse events.[12] Another phase I/II trial tested pertuzumab zuvotolimod in combination with other HER2-targeted therapies, including trastuzumab deruxtecan (NCT05091528).[13] Pertuzumab zuvotolimod showed limited activity in evaluable patients ($n = 14$; 1 patient with partial response, 3 with stable disease). However, significant immune-related toxicity, mostly cytokine release syndrome, made its use in combination with pertuzumab or increasing the dose[14] as a monotherapy not safe, leading to drug development discontinuation.[15] A sponsor strategic re-alignment also led to the withdrawal of SBT6290, an anti-NECTIN4 antibody conjugated with a

TLR8 agonist (NCT05234606).[16] As such, these agents appear still to present the safety issues that TLR systemic administration brings in term of dysregulated immune activation, and optimized technologies are required for the safest clinical use. Another agent BDC-1001 (trastuzumab imbotolimod) is an anti-HER2 ISAC conjugating a trastuzumab biosimilar with a TLR7/8 dual agonist via a non-cleavable linker; it has been tested in HER2-expressing tumors with or without nivolumab. BDC-1001 development was discontinued due to a sponsor's decision, despite the limited toxicity, as the preliminary clinical activity was not deemed promising amidst many competitive ADCs anti-HER2 in development.[17] Additionally, NJH395 consisting of an anti-HER2 antibody conjugated to a TLR7 agonist was investigated in a phase I trial enrolling patients with HER2-expressing non-breast advanced solid tumors. Pharmacokinetic and pharmacodynamic analyses demonstrated that the TLR7-agonist payload is delivered to tumor cells, also showing an effective immune system modulation, as suggested by the induction of type I interferon response. Although manageable, cytokine release syndrome was a common adverse event, together with neuroinflammation (meningitis), highlighting that ISACs' side effects remain one of the main issues to face in the development of this drug class. Due to side effects, limited efficacy with no complete nor partial response observed, and the high antidrug immunogenicity (as suggested by the ADA formation in treated patients), NJH395 development was discontinued in phase I.[7] TAC-001 is a TLR agonist antibody conjugate composed of a TLR9 agonist bound to an anti-CD22 antibody. In preclinical models, by binding the CD22 receptor on B cells and favoring the antigen presentation through the TLR9 agonism, TAC-001 was demonstrated to stimulate the activation of both the innate and the acquired immune systems to favor anti-tumor activity.[18] Clinical TAC-001's activity is currently under evaluation in a phase II trial. Several TLR-based ISACs are under investigation in both preclinical and clinical settings, and a selection of them is reported in Table 1.

Stimulator of interferon genes. STING is a protein located in the endoplasmic reticulum and involved in initiating the IFN type I-dependent innate immunity and is a key component of the cyclic GMP–AMP synthase (cGAS)–STING–IFN pathway. cGAS interacts with dsDNA from pathogens or apoptotic cancer cells and is subsequently recognized by STING, activating the downstream signaling that finally leads to the IFN induction.[19] Preclinical studies testing ISACs with STING agonists as payload showed promising results in terms of antitumor activity.[20] TAK-500 is a STING agonist (TAK-676) conjugated to a human IgG1 anti-CC-chemokine receptor 2 antibody that activates the innate and adaptive immune system in murine models, especially when associated with an anti-PD1 antibody. These results represented the rationale for designing a phase I/II trial testing TAK-500 with or without pembrolizumab in patients with advanced solid tumors.[21] Another promising agent is XMT-2056, an anti-HER2 ISAC conjugated with a STING agonist (dimeric amidobenzimidazole) that demonstrated an intense anti-tumor efficacy in mouse models in a dose- and target-dependent manner.[22] XMT-2056 is under clinical investigation in a phase I trial enrolling patients with HER2-expressing advanced tumors (NCT05514717). Due to a fatal event at the first dose level, the study was temporarily put on clinical hold and restarted after lowering the dose, suggesting that even dose finding is extremely challenging for ISACs targeting STING, and innate immunity more broadly.

Proteolysis targeting chimeras-conjugated ADCs

A new class of anti-cancer drugs is composed of targeted protein degraders (TPD). TPD are bifunctional compounds that have the ability to bind to a protein of interest (POI) at one end and to E3 ligases at the other end. This induces ubiquitylation of the POI and its subsequent degradation by the ubiquitin–proteasome system.[23] Presently, there are two main types of TPD molecules: molecular "glues" and hetero-bifunctional "degraders," also known as proteolysis targeting chimeras (PROTACs). Molecular glues are typically small, low molecular weight compounds that bind to both the target protein and the E3 ligase at the same time, inducing a conformational change that promotes their interaction (such as thalidomide); PROTACs are heterobifunctional molecules—chimeras consisting of an E3 binding moiety coupled to a specific substrate protein binding moiety to enable the interaction between a desired target protein and the proteasome machinery, forming a ternary complex that leads to targeted-protein degradation.[24] Virtually, well-designed PROTAC can target any oncogene and induce degradation of major cancerogenesis

Table 1. An overview of the landscape of drug development of ADCs conjugated with novel, non-chemotherapy payloads.

Agent	Target	Payload	Latest trial phase	Setting	Trial number
TAK500	CCR2	STINGa	Phase Ia/b	Advanced solid tumors	NCT05070247
XMT-2056	HER2	STINGa	Phase I	Advanced solid tumors	NCT05514717
HE-S2	PDL1	TLR7-8a	Preclinical	Advanced solid tumors	Preclinical
PERTUZUMAB ZUVOLIMOD | SBT-6050	HER2	TLRa	Discontinued	Advanced solid tumors	NA
BDC-1001	HER2	TLR7-8a	Discontinued	Advanced solid tumors	NA
NJH395	HER2	TLR7a	Discontinued	Advanced solid tumors	NA
TAC 001	CD22	TLR9a	Phase II	Advanced solid tumors	NCT05399654
BMS-986497	CD33	GSPT1 degrader	Phase I	Acute myeloid leukemia	NCT06419634
ORM5029	HER2	GSPT1 degrader	Phase I	Advanced solid tumors	NCT05399654
Moxetumomab pasudotox	CD22	PE38	FDA approval	Refractory HCL	
DT2219ARL	CD19 and CD22	Diphtheria toxin	Phase II (concluded)	Hematological malignancies	NCT02370160
WTX212	PD1	Erythrocyte	Phase I	Advanced solid tumors	NCT06026605
BMS-986288	CTLA-4	Masking peptide	Phase I/II	Advanced solid tumors	NCT03994601
HDP-101	BCMA	α-Amanitin	Preclinical	Multiple myeloma	
MIRZOTAMAB CLEZUTOCLAX | ABBV-155	CD276	Clezutoclax (Bcl2-Xli)	Phase I	Lung cancer	NCT03595059
CETUXIMAB SAROTALOCAN	EGFR	Photo-sensitizer	FDA approval	Unresectable head and neck cancer	jRCT2031200133
CD184-DASATINIB	CD184	Dasatinib	Preclinical	Immunosuppression	NA
KSI 301	VEGFR	Biopolymer	Phase III (completed)	Retinal disorders	NCT04592419
ABBV-319	CD19	Glucocorticoid Rec modulator	Phase I	Hematological malignancies	NCT05512390
EDC1	Dysadherin	Steroidal glycoside	Preclinical	Solid tumors	NA
EDC9	CD20	Steroidal glycoside	Preclinical	Hematological malignancies	NA

ADC, antibody–drug conjugates; BCMA, B-cell maturation antigen; CCR2, C-C chemokine receptor type 2; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; EGFR, epidermal growth factor receptor 2; GSPT, G1-to-S-phase transition 1; HER-2, human epidermal growth factor receptor 2; PDL1, programmed death ligand 1; PE, *Pseudomonas* Exotoxin A; STING, stimulator of interferon genes; TLR, Toll-like receptor; VEGFR, vascular endothelial growth factor receptor 2.

drivers, representing one of the most appealing technologies of the last two decades. Precisely due to their composition and physicochemical properties, these heterobifunctional degraders feature poor drug metabolism and pharmacokinetic properties, such as low oral bioavailability and/or rapid in vivo clearance.[25] Another drawback of PROTACs is that although they are highly efficient degraders, they are generally not tissue-specific, since they exploit E3 ligases with broad expression profiles, causing a small therapeutic window and potential more side effects when targeting universal cellular targets. One strategy to improve the in vivo bioavailability is to conjugate PROTACs with monoclonal antibodies and exploit them as a payload. Monoclonal antibodies have the ability to recognize specific antigens and deliver degrading molecules to specific tumor cells, enhancing the in vivo delivery of chimeric degraders with poor physicochemical or drug metabolism characteristics. This new entity composed of monoclonal antibody and PROTAC is defined as novel Degrader–Antibody Conjugates (DAC).[26] One of the main differences with ADC is that the toxic payload of traditional ADCs is broadly toxic to many cells, whereas DACs are usually not. This is because the selectivity toward the target of the degrader is added to the selectivity of monoclonal antibody to its target. This mechanism also overcomes some limitations of PROTACs: tissue-specific degradation could enable optimization of the therapeutic window and minimize side effects for broad-spectrum PROTACs, increasing their potential as drugs or chemical tools.[27] As for the ADC, even DACs can enhance therapeutic monoclonal antibodies, such as trastuzumab or pertuzumab. Several DACs are currently being investigated. Some in vitro studies have demonstrated potential use in breast cancer, using PROTACs against Bromodomain-containing protein 4, or to degrade estrogen receptor alpha (ERα), TGFβR2, chromatin-remodeling complex regulating proteins such as BRM, and other targets.[28] One of the most targeted proteins is the G1-to-S-phase transition 1 (GSPT1), also known as eukaryotic release factor 3a. This G-loop degron-containing protein is a crucial translational termination factor[29] and is dysregulated in various tumor cell types. GSPT1 is significantly overexpressed in various cancers, including colon cancer,[30] acute myeloid leukemia (AML),[31] gastric cancer,[32] liver cancer,[33] and breast cancer.[34] ORM5029 is one such DAC. ORM-5029 (Orum Therapeutics USA, Inc., Lexington, MA) is a new antibody neo-Degrader conjugate composed of pertuzumab, an antibody directed against HER-2 conjugated, via a Val-Cit PABc cleavable linker, to SMol006, a selective molecular "glue" degrader of GSPT1. SMol006 specifically targets and binds to GSPT1, leading to GSPT1 degradation via the E3 ubiquitin ligase pathway. GSPT1 PROTAC-induced loss results in activation of the integrated stress response, which ultimately leads to apoptosis of the targeted cancer cells.[26] ORM5029 uses a Dual-Precision TPD (TPD2) approach combining the catalytic mechanism of TPDs with the precision of tumor-targeting therapeutic antibodies. Preclinical studies showed that HER2-positive breast cancer cell lines display a higher sensitivity to GSPT1 degradation than all other cell lines tested, suggesting a possible setting for clinical implementation.[26] ORM-5029 is currently in clinical development for the treatment of HER2-expressing solid tumor (such as breast cancer, gastric, or gastroesophageal junction adenocarcinoma) or tumors with HER2 amplification or mutations (e.g., colorectal, bile duct, ovarian, bladder, non-small cell lung). In the BT474 xenograft model, treatment with ORM-5029 demonstrated single-dose activity superior to T-DM1, and comparable activity to Trastuzumab Deruxtecan when given at an equivalent dose.[26] The ongoing trial phase I, first-in-human, clinical study of ORM-5029 (NCT05511844) started to enroll patients in October 2022 to evaluate the safety, tolerability, and efficacy of ORM-5029 administered by intravenous infusion in patients with HER2-expressing advanced solid tumors. Each cohort has enrolled >3 patients with breast cancer with at least HER2 expression of at least 1+ (HER2 low) or greater by immunohistochemistry or positive by in situ hybridization.[35] Data are awaited, to understand if the preclinical promising potential will translate into benefits for patients. Another GSPT1-directed DAC in development is BMS-986497 (ORM 6151) (Bristol Myers Squibb, USA, Inc., Princeton, NJ), which is composed of a CD33-targeting antibody (OR000283) conjugated to SMol006, a highly potent GSPT1 degrader, via a novel β-glucuronide releasable linker. ORM-6151 has been designed for AML. In pre-clinical studies, it has shown potent activity against CD33-expressing cell lines and primary relapsed/refractory AML patient blasts, as well as robust efficacy in vivo. ORM-6151 also exhibited picomolar potency in in vitro cytotoxicity to primary relapsed/refractory AML patient blasts, with better potency than GSPT1-inhibitors CC-90009

and the CD33-directed ADC Gemtuzumab ozogamicin. This suggests that the DAC technology may enhance the activity of co-targeting with two drugs. Moreover, ORM-6151 showed minimal cytotoxic activity to non-cancerous hematopoietic progenitor cells, with 10–10,000-fold less toxicity than CC-90009 or Gemtuzumab.[35] The ongoing phase I trial is testing BMS-986497 (ORM-6151) in Subjects with CD33-positive relapsed or refractory AML or myelodysplastic syndrome (NCT06419634) and began to enroll patients in May 2024. Orum therapeutics is currently studying other ADC-PROTAC-GSPT degraders such as ORM 1023 which appears to have been designed against small-cell lung cancer and neuroendocrine tumors, and ORM-1153, whose characteristics are not yet disclosed.

Bacterial and other "otherwise too potent for systemic administration" toxins

Bacterial toxins as payloads introduce a novel and highly potent mechanism for inducing cell death. These toxins, such as exotoxins and endotoxins, possess unique properties that can disrupt cellular processes with high specificity and potency. These features have been highly selected during evolution of bacterial pathogenicity. When conjugated with antibodies, bacterial toxins can be selectively delivered to cancer cells, thereby minimizing systemic toxicity while maximizing therapeutic efficacy. While broadly classified under ADCs, these agents are formally immunotoxins, and in general, the toxin moiety is fused, not "linked" to the ADC backbone. The use of bacterial toxins in ADCs capitalizes on their ability to interfere with essential cellular functions, leading to rapid and irreversible cell death. Antibodies conjugated to bacterial toxins represent a fascinating aspect of targeted cancer therapy. This specific subset of ADCs is defined as immunotoxin and consists of a toxin, that is of proteic nature, conjugated to a specific targeting fraction. The targeting component is typically an antibody or ligand, such as a monoclonal antibody, antibody fragment, cytokine, or growth factor. The toxins generally originate from plant toxins, bacterial toxins, or cytotoxic elements derived from human sources.[36] Generally, cancer cell-killing by immunotoxins can be summarized as the following three steps: first, binding to the target cells, the immunotoxin's antigen or receptor binding domain attaches to the corresponding antigen or receptor on the surface of the target cells. Endosomes, lysosomes, and the endoplasmic reticulum are crucial in determining the fate of these molecules. However, unlike some ADCs, protein toxins must remain intact and evade lysosomal degradation.[37] Finally, the catalytic domain of the immunotoxin acts within the target cells, inducing cell death either by halting target cell protein synthesis or by activating critical apoptotic proteins. Among bacteria, Diphtheria Toxin (DT) and *Pseudomonas* Exotoxin A (PE) are the most common toxins used in immunotoxin development.[38]

Pseudomonas aeruginosa's *toxin.* PE facilitates the transfer of ADP ribose from nicotinamide adenine dinucleotide to elongation factor 2 (EF2).[39] This transfer causes irreversible modification of EF2, rendering it inactive and resulting in cessation of protein translation and induction of cell death through apoptosis.[40] Several trials evaluated the efficacy of PE-based immunotoxins, among them the most important therapeutic success was achieved by Moxetumomab pasudotox (CAT-8015, HA22) in the treatment of hairy cell leukemia (HCL). This immunotoxin is composed of a truncated *Pseudomonas exotoxin A* (PE38) attached to a high-affinity anti-CD22 fragment variable (Fv). This compound recognizes and binds to CD22-positive HCL cells, then transfers into the cell and releases its toxin, leading to the apoptosis of the target cell. Moxetumomab pasudotox was tested in a pivotal trial evaluating 80 patients with relapsed HCL, in which the observed complete response (CR) rate was 41% and the overall response rate was 75%.[41] This specific trial led to FDA approval of Moxetumomab pasudotoxin for the treatment of patients with relapsed/refractory HCL who have not responded to at least two prior treatments, including a purine nucleoside analog.[42] However, the role of PE-based immunotoxins in solid tumors needs to be further explored. Patients with solid tumors are characterized by a more effective immune system which strongly limits immunotoxin activity. With the aim to overcome this limitation, a clinical trial evaluated the efficacy of the combination of immune-modulating chemotherapies with SSP1, a PE-based immunotoxin that targets mesothelin, in patients with mesothelioma.[43] Notably, of 10 patients with chemotherapy-refractory mesothelioma, 3 experienced major tumor regressions, with 2 ongoing at 15 months, and 2 others responded to chemotherapy after discontinuing immunotoxin therapy.[44] Furthermore, a phase I clinical trial showed efficacy by the intra-tumor injection of VB4-845, targeting EpCam, in 20 patients with squamous cell carcinoma of the head and neck.[45] The

immunotoxin led to complete regression in four tumors, partial regression in six, and stabilization in four of them. Similarly, in another trial, 11 patients with cutaneous non-melanoma cancers were treated with intra-tumor scFv (FRP5)-ETA immunotoxin, which targets *ErbB2* (HER2/Neu). All patients received daily treatments for 7–10 days. Complete regression of injected tumors occurred in four patients, and partial regression was observed in three.[46] In both studies, the most common reported site effects were site edema, redness, and pain in the injected area. Furthermore, preclinical studies evaluated the use of immunotoxins as immunotherapy testing them in combination with ICIs in murine models. It was observed that immunotoxins can stimulate antitumor immunity and can be used locally to prepare tumors for an immune response triggered by anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). Zhang et al. obtained interesting results in a study that evaluated an immunotoxin targeting the prolactin receptor (PRLR), N8-PE24, using in vivo and in vitro methods. Exogenous or PRL overexpression cell models were employed to investigate the role of the activated PRLR pathway in mediating tamoxifen insensitivity. Their results highlighted the potential role of N8-PE24 in inhibiting breast cancer cell growth and in promoting drug sensitivity of PRLR-positive breast cancer cells to tamoxifen and paclitaxel.[47] Of note, several trials are ongoing with the aim of identifying the ideal combination strategy (NCT03258593, NCT03644550).

Corynebacterium diphtheriae's *toxin.* DT is a single chain, 62-kDa protein consisting of 535 amino acid residues that are produced by *Corynebacterium diphtheriae* containing lysogenic beta phage.[48] Truncated versions of DT have been effectively utilized to create recombinant immunotoxins for cancer treatment.[49] DT undergoes proteolytic cleavage outside the cell resulting in two domains linked by a disulfide bond. After binding to its cell surface receptor, DT is internalized via clathrin-coated vesicles. The acidic environment in the endosome triggers a conformational change, leading to the reduction of the disulfide bond. This allows the toxin to insert into the endosome membrane, forming a channel for the enzymatic fragment to translocate to the cytosol. The cell is then led to death through enzymatic ADP-ribosylation of EF2 in the cytosol.[50] DT is highly toxic, with a single molecule of its enzymatic fragment sufficient to induce cell death. Utilizing this potency, DT-based immunotoxins have been developed by fusing the first 388 amino acids of the toxin to a targeting moiety, allowing for targeted cancer therapy.[51] Denileukin diftitox is the first FDA-approved immunotoxin, consisting of IL-2 fused with a truncated form of DT (DAB389), used for treating recurrent cutaneous T-cell lymphoma.[52] More recently, a study conducted by Franket et al. demonstrated the efficacy of Tagraxofusp, which is a first-in-class CD123-directed conjugate of an amended DT platform and recombinant interleukin 3, in patients affected by the rare CD123-positive plasmacytoid dendritic cell neoplasm (BPDCN). Forty-four patients were included and in the 29 newly diagnosed patients who received the 12 μg/kg/day dose, the combined CR and CR with rescue of the hematological peripheral blood values (CRc) rate was 72% (95% confidence interval, 53%–87%), with a median of 43 days (range, 14–131 days) to response. These results led to the FDA approval of Tagraxofusp for adult and pediatric patients aged 2 years and older with BPDCN, in 2018.[53] Promising results were also obtained by a bispecific ligand-directed toxin called DT2219ARL consisting of two scFv ligands recognizing CD19 and CD22 and catalytic DT390 was genetically enhanced for superior in vivo anti-leukemia activity. Studies demonstrated that DT2219ARL exhibited high potency (IC50s 0.06–0.2 nM range) and its effects were selectively inhibitable.[54] An update on the phase I/II trial for hematological malignancies with this drug has been recently reported (on ClinicalTrial.gov: NCT02370160). The update concerned the first 18 patients enrolled, at two dose levels of the drug, and of the phase II expansion, showing an overall response rate between 41.7% and 59.5%, corresponding to a 1-year disease-free survival of 77.8%–100%, with a relapse-free survival of 27.3–42 months. Under the safety profile, five patients in total had serious adverse events, mostly infections and capillary leak syndrome. Non-serious events included fever, gastrointestinal and hematological toxicities, and high rates of transaminitis. Studies are ongoing in order to evaluate the efficacy and safety of this compound in hematological malignancies. Additionally, a novel toxin consisting of a truncated form of DT and a HER3-binding affibody domain showed promising results; preclinical evidence could potentially offer a new treatment avenue for HER3-positive cancer thanks to its ability to specifically bind to HER3-positive cells.[55]

α-*Amanitin.* Maleimide-amanitin conjugates are a promising area of research in cancer therapy,

particularly as components of ADCs.[56] *Amanita phalloides*, known for causing fatal mushroom poisoning, contains the toxin α-amanitin, which inhibits RNA polymerase II and I, reducing overall transcription levels within cells and killing cells regardless of their proliferative state. Additionally, α-amanitin is not enzymatically degraded and demonstrates good stability in plasma, lacks immunogenicity; α-amanitin-based ADCs are currently in development, with preclinical studies being conducted on various tumor models. Of note, in 2012, Moldenhauer et al.[57] developed a new compound conjugating α-amanitin to the anti-EpCAM antibody chiHEA125, thus creating chiHEA125-Ama; they conducted in vivo and in vitro experiments demonstrating interesting results in inhibiting proliferation of EpCAM-expressing cancer cell lines. Moreover, α-amanitin-conjugated trastuzumab (T-Ama) was developed and tested for HER2-low breast cancer, particularly HER2-low triple-negative breast cancer (TNBC). According to data from The Cancer Genome Atlas and the Molecular Taxonomy of Breast Cancer International Consortium, heterozygous loss of 17p occurs in 51.6% of breast cancer patients, including 41.9% in TNBC. On the basis of this data, T-Ama was tested in vitro in 17p loss TNBC cell lines with low HER2 expression, demonstrating higher cytotoxicity and extended survival than ado-trastuzumab emtansine (T-DM1).[57] In contrast, no improvements in terms of tumor growth inhibition or overall survival rates were observed in those 17p intact/HER2-low cell lines,[57] suggesting a selective effect. In recent times, α-amanitin was also evaluated in FGFR1-positive tumors, conjugated with Monomethyl auristatin E and FGF2 dimer, demonstrating promising activity in preclinical models with a potential role of FGF2 as an alternative antibody-targeting carrier.[58] Moreover, encouraging findings were observed in multiple myeloma testing HDP-101 which conjugates α-amanitin with an anti-B-cell maturation antigen.[59] This study emphasizes the therapeutic potential of HDP-101 in treating multiple myeloma. The specific targeting of this ADC, along with the strong cell-killing effect of α-amanitin, which operates regardless of the cell cycle, underscores the promise of this ADC for further investigation.[59]

Photoimmunotherapy

Photoimmunotherapy (PIT) is a recently developed application of ADC technology for cancer treatment which uses light-activable payload dyes conjugated to tumor-specific antibodies to mediate selective cytotoxicity.[3,4] Specifically, near-infrared photoimmunotherapy (NIR-PIT) is a phototherapy based on the employment of ADCs employing a near infrared sensitive payload, such as silicon-phthalocyanine derivative IRdye700DX(IR700). Subsequent local exposure to NIR electromagnetic radiation triggers photochemical membrane rupture and immunogenic cell death. This mechanism is highly target selective, causing few side effects and allowing rapid healing.[5] Cetuximab sarotalocan is composed of cetuximab, a monoclonal antibody directed against the epidermal growth factor receptor, conjugated to previously mentioned IR700. Its potential use as a NIR-PIT ADC agent is under investigation for the treatment of locally advanced head-and-neck squamous cell cancer in clinical trial (NCT03769506).

Other conjugate-based therapies

WTX212. A growing category of conjugate therapies now includes alternatives to traditional antibody-based treatments, such as Erythro-Drug Conjugates like WTX212.[60] These therapies utilize engineered red blood cells conjugated with therapeutic agents, such as the anti-PD-1 inhibitor pembrolizumab. The natural biological functions of red blood cells facilitate targeted distribution of pembrolizumab within spleen tissue, subsequently activating T cells. This mechanism suggests that WTX212[60,61] may address the challenge of acquired resistance to immunotherapy. The ongoing clinical trial NCT05707325 is a first-in-human study evaluating the efficacy of WTX212 in patients with advanced solid tumors and refractory lymphomas. Data presented at the American Society of Clinical Oncology 2024 conference indicated that, among the seven patients with metastasized solid tumors, five achieved disease control, resulting in a disease control rate of 71%. These results confirm the potential of Erythro-Drug Conjugates to enhance the efficacy of immunotherapeutic approaches and overcome resistance in several types of cancer.

BMS-986288. Another new class of ADCs includes those linked to immunotherapeutic agents to alter their pharmacodynamics, such as BMS-986288, which is based on a modified version of ipilimumab.[62] BMS-986288 is composed of a probody, a modified ipilimumab variant, targeting the human T-cell receptor CTLA-4 linked

to a proprietary masking peptide that conceals the active antigen-binding site of the antibody through a protease-cleavable linker, thereby offering potential immune checkpoint inhibitory and antineoplastic activities.[63] The ongoing clinical trial NCT03994601 is evaluating the efficacy of BMS-986288, both as a monotherapy and in combination with nivolumab, in patients with selected advanced solid cancers.[63]

Mirzotamab clezutoclax. Mirzotamab clezutoclax (Mirzo-C; ABBV-155) is an anti-B7-H3 monoclonal antibody conjugated to a BCL-XL inhibitor payload. Preclinical studies have shown that targeting BCL-XL can enhance the effects of other anticancer treatments. Data from the Japanese cohorts in the phase 1 study (posted on ClinicalTrial.gov: NCT03595059) were reported for Mirzo-C as monotherapy or in combination with paclitaxel. Among 16 patients receiving Mirzo-C, 7 in the monotherapy group and 9 in the combination therapy group, the median treatment duration was 78 and 56 days, respectively. The most common side effects were elevated levels of aspartate aminotransferase (57%) in the monotherapy group and anemia (56%) in the combination therapy group. No dose-limiting toxicities or fatal side effects were reported. Stable disease was observed in 57% of monotherapy patients and 44% of combination therapy patients, while 43% and 56%, respectively, experienced disease progression. No complete or partial responses were observed.

Extracellular-drug conjugates. An extracellular-drug conjugate (EDC) differs from most ADCs in that it requires no internalization. Rather, EDCs target cell surface proteins that are expressed on a target cell, while the cytotoxic agent kills the targeted cells by affecting a protein or an enzyme that is different from the protein or enzyme bound by the mAb but that is closely associated with the target protein or enzyme.

Cardiac glycosides as anticancer agents. Cardiac glycosides, also known as cardiotonic steroids, selectively inhibit Na+/K+ ATPase and have long been used to treat cardiac congestion and several types of arrhythmias. Preclinical evidence suggested they might also play a role in cancer treatment, as these compounds typically inhibit cancer cell proliferation at nanomolar concentrations.[64] However, there is evidence that their antiproliferative effects may not be selective for tumor growth and could result from these compounds' ability to kill human cells at lower concentrations than rodent cells. After multiple clinical trials, no significant benefit in clinical terms was found for any of these compounds in the setting of cancer treatment. A major reason for these has been suggested to be their narrow therapeutic index. Research was then set out to determine whether these safety limitations could be overcome with precise targeting treatment through the use of EDC technology.

EDC1 and EDC9. EDC1 is a novel type of ADC which binds and inhibits the Na+/K+ ATPase on the surface of cancer cells expressing dysadherin—a cancer-associated cell membrane glycoprotein, through the action of CEN-106n, a novel steroidal glycoside, leading to cell swelling and death.[65] A 2017 study has shown showed selective inhibition of growth in thyroid cancer cells with moderate to high expression of dysadherin at nanomolar concentrations, thus warranting further investigation. EDC9 is a rituximab-steroidal glycoside conjugate currently in preclinical stages of development. It has shown promising activity in primate models.[66]

Immunosuppressive ADC

CD184-DASATINIB. Recent advances have extended the ADC strategy to other therapeutic areas, including immunosuppression. A notable example is the CD184-dasatinib ADC, which innovatively targets the selective delivery of kinase inhibitors beyond cancer treatment.[67] Dasatinib, a well-established kinase inhibitor, has been clinically utilized for the treatment of BCR-ABL-dependent chronic myelogenous leukemia. Its mechanism of action involves the inhibition of multiple tyrosine kinases, including those in the Src family such as Lck and Fyn.[68] These kinases are integral to T-cell receptor signaling, and their inhibition suggests a potential role for dasatinib as an immunosuppressive agent. However, dasatinib's lack of selectivity across various kinases results in significant off-target effects and severe side effects, thus limiting its broader clinical application. CD184-dasatinib seeks to mitigate these limitations through targeted delivery. It is based on a humanized antibody which selectively binds with high affinity to CXCR4, an antigen that is selectively expressed on hematopoietic cells, nonspecifically conjugated to dasatinib derivatives. Employing the ADC strategy, dasatinib can be selectively delivered to human T cells.[69]

Radioimmunoconjugates

Radioligands represent another innovative class of ADC payloads, combining the precision of antibody targeting with the cytotoxic power of ionizing radiation. Radioligand-conjugated antibodies deliver radioactive isotopes directly to tumor cells, enabling localized radiation therapy that spares surrounding healthy tissue. This approach offers a dual mechanism of action, where the antibody component provides specificity, and the radioligand delivers lethal doses of radiation to induce DNA damage and apoptosis. The development of radioligand ADCs holds the potential for treating cancers that are resistant to conventional therapies, or to potentiate active systemic therapies. Radioimmunoconjugates, monoclonal antibodies linked to a radionuclide, have the potential to be theranostic tools, that is, to couple diagnostic, cancer mapping, and treatment capacity at once. Radiolabeled antibodies can be employed for both diagnostic and therapeutic purposes in scintigraphy imaging and radioimmunotherapy (RIT), respectively.[70,71] Some radionuclides commonly used for labeling mAbs for both therapeutic and diagnostic purposes include the alpha-emitters actinium-225 (^{225}Ac), astatine-211 (^{211}At), bismuth-213 (^{213}Bi) and lead-212 (^{212}Pb), the beta-emitters yttrium-86 (^{86}Y), yttrium-90 (^{90}Y), lutetium-177 (^{177}Lu), zirconium-89 (^{89}Zr), the gamma-emitters indium-111 (^{111}In), iodine-123 (^{123}I), iodine-124 (^{124}I), iodine-131 (^{131}I), technetium-99m (^{99m}Tc), the positron-emitters (beta+) copper-64 (^{64}Cu) and gallium-68 (^{68}Ga).[72] Therapeutic radionuclides can be classified on the basis of their mode of decay into Alpha-particles emitters, Beta-particles emitters, and Non-energetic particles emitters. Beta-negative particles are electrons emitted from a neutron in a decaying nucleus. They have low linear energy transfer (LET) and low range. They have historically been the most common option for RIT. Alpha-particles, consisting of two protons and two neutrons bound together, have higher energies, higher LET, and very short path lengths.[73] Some of the most actively studied alpha emitters are ^{225}actinium, ^{212}lead, and ^{211}astatine.[74] Radioimmunoconjugates have demonstrated utility in both tumor detection and therapy, and can be combined with conventional treatments to enhance the therapeutic efficacy of monoclonal antibodies (mAbs). Additionally, mAb radioconjugates serve as imaging theranostics, enabling real-time antigen quantitation, detection of heterogeneity, and dynamic changes in antigen expression. These capabilities are crucial for guiding and monitoring therapeutic responses, as well as for drug development, providing critical information on mAb pharmacokinetics, patient selection, and required therapeutic doses. While RIT has shown therapeutic efficacy in hematological malignancies, similar benefits have not yet been observed in solid tumors, representing a primary challenge for future research. Currently, to our knowledge all approved radiolabeled mAbs for RIT employ radionuclides that mostly undergo Beta-decay. 90Y-ibritumomab tiuxetan (90Y-IT) was the first radiolabeled mAb for RIT to receive FDA approval in hematologic malignancies in February 2002 for the treatment of CD20-positive, relapsed or refractory (R/R), low-grade, or follicular B-cell non-Hodgkin lymphoma, expanded in 2009 to include patients with previously untreated follicular non-Hodgkin lymhpoma who had achieved a partial or CR to first-line therapy. 90Y-IT is composed of three main components: a CD20-targeting antibody (ibritumomab), a metal ion chelator and linker (tiuxetan), and the previously mentioned radionuclide Yttrium-90 (^{90}Y).[75] Similarly, ^{131}I-tositumomab an anti-CD20 mAb regimen, received FDA approval in June 2003 for the treatment of CD20-positive relapsed/refractory follicular NHL.[75] Alpha emitters also are currently used in clinical practice. Following the success of the ALSYMPCA trial[76] in prolonging overall survival in patients with bone metastases, alpha-emitting radionuclides have gained significant attention as potential treatments for solid tumors, particularly in the settings prostate cancer and neuroendocrine tumors, as well as hematological malignancies. Because of ADC ability to selectively delivering radionuclides, many pharmaceutical companies and academic institutions have started Target Alpha-Therapy trials.[74] However, TAT poses some unique challenges and risks in its clinical application, and some concerns must be addressed for their clinical implementation.

Discussion

With the advent of precise cell-directed delivery strategies, the potentials of cancer treatments have incredibly expanded. ADCs have redefined the concepts of treatment approach and targetability, showing that cancer cells' antigens can become entry points for virtually any active compounds, and usually regardless of the actual antigen expression. While a minimum quantity—a so-called *quantum (6)*—may still be required to trigger biological activities, novel ADCs exerting bystander effects may overcome such this problem and be effective by proximity on antigen-negative cancer cells. With

the current paradigm of ADC development, however, the chemo-compounds utilized are usually directed toward classic targets of the systemic chemotherapy, resulting in cross-resistance. To overcome such a clinical unmet need, novel payloads have been developed and conjugated to ADC-like molecules. The intent is usually similar, *à la* Paul Ehrlich: "*corpora non agunt nisi fixata*" (meaning "to be effective, you need to deliver targeted agents within cancer cells"). As such, having the availability of broader potential can expand the anticancer effect, while optimizing the opportunity to sequence ADCs and combine as exerting synergistic, non-overlapping effects. Initial promising approaches have been directed toward the innate immune, including TLR- and STING-directed molecules, delivered with ISACs. The problem with innate immunity is that nowadays it remains insufficiently specific for cancer often resulting in unacceptable immune dysregulation and immune-related toxicity. As of now, these agents are broadly unsafe for clinical use. Importantly, ISACs can also be directed toward the immune-specific axis, via tumor-specific immune activation or engagement, and even with immune-suppression (for hematological malignancies). More interestingly, ADCs' targetability potentials can be duplicated when they are conjugated to novel targeted agents such as PROTACs. These molecules are molecular disruptors, which can target multiple cellular drivers of the cancer growth, including so-called "undruggable" targets. With the combination of ADC and PROTAC, the antineoplastic effect can be tremendously tailored and directed toward key mechanisms, as opposed to being chemo-like non-specific, cytotoxic approaches. Perhaps, with the double ADC-PROTAC compounds, much of the systemic toxicities of the classic ADCs may be spared, through better selectivity and more elegant mechanisms of action. Surely, leveraging highly potent toxins preserved during phylogenesis of bacteria, plants, and yeasts is appealing: these toxins are usually unacceptably toxic for use as systemic agents in cancer treatment and would result in fatal outcomes. However, their combination with highly selective Trojan horses may represent the key for the future of cancer treatment. We reported that two of such compounds are FDA approved, one from *Pseudomonas* and the other DTs. The caveats of these agents are in the bacterial, "non-self" nature of the compounds, which may require engineering optimization to avoid immune adverse, severe reactions. The landscape of radioconjugates is probably worthwhile a separate dissertation because it is expanding to an unprecedented level, as of now. We displayed an overview of the portfolio, which is rich and attracts interest for the theranostic potential beyond the "see and treat" classic approach of nuclear medicine, exploring the more articulated paths of precision medicines. Similarly, radioconjugates are now being intended as added-on to cancer therapies, or to be combined with each other, including to enhance the anticancer effects through alpha, and beta emissions. Perhaps, time for reconciliation of those radiations, so far kept very separate. Interestingly, no ADC aimed at improving safety and tolerability of chemotherapeutic treatment has been found in our research. This could be due to the "magic bullet" concept, the main driving idea behind development of ADCs and other target therapies. We think the idea of a drug with a "magic shield," skipping healthy cells and/or arming them with the means to survive systemic cytotoxic therapy is also a concept worth exploring. Antibody–drugs conjugates could provide a way to explore such opportunities by selectively binding to antigens downregulated in cancer and delivering compounds antidotal to concomitant systemic cytotoxic or target therapeutic agents.

Conclusion

Overall, with the advent of new payloads, and their conceptualization as targeted agents, and not only as chemotherapy compounds passively transported to cancer cells, but ADCs are also becoming co-starring. Probably, the future of ADCs is intrinsically linked to innovations of payloads, expecting to transform the landscape. Now that ADCs per se are in saturation, plateauing phase spreading in all disease areas—payload research will be transformative to eventually result in more active, more precise ADC technology—and ultimately, patient-improved clinical benefits. Perhaps a "magic shield," in addition to a "magic bullet," could be a way toward optimized cancer treatments.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contributions

Davide Izzo: Conceptualization; Data curation; Formal analysis; Software; Validation;

Visualization; Writing – original draft; Writing – review & editing.

Liliana Ascione: Formal analysis; Validation; Visualization; Writing – original draft; Writing – review & editing.

Lorenzo Guidi: Formal analysis; Writing – original draft; Writing – review & editing.

Renato Maria Marsicano: Formal analysis; Writing – original draft; Writing – review & editing.

Chrysanthi Koukoutzeli: Formal analysis; Writing – original draft; Writing – review & editing.

Dario Trapani: Conceptualization; Data curation; Formal analysis; Software; Validation; Visualization; Writing – original draft; Writing – review & editing, Methodology; Project administration; supervision.

Giuseppe Curigliano: Conceptualization; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing – original draft; Writing – review & editing.

Acknowledgements
None.

Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

Competing interests
G.C. received honoraria for speaker, consultancy, or advisory rule from AstraZeneca, Roche, Pfizer, Novartis, Seattle Genetics, Lilly, Ellipses Pharma, Foundation Medicine, Daiichi Sankyo, and Samsung. The other authors declare no competing interests.

Availability of data and materials
Not applicable.

ORCID iD
Dario Trapani https://orcid.org/0000-0003-1672-9560

References
1. Waldman AD, Fritz JM and Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* 2020; 20(11): 651–668.
2. Rolfo C, Giovannetti E, Martinez P, et al. Applications and clinical trial landscape using Toll-like receptor agonists to reduce the toll of cancer. *NPJ Precis Oncol* 2023; 7(1): 26.
3. Milhem M, Zakharia Y, Davar D, et al. 304 Intratumoral injection of CMP-001, a toll-like receptor 9 (TLR9) agonist, in combination with pembrolizumab reversed programmed death receptor 1 (PD-1) blockade resistance in advanced melanoma. *J Immunother Cancer* 2020; 8(Suppl. 3): A186–A187.
4. Fu C, Tong W, Yu L, et al. When will the immune-stimulating antibody conjugates (ISACs) be transferred from bench to bedside? *Pharmacol Res* 2024; 203: 107160.
5. Ackerman SE, Pearson CI, Gregorio JD, et al. Immune-stimulating antibody conjugates elicit robust myeloid activation and durable antitumor immunity. *Nat Cancer* 2021; 2(1): 18–33.
6. van Brummelen EMJ, Ros W, Wolbink G, et al. Antidrug antibody formation in oncology: clinical relevance and challenges. *Oncologist* 2016; 21(10): 1260–1268.
7. Janku F, Han SW, Doi T, et al. Preclinical characterization and phase I study of an anti-HER2-TLR7 immune-stimulator antibody conjugate in patients with HER2+ malignancies. *Cancer Immunol Res* 2022; 10(12): 1441–1461.
8. Shastry M, Gupta A, Chandarlapaty S, et al. Rise of antibody-drug conjugates: the present and future. *Am Soc Clin Oncol Educ Book* 2023; 43: e390094.
9. Li D and Wu M. Pattern recognition receptors in health and diseases. *Signal Transduct Target Ther* 2021; 6(1): 1–24.
10. Fenis A, Demaria O, Gauthier L, et al. New immune cell engagers for cancer immunotherapy. *Nat Rev Immunol* 2024; 24(7): 471–486.
11. Gadd AJR, Greco F, Cobb AJA, et al. Targeted activation of Toll-like receptors: conjugation of a Toll-like receptor 7 agonist to a monoclonal antibody maintains antigen binding and specificity. *Bioconjug Chem* 2015; 26(8): 1743–1752.
12. Metz H, Childs M, Brevik J, et al. SBT6050, a HER2-directed TLR8 therapeutic, as a systemically administered, tumor-targeted human myeloid cell agonist. *J Clin Oncol* 2020; 38(15_Suppl.): 3110.
13. Klempner S, Strickler J, Gourley L, et al. 393 A phase 1/2 study of SBT6050 combined with trastuzumab deruxtecan (T-DXd) or trastuzumab and tucatinib with or without capecitabine

in patients with HER2-expressing or HER2-amplified cancers. *J Immunother Cancer* 2021; 9(Suppl. 2): A426.

14. Loriot Y, Marabelle A, Guégan JP, et al. Plasma proteomics identifies leukemia inhibitory factor (LIF) as a novel predictive biomarker of immune-checkpoint blockade resistance. *Ann Oncol* 2021; 32(11): 1381–1390.
15. Conilh L, Sadilkova L, Viricel W, et al. Payload diversification: a key step in the development of antibody–drug conjugates. *J Hematol Oncol* 2023; 16(1): 3.
16. Savarese F, Gollner A, Rudolph D, et al. Abstract 1271: In vitro and in vivo characterization of BI 1823911—a novel KRAS G12C selective small molecule inhibitor. *Exp Mol Ther* 2021; 81(13_Suppl.): 1271.
17. Li BT, Pegram MD, Lee KW, et al. A phase 1/2 study of a first-in-human immune-stimulating antibody conjugate (ISAC) BDC-1001 in patients with advanced HER2-expressing solid tumors. 2023; 41(16_suppl): 2538.
18. Jacobs CR, Rapoport BL, Cohen GL, et al. Abstract CT143: pembrolizumab bioavailability after subcutaneous administration: analysis from the KEYNOTE-555 Cohort A in metastatic melanoma. *Cancer Res* 2021; 81(13_Suppl): CT143.
19. Decout A, Katz JD, Venkatraman S, et al. The cGAS-STING pathway as a therapeutic target in inflammatory diseases. *Nat Rev Immunol* 2021; 21(9): 548–569.
20. Wu YT, Fang Y, Wei Q, et al. Tumor-targeted delivery of a STING agonist improves cancer immunotherapy. *Proc Natl Acad Sci U S A* 2022; 119(49): e2214278119.
21. Yan XQ, Ye MJ, Zou Q, et al. Toripalimab plus axitinib versus sunitinib as first-line treatment for advanced renal cell carcinoma: RENOTORCH, a randomized, open-label, phase III study. *Ann Oncol* 2024; 35(2): 190–199.
22. Cipriani B, Miller D, Naylor A, et al. Abstract 2162: inhibition of GPR65 counteracts low pH induced immunosuppressive polarization of macrophages: in vitro and in vivo characterization of potent, selective and orally bioavailable small molecule GPR65 antagonists. *Cancer Res* 2022; 82(12_Suppl.): 2162.
23. Békés M, Langley DR and Crews CM. PROTAC targeted protein degraders: the past is prologue. *Nat Rev Drug Discov* 2022; 21(3): 181–200.
24. Dragovich PS. Degrader-antibody conjugates. *Chem Soc Rev* 2022; 51(10): 3886–3897.
25. Rej RK, Allu SR, Roy J, et al. Orally bioavailable proteolysis-targeting chimeras: an innovative approach in the golden era of discovering small-molecule cancer drugs. *Pharmaceuticals* 2024; 17: 494.
26. Palacino J, Bai C, Yi Y, et al. Abstract 3933: ORM-5029: a first-in-class targeted protein degradation therapy using antibody neodegrader conjugate (AnDC) for HER2-expressing breast cancer. *Cancer Res* 2022; 82(12_Suppl.): 3933–3933.
27. Lambert JM and Berkenblit A. Antibody–drug conjugates for cancer treatment. *Annu Rev Med* 2018; 69: 191–207.
28. Maneiro M, Forte N, Shchepinova MM, et al. Antibody-PROTAC conjugates enable HER2-dependent targeted protein degradation of BRD4. *ACS Chem Biol* 2020; 15(6): 1306–1312.
29. Zhouravleva G, Frolova L, Le Goff X, et al. Termination of translation in eukaryotes is governed by two interacting polypeptide chain release factors, eRF1 and eRF3. *EMBO J* 1995; 14(16): 4065–4072.
30. Long X, Zhao L, Li G, et al. Identification of GSPT1 as prognostic biomarker and promoter of malignant colon cancer cell phenotypes via the GSK-3β/CyclinD1 pathway. *Aging* 2021; 13(7): 10354–10368.
31. Matyskiela ME, Lu G, Ito T, et al. A novel cereblon modulator recruits GSPT1 to the CRL4(CRBN) ubiquitin ligase. *Nature* 2016; 535(7611): 252–257.
32. Malta-Vacas J, Aires C, Costa P, et al. Differential expression of the eukaryotic release factor 3 (eRF3/GSPT1) according to gastric cancer histological types. *J Clin Pathol* 2005; 58(6): 621–625.
33. Li JJ, Lu ZL, Kou WR, et al. Long-term effects of Xuezhikang on blood pressure in hypertensive patients with previous myocardial infarction: data from the Chinese Coronary Secondary Prevention Study (CCSPS). *Clin Exp Hypertens* 2010; 32(8): 491–498.
34. Miri M, Hemati S, Safari F, et al. GGCn polymorphism of eRF3a/GSPT1 gene and breast cancer susceptibility. *Med Oncol* 2012; 29(3): 1581–1585.
35. Hurvitz SA, Hamilton EP, Spira AI, et al. A phase 1, first-in-human, open label, escalation and expansion study of ORM-5029, a highly potent GSPT1 degrader targeting HER2, in patients with HER2-expressing advanced solid tumors. *J Clin Oncol* 2023; 41(16_Suppl): TPS1114.

36. Mei X, Chen J, Wang J, et al. Immunotoxins: targeted toxin delivery for cancer therapy. *Pharmaceutical Fronts* 2019; 01(01): e33–e45.

37. Wayne AS, FitzGerald DJ, Kreitman RJ, et al. Immunotoxins for leukemia. *Blood* 2014; 123(16): 2470–2477.

38. Akbari B, Farajnia S, Ahdi Khosroshahi S, et al. Immunotoxins in cancer therapy: review and update. *Int Rev Immunol* 2017; 36(4): 207–219.

39. Li M, Dyda F, Benhar I, et al. Crystal structure of the catalytic domain of *Pseudomonas* exotoxin A complexed with a nicotinamide adenine dinucleotide analog: implications for the activation process and for ADP ribosylation. *Proc Natl Acad Sci U S A* 1996; 93(14): 6902–6906.

40. Qin S, Xiao W, Zhou C, et al. *Pseudomonas aeruginosa*: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduct Target Ther* 2022; 7(1): 1–27.

41. Kreitman RJ, Dearden C, Zinzani PL, et al. Moxetumomab pasudotox in relapsed/refractory hairy cell leukemia. *Leukemia* 2018; 32(8): 1768–1777.

42. Hairy cell leukemia treatment approved. *Cancer Discov* 2018; 8(11): OF1.

43. Hagerty BL, Pegna GJ, Xu J, et al. Mesothelin-targeted recombinant immunotoxins for solid tumors. *Biomolecules* 2020; 10(7): 1–18.

44. Hassan R, Miller AC, Sharon E, et al. Major cancer regressions in mesothelioma after treatment with an anti-mesothelin immunotoxin and immune suppression. *Sci Transl Med* 2013; 5(208): 208ra147.

45. MacDonald GC, Rasamoelisolo M, Entwistle J, et al. A phase I clinical study of intratumorally administered VB4-845, an anti-epithelial cell adhesion molecule recombinant fusion protein, in patients with squamous cell carcinoma of the head and neck. *Med Oncol* 2009; 26(3): 257–264.

46. Azemar M, Djahansouzi S, Jäger E, et al. Regression of cutaneous tumor lesions in patients intratumorally injected with a recombinant single-chain antibody-toxin targeted to ErbB2/HER2. *Breast Cancer Res Treat* 2003; 82(3): 155–164.

47. Zhang J, Liu J, Yue Y, et al. The immunotoxin targeting PRLR increases tamoxifen sensitivity and enhances the efficacy of chemotherapy in breast cancer. *J Exp Clin Cancer Res* 2024; 43(1): 1–18.

48. Holmes RK. Biology and molecular epidemiology of diphtheria toxin and the tox gene. *J Infect Dis* 2000; 181(Suppl. 1): S156–S167.

49. Shapira A and Benhar I. Toxin-based therapeutic approaches. *Toxins (Basel)* 2010; 2(11): 2519–2583.

50. Yamaizumi M, Mekada E, Uchida T, et al. One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell. *Cell* 1978; 15(1): 245–250.

51. Dosio F, Stella B, Cerioni S, et al. Advances in anticancer antibody–drug conjugates and immunotoxins. *Recent Pat Anticancer Drug Discov* 2014; 9(1): 35–65.

52. Mahmoudi R, Dianat-Moghadam H, Poorebrahim M, et al. Recombinant immunotoxins development for HER2-based targeted cancer therapies. *Cancer Cell Int* 2021; 21(1): 470.

53. Teicher BA and Fricker SP. CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clin Cancer Res* 2010; 16(11): 2927–2931.

54. Vallera DA, Chen H, Sicheneder AR, et al. Genetic alteration of a bispecific ligand-directed toxin targeting human CD19 and CD22 receptors resulting in improved efficacy against systemic B cell malignancy. *Leuk Res* 2009; 33(9): 1233–1242.

55. Jogam P, Sandhya D, Alok A, et al. A review on CRISPR/Cas-based epigenetic regulation in plants. *Int J Biol Macromol* 2022; 219: 1261–1271.

56. Ning D, Xue J, Lou X, et al. Transforming toxins into treatments: the revolutionary role of α-amanitin in cancer therapy. *Arch Toxicol* 2024; 98(6): 1705–1716.

57. Moldenhauer G, Salnikov AV, Lüttgau S, et al. Therapeutic potential of amanitin-conjugated anti-epithelial cell adhesion molecule monoclonal antibody against pancreatic carcinoma. *J Natl Cancer Inst* 2012; 104(8): 622–634.

58. Nawrocka D, Krzyscik MA, Sluzalska KD, et al. Dual-warhead conjugate based on fibroblast growth factor 2 dimer loaded with α-amanitin and monomethyl auristatin E exhibits superior cytotoxicity towards cancer cells overproducing fibroblast growth factor receptor 1. *Int J Mol Sci* 2023; 24(12): 10143.

59. Figueroa-Vazquez V, Ko J, Breunig C, et al. HDP-101, an anti-BCMA antibody–drug conjugate, safely delivers amanitin to induce cell death in proliferating and resting multiple myeloma cells. *Mol Cancer Ther* 2021; 20(2): 367–378.

60. Xiaoqian N, Liu Y, Chen Y, et al. WTX212, an erythrocyte-anti-PD1 antibody conjugate, to demonstrate anti-tumor activities in tumor

models and patients with cancer with acquired resistance to immunotherapy. *J Clin Oncol* 2023; 41(16_suppl): e14529.

61. Nie X, Yang L, Liu Y, et al. Erythrocyte-αPD-1 conjugates overcome resistance to checkpoint blockade immunotherapy: a first-in-human study. *J Clin Oncol* 2024; 42(16_suppl): 2557.
62. BMS986288 | BMS-986288. *ADC Review*, https://www.adcreview.com/drugmap/bms-986288/ (2024, accessed 16 July 2024).
63. Jhatakia A, Nasser M, Mukhopadhyay A, et al. Abstract 1351: preclinical characterization of BMS-986288, a novel non-fucosylated (NF) anti-cytotoxic T lymphocyte antigen-4 (anti-CTLA-4) Probody® therapeutic. *Cancer Res* 2024; 84(6_Suppl.): 1351.
64. Calderón-Montaño JM, Burgos-Morón E, Orta ML, et al. Evaluating the cancer therapeutic potential of cardiac glycosides. *Biomed Res Int* 2014; 2014(1): 794930.
65. Ivanov AV, Valuev-Elliston VT, Tyurina DA, et al. Oxidative stress, a trigger of hepatitis C and B virus-induced liver carcinogenesis. *Oncotarget* 2017; 8(3): 3895–3932.
66. Prudent JR, Hall CA, Marshall DJ, et al. Abstract 2964: an anti-CD20 extracellular antibody–drug conjugate for the treatment of B-cell malignancies. *Cancer Res* 2016; 76(14_Suppl.): 2964–2964.
67. Wang RE, Liu T, Wang Y, et al. An immunosuppressive antibody–drug conjugate. *J Am Chem Soc* 2015; 137(9): 3229–3232.
68. Wang B, Wu H, Hu C, et al. An overview of kinase downregulators and recent advances in discovery approaches. *Signal Transduct Target Ther* 2021; 6(1): 1–19.
69. Yallapu M, Gonzalez D, Conlan RS, et al. An overview of the development and preclinical evaluation of antibody–drug conjugates for non-oncological applications. *Pharmaceutics* 2023; 15: 1807.
70. Parakh S, Lee ST, Gan HK, et al. Radiolabeled antibodies for cancer imaging and therapy. *Cancers* (*Basel*) 2022; 14(6): 1454.
71. Lapi SE, Scott PJH, Scott AM, et al. Recent advances and impending challenges for the radiopharmaceutical sciences in oncology. *Lancet Oncol* 2024; 25(6): e236–e249.
72. Sgouros G, Bodei L, McDevitt MR, et al. Radiopharmaceutical therapy in cancer: clinical advances and challenges. *Nat Rev Drug Discov* 2020; 19(9): 589–608.
73. Kassis AI. Therapeutic radionuclides: biophysical and radiobiologic principles. *Semin Nucl Med* 2008; 38(5): 358–366.
74. Jang A, Kendi AT, Johnson GB, et al. Targeted alpha-particle therapy: a review of current trials. *Int J Mol Sci* 2023; 24(14): 11626.
75. Durando M, Gopal AK, Tuscano J, et al. A systematic review of clinical applications of anti-CD20 radioimmunotherapy for lymphoma. *Oncologist* 2024; 29(4): 278–288.
76. Parker C, Nilsson S, Heinrich D, et al.; ALSYMPCA Investigators. Alpha emitter radium-223 and survival in metastatic prostate cancer. *N Engl J Med* 2013; 369(3): 213–223.