Chapter 15
Diphtheria Toxin Based Molecules as Therapeutic Approaches

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Abstract Malignant diseases are still one of the main causes of death and due to this high mortality rate new therapy approaches are needed. During the last decades cancer specific antigens on the cell surface were identified. This fundamental discovery was the important step to develop ligand-directed-toxins and antibody-drug-conjugates. They consist of an antigen binding domain plus an effector moiety like bacterial or plant toxins, respectively. These kinds of new molecules achieved a higher selectivity and efficacy, eliminated side effects and ensured better drug delivery as the standard approaches in cancer therapy. In consequence, both types of molecules are promising candidates for further development to extend the existing chemotherapeutic and radioactive armamentarium. In this review, the existing approaches to apply diphtheria toxin in this respect are summarized.

Keywords Antibody-drug-conjugates · Ligand-directed-toxins · Lymphoma

15.1 Introduction

The 'magic bullet' concept introduced by Paul Ehrlich, inspired scientists to develop agents with the ability to bind target cells with a higher selectivity. Ligand-directed-toxins (LDTs) and antibody-drug-conjugates (ADCs) are fusion proteins consisting of a binding domain (antibody, antibody derived binding head, cytokine or chemokine), which is specific for a target cell, and a toxic component. The binding heads of these molecules allow to target the malignant cell with an increased efficacy. After binding to the target cell, these molecules are internalized by endocytosis and induce cell death. Over time, several different toxins have been used in LDTs and ADCs, such as *Pseudomonas* Exotoxin A (ETA), diphtheria toxin (DT), anthrax, ricin and saporin (Blythman et al. 1981; Frankel et al. 2002; Thorpe et al. 1985;

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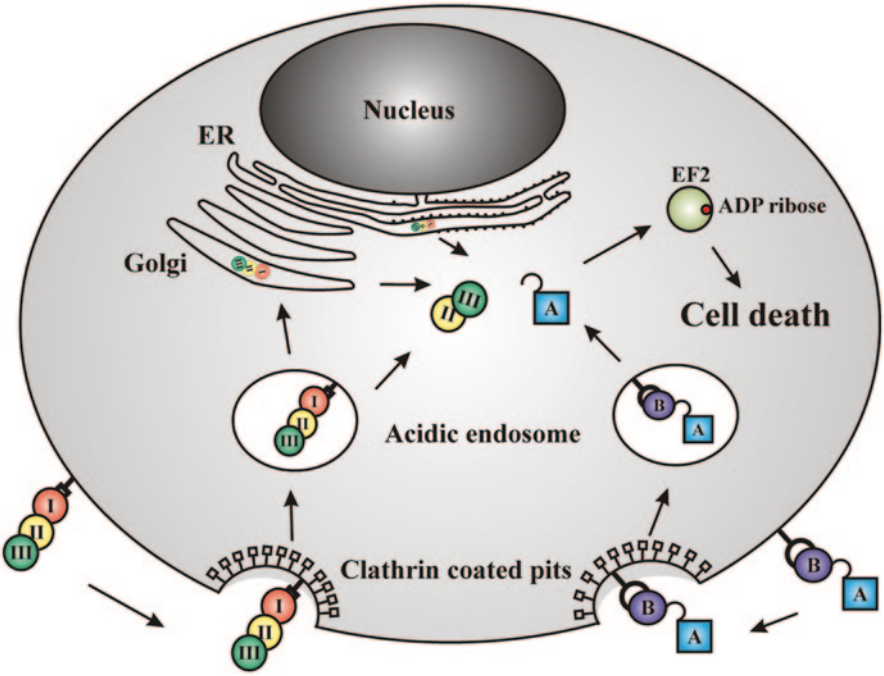

Fig. 15.1 Mechanism of action of *Pseudomonas* Exotoxin A (ETA) and diphtheria toxin (DT). After binding to a receptor, the toxin is internalised through clathrin coated pits into an endosome. Unfolding of the protein, cleavage by furin and the release of the catalytic domain into the cytosol is induced by low pH. Both toxins inhibit protein synthesis by ADP ribosylation of the elongation factor 2 which mediates cell death. *I* binding domain of ETA; *II* furin cleavage site of ETA; *III* catalytic domain of ETA; *B* binding moiety of DT; *A* enzymatic domain of DT; *ER* endoplasmatic reticulum; *EF2* elongation factor 2

Wels et al. 1992; Youle et al. 1986). The first generation of immunotoxins, developed 35 years ago, consisted of a toxic component chemically-linked to an antibody (ab). They showed tumour regression in some lymphoma patients, but, due to their size, they were ineffective in infiltrating solid tumours (Ghetie and Vitetta 2001).

After binding the target cell, ADCs and LDTs enter the cell via receptor-mediated endocytosis. Unfolding of *Pseudomonas* endotoxin A and diphtheria toxin is induced by the low pH in the endosome. Then, the toxins are proteolytically cleaved and the catalytic domains are released into the cytosol (Gonzalez and Wisnieski 1988), where they inhibit protein biosynthesis, leading to death like the unmodified toxins (Fig. 15.1).

15.1.1 Targeted Therapy Using Diphtheria Derived Ligand-Directed-Toxins (LDT) and Antibody-Drug Conjugates (ADCs)

During the last three decades, different kinds of ADCs and LDTs have been constructed on the basis of the toxic moiety of *Corynebacterium diphtheriae*. Most ligand-directed-toxins contain a cytokine as binding domain for the target cell (Table 15.1) and an antibody or an antibody-derivative (e.g. single chain Fragment variable; scFv) mediates binding of the ADCs (Table 15.2). Diphtheria toxin is a protein 535 amino acids in length with three domains. The enzymatic A domain, the binding B domain and the transmembrane domain which is located in the centre of the molecule. To improve of specificity of the ADCs and LDTs, they were designed with recombinant toxic components in which the binding domain was truncated or site-specifically mutated. A full-length version of diphtheria toxin would contain mutations at position 390 and 525 which inhibit its binding ability (Greenfield et al. 1987). Truncated versions in which the B domain had been deleted include DAB_{486}, DT388 and DAB_{389} (Chaudhary et al. 1991; Williams et al. 1987; Williams et al. 1990). Applications of combinations of different targeting moieties and toxin molecules are described below.

15.2 Ligand-Directed-Toxins (LDT)

A number of different ligands has been used to direct toxin molecules to target cells, including interleukins, growth factors and other peptides.

15.2.1 Binding Moiety Interleukin

DAB_{389}-IL6 Jean and co-workers replaced the portion of the diphtheria toxin which encodes the receptor binding domain with human IL-6. The resulting fusion protein DAB_{389}-IL-6 is selectively cytotoxic for eukaryotic cells bearing the interleukin 6 receptor, while cells, which are devoid of the IL-6 receptor, are resistant to the action of this recombinant toxin (Jean and Murphy 1991).

DAB_{389}-IL4 IL-4 is a cytokine which affects the growth and differentiation of various cell types. One of the common AIDS symptoms is the so-called AIDS-related Kaposi's sarcoma (KS). These KS infected cells express high affinity IL-4 binding sites. Recombinant human IL-4 minimally inhibited Kaposi's sarcoma cell growth and expression of IL-6. Investigators studied the effects of a fusion toxin DAB_{389}IL4 which mediates cellular toxicity only on cells expressing the IL-4 receptor (IL-4R). DAB_{389}IL4 inhibited protein synthesis in Kaposi's sarcoma cells at low concentrations and this effect was neutralized with an IL-4 specific antibody. They concluded

Table 15.1 Ligand-directed-toxins

Name	Binding moiety	Indication	Reference
DAB_{389}-IL-6	Interleukin 6 (IL-6)	Myeloma	(Jean and Murphy 1991)
DAB_{389}IL4	Interleukin 4 (IL-4)	AIDS-related Kaposi sarcoma	(Cai et al. 1997)
DAB_{389} sIL15	Simian interleukin 15 (sIL-15)	IL-2 and IL-15 dependent diseases	(vanderSpek et al. 1995)
DT_{388}IL3	Interleukin 3 (IL-3)	Acute myeloid leukemia (AML)	(Urieto et al. 2004)
DT_{390}IL3	Interleukin 3 (IL-3)	*ex vivo* purging of leukemia cells; autologous bone marrow transplantation	(Vallera et al. 1999)
DAB_{389}IL7	Interleukin 7 (IL-7)	Hematologic malignancies	(Sweeney et al. 1998)
DT_{390}IL18	Interleukin 18 (IL-18)	Autoimmune encephalomyelitis	(Jia et al. 2008)
DAB_{389} IL2 (Denileukin diftitox)	Interleukin 2 (IL-2)	cutaneous T cell lymphoma (CTCL)	(Manoukian and Hagemeister 2009)
DAB_{389}GRP	Gastrin-releasing peptide (GRP)	small cell lung cancer	(vanderSpek et al. 1997)
DAB_{389}-NT4	Rat neurotrophin-4/5	NT-4/5 nerve cell depletion	(Negro and Skaper 1997)
DAB_{389}EGF	Epidermal growth factor (EGF)	Several malignancies	(Shaw et al. 1991)
DT_{389}-bFGF	Basic fibroblast growth factor (bFGF)	Several malignancies	(Zhang et al. 2006)
DTGM	Granulocyte–macrophage colony-stimulating factor	Acute myeloid leukemia (AML)	(Frankel et al. 1999)
VEGF-DT385	Vascular endothelial growth factor (VEGF)	Several malignancies	(Olson et al. 1997)
DTAT	Aminoterminal (AT) fragment of the urokinase-type plasminogen activator	Glioblastoma multiforme (GBM)	(Vallera et al. 2002)
DTAT13	Aminoterminal (AT) fragment of the urokinase-type plasminogen activator; interleukin 13 (IL-13)	Glioblastoma multiforme (GBM)	(Rustamzadeh et al. 2006)
DTEGF13	Epidermal growth factor (EGF); interleukin 13 (IL-13)	Glioblastoma	(Oh et al. 2009)

Table 15.2 Antibody-drug conjugates

Name	Specificity	Indication	Reference
DT_{388}-anti-Tac(Fv)	Interleukin 2 receptor (IL-2R)	Chronic lymphocytic leukemia (CLL)	(Kreitman et al. 1992)
DTM1-E6scFv-PE40	Transferrin receptor (TfnR)	Several malignancies	(Nicholls et al. 1993)
A-dmDT390-bisFv(UCHT1)	CD3ε	T cell malignancies	(Woo et al. 2008)
A-dmDT390-scfbDb(C207)	Monkey CD3	T cell depletion in monkeys	(Kim et al. 2007)
A-dmDT390biscFv(2-6-15)	Porcine CD3	T cell depletion	(Wang et al. 2011)
DT2219	CD22/CD19	B cell leukemia and lymphoma	(Vallera et al. 2005)

that the expression of a functional IL-4R could serve as a target for novel therapy with agents such as DAB_{389}IL4 (Cai et al. 1997).

DAB_{389}-sIL15 The fusion toxin DAB_{389}-sIL15 is directed against simian interleukin 15 (sIL-15). The sIL-15 domain of the molecule binds to the IL-2/IL-15 receptor and is involved in signal transduction leading to DNA and protein synthesis. This recombinant molecule is internalized by receptor-mediated endocytosis and the catalytic domain of diphtheria toxin is delivered to the cell cytosol, where it kills the target cell. DAB_{389}-sIL15 could be a potential therapeutic agent in cases where patients have proven refractory to treatment with IL-2 specific toxins (vanderSpek et al. 1995).

DT_{388}-IL3 Acute myeloid leukemia (AML) is the most common leukemia in adults and the second most common leukemia in children. Interleukin 3 (IL-3) is needed for proliferation and differentiation of multi-potential and committed myeloid and lymphoid progenitors. A major advantage of therapies with IL-3 specific agents is, that the receptor is absent from mature myeloid cells whereas myeloid leukemia progenitors over-express the IL-3 receptor. A recombinant toxin consisting of truncated diphtheria toxin and the human IL-3 showed potent and selective killing of IL-3 receptor expressing AML cell lines and patient leukemic progenitors (Alexander et al. 2001). Therapy of SCID mice bearing human leukemia cells revealed *in vivo* efficacy (Black et al. 2003). The safety of DT_{388}-IL3 was tested in rodents and monkeys and was remarkable (Urieto et al. 2004).

DT_{390}-IL3 Another LDT directed against IL-3 is the molecule DT_{390}-IL3 with murine IL-3 as binding moiety. DT_{390}-IL3 was applied as an anti-leukaemic drug to mice. However, it was already toxic when administrated at low doses. Due to the high toxicity they alternatively tried to use this recombinant molecule *ex vivo* for purging of contaminated leukemic cells from bone marrow grafts in an autologous transplantation assay. Mice given treated bone marrow survive over 100 days while mice given untreated cells did not survive. So DT_{390}-IL3 may rather prove to be a useful agent for *ex vivo* purging of bone marrow grafts (Vallera et al. 1999).

DAB_{389}-IL7 Interleukin 7 (IL-7) is important for cell growth in haematological malignancies such as acute lymphoid leukaemia (ALL), chronic lymphoid leukaemia (CLL), AML, and Sezary syndrome. A recombinant fusion protein, DAB_{389}-IL-7, composed of the toxic domain of diphtheria toxin fused to IL-7, was constructed and tested. This fusion protein selectively inhibited protein synthesis in IL-7 receptor (IL-7R) positive cells. Due to the high expression of IL-7R on a variety of haematopoietic neoplasms, DAB_{389}-IL-7 may serve as a therapeutic agent for patients with IL-7R positive leukaemia and lymphoma (Sweeney et al. 1998).

DT_{390}-IL18 Antigen-presenting cells (APCs) expressing interleukin-18 receptor (IL-18R) were shown to be crucial for establishing and maintaing experimental autoimmune encephalomyelitis (EAE) in an animal model for multiple sclerosis. This LDT showed good results *in vivo*; EAE mice treated with the agent showed a delayed manifestation of EAE and decreased symptoms (e.g. infiltration of inflammatory cells into the brain) in comparison to the control group (Jia et al. 2008).

$DAB_{389}IL2$ DAB_{389}IL2, also known as denileukin diftitox, is the first commercially available recombinant fusion protein which is capable of delivering a cytotoxic agent directly to specific intracellular targets. Denileukin diftitox is composed of the full-length sequence of interleukin 2 (IL-2) and a modified cytotoxic DT. It binds to cells expressing the interleukin 2 receptor (IL-2R) and inhibits protein synthesis due to the diphtheria toxin fragment. In a mouse model with an IL-2R-expressing malignancy, denileukin diftitox prolonged survival of the treated group compared with controls. During the first in man study with patients bearing IL-2R-positive cutaneous T cell lymphoma (CTCL), the overall response rate was 36 % after administration of 18 μg/kg/day for 5 days every 3 weeks (Figgitt et al. 2000). Denileukin diftitox is FDA-approved only for CTCL, but other applications might be beneficial as well. Several clinical studies are in progress for different indications, but data are still pending (Manoukian and Hagemeister 2009).

Different groups showed a correlation between the presence of regulatory T-cells (Treg) and the efficacy of tumour vaccination, as Treg cells have been shown to inhibit anti-tumour immune responses. It was shown that the depletion of Treg using the IL-2 fusion protein denileukin diftitox decreased Treg function and increased antigen-specific T-cell response to a cancer vaccine in mice (Hobeika et al. 2012). *In vitro* studies demonstrated that canine Treg cells are a target of denileukin diftitox. Suppression of T-cell proliferation due to Treg was corrected by addition of denileukin diftitox. Application of the toxin resulted in a depletion of Treg which was followed by an increase in the immune response *in vitro* (Knueppel et al. 2012).

15.2.2 Growth Factors and Other Peptides as Binding Domain

$DAB_{389}GRP$ Gastrin-releasing peptide (GRP) is a bombesin-like peptide involved in the regulation of a large number of different cellular processes including e.g. exocrine secretion and smooth muscle contraction. GRP also functions as autocrine

growth factor on a number of neoplastic tissues, including small cell lung cancer cells (SCLC), a property which has been exploited for toxin delivery. The fusion protein DAB_{389}GRP showed receptor specific cytotoxicity which could be inhibited by an excess of GRP and anti-GRP antibody, respectively. A number of SCLC cell lines were tested and the LDT showed inhibition of protein synthesis in all of them (vanderSpek et al. 1997).

DAB_{389}-NT4 Neutrophins are a family of structurally related proteins which play a role in the development of the nervous system. The DAB_{389}NT4 is a recombinant molecule with a truncated version of diphtheria toxin serving as effector and the rat neutrophin-4/5 (NT-4/5) as binding domain. This fusion protein was cytotoxic for different neural cell lines. To study the role of neutrophins, gene knockouts can be used, but in a conventional knockout the protein is absent in all tissues during embryonic development. So DAB_{389}NT4 could be a useful agent for the selective depletion of NT-4/5 positive nerve cells *in vivo* (Negro and Skaper 1997).

DAB_{389}EGF A characteristic of several malignancies including those of the breast, bladder, prostate, lung, and neuroglia is the expression of the receptor for the epidermal growth factor (EGF). The fusion protein DAB_{389}EGF was constructed for treatment of these malignancies. The toxin binds specifically to the EGF receptor and inhibits protein synthesis in different EGF receptor expressing tumour cell lines. Studies of the kinetics of poisoning showed a fast effect which resulted in complete protein synthesis inhibition after a 15 min exposure of an EGF receptor positive cell line to DAB_{389}EGF. The ability of the toxin to effectively kill tumour cells at extremely low concentrations and the selectivity and rapid kinetics of cytotoxic action suggest that this molecule could be an efficient anti-tumour agent (Shaw et al. 1991).

DT_{389}-bFGF The high-affinity receptor for basic fibroblast growth factor (bFGF), fibroblast growth factor receptor (FGFR), is an important tumour-associated growth factor that contributes to proliferation and angiogenesis, and it is over-expressed in a number of tumour cell lines. A recombinant immunotoxin containing a truncated version of diphtheria toxin and human bFGF was designed, expressed and purified. The activity and potential anti-tumour effect of DT_{389}-bFGF was evaluated by testing the cytotoxicity on a human ovarian teratocarcinoma cell line with high-level expression of FGFR. The immunotoxin showed significant cytotoxicity and the effect could be blocked by an excess of bFGF and anti-bFGF antibodies, respectively. Additionally, cell lines with low expression of FGFR were found to be resistant to the recombinant molecule. FGFR could be an interesting target for tumour therapy and the FGFR targeting molecules might be a promising approach in the treatment of cancers (Zhang et al. 2006).

DTGM Frankel et al. constructed an AML-selective fusion toxin consisting of human granulocyte–macrophage colony-stimulating factor (GM-CSF) as binding domain fused to the catalytic domain of diphtheria toxin, the so-called DTGM. The receptor for GM-CSF is selectively expressed on mature and immature monocytes, granulocytes, or macrophages and on malignant myeloid leukemias (Cannistra et al. 1990).

Treatment failure of patients with AML is due to the development of multidrug resistant phenotypic blasts. The fusion toxin DTGM selectively inhibited protein synthesis and inducted apoptosis in receptor-positive cells (Burbage et al. 1997). Frankel and his group designed a high-level expression vector and a purification protocol for a phase I clinical trial with DTGM (Frankel et al. 1999).

VEGF-DT385 Tumour-derived vascular endothelial growth factor (VEGF) plays an important role in neovascularisation and in the development of tumour stroma. The VEGF receptors (VEGFR) are over-expressed on tumour cells of endothelial origin and nearly undetectable in the endothelium of normal tissues. This expression profile offers a selective advantage for targeting VEGFR positive tumours. VEGF-DT385 was selectively toxic to endothelial cell lines and inhibited neovascularisation. Athymic nude mice with established subcutaneous tumours were treated with daily injections of VEGF-DT385 or with free toxin. The conjugate-treated animals displayed a significant inhibition of tumour growth in comparison to the control animals treated with the toxin alone. Analysis of the tumours from VEGF-DT385 animals revealed haemorrhagic necrosis. In contrast, highly vascularised normal tissues from the same animals demonstrated no evidence of haemorrhage or tissue injury. This recombinant protein illustrates the selectively anti-tumour activity of a VEGF conjugate (Olson et al. 1997)

DTAT Patients with brain malignancies have a poor prognosis and new, selective therapies are urgently needed. The urokinase-type plasminogen activator (uPA) receptor (uPAR) is expressed on the surface of glioblastoma (GB) and could be a potential target for GB treatment. The recombinant fusion protein DTAT, which contains the catalytic portion of diphtheria toxin fused to the non-internalizing amino-terminal (AT) fragment of uPA, was highly potent and selective in killing uPAR-expressing glioblastoma cells. DTAT caused a statistically significant regression of cell-induced tumours in all mice in comparison with the control group. To investigate the molecule's potential toxicity, DTAT was given to tumour-free mice. DTAT had only small effects on kidney, liver, heart, lung and spleen. Due to its ability to target tumour cells and tumour vasculature simultaneously, combined with the lack of systemic effects, DTAT maybe a novel agent for glioblastoma therapy (Vallera et al. 2002).

15.2.3 Dual-Targeting Ligand-Directed-Toxins

DTAT13 DTAT13, another recombinant LDT consisting of truncated diphtheria toxin, an AT-fragment and a fragment of IL-13 was constructed to target receptors on glioblastoma cells. To determine the properties of a dual-targeting DTAT13, pharmacokinetic and bio-distribution experiments were performed. Binding analysis revealed that the IL-13 domain functioned independently of the AT and that the equilibrium constant for each binding domain was essentially the same as in the monovalent DTIL13 and DTAT molecules. Flow cytometry studies indicated that

the bivalent DTAT13 bound better than the monovalent control molecules. DTAT13 inhibits the rate of protein synthesis in double positive cells faster than DTAT, but in a same order of magnitude as DT13. This study showed that DTAT13 has properties encompassing those of both DTIL13 and DTAT and warrants further consideration for clinical development (Rustamzadeh et al. 2006).

DTEGF13 A bispecific ligand-directed toxin (BLT) consisting of IL-13, EGF and a truncated version of DT was constructed to target glioblastoma cells. The corresponding molecule, DTEGF13, selectively killed a human glioblastoma cell line. This recombinant BLT has greater activity than the monospecific controls or their mixture. Aggressive brain tumours were established in nude rats with the U87 cell line genetically marked with a luciferase reporter gene. These were treated with two injections of the dual-targeting BLT, resulting in tumour eradication in 50% of the rats. In contrast a combination of the monospecific LDTs DTEGF and DTIL13 was not able to inhibit tumour growth. Anti-DT antibodies were not generated in normal immune-competent rats given the identical intracranial DTEGF13 therapy. These observations led to the conclusion that DTEGF13 is safe and could be an alternative drug for glioblastoma therapy (Oh et al. 2009).

15.3 Antibody-Drug Conjugates (ADCs)

15.3.1 Monospecific and Monovalent ADCs

DT_{388}-anti-Tac(Fv) The monoclonal antibody anti-Tac is specific for the IL-2 receptor, and in the fusion protein DT_{388}-anti-Tac(Fv), the variable domain of this antibody is fused to the C-terminus of a truncated diphtheria toxin. Peripheral blood mononuclear cells (PBMCs) from 14 chronic lymphoid leukaemia (CLL) patients were incubated with the recombinant ADC. It was active against eleven of the 14 samples tested, while an LDT consisting of the truncated diphtheria toxin and the human IL-2 as binding moiety was only cytotoxic for four of these samples. The patient samples, which were sensitive to the ADC, contained between 400 and 2,500 binding sites per cell. These data showed that DT_{388}-anti-Tac(Fv) is able to kill CLL cells even when there is only a low number of IL-2R (Kreitman et al. 1992)

DTM1-E6scFv-PE40 DTM1-E6scFv-PE40 is a tripartite protein, consisting of the diphtheria toxin mutant M1 (containing two mutations in the binding domain), the E6scFv directed against the human transferrin receptor (TfnR) followed by the *Pseudomonas* Exotoxin PE40 (lacking the binding domain).

The expression pattern of TfnR is limited in normal tissue, but it is widely distributed on cells from several malignancies. Therefore it could be a suitable target for therapy. DTM1-E6scFv-PE40 showed cytotoxicity to TfnR-positive cells. Although this molecule contains two bacterial toxin components, the cell death mediated was very similar to that mediated by the mono-toxin E6scFv-PE40. This would

suggest that the main effect is due to the PE40 domain. However, this molecule showed that it is possible in principle to create a fusion protein with two effector domains (Nicholls et al. 1993).

15.3.2 Monospecific, Bivalent ADCs

A-dmDT390-bisFv(UCHT1) The bivalent ADC A-dmDT390-bisFv(UCHT1) for treatment of patients with T cell malignancies is a fusion protein composed of the catalytic domain of DT fused to two tandem scFvs. The scFv(UCHT1) is specific for human CD3ε and the fusion molecule was able to selectively kill CD3ε positive T cells. For evaluation of the maximum tolerated dose, pharmacokinetics and immunogenicity of the ADC, studies with rats and squirrel monkeys were performed by Woo and colleagues. The administration of the toxin did not affect liver function, renal function, the haemogram, nor did it produce serious organ histopathology in the animals tested. A-dmDT390-bisFv(UCHT1) had a plasma half life of 23 min on average in the two species. Due to these data, this ADC appears to be a promising drug for patients with CD3-positive T cell malignancies (Woo et al. 2008).

A-dmDT390-scfbDb(C207) T cell depletion in non-human primates is important for models of transplantation tolerance and autoimmune disease therapy. A-dmDT390-scfbDb(C207) consists of a truncated version of DT and a diabody directed against monkey CD3. The ADC format showed a 7-fold increased binding to T cells in comparison to the parental scFv(C207). It mediated 5- to 7-fold more bioactivity over the A-dmDT390bisFv(C207) and the diabody format was able to deplete T cells in monkeys (Kim et al. 2007), providing a putative animal model.

A-dmDT390-biscFv(2-6-15) Another molecule for T cell depletion is the ADC A-dmDT390-biscFv(2-6-15). This fusion protein is directed against porcine CD3 and it induces a profound but transient T-cell depletion which allows to investigate transplantation tolerance models and autoimmune disease therapies in pigs. A-dmDT390-biscFv(2-6-15) was tested in four animals and was able to deplete the T cell population without any detectable clinical toxicity. This monospecific, bivalent molecule could be utilized in experimental porcine models of transplantation tolerance, autoimmune disease therapy and treatment of T-cell leukaemia (Wang et al. 2011).

15.3.3 Dual-Targeting ADC

DT2219 A dual-targeting immunotoxin called DT2219, consisting of two scFvs recognizing CD19 and CD22 and the catalytic DT_{390} fragment as death effector, showed strong *in vivo* anti-leukaemic activity in a murine xenograft model. Treatment of transplanted mice with this agent resulted in long-term tumour-free survival,

measured in a bioluminescent xenograft imaging model, in which the propagation of Raji Burkitt's lymphoma cells was tracked in real time (Vallera et al. 2005).

15.4 Conclusions

The various diphtheria toxin based molecules described above showed promising results *in vitro* and *in vivo*; they selectively killed the target cells and displayed only marginal or no clinical cytotoxicity in animal models. However, only one fusion protein, denileukin diftitox, has been approved by the FDA for use in CTCL up to now. Ongoing studies suggest the potential value in therapy of other diseases which express IL-2R, and investigators have demonstrated that it may be combined with other drugs to achieve synergistic effects (Manoukian and Hagemeister 2009). A newly designed phase II study showed that denileukin diftitox has significant clinical activity in unresectable stage IV melanoma patients due to T cell depletion and CD8-positive T cell expansion (Telang et al. 2011).

However, it is noticeable that many groups, which have worked with diphtheria toxin in the past and which have achieved remarkable results *in vivo* like Vallera and co-workers with DT2219, have now switched to *Pseudomonas* exotoxin as death domain. One reason could be to avoid immunogenicity and to achieve a higher potency of the agent (Vallera et al. 2010). In the late 90s, Brinkmann et al. investigated the effect of BCL-2 expression on the sensitivity of cancer cell lines to different immunotoxins. BCL-2, a mitochondrial membrane protein, has been shown to inhibit apoptosis (Sentman et al. 1991). Some malignant cells are relatively resistant to apoptosis due to overexpression of BCL-2 (Tsujimoto et al. 1985). ADCs with *Pseudomonas* exotoxin as effector domain were the only molecules which were able to induce cell death independent of the BCL-2 level (Brinkmann et al. 1997).

In summary, DT-derived LDTs and ADCs described here proved which molecule formats could lead to promising new therapies for several diseases. But, one has to keep in mind that it is important to identify and verify which combination of toxin and target is the best for each individual ailment.

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