Chapter 1

Antibody–Drug Conjugate (ADC) Clinical Pipeline: A Review

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Abstract

Biological therapies play an increasing role in cancer treatment, although the number of naked antibodies showing clinical efficacy as single agent remains limited. One way to enhance therapeutic potential of antibodies is to conjugate them to small molecule drugs. This combination is expected to bring together the benefits of highly potent drugs on the one hand and selective binders of specific tumor antigens on the other hand. However, designing an ADC is more complex than a simple meccano game, requiring thoughtful combination of antibody, linker, and drugs in the context of a target and a defined cancer indication. Lessons learned from the first-generation antibody–drug conjugate (ADC) and improvement of the technology guided the design of improved compounds which are now in clinical trials. Brentuximab vedotin (Adcetris®), an anti-CD30 antibody conjugated to a potent microtubule inhibitor for the treatment of Hodgkin's lymphoma and anaplastic large cell lymphomas, is the only marketed ADC today. A total of 27 ADC are currently undergoing clinical trials in both hematological malignancies and solid tumor indications. Among them, T-DM1 (trastuzumab emtansine), an ADC comprised of trastuzumab conjugated to DM1, via a non-cleavable linker, is showing very promising results in phase III for the treatment of HER2-positive refractory/relapsed metastatic breast cancer. Other compounds, such as CMC-544, SAR3419, CDX-011, PSMA-ADC, BT-062, and IMGN901 currently in clinical trials, targeting varied antigens and bearing different linker and drugs, contribute to the learning curve of ADC, as do the discontinued ADC. Current challenges include improvement of the therapeutic index, linked to a careful selection of the targets, a better understanding of ADC mechanism of action, the management and understanding of ADC off-target toxicities, as well as the selection of appropriate clinical settings (patient selection, dosing regimen) where these molecules can bring highest clinical benefit.

Key words Antibody–drug conjugate, Cancer, Cytotoxic, Linker, Antibody, Maytansine, Auristatin, Calicheamicin, T-DM1, SGN-35, CMC-544

1 Introduction

Decades of intensive research in oncology have been devoted to find drugs able to fight cancer and improve patient's life. Nowadays, cancer biologics (antibodies, peptides, and proteins) play an increasing role in the arsenal of therapeutic molecules, usually in combination with radiotherapy or chemotherapy. Despite clear

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advantages of antibodies compared to small molecules in terms of (a) exquisite selectivity towards antigen-positive cells, leading to decreased off-target toxicity and (b) long half-life, only 13 therapeutic antibodies are marketed today for the treatment of cancer [1], highlighting the difficulty to identify targets whose modulation will impact tumor growth as well as the difficulty to identify antibodies with clinical efficacy as single agent. Arming antibodies or antibody fragments with toxins, cytotoxic drugs, and radionuclides can be viewed as a means of enhancing tumor-cell killing while sparing normal cells. Several of such armed molecules are marketed, namely, denileukin diftitox (Ontak®), an engineered protein combining interleukin-2 (which binds to IL2R) and Diphteria toxin, for the treatment of persistent or recurrent cutaneous T cell lymphoma, ibritumomab tiuxetan (Zevalin®), and ^{131}I-tositumomab (Bexxar®), two murine anti-CD20 antibodies conjugated to ^{90}Y and ^{131}I, respectively, for the treatment of relapsed/refractory follicular lymphoma, as well as the antibody–drug conjugate (ADC) brentuximab vedotin (Adcetris®), an anti-CD30 antibody conjugated to a potent microtubule inhibitor for the treatment of Hodgkin's lymphoma and anaplastic large cell lymphomas.

The concept of arming antibodies is not recent, as the use of ADC in animal models was already described in the literature in the 1970s, and clinical trials with murine IgG-based ADC were conducted in the 1980s, although with limited success. This is only in 2000 that the first ADC, gemtuzumab ozogamicin (Mylotarg®), an anti-CD33 antibody conjugated to calicheamicin (a very potent DNA binding drug), was approved in the USA for the treatment of acute myelocytic leukemia (AML), based on clear evidence of blast decrease in patient bone marrows [2, 3]. In 2010, the product was withdrawn from the market by the developer, Pfizer, following interim results from post-approval study (SWOG S0106), because of serious concerns about product's safety and failure to demonstrate clinical benefit [4].

This review will focus on ADC which are undergoing clinical trials (cf. Table 1). Lessons learned from first-generation ADC and improvement of the technology, both described in the first section, guided the design of improved compounds which are currently at different stages of clinical development. Adcetris® and the most advanced ADC in clinical trials will be described in a second section. The third section covers explored areas of improvement based on a thorough understanding of key parameters for ADC safety and efficacy retrieved from preclinical and clinical trials. The growing number of ADC in the clinic reflects the interest and confidence of clinicians and pharmaceutical companies that this approach can bring high benefit to cancer patients.

Table 1
ADC in clinical trials and launched

Drug names	Company	MAb mode	Target	Drug	Linker	Highest phase[a]	Indications
Adcetris® Brentuximab vedotin SGN-35	Seattle Genetics/ Takeda (Millenium)	Chimeric	CD30	Auristatin (MMAE)	vc	Launched 8.2011	Hodgkin's lymphoma ALCL
Inotuzumab ozogamicin CMC-544	UCB (Celltech) Pfizer	Humanized	CD22	Calicheamicin	Hydrazone AcBut	Phase 3 04.2011	ALL NHL (DLBCL)
T-DM1 Trastuzumab emtansine	Roche (Genentech) ImmunoGen	Humanized	Her-2	Maytansine (DM1)	SMCC	Phase 3 3.2009	Her2+ breast Gastric
Glembatumumab-vedotin CDX-011 CR-011-vcMMAE	Celldex (Curagen) Amgen (Abgenix)	Fully human	GPNMB (osteoactivin)	Auristatin (MMAE)	vc	Phase 2 4.2008	Breast Melanoma
IMGN-901 Lorvotuzumab mertansine	ImmunoGen	Humanized	CD56	Maytansine (DM1)	SPP	Phase 2 3.2012 SCLC	MCC, SCLC
SAR3419 HuB4-DM4	ImmunoGen/Sanofi	Humanized	CD19	Maytansine (DM4)	SPDB	Phase 2 9.2011	NHL, ALL
BT-062	Biotest	Chimerized	CD138 (Syndecan1)	Maytansine (DM4)	SPDB	Phase 1/2 8.2010	MM Solid tumors
IMMU-110 Milatuzumab-doxorubicin	Immunomedics	Humanized	CD74	Doxorubicin	Hydrazone	Phase 1/2 6.2010	Multiple myeloma
AGS-16M8F AGS-6MF	Astellas (Agensys)	Fully human	ENPP3	Auristatin (MMAF)	mc	Phase 1 8.2010	RCC

(continued)

Table 1
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Drug names	Company	MAb mode	Target	Drug	Linker	Highest phase[a]	Indications
AGS-22M6E ASG-22ME	Astellas (Agensys)	Fully human	Nectin-4	Auristatin (MMAE)	vc	Phase 1 5.2011	Solid tumors
AMG-172	Amgen	nd	nd	nd	nd	Phase 1 12.2011	Renal cancer
AMG-595	Amgen	Fully human	EGFRvIII	Maytansinoid	Non-cleavable	Phase 1 3.2012	Glioma
ASG-5ME AGS-5M2E	Astellas (Agensys)	Fully human	SLC44A4	Auristatin (MMAE)	vc	Phase 1 7.2010	Pancreas Prostate
BAY 94-9343	Bayer MorphoSys	Fully human	mesothelin	Maytansine (DM4)	SPDB	Phase 1 9.2011	Solid tumors
DEDN-6526A Anti-ETBR-vc-E	Roche (Genentech)	Humanized	ET8R (endothelin B)	Auristatin (MMAE)	vc	Phase 1 3.2012	Melanoma
IMGN 529 K7153A-SMCC-DM1	ImmunoGen	Humanized	CD37	Maytansine DM1	SMCC	Phase 1 2.2012	NHL
IMMU-130 hMN14-SN38	Immunomedics	Humanized	CEACAM5	SN-38	CL2	Phase 1 8.2011	Breast, colorectal, lung
MDX-1203	BMS (Medarex)	Fully human	CD70	MGBA	vc	Phase 1 7.2009	B-NHL ccRCC
PSMA-ADC PSMA-ADC-1301	Progenics	Fully human	PSMA	Auristatin (MMAE)	vc	Phase 1 9.2008	Prostate

RG-7450 DSTP-3086S	Roche (Genentech)	nd	nd	Auristatin	nd	Phase 1 3.2011	Prostate (CRPC)
RG-7458	Roche (Genentech)	nd	MUC16 (CA125)	Auristatin	nd	Phase 1 4.2011	Ovary
RG-7593 DCDT-2980S	Roche (Genentech)	Humanized	CD22	Auristatin (MMAE)	vc	Phase 1 10.2010	NHL
RG-7596 DCDS-4501A	Roche (Genentech)	nd	CD79b	Auristatin	nd	Phase 1 3.2011	CLL, NHL
RG-7598	Roche (Genentech)	nd	nd	Auristatin	nd	Phase 1 9.2011	MM
RG-7599	Roche (Genentech)	nd	MUC16 (CA125)	Auristatin	nd	Phase 1 7.2011	Ovary Lung (NSCLC)
RG-7600	Roche (Genentech)	nd	nd	Auristatin	nd	Phase 1 12.2011	Ovary Pancreas
SAR-566658 huDS6-DM4	ImmunoGen Sanofi	Humanized	Muc1 (CA6)	Maytansine (DM4)	SPDB	Phase 1 9.2010	Solid tumors
SGN-75 h1F6-vcMMAF	Seattle Genetics	Humanized	CD70	Auristatin (MMAF)	vc	Phase 1 11.2009	NHL RCC

[a]Current highest phase for cancer/first date/first indication
nd not disclosed

2 ADC Building Blocks

2.1 Definition of an ADC

An ADC can be defined as a prodrug. The antibody connected to the cytotoxic warhead (drug) via a linker serves as targeted delivery system to the tumor expressing the antigen/target recognized by the antibody. Ideally, in blood, after systemic administration, this prodrug is nontoxic. Upon binding of the antibody to the targeted tumor antigen and internalization of the complex into the cancer cell, the drug is then released in its active form and in sufficient quantity to kill the cell.

Designing an ideal ADC is more complex than a simple meccano game. On top of the careful choice of a target/antigen expressed in specific tumor indication, it requires finding the best combination between the antibody, the linker, and the drug, which, besides its own characteristics and constraints, are linked and impact each other.

2.2 Target/Antigen for ADC

The target/antigen is the starting point to build an ADC. It first determines which tumor indication will be targeted by the ADC and potentially impacts the choice of the conjugated drug. In addition, the target will also drive the criteria which will be defined for the selection of the targeted patient population within the tumor indication.

Many targets have been evaluated for an ADC approach across the years (for a review, *see* ref. 5), showing that a high variety of targets, either single or multiple transmembrane domains proteins or glycosylphosphatidylinositol (GPI)-anchored, can lead to ADC internalization and subsequent tumor growth delay and regression in preclinical mouse models.

The basis for the selection of the antigen is a high expression level in tumor tissues and a restricted normal tissue distribution, in order to limit on-target toxicity of the future ADC. However, tumor-specific antigens with no expression in normal tissues are rare, and most of the time, the antigen is expressed at the surface of epithelial cells in a subset of normal tissues/organs. The type of organ expressing the antigen (vital organs vs. reproductive organs, for example), the cellular subtype and cell-cycle status (dividing cells vs. differentiated quiescent cells), and the differential of expression between normal antigen-positive cells and tumor cells are to be considered for selection of the target.

It is important to notice that expression in normal organs may not always mean subsequent toxicity in clinical trials. Several ADC with normal tissue cross-reactivity have been well tolerated in patients, causing minimal or manageable and reversible toxicities, namely, cantuzumab mertansine/IMGN242 (targeting CanAg antigen, a glycotope on Mucin-like protein [6, 7]), BT-062 (targeting CD138; see below), or CDX-011 (targeting gpNMB;

Table 2
Discontinued ADC

Product name	Target name	Drug/linker	Reasons for discontinuation	Year	References
BAY79-4620	CAIX	MMAE/vc	Not disclosed	2011	Press release
IMGN388	Integrinαvβ3	DM4/SPDB	Change in business strategy	2011	Press release
MEDI547	EphA2	MMAF/mc	Safety issues: bleeding and coagulation events	2012	[122]
Mylotarg	CD33	Calicheamicin/ hydrazone	Failure to demonstrate clinical benefit	2010	[4]
BIIB015	Crypto1	DM4/SPDB	Not disclosed	2010	
IMGN242	CanAg	DM4/SPDB	Not disclosed	2009	Press release
AVE9633	CD33	DM4/SPDB	Lack of clinical efficacy	2008	[12]
MLN2704	PSMA	DM1/SPP	Not disclosed	2006	[94, 123], Press release
CMD-193	Le^Y carbohydrate	Calicheamicin/ hydrazone	Not disclosed	2006	ClinicalTrials.gov
Bivatuzumab mertansine	CD44v6	DM1/SPP	Safety issues: fatal case of toxic epidermal necrolysis	2006	[9, 11]
SGN-15	Le^Y carbohydrate	Doxorubicin/ hydrazone	Change in business strategy	2005	Press release
CMB-401	MUC1	Calicheamicin/ hydrazone	Lack of clinical efficacy	1999	[124, 125]

see below). Inversely it has clearly been demonstrated in the case of bivatuzumab mertansine (targeting CD44v6), whose trial was prematurely stopped in phase I (cf. Table 2), that the expression of the CD44v6 target in skin keratinocytes [8] led to severe skin toxicity, including a fatal case of toxic epidermal necrolysis [9–11].

While expression of the target should remain limited and at low level in normal tissues, on the contrary, the level of expression (antigen density) at the surface of cancer cells should be high and combined to the ability of the antigen/antibody complex to internalize and be processed in the right subcellular compartments, in order to release enough quantity of the active drug in the cytosol. The use of tumor models mimicking the target expression pattern and level found in patient biopsies is a very critical element to translate preclinical data into clinical efficacy. AVE9633, an immunoconjugate targeting CD33 antigen, did not show clinical efficacy in phase I [12] in part because of too limited antigen expression on the malignant cell population, suggesting an insufficient delivery of

molecules in the cytoplasm to achieve cell death. In contrast, preclinical models showed good response to AVE9633 [13] but displayed a much higher CD33 antigen level than the one measured in patient biopsies (unpublished internal data, sanofi, 2009).

2.3 Drugs and Linkers

Many conventional therapeutic agents have been conjugated to antibodies, but it soon became clear that they were not potent enough, when conjugated, to achieve antitumor activity in the clinic [14–16]. Efforts have then been turned towards natural small cytotoxic molecules with higher potency but which have been found too toxic as free drug in clinical trials. Currently, only few highly potent natural cytotoxics, derivatives, or synthetic analogues have been conjugated to antibodies and progressed to the clinic. They fall into the following two classes: microtubule destabilizing agents (auristatin derivatives, MMAE and MMAF and maytansinoid derivatives, DM1 and DM4) and DNA minor groove binders (calicheamicin and duocarmycin derivatives). Both classes are extremely potent towards proliferating tumor cell lines [16]. IC_{50} of proliferation/viability of tumor cell lines are in the range of 10^{-10}–10^{-12} M for DM1/DM4 maytansinoid derivatives [17, 18], 10^{-7}–10^{-10} M for MMAF/MMAE auristatin derivatives [19], around 10^{-10} M for *N*-acetyl-γ calicheamicin DMH [20], and 10^{-11}–10^{-12} M for DC1 and CC-1065 duocarmycin precursors [14, 21].

Importantly, the engineered linker connecting the cytotoxic molecule to the antibody has been deeply studied as it is considered to be an important parameter for preclinical, clinical efficacy and safety of ADC: linkers must be stable enough in circulation since release of the cytotoxic payload may be associated with undesired and untargeted toxicities, but they must also be able to efficiently release cytotoxics in their active form in the cytosol of the target cell following internalization and trafficking in specific subcellular compartments [16, 22, 23]. Indeed, upon binding of the ADC to its target, and subsequent internalization of the antigen/ADC complex by receptor-mediated endocytosis, the ADC is trafficked in acidifying endosomal and then in lysosomal vesicles, a compartment rich in proteolytic enzymes. Due to the chemical environment or to the metabolic properties of these intracellular compartments, the ADC is activated/metabolized. This metabolization depends on the type of linker connected to the drug:

– The acid labile hydrazone linkers are relatively stable at neutral pH (pH 7.3–7.5, pH of the bloodstream) but undergo hydrolysis once the ADC is internalized into acidic endosomes (pH 5–6.5) and lysosomes (pH 4.5–5). They have been conjugated to doxorubicin, calicheamicin, and auristatin. Their relative stability depends on the antibody part attached, but they have

been associated with high nonspecific release of the drug in circulation in preclinical studies [24].

- The disulfide-based linkers have been combined with DM1 and DM4 maytansinoids. The corresponding ADC is activated by lysosomal degradation of the antibody part, resulting in metabolites consisting of intact maytansinoid drug and linker attached to lysines [23, 25]. Linkers are subsequently reduced with more or less efficiency, depending on the level of steric hindrance at carbon atoms adjacent to the disulfide linkage, optimized linkers being the best compromise between high ADC plasma stability and efficient metabolization/release of the metabolites in tumor cells [26].
- The peptide-based linkers, already used for a number of years with doxorubicin, mitomycin C, camptothecin, and talysomycin [16], have been designed for the auristatin and the duocarmycin derivatives. The type of linker which has been progressed to clinical stage is composed of a valine–citrulline dipeptide selectively hydrolized by cathepsin B and plasmin enzymes, a self immolative spacer that spatially separates the drug from the site of enzymatic cleavage, and the auristatin E microtubule disruptive agent or duocarmycin prodrug derivative. In the case of an auristatin E conjugate, the membrane-permeable monomethyl auristatin E accounts for the only detectable metabolite found in antigen-positive cells [27].
- Contrary to the above linker types, which are considered as "cleavable," thioether bond containing linkers are considered as "non-cleavable," and the corresponding ADC have been clinically tested with DM1 and MMAF cytotoxics. In this case, the degradation of the mAb component into the lysosomes releases the drug still attached to the linker via a Lys or Cys residue of the antibody. These charged entities are not able to cross membranes with high efficiency, by contrast to metabolites of maytansine and auristatin ADC conjugated to "cleavable" linkers. In this case, the diffusion of metabolites induces killing of surrounding cells, a property named "bystander effect" [27–29].

2.4 Antibody Selection

All ADC currently in oncology clinical trials are canonical (i.e., full length) IgG molecules, mostly of the IgG1 isotype. They are either chimeric, humanized, or fully human antibodies (cf. Table 1). The generation of an immune response to these ADC has remained very limited, highlighting the benefit of antibody engineering technologies over the last decades, as well as the fact that small molecule cytotoxics, contrary to natural toxins, are not immunogenic.

Attention has also been focused on drug conjugation technologies on the selected antibodies. On top of the fact that drug

conjugation should not disturb antigen/antibody interaction, the localization, the number, and the nature of the attachment between linker and antibody have been shown to influence pharmacokinetics, tumor exposure, and ADC plasma stability [30, 31]. So far, the two conjugation technologies which progressed to clinical trials are based on the following two principles: either conjugation through Lysine side chain amines (with drugs such as DM1, DM4, or calicheamicin) or conjugation through cysteine sulfhydryl groups activated by reducing interchain disulfide bonds (with drugs such as MMAE, MMAF, or duocarmycin) of the antibody. Both processes give more or less heterogeneous mixtures of ADC with variable drug load per antibody and variable sites of conjugation to the protein. This heterogeneous mixture is defined by an average drug–antibody ratio (DAR) and is challenging from a development point of view, although robust analytical technologies and processes are available to ensure constant quality control of the final product [32].

3 Current Clinical Results of Antibody–Drug Conjugates

A total of 27 ADC are currently in clinical trials, 20 in phase I, 5 in phase II, 2 in phase III, and 1 launched ADC (cf. Table 1). A total of 12 ADC have been stopped and are listed in Table 2.

3.1 Brentuximab Vedotin (Adcetris®) Clinical Overview

CD30, a type II transmembrane protein belonging to the TNF (tumor necrosis factor) superfamily, is abundantly and selectively expressed on the surface of Hodgkin's lymphoma (HL), Reed–Sternberg (RS) cells, anaplastic large cell lymphomas (ALCL), and other lymphoid malignancies as well as on several nonlymphoid malignancies [33]. RS cells and ALCL cells express high levels of CD30, but the downstream signalling of CD30 may differ between both diseases [34, 35]. In non-pathological conditions, CD30 expression is highly regulated and restricted to activated B and T lymphocytes and NK cells, low expression being also noticed in monocytes and eosinophils (for review, *see* refs. 34, 36), making it a good candidate target for an ADC strategy.

HL is considered as one of the most curable cancers, with a 5-year survival rate of above 85 % although up to 20 % of patients are refractory and advanced-stage patients often relapse [37]. In frontline systemic ALCL treatment, disease recurs in 40–65 % of patients [38].

Clinical trials have been reported for unconjugated anti-CD30 antibodies [39]. Acceptable safety profile but modest antitumor clinical activity precluded further development as naked but supported exploration and development of a conjugated version: SGN-35. SGN-35 (Adcetris®, brentuximab vedotin) is an ADC comprised of a chimeric anti-CD30 antibody (cAC10) conjugated

through interchain disulfide bonds to monomethyl auristatin E (MMAE) via a valine–citrulline dipeptide cleavable linker, with an average DAR of 4 [40].

Based on preclinical data showing good efficacy of SGN-35 at low doses in lymphoma models [40], a phase I study was conducted in 2006. Forty-five patients (42 HL, 3 ALCL) were enrolled, in a dose escalation study ranging from 0.2 to 3.6 mg/kg with intravenous (IV) administration once every 3 weeks (q3w) [41]. The maximum tolerated dose (MTD) was found to be 1.8 mg/kg, and drug-related dose-limiting toxicities (DLT) were febrile neutropenia and hyperglycemia. At the MTD, objective clinical responses were observed, with an objective response rate (ORR) of 38 %, including 4 complete responses (CR) and 2 partial responses (PR) out of 12 patients. In terms of pharmacokinetics (PK), terminal half-life of the ADC and MMAE, at 1.8 mg/kg, was estimated to be 4–6 and 3–4 days, respectively [41]. In a second phase I study, enrolling 44 patients, a more frequent regimen was investigated, at doses ranging from 0.4 to 1.4 mg/kg administered weekly for 3 out of 4 weeks, for a total of four cycles. The MTD was 1.2 mg/kg and the ORR was 59 %, with 34 % CR. Most common grade 3 adverse events (AE) were peripheral sensory neuropathy (14 %), anemia (9 %), neutropenia (7 %), peripheral motor neuropathy (7 %), and hyperglycemia, diarrhea, and vomiting (5 % each). Overall, 32 patients (73 %) experienced one or more events of peripheral neuropathy. Compared to the q3w schedule, there was a marked increase in neuropathy which led to the adoption of the q3w schedule for further clinical studies [42].

In a phase II study, 102 heavily pretreated relapsed or refractory HL patients were treated at the dose of 1.8 mg/kg in a q3w schedule [43]. The ORR was 75 % including 34 % CR and 40 % PR. The more severe AE were grade 3 neutropenia (14 %), peripheral sensory neuropathy (5 %), fatigue and hyperglycemia (3 % each), grade 4 hematological toxicities (neutropenia 4 %; thrombocytopenia 1 %), and pulmonary embolism and abdominal pain (1 % each). In a second phase II trial, 58 patients with relapsed systemic ALCL were treated with 1.8 mg/kg of with a q3w schedule [38]. The ORR was 86 % with 53 % achieving CR. Grade 3–4 AE were similar to the previous studies.

Based on these outstanding data, SGN-35 has been granted accelerated approval by the FDA in August 2011 for the treatment of HL that had relapsed after autologous stem cell transplant (ASCT) and for the management of relapsed ALCL, making it the first approved drug over 30 years in HL. In July 2012, a positive opinion was issued in the EU, recommending conditional marketing authorization for treatment of adults with relapsed or refractory CD30-positive HL following ASCT or following at least two prior therapies when ASCT or multi-agent chemotherapy is not a treatment option as well as for the treatment of adults with relapsed

or refractory systemic ALCL. SGN-35 is currently evaluated in a phase III randomized, double-blind, placebo-controlled study (AETHERA) in HL patients following autologous stem cell transplant [35]. Interim results show that 75 % of patients responded to the drug, including 34 and 40 % achieving CR and PR, respectively [44]. Future results of the AETHERA trial expected to be completed in June 2013 will form the basis for full FDA approval. Other trials are ongoing, including another phase III trial evaluating SGN-35 versus methotrexate or bexarotene in patients with CD30-positive cutaneous T cell lymphomas [44].

3.2 Trastuzumab-DM1 (T-DM1) Clinical Overview

ErbB2/neu/HER2 is a member of the ErbB receptor tyrosine kinase family which is involved in cell growth, survival, and differentiation [45]. Breast cancer accounts for 28 % of all new cases of cancer in women, and 15–25 % of these new cases contain gene amplification or overexpression of HER2 [46]. The humanized anti-HER2 monoclonal antibody trastuzumab (Herceptin®; Genentech), and the dual epidermal growth factor EGFR/HER2 tyrosine kinase inhibitor lapatinib (Tykerb®, GSK), in combination with chemotherapy, prolongs survival of HER2-positive breast cancer patients in metastatic and adjuvant settings. However, a significant portion of these patients relapse and finally die from their cancer, highlighting the need for new therapeutic approaches [47, 48].

T-DM1 (trastuzumab emtansine) is an ADC comprised of trastuzumab conjugated through lysines to DM1, via a non-cleavable thioether linker (*N*-succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate, SMCC), with an average DAR of 3.5 [49].

Preclinical studies of T-DM1 suggested that the ADC retained all activities of unconjugated trastuzumab, inhibition of PI3K/AKT signalling, inhibition of HER2 shedding, and Fcγ receptor engagement triggering ADCC [50]. Moreover, T-DM1 showed a strong growth inhibitory effect on trastuzumab-resistant breast cancer cell lines in vitro, as well as a significant inhibition of tumor growth when administered in trastuzumab and lapatinib resistant tumor-bearing mice [49, 51].

Four phase I/II studies evaluated T-DM1 as single agent for the treatment of HER2-positive refractory/relapsed metastatic breast cancer. In 2006, 24 patients were enrolled in a phase I dose escalation study, with doses ranging from 0.3 to 4.8 mg/kg, in a q3w schedule [52]. T-DM1 MTD was identified at 3.6 mg/kg without cardiotoxicity or neuropathy. Transient grade 4 thrombocytopenia was the most common adverse event and was defined as the DLT [52]. Encouraging antitumor activity was observed: out of 15 patients enrolled in the 3.6 mg/kg group, four had a confirmed objective partial response. One confirmed PR was also observed in the 2.4 mg/kg group [52]. A phase I weekly dosing [53] reported MTD at 2.4 mg/kg, with thrombocytopenia being also the DLT

and showing the same range of activity. Different phase II studies evaluated T-DM1 at 3.6 mg/kg, q3w (for review, *see* refs. 54, 56). In one study [57] a median of seven doses was administered to 112 patients with HER2-positive metastatic breast cancer previously treated with chemotherapy and progressed under trastuzumab therapy. The ORR evaluated by independent review was 25.9 %, all PR. Interestingly, in the group of tumors confirmed HER2-positive in a retrospective central testing by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH), the ORR was 33.8 % versus 4.8 % for the group of tumors with normal HER2 expression. The most common grade 3 or 4 AE were hypokalemia (8.9 %), thrombocytopenia (8.0 %), and fatigue (4.5 %). PK parameters showed a terminal half-life of T-DM1 of around 4 days, which was found to be lower than the total trastuzumab half-life. No accumulation of T-DM1 was reported [57]. In a second study, T-DM1 was administered in 110 patients with metastatic breast cancer previously treated with an anthracycline, a taxane, and capecitabine, as well as lapatinib and trastuzumab [58]. The ORR by independent review was 34.5 % without CR and again rose to 41.3 % for patients with tumors centrally confirmed for HER2-positivity (FISH and IHC) compared to 20 % in the patient group displaying HER2-normal expression levels. The most common grade 3 and 4 AE were thrombocytopenia (9.1 %), fatigue (4.5 %), and cellulitis (3.6 %). In the different studies, thrombocytopenia was one of the most reported grade 3 or 4 abnormalities, but the decrease in platelets was generally reversible and not associated with serious hemorrhage [56–58]. Increased serum concentrations of hepatic enzymes was observed [56]. T-DM1 exposure did not correlate with clinical responses, grade 3 thrombocytopenia or grade 3 increase in hepatic enzymes serum concentrations [59]. The comparison of pharmacokinetics data from phase I and phase II studies, as single agent, demonstrated a positive correlation between DM1 and T-DM1 exposure with neither accumulation of T-DM1 nor DM1 [59, 60]. At the MTD, T-DM1 showed a median terminal half-life of 4.5 days which is shorter than the one from total trastuzumab (around 9 days) [59, 60]. The PK profile of T-DM1 was not affected by circulating levels of HER2 or residual trastuzumab [59, 60]. On a total of 286 patients, 4.5 % developed an antibody response to T-DM1 but no impact on PK parameters, safety or efficacy profiles were observed [59].

Interestingly a randomized phase II study was conducted to compare T-DM1 versus trastuzumab plus docetaxel [55] in the first-line treatment of HER-2-positive locally advanced or metastatic breast cancer. A total of 137 patients, with no prior chemotherapy for metastatic disease, were randomized to T-DM1 (3.6 mg/kg, q3w) or trastuzumab (8 mg/kg first cycle, then 6 mg/kg) plus docetaxel (75 or 100 mg/m^2). Assessment by investigators showed equivalent ORR of 47.8 % with T-DM1 and

41.4 % with docetaxel plus trastuzumab [61] but with improved therapeutic ratio in the case of T-DM1. Primary efficacy and update on safety results were presented at ESMO 2011 [62] with a significant improvement of progression-free survival (PFS) in the T-DM1 population (14.2 months vs. 9.2 months) and a confirmed favorable safety profile with grade 3 AE reported less frequently in the T-DM1 arm (46.4 % vs. 89.4 %). The most frequent AE were also different between the two arms, with increased level of liver enzymes, fatigue and thrombocytopenia in the T-DM1 arm versus alopecia, neutropenia, fatigue, and diarrhea in the trastuzumab/docetaxel arm.

In addition, three phase III trials (EMILIA, MARIANNE and TH3RESA) are ongoing [55]. EMILIA is a randomized trial designed to evaluate the safety and efficacy of T-DM1 in comparison to lapatinib plus capecitabine in patients with HER-2 positive, unresectable, locally advanced breast cancer or metastatic breast cancer, following prior trastuzumab and taxane containing chemotherapies. Recent publication of the first and second interim analysis of the 991 patients enrolled indicates that T-DM1 significantly improves PFS (9.6 months vs. 6.4 months) and overall survival (OS) (30.9 months vs. 25.1 months) as compared to lapatinib plus capecitabine treatment [63]. In addition, as previously shown in phase II, the safety profile of T-DM1 was different and more favorable than lapatinib plus capecitabine, as shown by the reduced incidence of grade 3 and 4 AE (40.8 % vs. 57.0 %). Thrombocytopenia (12.9 %) and elevated AST (4.3 %) were the most commonly reported AE for T-DM1, while diarrhea (20.7 %), palmar–plantar erythrodysesthesia (16.4 %), vomiting (4.5 %), and neutropenia (4.3 %) were the ones reported for lapatinib plus capecitabine [63]. On the basis of this study, a Biologics License Application (BLA) was filed in August 2012. MARIANNE is a randomized trial of T-DM1 with or without pertuzumab compared with trastuzumab plus taxane for first-line treatment of HER2-positive, progressive, or recurrent locally advanced or metastatic breast cancer. TH3RESA is a randomized trial to evaluate the efficacy of T-DM1 compared with treatment of physician's choice in patients with HER2-positive metastatic breast cancer who have received at least two prior regimens of HER2-directed therapy.

3.3 CMC-544 (Inotuzumab Ozogamicin) Clinical Overview

CD22 is a glycoprotein belonging to the sialic-acid-binding immunoglobulin-like lectins (siglecs) expressed at the surface of normal immature and mature B cells but neither on hematopoietic stem cells nor on memory B cells. Its function is still unclear, but it is thought to be involved in cellular adhesion, B -cell homing, and B-cell activation [64]. CD22 has been shown to be rapidly internalized upon ligand binding, an attractive property supporting CD22 as target for ADC [65]. CD22 is expressed in more than 90 % of diffuse large B-cell non-Hodgkin lymphomas

(DLBCL) and follicular lymphomas (FL) [66]. It is also expressed in up to 100 % of mature B-cell acute lymphoblastic leukemia (B-ALL) [67].

CMC-544 (Inotuzumab ozogamicin) is an ADC comprised of a humanized anti-CD22 IgG4 monoclonal antibody (G544) conjugated through a cleavable acid-labile hydrazone linker to calicheamicin with an average DAR of 6 (73 μg calicheamicin/mg of antibody) [4]. G544 binds to CD22 with subnanomolar affinity and has no effector functions and no antitumor activity as naked monoclonal antibody [4].

Based on encouraging preclinical data [68], two phase I single agent studies were conducted in relapsed/refractory B-cell NHL. The first phase I enrolled 36 patients in the dose escalation phase with a q3w schedule and 43 patients in the expanded MTD cohort [69]. In the dose escalation phase, DLT were grade 4 thrombocytopenia and grade 4 neutropenia, and the MTD was declared 1.8 mg/m^2 (0.048 mg/kg) in a q4w schedule in order to allow platelets recovery. Among the 49 patients treated at the MTD, the common grade 3 or grade 4 AE were thrombocytopenia (63.3 %) and neutropenia (34.7 %). At MTD, the ORR was 68 % for FL and 15 % for aggressive DLBCL with CR observed [69]. Drug disposition for CMC-544 and total calicheamicin was nonlinear with dose or number of doses suggesting an accumulation of the drug, which could be explained by the decrease in CD22 target after the first dose [4]. After the first cycle, terminal half-life of CMC-544 at the MTD was 17.1 h, increasing to 34.7 h at the second cycle [69]. The second phase I dose escalation study was conducted in 13 Japanese patients with relapsed/refractory FL. The MTD was confirmed at 1.8 mg/m^2 q4w, with most common grade 3 and 4 AE being also thrombocytopenia (54 %) and neutropenia (31 %). The ORR was 80 %, CR included [70]. PK parameters were similar to what was observed previously.

Based on preclinical studies suggesting superior activities of CMC-544 with rituximab [71], several phases I/II studies have been initiated in recurrent/refractory FL or DLBCL [4, 66]. The MTD was determined at 375 mg/m^2 rituximab given on day 1 and 1.8 mg/m^2 CMC-544 given on day 2 every 28 days for four cycles [72, 73]. Pharmacokinetic and safety profile of CMC-544 were shown to be equivalent to single-agent, dose-limiting toxicities being again thrombocytopenia and neutropenia [66]. In one of the study [72, 74], enrolling 110 patients treated at the combination MTD, the ORR of relapsed FL and DLBCL were 84 and 80 %, respectively. Response to rituximab in prior treatment appeared to be a very strong prognostic of response to the combination as when rituximab-refractory patients were considered; the ORR was only 20 % [66, 72, 74]. A randomized open-label phase III trial is now recruiting, comparing rituximab plus CMC-544 to rituximab plus gemcitabine or bendamustine in relapsed/refractory aggressive B-cell NHL [4].

CMC5-44 was also explored in refractory/relapsed acute lymphoblastic leukemia (ALL) patients. The first published report evaluating CMC-544 at 1.8 mg/m^2 in a q3w schedule was promising, as the ORR was 56 % [75]. A phase II trial has therefore been undertaken in patients with refractory/relapsed ALL with the same dosing schedule [76]. A total of 49 patients were treated, with CD22 expressed in more than 50 % of blasts in all patients. The ORR was 57 % with 18 % complete marrow response of short duration and 39 % with no platelets or incomplete blood cell count recovery. Thrombocytopenia was, like in NHL, a notable adverse event, but based on the leukemia risk, treatment was not delayed. Grade 3–4 fever was the most common AE (31 %). Further clinical evaluation in ALL is ongoing with a weekly schedule [76].

3.4 Other ADC in Early Clinical Trials

Beside SGN-35, T-DM1, and CMC-544, 24 other ADC are currently being evaluated in phase I and II (cf. Table 1). The more advanced ones, for which efficacy data are available, are described below.

SAR3419: CD19 is a type I transmembrane glycoprotein of the immunoglobulin (Ig) superfamily, expressed from the earliest stages of pre-B-cell development until terminal B-cell differentiation into plasma cells. CD19 expression covers all types of B-lymphomas and non-T acute lymphoblastic leukemia, with moderate to high homogeneous expression [77]. SAR3419 is composed of a humanized IgG1 monoclonal anti-CD19 antibody, huB4, conjugated via a cleavable disulfide linker to DM4 (huB4-SPDB-DM4). In a first phase I study with refractory or relapsed B-cell NHL (R/R NHL) [78], SAR3419 was evaluated in a q3w schedule. The MTD was determined at 160 mg/m^2 (4.3 mg/kg), and the DLT was reversible severe blurred vision associated with microcystic epithelial corneal changes. Tumor reduction from baseline was observed in 74 % of patients bearing a variety of lymphoma subtypes including DLBCL. The ORR was of 23.5 % at MTD [78]. A second dose escalation study was performed with a weekly schedule, again in R/R NHL patients. The regimen consisting of 4 weekly doses of 55 mg/m^2 followed by four additional doses administered every 2 weeks showed a favorable safety profile and was therefore retained for further clinical studies. In particular there was no grade 3 or 4 ocular toxicity observed and hematotoxicity incidence was low, allowing potential combination of SAR3419 with other agents used to treat NHL. In addition, no dose-limiting cumulative side effects were observed in this cohort of 21 patients [79]. In this heavily pretreated patient population, antitumor activity with around 30 % ORR in both aggressive (e.g., DLBCL) and indolent (e.g., FL) subtypes of NHL was obtained. A phase II program in patients with R/R DLBCL is underway testing the drug as a single agent and also in combination with rituximab (NCT01472887 and NCT01470456, respectively) in order

to confirm the clinical benefit of SAR3419 in a more homogeneous population. Based on encouraging preclinical data, the activity of SAR3419 is also explored in adult patients with R/R ALL [80].

CDX-011 (glembatumumab vedotin): gpNMB (glycoprotein nonmetastatic melanoma protein b/osteoactivin) is a type I transmembrane glycoprotein identified in melanoma cell lines and shown to be expressed in several tumor indications including melanoma and breast [81, 82]. CDX-011 is an ADC comprising a fully human IgG2 anti-gpNMB antibody conjugated to MMAE via the cleavable protease-sensitive valine–citrulline linker [83]. A phase I/II was undertaken in 117 unresectable, stage III/IV melanoma patients treated with a q3w schedule, or with more frequent dosing regimens, q2/3w and weekly. In the q3w dose escalation, DLT were grade 3 rash and desquamation [83]. The MTD were 1.88, 1.5, and 1 mg/kg, respectively [84], with most common grade 3 or 4 AE being rash (20 %) and neutropenia (15 %) across the studies. At 1.88 mg/kg q3w, the half-life of CDX-011 was around 28 h and the half-life of the total antibody was 40 h [83, 85]. At MTD, the ORR of the q3w, q2/3w, and qw were 15 % (5/34), 33 % (2/6), and 29 % (2/7), respectively, and a clear correlation of skin rash with outcome was observed [83, 84]. Another phase I/II was completed in 42 locally advanced or metastatic breast cancers. Among the 34 patients, without preselection of gpNMB expression, treated at 1.88 mg/kg q3w, ORR was 13 % [81, 83, 86]. In the subgroup of patients expressing gpNMB, the ORR reached 29 %. A phase II clinical study with breast cancer patients expressing high gpNMB measured by IHC is ongoing [81]. It is interesting to note that one of the most common treatment-related toxicities with the melanoma and breast cancer studies were dermatologic events (pruritus, rash, alopecia). The AE could be linked to the expression of gpNMB in normal melanocytes [87].

PSMA-ADC (PSMA-vc-MMAE): PSMA (prostate-specific membrane antigen) is a type II transmembrane glycoprotein displaying carboxypeptidase activity and expressed mainly in normal prostate epithelium [88, 89]. PSMA has been shown to be highly expressed at the membrane of prostate cancer cells [90–92] providing a rationale for the design of PSMA-ADC. The PSMA-vc-MMAE ADC is a fully humanized IgG1 antibody, linked to MMAE via the cleavable valine–citrulline linker [93]. It is the second PSMA ADC to be evaluated in the clinic. The first one (PSMA-SPP-DM1/MLN2704) was stopped in 2008 (Table 2). Clinical data of MLN2704 showed low efficacy and limiting peripheral neuropathy [94]. A phase I, dose escalation trial with PSMA-vc-MMAE, is being conducted in men with taxane-refractory metastatic castration-resistant prostate cancer in a q3w schedule for up to four cycles [95, 96]. As of today 26 patients have been enrolled in a dose escalation study up to 2.0 mg/kg, and the MTD has not been reached [95]. Evidence for antitumor activity, as

reflected by declines in PSA, circulating tumor cells and/or bone pain, has been observed in 4 of 12 subjects treated at 1.6 or 1.8 mg/kg. Dose proportional increases in serum concentrations of PSMA ADC have been seen with half-life of around 50 h [96]. From the last EORTC update, dose escalation has been completed and 2.5 mg/kg has been identified as the MTD. DLT observed at 2.8 mg/kg were neutropenia and reversible liver function alteration [97].

BT-062: CD138 (Syndecan-1) is a member of the family of transmembrane heparan sulfate proteoglycans overexpressed in various solid tumors and hematological malignancies. In the normal human hematopoietic compartment, CD138 expression is restricted to plasma cells [98], and in malignant hematopoiesis, CD138 is expressed on the majority of multiple myeloma (MM) cells making it a good candidate antigen for this indication [99]. BT-062 is an antibody–drug conjugate, comprised of the anti-CD138 chimeric IgG4 antibody conjugated to DM4 via a cleavable disulfide linker. In a phase I trial enrolling a total of 32 MM patients, receiving 1 of 7 dose levels ranging from 0.27 to 5.4 mg/kg in a q3w schedule, the MTD was defined at 4.3 mg/kg, with mucositis and palmar–plantar erythrodysesthesia syndrome being the DLTs [100]. Mucositis side effect could be correlated with the target expression observed in stratified squamous epithelium (mucosa) of the esophagus [99]. Of the 28 patients who were evaluated for response, 4 % achieved a PR. A phase I/IIa study in MM has been initiated to further evaluate the safety and efficacy of BT-062 using a more frequent dosing regimen of three weekly doses [100]. Combination trials with lenalidomide and dexamethasone are also ongoing.

IMGN901 (lorvotuzumab mertansine): CD56 antigen, a neural cell adhesion molecule implicated in cell–cell adhesion, neurite outgrowth, and other brain functions is overexpressed in a variety of cancers including small-cell lung cancer (SCLC), neuroblastoma and other neuroendocrine malignancies, multiple myeloma, and ovarian cancers. The expression of CD56 on normal tissues is restricted to NK cells and a subset of T lymphocytes [101]. IMGN901 is an anti-CD56 IgG1 antibody conjugated to DM1 via a hindered disulfide cleavable SPP linker. It has been evaluated in several phase I trials in patients with SCLC, MM, or other neuroendocrine tumors. A phase I dose escalation trial in 32 patients with MM established the MTD at 112 mg/m^2 (3 mg/kg) when the ADC was administered weekly for 2 consecutive weeks every 3 weeks [102]. DLT was grade 3 fatigue in 2 out of 6 patients treated at 140 mg/m^2. One sustained PR was documented in a patient treated at 140 mg/m^2/week. In a small phase I trial enrolling 6 patients with Merkel cell carcinoma, the MTD was established at 75 mg/m^2 (2 mg/kg) when the ADC was administered daily for 3 consecutive days every 3 weeks [103]. In this trial,

DLTs were grade 3 myalgia, headache, and back and shoulder pain. Out of 6 patients, there was 1 CR and 1 PR. A similar schedule of administration was used during another phase I on CD56-positive solid tumors from different types [104]. The MTD was also established at 75 mg/m^2/day, and DLT were grade 3 headache, neuropathy, fatigue, and myalgia, as previously reported. Half-life of IMGN901 at MTD was 34 h. Evidence of activity was observed with 1 CR and 1 PR in MCC and 1 unconfirmed PR in SCLC. Combination trials were also initiated. Escalating doses of IMGN901, given weekly for 3 weeks in a 4-week cycle, were evaluated in combination with lenalidomide/dexamethasone at their usual doses in patients with R/R CD56-expressing MM. Among the 12 patients enrolled, all had previously been treated with chemotherapy, with 42 % having received prior lenalidomide. No DLT has been reported and no grade 4 toxicities have been observed. One serious AE and 7 grade 3 toxicities related to combination treatment have been observed in four patients. On 12 efficacy-evaluable patients, 2 had a very good PR (VGPR) [105], and 4 had a PR. A phase I/II study to assess the safety and efficacy of IMGN901 in combination with carboplatin/etoposide in patients with advanced solid tumors including extensive stage small-cell lung cancer is ongoing. The NORTH trial is the phase II portion of this trial in which the ADC is administered for 3 consecutive days every 21 days at the dose of 60 mg/m^2/day (IMGN website, clinicaltrial.gov). Another phase I/II combination study with panobinostat and carfilzomib is currently ongoing in patients with R/R multiple myeloma [106].

4 Challenges and Perspectives

ADC have made tremendous progress over the last decades as demonstrated by the outstanding clinical efficacy observed in both hematological malignancies, with Adcetris® for the treatment of Hodgkin's lymphoma, and solid tumors, with T-DM1 for the treatment of metastatic breast cancer. The conjunction of the evolution of monoclonal antibodies from murine to humanized and human versions and the technological advances in the conjugation of highly potent non-immunogenic small molecules have been the pillars of these progresses. The increasing number of ADC reaching the clinic, targeting different antigens, and bearing different linkers and cytotoxics have contributed to the learning curve and stepwise progress of ADC. Lessons learned from the past experience of successful and stopped ADCs (*see* Tables 1 and 2) highlight the major axes that shall guide the development of future ADC.

Targets are at the heart of ADC development. Through their expression in some normal organs/tissues, they can contribute to

"on-target" toxicity and thereby decrease the therapeutic index and compromise clinical benefit. Although several ADC, such as IMGN242, MLN2704, and T-DM1, targeting epithelial antigens known to be expressed in some normal tissues have been well tolerated in clinical trials, with no antigen-positive related normal tissues toxicity, it has clearly been demonstrated in the case of bivatuzumab mertansine that the expression of the CD44v6 target in the skin can lead to severe toxicity. In the same direction, the skin-related AE observed for CDX-011 could be linked to the expression of gpNMB in normal melanocytes, which highlights skin as a particularly sensitive tissue to tubulin binders cytotoxics-ADC.

In parallel, targets contribute to efficacy by their level and homogeneity of expression. AVE9633, targeting CD33 antigen, was stopped in phase I due to lack of efficacy signal, in part driven by the low expression of CD33 on AML blasts. T-DM1 ORR in clinic does not correlate with exposure but is clearly linked to the level of target expression on breast cancer cells, as ORR is higher in the group of patients whose tumors have confirmed HER2 overexpression. Similarly, preliminary data show a correlation trend between gpNMB expression level in tumors and ORR for CDX-011 [86]. In addition to antigen density, the target has to be understood and documented in the context of the pathology itself, including antigen turnover and trafficking to the "right" subcellular compartments, morphological aspects of the tumor with regard to polarized versus depolarized target expression, but also, proliferation index and intrinsic sensitivity of the tumor to the selected cytotoxics. Leveraging this knowledge will help to better select future targets.

If clinical exploration of ADC directed towards epithelial antigens has proven the value of the strategy, future ADC could also be directed towards vascular, stromal, and cancer stem cell targets [107–110].

In link with target expression features, progress of future ADC will require the capacity to better define the patient population which will benefit from the treatment. The development of improved companion diagnostics for the evaluation of target expression level and distribution in human tumor biopsies will be a critical asset.

On top of the right target choice, developing and optimizing cytotoxics and linkers to improve efficacy and safety profile is mandatory but remains very complex and therefore challenging. ADC have, unlike naked antibodies, an "off-target" driven intrinsic toxicity linked to their cytotoxic moiety. Whether it is due to plasmatic release of the cytotoxic payload, modulated by the type of linker used, or to target-independent internalization (endopinocytosis, FcR-driven internalization) by normal cells, in some body compartment(s), remains to be analyzed on a case by case basis. Progress in deciphering the origin of observed AE, like the recently published

data demonstrating the impact of T-DM1 on platelet production inhibition, leading to the observed thrombocytopenia toxicity in patients [111], will help improving the design of future ADC.

Other explorations in preclinical studies include:

1. Deciphering metabolite properties: increasing metabolite accumulation in tumor cells to improve efficacy, like the design of PEGylated linkers to decrease multidrug-resistance recognition of metabolites [112]. On the same note, the chemical nature of the linker will influence not only the plasma pharmacokinetics and biodistribution of the ADC but also the type and properties of the metabolites within the tumor and the liver [113–115]. As an example, bystander effect may be wished to amplify the tumor response, although it may also bring more toxicity on normal organs.
2. Decreasing the heterogeneity of current ADC by better controlling the DAR. ADC are produced as complex mixtures whether they arise from lysine or cysteine conjugation. The different components of these mixtures might behave differently. Indeed, it has been shown that the level of cytotoxics attached to the antibody impact pharmacokinetics, efficacy, and safety [30]. Since few years, different options are explored to better monitor the DAR, like introduction in the antibody backbone of defined sites for conjugation. For example, cysteine engineering has been developed by different groups, and some thiomab-ADC have demonstrated equivalent if not better preclinical in vivo efficacy and tolerability [116, 117]. No clinical data using thiomabs has yet been published.
3. Improving physicochemical properties of the ADC, including solubility [112, 118] and capacity to aggregate. These modifications may concern the antibody backbone, as well as the linker and the drug itself.
4. Developing novel cytotoxics with different mechanisms of action, like the recently published alpha-amanitin-ADC [119]. This might help to improve therapeutic index and certainly offers new options of treatment for a larger panel of tumor indications.
5. Improving tumor penetration by using antibody fragments or protein scaffolds. Preclinical studies with anti-CD30 diabodies conjugated to auristatin demonstrated efficacy in tumor models [120]. But the balance between size, affinity, and pharmacokinetics properties has to be carefully explored to achieve optimal accumulation in tumors [121], and today no clinical exploration of ADCs with backbone different from IgG has been started.

Finally, ADC being a prodrug, understanding ADC metabolism/catabolism and properties and fate of metabolites is also essential to modulate efficacy and toxicity [59, 113–115]. Integrating quantitative and predictive understanding of PK/PD relationship will surely contribute to the optimization of all three components of the ADC in relation to target/disease properties, as well as administration regimen [115].

ADC design will be based on thoughtful combination of antibody, linker, and drugs in the context of a target and a defined cancer indication and a thorough understanding of the behavior of each ADC, with the ultimate goal to kill cancers while improving patients quality of life.

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