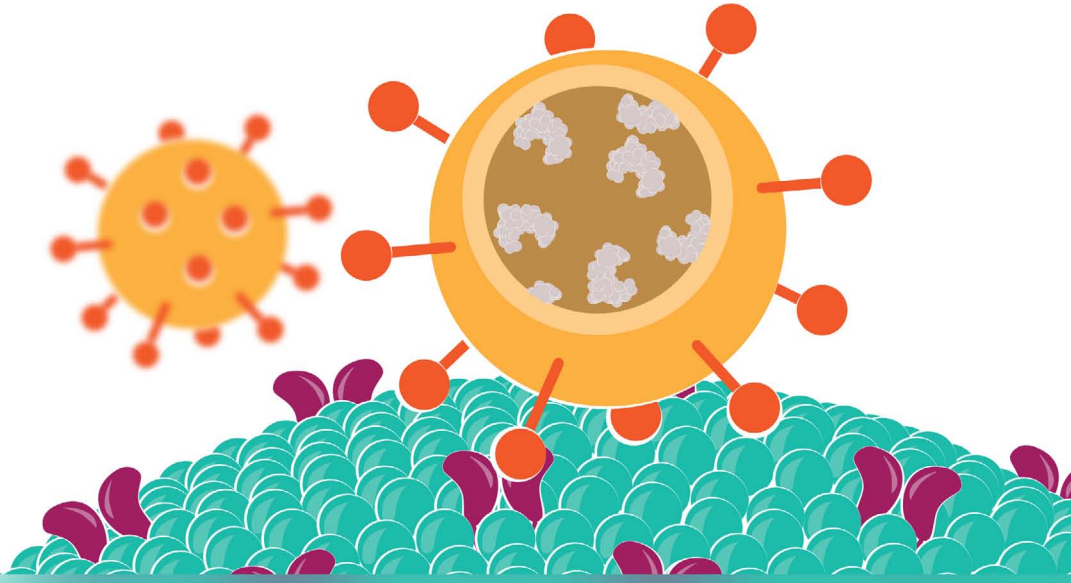
Drug Delivery in Oncology



Alejandro D Ricart

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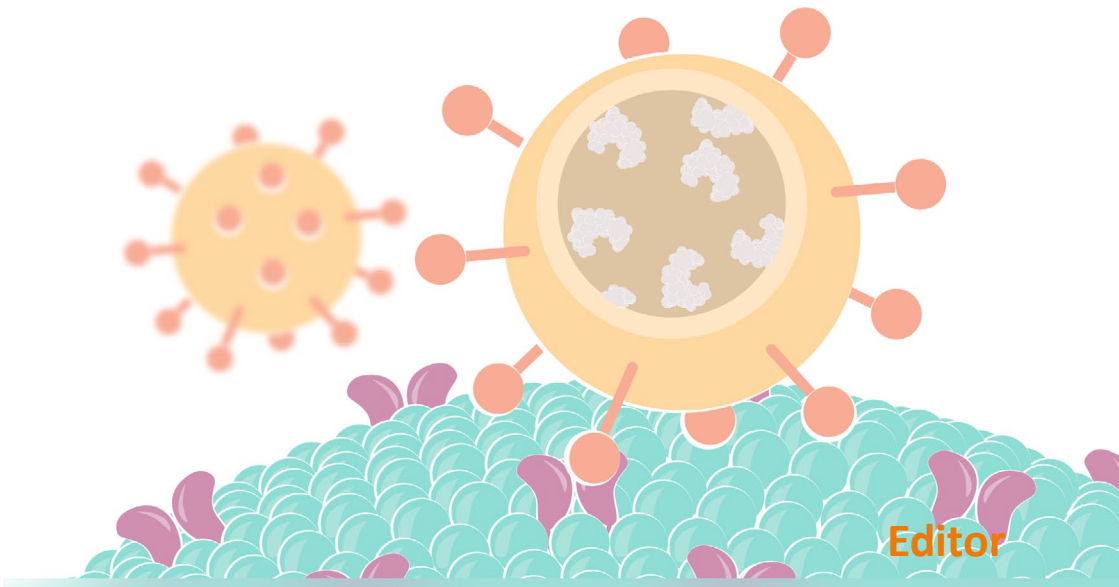

Editor

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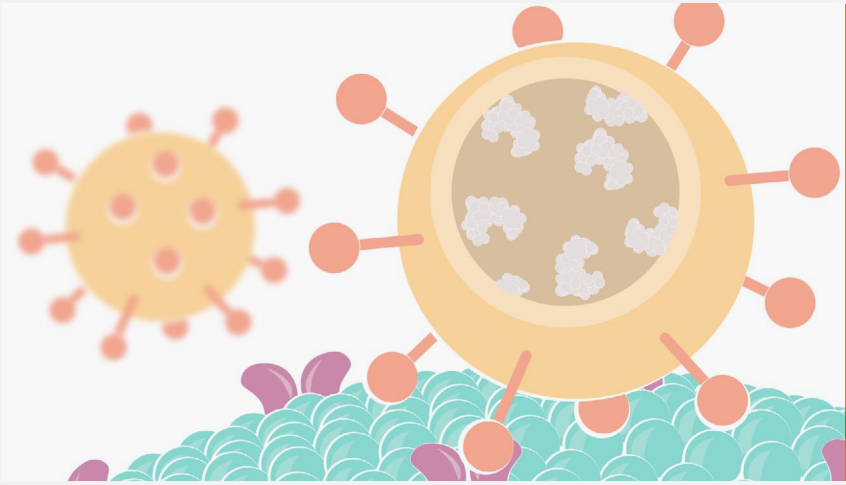

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About the Editor

Alejandro D Ricart

Alejandro D Ricart is Consultant Medical Oncologist and Senior Clinical Investigator in the Division of Solid Tumors at Henry Moore Institute of Oncology (Buenos Aires, Argentina). He received his MD from the University of Buenos Aires School of Medicine (Argentina) and completed his residency training at the Hospital Militar Central (Buenos Aires, Argentina). He was a fellow at the Department of Medical Oncology at the Alexander Fleming Institute (Buenos Aires, Argentina), where he first developed an interest in developmental therapeutics. He was Consultant Oncologist at Cvitkovic & Associés Consultants SA (Paris, France). He also served as Assistant Professor of Pharmacology at the University of Buenos Aires, and as Professor of Pharmacology at the Hebrew University Argentina Bar-Ilan (Buenos Aires, Argentina) until 1999. He completed an advanced oncology drug development/medical oncology program at the Institute for Drug Development, Cancer Therapy and Research Center (TX, USA) and at the Division of Medical Oncology, University of Texas Health Science Center at San Antonio (USA). There, he was introduced to multiple new therapeutic agents targeting cancer in a variety of novel ways. He has been an investigator in over 50 trials on cancer, including several studies of monoclonal antibodies, signal transduction inhibitors and tyrosine kinase inhibitors, in all phases of clinical development. His honors include receipt of the American Society of Clinical Oncology Foundation Merit Award in 2006. He joined Pfizer Inc. (CA, USA) in October 2006 and participated in the clinical development of several investigational compounds, including axitinib and bosutinib. Later, he was Director of Biotechnology and Oncology Clinical Research at Pfizer Inc. in San Diego. He has published more than 80 journal articles, abstracts, reviews and book chapters.

Foreword

Drug-delivery systems in cancer therapy

Alejandro D Ricart

Drug-delivery systems refer to formulations and technologies for preferentially transporting a drug to its intended target. In general, drug-delivery systems drastically change the pharmacokinetics of the transported drug. In the treatment of cancer, delivery systems attempt to improve the therapeutic index of anticancer agents by efficiently directing them to tumor cells. This can be achieved by active or passive targeting. Other advantages of delivery systems in oncology may include reducing toxic effects to normal tissues, simplifying drug administration and improving patient's compliance.

Chapter 1 addresses the basic aspects of the biology of cancer and the essentials of the pharmacology of drug-delivery systems in oncology. This chapter briefly mentions the variation in the lethal effect of many cytotoxic drugs during the cell cycle, one of the fundamental considerations to understand the importance of nanomedicine in oncology. **Chapter 2** describes antibody–drug conjugates that have obtained regulatory approval, with a brief account of those in clinical development. In **Chapter 3**, Dalia and Bruno discuss fusion proteins, a very interesting drug-delivery class for their theoretical lack of cross-resistance with cytotoxic chemotherapy. In **Chapter 4**, Mulet-Margalef *et al.* review the use of polymer–drug conjugates, with a comprehensive examination of experimental agents. Peters *et al.* review nab-paclitaxel in their chapter 'Drug protein-bound particles and polysaccharide–drug conjugates' **(Chapter 5)**. Finally, Lewi *et al.* describe several liposomal formulations in **Chapter 6**, including approved and experimental agents.

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There is no other specialty so closely tied to pharmacologic considerations as clinical oncology. This book attempts to provide essential information about the clinical pharmacology of drug-delivery systems, with an emphasis on those systems that have achieved regulatory approval. I strongly believe that subsequent editions of this book will narrate new and much more clinical successes against cancer. As an example, the US FDA expanded the approved uses of nab-paclitaxel to treat patients with metastatic pancreatic cancer while this foreword was being written.

The authors and I hope this effort will be a source of inspiration and encouragement to continue the fight to cure this disease.

Acknowledgements

This book has involved the efforts of many knowledgeable and dedicated people. AD Ricart would like to thank his colleagues and friends who contributed to this book, as well as his family for their support. Finally, he would like to thank his colleagues at Future Science Group, G D'Oca, Managing Editor, and N Zaman, Editorial Administrative Assistant, for their patience.

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Alejandro D Ricart is Consultant Medical Oncologist and Senior Clinical Investigator in the Division of Solid Tumors at Henry Moore Institute of Oncology (Buenos Aires, Argentina). He received his MD from the University of Buenos Aires School of Medicine (Argentina) and completed his residency training at the Hospital Militar Central (Buenos Aires, Argentina). He was a fellow at the Department of Medical Oncology at the Alexander Fleming Institute (Buenos Aires, Argentina), where he first developed an interest in developmental therapeutics. He was Consultant Oncologist at Cvitkovic & Associés Consultants SA (Paris, France). He also served as Assistant Professor of Pharmacology at the University of Buenos Aires, and as Professor of Pharmacology at the Hebrew University Argentina Bar-Ilan (Buenos Aires, Argentina) until 1999. He completed an advanced oncology drug development/medical oncology program at the Institute for Drug Development, Cancer Therapy and Research Center (TX, USA) and at the Division of Medical Oncology, University of Texas Health Science Center at San Antonio (USA). There, he was introduced to multiple new therapeutic agents targeting cancer in a variety of novel ways. He has been an investigator in over 50 trials on cancer, including several studies of monoclonal antibodies, signal transduction inhibitors and tyrosine kinase inhibitors, in all phases of clinical development. His honors include receipt of the American Society of Clinical Oncology Foundation Merit Award in 2006. He joined Pfizer Inc. (CA, USA) in October 2006 and participated in the clinical development of several investigational compounds, including axitinib and bosutinib. Later, he was Director of Biotechnology and Oncology Clinical Research at Pfizer Inc. in San Diego. He has published more than 80 journal articles, abstracts, reviews and book chapters.

Chapter 1

Drug-delivery considerations in oncology

Alejandro D Ricart

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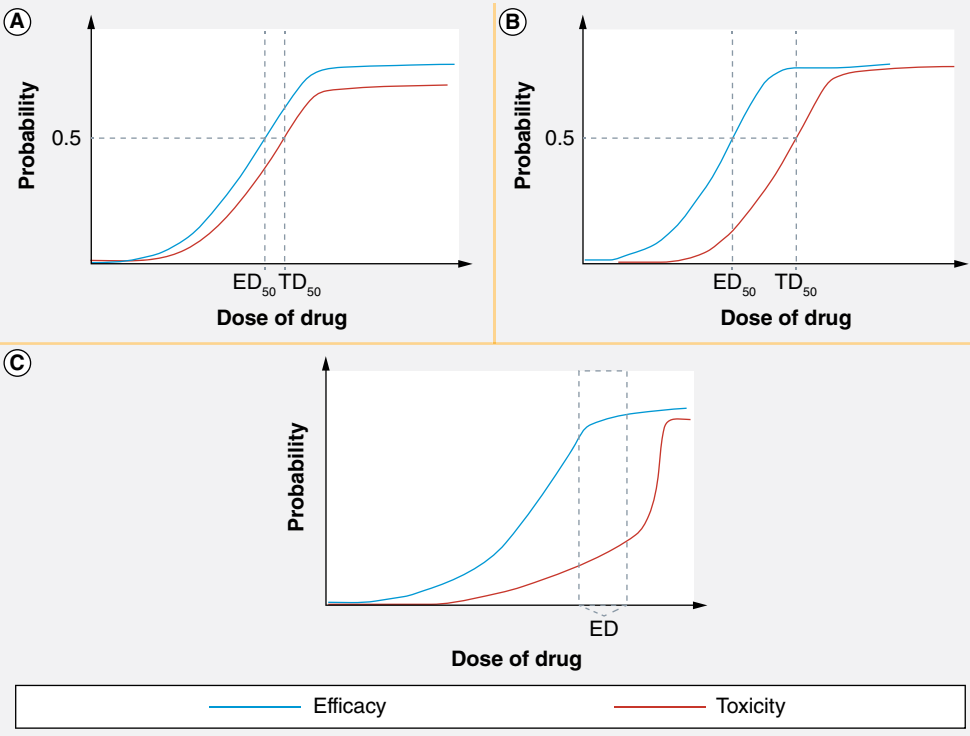
Optimal cytotoxic treatment scheduling is difficult to apply in clinical oncology. The main reasons conspiring against optimal scheduling include rapid loss of synchrony (variation of cell-cycle times) and heterogeneity of tumor cell populations, drug toxicity to normal tissues and deficient drug delivery to tumors [1–10]. All cytotoxic drugs have toxic effects to normal tissues. As a general rule, this toxicity limits the dose that can be administered to patients. In addition, cytotoxic drugs lack selectivity for tumor cells. Consequently, a clear approach is to increase the therapeutic index of a certain cytotoxic drug by preferentially directing the drug to tumor cells and/or reducing the toxic effects to normal tissues [11]. Delivery systems attempt to improve the therapeutic index of cytotoxic drugs.

Therapeutic index

The therapeutic index is the ratio given by the dose of a drug that produces a defined level of damage to a normal tissue (toxicity) divided by the dose that produces a defined level of effect (antitumor activity). Hence, the therapeutic index is a measure of the relative efficacy of a drug against a tumor compared with the toxicity caused **(Figure 1.1)**. It is quite obvious from **Figure 1.1B** that if the effective curve is shifted to the left away from the toxicity curve, the drug may be said to be a more effective anticancer agent. Most cytotoxic drugs have a narrow therapeutic index and are dosed close to the maximum tolerated dose found in dose-escalating studies [12–14]. As previously mentioned, a method for increasing the therapeutic index is to preferentially direct drugs to tumor cells and/or to reduce their toxicity.

Figure 1.1. Relationship between toxicity and antitumor effect.



(A) An example of a classical cytotoxic drug. Therapeutic index = TD_{50}/ED_{50} **(B)** Better therapeutic index when the effect curve is displaced to the left (ED_{50} is a much lower dose compared with the TD_{50}). Therapeutic index = TD_{50}/ED_{50}. **(C)** Increase the therapeutic index by preferentially direting the drug to tumor cells and or reducing the toxic effects.

This book describes several common approaches currently used in the clinic: linking them to a carrier (antibodies); binding them to proteins; conjugating them to polymers; and entrapping them in liposomes.

Most cytotoxic drugs have a narrow therapeutic index and are dosed close to the maximum tolerated dose found in dose-escalating studies.

Factors regulating drug penetration in solid tumors

Tumor nodules *in vivo* have unique biophysical properties that can create barriers to drug delivery **(Box 1.1)** [7,8]. These properties are the result of complex interactions among growing tumor cells, the blood vessels generated inside the tumor, the surrounding normal tissues and circulating cells from the bone marrow [7,8,15,16]. This complex network precludes a thorough study by *in vitro* work. There are clear differences in transport variables between tumors and normal tissues, but the extent of this chapter impedes a detailed description. An essential explanation is summarized below.

Influence of tumor microenvironment

Factors that have influence in drug delivery to cells within tumor masses include: the chaotic arrangement of tumor blood vessels and their particular permeability, the variability in blood flow and the interstitial fluid pressure in the tumor microenvironment [7,8]. The volume of the interstitial space is generally higher in tumor models than in normal tissues. The extracellular matrix composition depends on the stage and type of tumor. In several tumor types, the tumor interstitial space has a greater amount of type IV collagen and glycosaminoglycans, and extremely acidic conditions compared with the normal interstitial space [8]. Tumor masses display a remarkable degree of metabolic diversity. In experimental models, glucose deprivation significantly changes the energetic status of the tumor cells. However, therapies directed to alter the tumor microenvironment have been largely ineffective in the clinic.

Box 1.1. Potential barriers to drug delivery in solid tumors.

- Imperfect vascular supply
- Permeability of blood vessel walls to anticancer drugs
- Variability in blood flow
- Increased interstitial fluid pressure
- Composition and structure of extracellular matrix
- Acidic conditions in tumor microenvironment
- Shedding of targeted cancer antigen (in the case of monoclonal antibodies)
- Proteolytic cleavage by metalloproteinases (in the case of monoclonal antibodies)

Aa Cell cycle-phase-dependent cytotoxic drugs: show variation in their lethal effect during the cell cycle (e.g., antimetabolites, topoisomerase I inhibitors, doxorubicin and vinca alkaloids). For these agents, that act preferentially in specific phases of the cell cycle, duration of exposure (e.g., $AUC_{0\text{-}INF}$) is relatively more important than maximum plasma concentration.

The ways solutes move through spaces are convection, diffusion and transcytosis [17]. Several lines of investigation have shown that tumor blood vessels are leaky to large protein molecules [4,18]. Analyses of interstitial fluid have shown that there is a high protein content in this tumor compartment. This, along with the lack of functioning lymphatic vessels, contributes to an elevated interstitial pressure in most tumor masses [11]. In the interstitial space of a tumor, large molecules (those with a molecular weight greater than 800 Da) move mainly by convection, while smaller molecules depend mainly on diffusion [11]. Consequently, tumor penetration of large molecules (i.e., intact monoclonal antibodies [mAbs]) seems to be inferior compared with smaller molecules (please refer to the section entitled 'Factors regulating antibody drug conjugate-based therapy' in **Chapter 2**).

Taking all these facts into account, it is fair to conclude that effective treatment of cancer requires both sensitivity to the drugs used and penetration through the compartments to reach tumor cells distant from the blood vessels.

Pharmacologic factors

Systemic drug exposure is a function of drug concentration and time. In clinical oncology, the area under the concentration versus time curve (AUC) is usually the pharmacokinetic (PK) parameter used [12,19]. However, drug concentration parameters such as maximum plasma concentration (C_{max}) and steady-state concentration or drug concentrations above a threshold have been used for pharmacodynamic (PD) models [19]. The critical factor that controls drug exposure is the schedule of administration (i.e., the dose, the interval of the administration; and in the case of intravenous infusion, its duration). Thus, clearance is a key PK parameter because it relates drug dose to AUC, see **Equation 1.1** [19]:

$$Clearance = Dose/AUC_{0-INF} \quad \textbf{(Equation 1.1)}$$

where AUC_{0-INF} indicates the AUC from time zero to infinity. As aforementioned, dose and frequency of cytotoxic drug administration are influenced by tumor cytokinetics, but limited by toxicity to normal tissues [1,9]. These dynamics make most cytotoxic drugs highly schedule-dependent agents. For example, C_{max} is usually more critical than duration of exposure for drugs that are non-cell-cycle-phase dependent (e.g., alkylating agents). However, most cytotoxic drugs

Aa Cytokinetics: the study of the kinetics of cellular growth.

show variation in their lethal effect during the cell cycle (e.g., antimetabolites, topoisomerase I inhibitors, doxorubicin and vinca alkaloids). For these agents that act preferentially in specific phases of the cell cycle **(Figure 1.2)**, the reverse case may apply: duration of exposure (e.g., $AUC_{0\text{-}INF}$) is relatively more important than C_{max}. Doxorubicin is a very interesting case. It has maximum antitumor effects on cancer cells that are synthesizing DNA (S phase cells), with some activity during other phases of the cell cycle. However, its cardiac toxicity appears to be more related to C_{max} than to overall exposure ($AUC_{0\text{-}INF}$). Thus, entrapment within liposomes could be more effective and less cardiotoxic [20]. Liposomal carriers allow extended drug exposure, one of its advantages for cytotoxic drugs, with both of the following potential benefits: affecting a higher proportion of cancer cells at S phase; and reducing C_{max}-related toxicity (see **Table 1.1** in **Chapter 6** entitled 'Theoretical benefits of liposomal formulations'). This book includes several examples where it is clear that delivery systems can improve the exposure of certain cytotoxic drugs and/or reduce their toxicity. Drug-delivery systems could also help to obtain a higher concentration of drug inside the tumor cell

Figure 1.2. Cytotoxic drugs show variation in their lethal effect during the cell cycle.

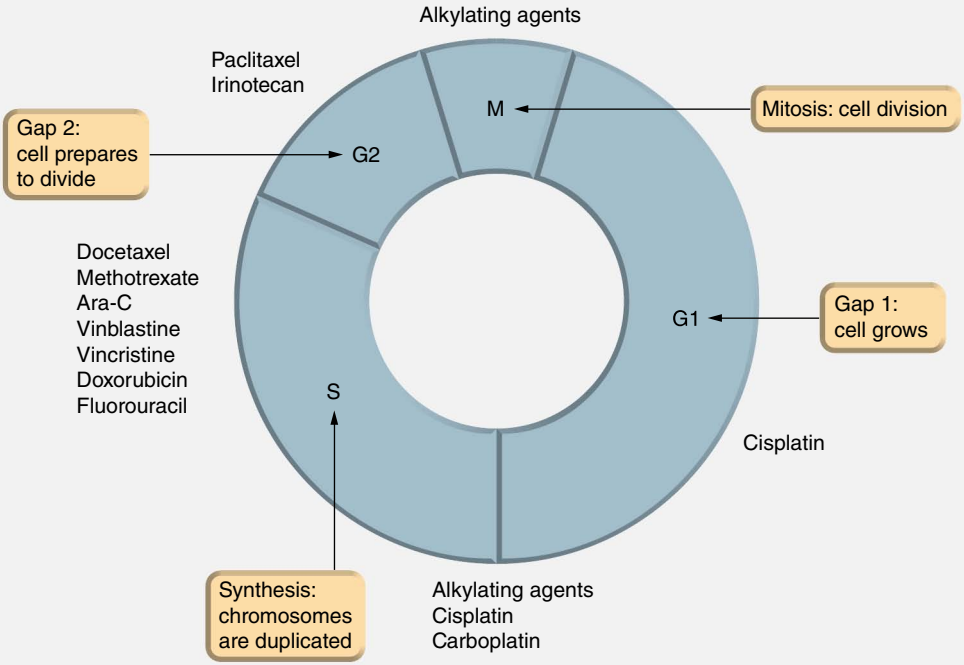


Table 1.1. Drug-delivery systems and their properties.

Class	Major properties			
	Type of cytotoxic drug delivered	***Pharmacokinetics***	***Pharmacodynamics***	***Clinical relevance***
ADCs	Drugs whose potencies are in the picomolar range (e.g., derivatives of calicheamicin, auristatin and maytansine)	Some ADCs display target-mediated drug disposition. Long $t_{1/2}$, low V_d. Usually, lower interpatient pharmacokinetic variability than oral drugs	Active targeting: improve the specificity of the cytotoxic mechanism. However, some show DILI (e.g., SOS)	There may be a need for dose adjustment after initial doses. Bodyweight or BSA might not be the best parameter for administration. Easy administration (on an outpatient basis)
Fusion proteins	Toxins (e.g., diphtheria toxin and pseudomonas exotoxin)	Immune response to the toxin may limit drug exposure with subsequent doses. Antibody fragments allow rapid elimination from blood and reduce 'vascular leak syndrome'	Theoretical lack of cross-resistance with cytotoxic drugs. Cell cycle-phase nonspecific	Effective against difficult-to-treat cancers (chemoresistant). Require clinical care to avoid severe 'vascular leak syndrome'
Polymer conjugates	Bioactive agent: peptides, proteins and cytotoxics with poor solubility (e.g., SN-38)	Prolong circulation time. Reduce immunogenicity, allow similar drug exposure with subsequent doses	Passive targeting: EPR effect. May circumvent some mechanisms of drug resistance	Less frequent administration. More effective and or less toxic than conventional counterpart. The prolonged effect may impose caution with concomitant drug administration
Liposomal drug carriers	Cytotoxic drugs that are cell cycle-phase-dependent	Prolonged plasma residence time. Significant inter-patient variability may remain due to differential clearance	Passive targeting: EPR effect. May circumvent some mechanisms of drug resistance	An alternative to prolonged infusions. More effective and or less toxic than conventional counterpart. Caution with concomitant drug administration

ADC: Antibody–drug conjugate; BSA: Body surface area; DILI: Drug-induced liver injury; EPR: Enhanced permeability and retention; SOS: Sinusoidal obstruction syndrome; V_d: Volume of distribution.

by improving drug transport across the cell membrane and/or reducing drug inactivation.

Organ dysfunction may alter drug disposition in the body and unfortunately is a common scenario in patients with advanced cancer. Drug-delivery systems may have a role in this situation, although specific clinical research is still rare.

Regional cytotoxic therapy

Regional therapy is an attempt to achieve high local concentration of a drug and hence to obtain a therapeutic advantage against tumor cells localized to a particular body region. Direct intrathecal, intraperitoneal, intravesical or topical administration may be advantageous over systemically administered drugs. In fact, all of these regional therapies have been studied extensively and clinical data support their use in certain circumstances in the clinic. Two examples of drug-delivery systems for regional therapy are oportuzumab monatox for intravesical administration (an investigational agent, see **Chapter 3**) and liposomal cytarabine for intrathecal administration **(Chapter 6)**. Intraarterial administration has also been used, mainly for intrahepatic therapy, where delivery systems also seem to have a future application.

PK & PD particularities of drug-delivery systems

Drug-delivery systems present particular PK and PD properties compared with conventional anticancer agents **(Table 1.1)**. Antibody–drug conjugates (ADCs) can be described as a strategy for improving the specificity of cytotoxic drugs and for enhancing the efficacy of naked mAbs [11,21]. The requirement for tumor-specific antigen expression is critical for the success of ADCs because internalization of the cytotoxic payload into nontumor cells may occur even with low antigen expression if the antigen is widely distributed amongst normal tissues [21]. The stability of ADCs in the bloodstream is also very important **(Chapter 2)**. In oncology, mAbs are mostly administered intravenously, although there are some subcutaneous formulations that are approved or in late clinical development. Antibodies can bind to the neonatal Fc receptor and remain in the central compartment for a long period (some have an elimination half-life [$t_{1/2}$] as long as 4 weeks). The distribution of these large molecules into tissues is slow, and volumes of distribution are numerically similar to the plasma volume [22]. Therefore, ADCs have a favorable PK disposition to treat hematologic malignancies that localize in the central compartment and in vascularized areas (circulating blood, bone marrow and lymph nodes). In solid tumors, heterogeneous antigen expression, imperfect vascular supply and increased

interstitial pressure can limit the distribution. Tumor penetration seems to be directly related to the size of the antibody molecules, with deeper and more uniform distribution of single-chain variable fragment (scFv) than of intact IgG. mAbs are metabolized in several tissues, specifically by their target-containing cells or by circulating phagocytic cells [11]. Both linear and nonlinear PK and PD have been described for mAbs. Nonlinear processes may result from target-mediated drug disposition (TMDD), a special case where a high proportion of a drug is bound with high affinity to a pharmacological target [23,24]. The best examples of TMDD are probably gemtuzumab ozogamicin, inotuzumab ozogamicin and naked trastuzumab **(Chapter 2)** [25]. For example, increased serum concentrations of gemtuzumabozogamicin were observed after the second dose probably due to a decrease in clearance by CD33-positive blast cells, a result of the lower peripheral CD33 count after the first dose [25]. For mAbs with TMDD, an open question remains: is there a better way to dose them? Although bodyweight and/or body surface area are generally related to clearance of these mAbs, clinical relevance is low when the advanced disease stage dictates the amount of pharmacological target [26]. Finally, cancer patients are a very heterogeneous population. They are usually older patients and suffer from a variety of comorbidities, including chronic diseases (e.g., diabetes and chronic obstructive pulmonary disease), nutritional deficits, organ dysfunctions, hypoalbuminemia and polymedication. Two facts may make mAbs easier to administer in the clinic than oral cancer drugs: they usually have a lower interpatient variability in exposure and metabolic drug–drug interactions are rare.

A significant number of fusion proteins have entered into clinical research in recent years as a result of modern protein engineering and the availability of potent toxins from bacteria and plants. The intention to use toxins as payloads is very attractive due to: the theoretical lack of cross-resistance with cytotoxic drugs; and the fact that they are cell cycle-phase-nonspecific agents [11]. Some toxins perform better for particular classes of target cells. Nevertheless, one obstacle to the success of this class of agents is the immune response to the toxin component, which may limit the treatment exposure. A second obstacle may be that immunotoxins with longer $t_{1/2}$ lead to increased vascular endothelial injury and consequently to severe 'vascular leak syndrome'. Rapid elimination from blood would allow for repeated, more frequent drug administration; potentially, even short continuous infusions could be used [11]. This is the reason why most immunotoxins are made with antibody fragments, principally with scFv. Complete human antibodies have prolonged $t_{1/2}$ owing to their ability to bind to the neonatal Fc receptor. This receptor is expressed on placenta

and blood vessel linings and protects serum IgG from degradation. As Fab and scFv fragments lack the Fc region, they are not protected by this receptor. There is also a difference in biodistribution kinetics between fragments and complete mAbs in experimental observations and mathematical models [11]. This may play a role in how effectively drug is delivered to solid tumors, although clinical data are limited.

The main intention of using polymers and liposomes is to improve the delivery of the active agent, in some cases having less adverse events. For instance, anticancer peptides and proteins have poor stability, are generally short-lived in circulation and could induce high immunogenicity. Polymer–protein conjugates aim to improve these features and could drastically change the PK of the bound drug. Polymeric carriers are also used to improve the solubility of cytotoxic small molecules. Several attempts are in early development for camptothecin analogs. Conjugates with prolonged circulation times and liposomes target tumors by the enhanced permeability and retention effect (see section entitled 'Passive targeting') [20,27]. Tumor blood vessels allow preferential extravasation of macromolecules, which stay for a long time in the interstitium owing to impaired lymphatic drainage in the tumor microenvironment. The uptake of polymer–drugs and liposomes by cancer cells and/or their intracellular metabolism may circumvent some mechanisms of drug resistance. Like other delivery systems, polymer–drug conjugates and liposomes have PK and PD particularities. Successful examples of these systems have good retention within the central compartment (prolonged plasma residence time) and minimal loss of bound or entrapped drug. From an administration perspective, this means that they could be an attractive alternative to prolonged infusions that usually require a portacath. However, significant intersubject variability in PK may remain. For some liposome formulations, the cause of the variability is a differential clearance during the initial α phase that could subdivide patients into two subsets ($+\alpha$ and $-\alpha$). The different α phases may be due to differential liposomal uptake by the reticuloendothelial system. Pegylation may protect liposomes from detection by the reticuloendothelial system [20]. The prolonged effect of these systems also imposes caution with concomitant drug administration. Pegfilgrastim (Neulasta®, Amgen Inc., CA, USA) must not be administered between 14 days before and 24 h after administration of cytotoxic chemotherapy; thus, this agent must not be used with any weekly schedule of chemotherapy. Likewise, the use of granulocyte colony-stimulating factor or granulocyte–macrophage colony-stimulating factor should be avoided for more than 24 h after the administration of cytotoxic liposomal formulations.

Several drug-delivery systems have been approved for the treatment of hematologic malignancies and solid tumors. They have particular characteristics that can help to improve the therapeutic index of cytotoxic drugs through better targeting of tumors and or reduced toxicity.

The last class of a delivery system approved in oncology is drug protein-bound particles. Some poorly water-soluble conventional cytotoxic drugs are formulated in oily vehicles, such as polyethoxylated castor oil (Cremophor EL). These vehicles require the use of premedication, restrict the infusion rate and may cause infusion-related reactions. The solvents may also adversely affect the PK of the active drugs owing to entrapment in micelles formed in the plasma, in some cases showing nonlinear PK. Nanoparticle technologies using drug protein-bound particles avoid the need of solvent oils and may have preferential transport and better concentration in tumors. The best example is nab-paclitaxel (see **Chapter 5**) that may have preferential transport by glycoprotein 60-mediated endothelial cell transcytosis and accumulation in the tumor by albumin binding to SPARC (secreted protein, acidic and rich in cysteine; or osteonectin).

Drug-delivery systems: differentials in tumor targeting

Active targeting

This term refers to the aiming at suitable (highly specific) targets on the tumor cell surface or in the tumor microenvironment [11]. It is best represented by mAbs that have been introduced in oncology practice since the 1990s. After several regulatory approvals of naked mAbs, we are now witnessing the approval and the extensive clinical development of several ADCs. The conjugation of mAbs with chemotherapeutic agents attempts to enhance the efficacy of cytotoxic drugs through specificity. In this regard, the tumor-specific antigen expression is critical for the success of ADCs (see the section entitled 'Factors Regulating Antibody Drug Conjugate-based Therapy' in **Chapter 2**). As **Chapter 2** states, ADC therapy is more favorable for the treatment of hematologic malignancies than for the treatment of solid tumors, with the only exception at present of HER2-positive tumors. However, it is fair to hope that the biotechnology available today will provide new candidates more suitable against solid tumors [11]. This new generation of agents should be developed with clinical designs based on the level of target expression (see the section entitled 'Conclusion' in **Chapter 2**). When there are no suitable targets for a particular tumor type, and we should assume this might happen even with a higher grade of bioinformatics and data mining, delivery systems with passive targeting may help to improve the efficacy of cytotoxic drugs.

Optimal cytotoxic treatment scheduling is difficult to apply in clinical oncology. The main reasons conspiring against optimal scheduling include rapid loss of synchrony (variation of cell-cycle times) and heterogeneity of tumor cell populations, drug toxicity to normal tissues and deficient drug delivery to tumors.

Passive targeting

Far from the concept of active targeting that represents specific molecular interactions, passive targeting represents an approach based on the so-called 'enhanced permeability and retention' effect [20,27]. This therapeutic approach originated after pivotal experiments conducted by Hiroshi Maeda showing that tumor microenvironment allows preferential accumulation of macromolecules [28]. Tumor blood vessels are markedly different to normal tissue blood vessels. In normal tissues, vessel organization is orderly and determined by the metabolic needs of the different regions. This organization can be described by 'fractal geometry'. Normal vessel walls consist of endothelial cells, pericytes and vascular smooth muscle cells. They regulate blood flow and vascular permeability. At the capillary and postcapillary level, the pericyte coverage varies with tissue function. Structurally, blood vessels in tumors are irregular in shape, tortuous, dilated and have large fenestrations [18]. They are leaky due to the high levels of VEGF present in the microenvironment and due to changes in perivascular cells and associations. The basement membrane is also irregular and differs in composition to the membrane in normal vessels. Findings in recent years have demonstrated the complex and unpredictable nature of the tumor microvasculature. The endothelial lining is patchy and may include tumor cells [18]. In addition, a new paradigm has emerged called 'vasculogenic mimicry', which describes vasculogenic-like networks by tumor cells [29]. Thus, tumor vasculature is more permeable for large molecules than normal vasculature. After permeating into the tumor interstitium, macromolecules are 'retained' owing to impaired lymphatic drainage in the microenvironment. However, higher accumulation in tumor interstitium may not automatically turned into higher efficacy for a particular cytotoxic drug. However, for those who have solubility issues, for example, SN-38, the conjugation with a polymer may give the dual benefit of improved PK profile and preferential accumulation in tumor microenvironment.

Aa Passive targeting: an approach based on the so-called 'enhanced permeability and retention' effect. Tumor blood vessels are markedly different to normal tissue blood vessels. Structurally, blood vessels in tumors are irregular in shape, tortuous, dilated and have large fenestrations. They are leaky due to the high levels of VEGF present in the microenvironment and due to changes in perivascular cells and associations. Thus, tumor vasculature is more permeable for large molecules than normal vasculature. After permeating into the tumor interstitium, macromolecules are 'retained' owing to impaired lymphatic drainage in the microenvironment.

Active targeting: the aiming at suitable (highly specific) targets on the tumor cell surface or in the tumor microenvironment.

Conclusion

Several drug-delivery systems have been approved for the treatment of hematologic malignancies and solid tumors. They have particular characteristics that can help to improve the therapeutic index of cytotoxic

drugs through better targeting of tumors and/or reduced toxicity. Modern technology will provide new candidates against difficult-to-treat tumors.

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Summary

- Most cytotoxic drugs have a narrow therapeutic index and are dosed close to the maximum tolerated dose found in dose-escalating studies. A method for increasing the therapeutic index is to preferentially direct drugs to tumor cells, and/or to reduce their toxicity. Delivery systems attempt to improve the therapeutic index of cytotoxic drugs. This book describes several common approaches currently used in the clinic: linking drugs to a carrier (antibodies); binding drugs to proteins; conjugating drugs to polymers; and entrapping drugs in liposomes.
- Drug-delivery systems present particular pharmacokinetic and pharmacodynamic properties compared with conventional anticancer agents.
- The term 'active targeting' refers to the aiming at suitable (highly specific) targets on the tumor cell surface or in the tumor microenvironment. It is best represented by monoclonal antibodies, which have been introduced in oncology practice since the 1990s.
- Far from the concept of active targeting that represents specific molecular interactions, passive targeting represents an approach based on the so-called 'enhanced permeability and retention' effect.

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Alejandro D Ricart is Consultant Medical Oncologist and Senior Clinical Investigator in the Division of Solid Tumors at Henry Moore Institute of Oncology (Buenos Aires, Argentina). He received his MD from the University of Buenos Aires School of Medicine (Argentina) and completed his residency training at the Hospital Militar Central (Buenos Aires, Argentina). He was a fellow at the Department of Medical Oncology at the Alexander Fleming Institute (Buenos Aires, Argentina), where he first developed an interest in developmental therapeutics. He was Consultant Oncologist at Cvitkovic & Associés Consultants SA (Paris, France). He also served as Assistant Professor of Pharmacology at the University of Buenos Aires, and as Professor of Pharmacology at the Hebrew University Argentina Bar-Ilan (Buenos Aires, Argentina) until 1999. He completed an advanced oncology drug development/medical oncology program at the Institute for Drug Development, Cancer Therapy and Research Center (TX, USA) and at the Division of Medical Oncology, University of Texas Health Science Center at San Antonio (USA). There, he was introduced to multiple new therapeutic agents targeting cancer in a variety of novel ways. He has been an investigator in over 50 trials on cancer, including several studies of monoclonal antibodies, signal transduction inhibitors and tyrosine kinase inhibitors, in all phases of clinical development. His honors include receipt of the American Society of Clinical Oncology Foundation Merit Award in 2006. He joined Pfizer Inc. (CA, USA) in October 2006 and participated in the clinical development of several investigational compounds, including axitinib and bosutinib. Later, he was Director of Biotechnology and Oncology Clinical Research at Pfizer Inc. in San Diego. He has published more than 80 journal articles, abstracts, reviews and book chapters.

Chapter 2

Antibody–drug conjugates

Alejandro D Ricart

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Drug development in oncology was mostly empirical until the late 1990s but, since then, the introduction of monoclonal antibodies (mAbs) into the clinic has established a new component of cancer treatment. The success of this therapy has relied predominantly on the ability to make a desired mAb and the characterization of suitable tumor targets, opening an unprecedented opportunity for targeted therapy [1]. It also offers an adaptable approach; antibodies can be engineered to carry moieties (payloads), such as radionuclides, cytotoxic drugs or toxins. Antibody–drug conjugation can be perceived as a strategy for improving the specificity of cytotoxic chemotherapy, and for enhancing the efficacy of passive immunotherapy, with the ambition of integrating the best characteristics of both therapeutic approaches [2]. Antibody–drug conjugates (ADCs) are built with cytotoxic drugs the potencies of which are in the picomolar range: derivatives of calicheamicin, auristatin and maytansine, among others **(Figure 2.1)** [3–6]. Thus, the efficacy of ADCs does not depend on the host immune system status and is not adversely affected by internalization of mAb–antigen complexes. However, conjugation to cytotoxic drugs

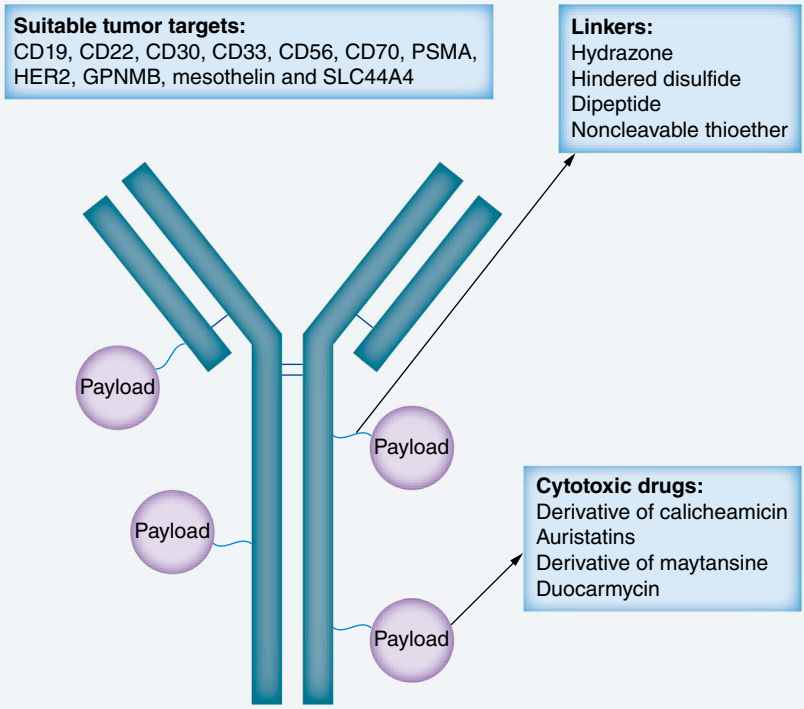
Aa Antibody–drug conjugates: a new class of biotherapeutic drugs designed with an unprecedented specificity to deliver cytotoxic drugs to cancer cells. Antibody–drug conjugates are large, complex molecules composed of a monoclonal antibody linked to cytotoxic drugs the potencies of which are in the picomolar range (e.g., derivatives of calicheamicin, auristatin and maytansine). The linkage is usually via a stable, chemical linker with labile bonds.

considerably increases the toxicity of mAb, best exemplified by the comparison of the naked trastuzumab and trastuzumab emtansine (T-DM1) safety profiles [7–9].

Factors regulating ADC-based therapy

Like mAbs, the efficacy of a particular ADC relies on different variables. These include not only the characteristics of the mAb itself (fine specificity, avidity and isotype), but also those of the targeted antigen (function, density, normal tissue distribution, the presence of secreted isoforms, internalization and its phenotypic expression in the cancer cell

Figure 2.1. Three components of an antibody–drug conjugate with examples of antigen targets, linkers and payloads.



GPNMB: Glycoprotein nonmetastatic melanoma protein B; HER2: Human EGF receptor 2; PSMA: Prostate-specific membrane antigen.

population). The requirement for tumor-specific antigen expression is critical for the success of ADCs because internalization of the cytotoxic payload into nontumor cells might occur even with low antigen expression if the antigen is widely distributed among normal tissues. This process, known as antigen sink, could manifest as adverse events (AEs) derived from anatomically distant organs and result in lack of antitumor activity [2]. The delivery of the payload can also be increased by several orders of magnitude targeting tumor antigens whose expression on the cell membrane is measured in the millions (e.g., human EGF receptor 2; HER2). The stability of the intact ADC in the bloodstream is also very important, as well as the selection of the payload (some mechanisms of action are more suitable for certain tumor types, such as microtubule polymerization inhibitors for metastatic breast cancer) [2].

Hematologic malignancies localize in areas readily accessible to ADCs, such as the circulating blood, bone marrow and lymph nodes. They possess antigens with the right grade of specificity, and the pharmacokinetic (PK) disposition of mAbs is favorable for their treatment, as distribution into other tissues is slow [10,11]. By contrast, significant obstacles prevent ideal targeting against solid tumors **(Box 2.1)**. In this case, intact mAbs have limitations due to their PKs: there is a relatively poor diffusion from the vasculature into and through the tumor and accordingly limited quantities are delivered [12]. Heterogeneous antigen expression and imperfect vascular supply are believed to be the main reasons. Impaired clearance of fluid from tumors (due to lack of lymphatic vessels) leads to increased interstitial pressure [13]. This elevated pressure in the centers of tumors opposes inward diffusion, leading to a net outward gradient from the tumor center and slowing the diffusion of IgG molecules from their extravasation site.

Box 2.1. Obstacles to achieving efficacy with antibody–drug conjugate therapy.

- Cell surface-targeted antigen density and rate of turnover
- Impaired monoclonal antibody distribution†
- Limited delivery to tumor masses†
- Insufficient trafficking of effector cells to tumor†‡
- Antigenic heterogeneity (intra- and inter-tumoral)†
- Shedding of targeted cancer antigen†
- Insufficient tumor specificity of target antigens†
- Immunogenicity: human anti-mouse and anti-chimeric antibody responses, immune response to peptide cytotoxins

†These obstacles are not seen or are less critical in hematologic malignancies.
‡In cases that the antibody–drug conjugate retains Fcγ receptor-mediated engagement of immune effector cells (e.g., trastuzumab emtansine).

Consequently, this gradient within solid tumors differentially inhibits the diffusion of larger molecules in comparison with smaller ones [14]. Experimentally, tumor penetration seems to be directly related to the size of the antibody molecules, with faster, deeper and more uniform tumor penetration of scFv than of intact IgG [13]. However, several questions remain unanswered in comparing the use of intact IgG versus fragments in the therapeutic application to solid tumors, as clinical experience is limited. Antigen shedding (soluble antigen in the extracellular fluid of tumors and in the bloodstream) can also limit delivery within the tumor and reduce the clinical activity of mAbs [15]. Teicher and Chari have recently described six steps for the payload to reach its intracellular target, with schematic calculations of efficiency [16].

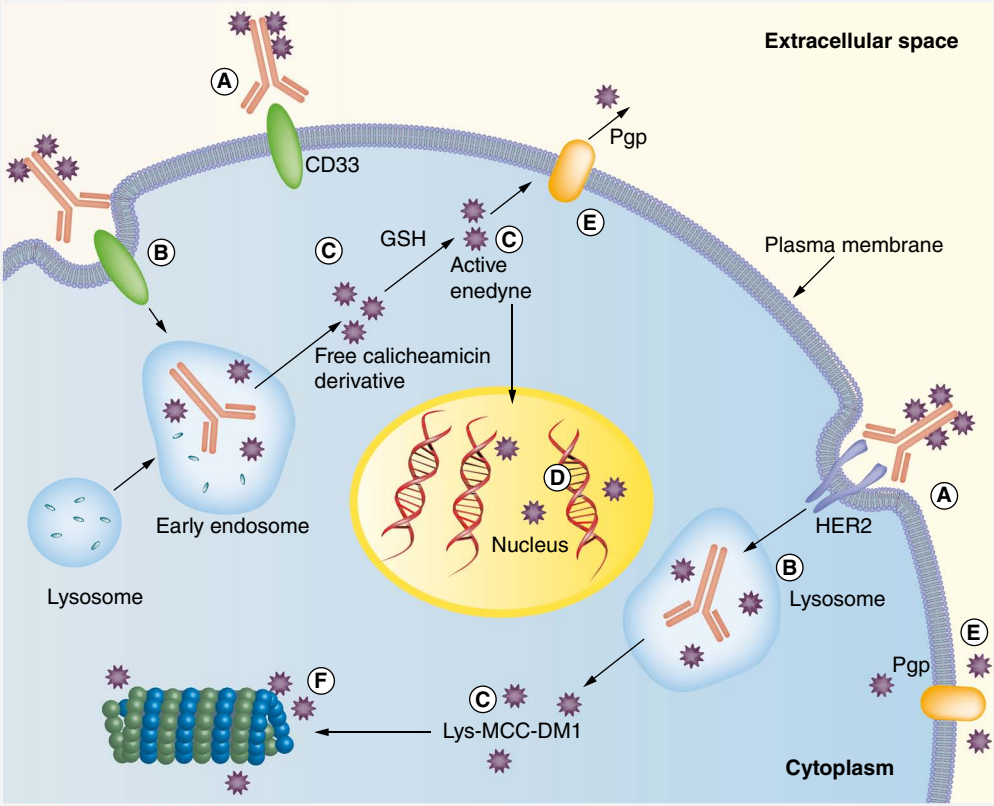
ADCs of calicheamicin derivative: gemtuzumab ozogamicin & inotuzumab ozogamicin

CD33 and CD22 are siglecs (sialic-acid-binding immunoglobulin-like lectins) restrictedly expressed on one or a few immune cell types. They are endocytic receptors that effectively carry the mAb payload inside the cell, and are thus ideal targets for an ADC strategy [17].

Gemtuzumab ozogamicin

Gemtuzumab ozogamicin (GO; Mylotarg®, Pfizer Inc., NY, USA) contains a semisynthetic derivative of calicheamicin (*N*-acetyl-γ calicheamicin 1,2-dimethyl hydrazine dichloride), a potent enediyne DNA-binding antibiotic, linked to a humanized mAb (an IgG_4) directed against CD33. On binding to CD33, GO is rapidly internalized, as determined by the decrease in maximal membrane binding [18]. The cytotoxic drug is attached to the mAb through a covalent linkage (condensation) of a bifunctional linker, 4-(4-acetylphenoxy) butanoic acid (AcBut linker), which allows an efficient payload release inside lysosomes [19]. The average loading of calicheamicin on the antibody is 2.5 mol/mol (drug loading range of 2–3 mol of calicheamicin per mole of antibody) [19]. *In vitro* studies using pulse labeling with GO showed a continuous renewal of CD33, which increased the internalization and thereby the intracellular accumulation of the cytotoxic agent [18]. Calicheamicin binds to the minor groove in the DNA and causes double-stranded DNA breaks, resulting in cell death (please refer to **Figure 2.2** to follow this sequence) [11]. Since cytotoxicity is dependent on the calicheamicin component, tumor cells exhibiting P-glycoprotein (Pgp) may be able to escape from the effect of GO. This is suggested by the correlation between clinical response and low Pgp activity in leukemic blast cells [20–22]. The PKs of GO were characterized in patients with acute myeloid leukemia (AML) [23]. Increased concentrations

Figure 2.2. Mechanisms of action of gemtuzumab ozogamicin and trastuzumab emtansine.



(A) Antibody–drug conjugate binding and internalization. **(B)** Antibody–drug conjugate intracellular trafficking from endosome to lysosome, with degradation of the monoclonal antibody and linker to **(C)** release the cytotoxic payload. **(D)** The active enedyne form binds to the minor groove in DNA and causes double-stranded DNA breaks, resulting in cell death. **(E)** Pgp-mediated efflux may be a mechanism of drug resistance in some tumor types. **(F)** DM-1 binds to tubulin and prevents microtubule polymerization, causing cell cycle arrest at the G2/M phase and leading to cell death.
DM1: *N*-methyl-*N*-[3-mercapto-1-oxopropyl]-L-alanine ester of maytansinol; GSH: Glutathione; HER2: Human EGF receptor 2; Pgp: P-glycoprotein.

were observed after the second dose, most probably due to a decrease in clearance by CD33-positive cells, a result of the lower peripheral leukemic burden after the first dose [23,24]. This clearly suggests a target-mediated disposition, which would explain why weight and body surface area do not affect the PKs of GO [25].

Aa Target-mediated drug disposition: corresponds to a particular pharmacological case wherein drug–target binding significantly influences pharmacokinetics. This drug–target interaction and its dose-dependent effects may have pharmacodynamic implications.

The major toxicity of GO is reversible myelosuppression, especially neutropenia and thrombocytopenia [4]. GO is easy to administer compared with conventional chemotherapy for relapsed AML. Although patients treated with GO in Phase II studies had relatively high incidences of myelosuppression, grade 3 or 4 hyperbilirubinemia (23%), and elevated hepatic transaminase levels (17%), rates of gastrointestinal toxicity and infections were relatively low. GO was administered on an outpatient basis in a good proportion of patients (38 and 41% for the first and second doses, respectively) and the median duration in hospital was 24 days [21]. Postmarketing reports of fatal anaphylaxis, adult respiratory distress syndrome (ARDS) and hepatotoxicity (especially sinusoidal obstructive syndrome; SOS) required labeling revisions and a registration surveillance program [26]. Hepatotoxicity was believed to be a nonspecific hepatocellular uptake rather than target-mediated effect, although a different hypothesis considers that GO might target CD33-positive cells in hepatic sinusoids (including Kupffer cells) [27,28]. At present, it is clear that GO has a slight association with SOS (incidence: 1–2%), but patients who undergo hematopoietic cell transplantation within a short interval after GO administration (≤3.5 months) are at increased risk [29–31]. It is recommended to reduce the leukocyte count to below 30,000/µl prior to GO administration to minimize the chances of tumor lysis and ARDS [26]. Furthermore, reduction of the leukemic cell burden prior to GO administration could optimize distribution into the bone marrow [32].

Three open-label trials evaluated the efficacy and safety of single-agent GO in patients with AML in first relapse **(Tables 2.1 & 2.2)**. The remission rate was 30%, the definition being ≤5% blasts in the marrow, recovery of neutrophils to at least 1500/µl and transfusion independence [21]. The US FDA granted marketing approval under the Accelerated Approval regulations in 2000 [26], with an indication for patients with CD33-positive AML in first relapse who were 60 years of age or older and who were not considered candidates for cytotoxic chemotherapy. GO was later approved with a similar indication in Japan and Europe. A final report covering a total of 277 patients showed similar results [30]. Several Phase II studies followed **(Tables 2.1 & 2.2)**, suggesting: acceptable activity with tolerable toxicity in AML, opportunity for first-line therapy and remarkable activity in acute promyelocytic leukemia. Prolonged molecular responses in acute promyelocytic leukemia, without reports of SOS [33–38], are particularly interesting, since this AML type has a high surface expression of CD33 with low levels or absence of Pgp [39–41].

However, the required postapproval study (SWOG S0106), combining GO with standard chemotherapy in first-line AML patients under the age of 61 years, failed to confirm clinical benefit. This study was stopped based

Table 2.1. Relevant gemtuzumab ozogamicin single-agent studies in adult patients.

Study (year)	Sample size (n)	Median age, years (range)	Dose and schedule	AML status	CR/ CRp (%)	Median RFS	Ref.
Sievers *et al.* (2001)	142	61 (22–84)	9 mg/m^2 days 1 and 15	First-relapse AML	16/13	6.8 months	[21]
Taksin *et al.* (2007)	57	64 (22–80)	3 mg/m^2 days 1, 4 and 7	First-relapse AML	26/7	11.0 months	[70]
Piccaluga *et al.* (2004)	24	63 (20–75)	6 or 9 mg/m^2 for two to three doses	Relapsed, refractory AML	13/8	6.0 months[†]	[71]
Lo-Coco *et al.* (2004)	16	52 (17–77)	6 mg/m^2 for two to three doses	Molecularly relapsed APL	88/0	15 months[‡]	[36]
Amadori *et al.* (2010)	56[§]	78 (62–86)	Arm A: 3 mg/m^2 days 1, 3 and 5 Arm B: 6 mg/m^2 days 1 and 8	Untreated AML	21/0 18/4	Not reported Not reported	[72]
Amadori *et al.* (2005)	40	76 (61–89)	9 mg/m^2 days 1 and 15	Untreated AML	10/7	6.1 months[¶]	[73]
Nabhan *et al.* (2005)	12	75 (66–79)	9 mg/m^2 days 1 and 15	Untreated AML	27/0	Not reported	[74]

This table includes published studies only. Phase II studies with heterogeneous diagnosis and/or mixed AML status were excluded.
[†]Median duration of response.
[‡]Seven patients who remained in sustained molecular remission for a median of 15 months.
[§]Randomized Phase II study. Patients in arm C (n = 28) received best supportive care. The rate of disease nonprogression, the primary end point, was 38% in arm A versus 63% in arm B; the days 1 + 8 schedule met the statistical criteria for Phase III comparison.
[¶]Patients with CR.
AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; CR: Complete remission; CRp: Complete remission with incomplete platelet recovery; RFS: Relapse-free survival.

on interim results showing no improvement in complete response rate or survival, with a significantly higher rate of fatal induction toxicity (5.8% in the GO–chemotherapy arm vs 0.8% in the chemotherapy-alone arm; p = 0.002) [42]. The most common fatal AEs were hemorrhage, infection and ARDS. One death in the GO–chemotherapy arm was attributable to SOS. The AML 15 study also failed to show improvement in clinical benefit in the intent-to-treat population with the addition of GO, but there was no significant additional toxicity. A subset analysis by cytogenetics showed highly significant interaction with induction GO (p = 0.001), with significant survival benefit for patients with favorable cytogenetics [43]. Pfizer Inc. voluntarily withdrew the New Drug Application for GO (Mylortag®) in the USA

Table 2.2. Relevant gemtuzumab ozogamicin studies in combination therapy in adult patients.

Study (year)	Sample size (n)	Median age, years (range)	GO dose and schedule	AML status	Combined with	CR/CRp (%)†	Ref.
Single-arm studies							
Stone *et al.* (2011)	37	64 (55–70)	9 mg/m^2 day 7	Relapsed, refractory AML	High-dose Ara-C	32/3	[75]
Chevallier *et al.* (2008)	62	56 (16–71)	9 mg/m^2 day 4	Relapsed, refractory AML	Ara-C and mitoxantrone	50/13	[76]
Tsimberidou *et al.* (2003)	32	53 (18–78)	6 mg/m^2 day 1	Relapsed, refractory AML	Fludarabine, Ara-C, cyclosporine	28/6	[77]
Ravandi *et al.* (2009)	25‡	47 (14–81)	9 mg/m^2 day 1	Untreated high-risk APL	ATRA and ATO	81/0	[33]
Estey *et al.* (2006)	15	45 (NR)	9 mg/m^2 day 1	Untreated high-risk APL	ATRA and ATO	73/0	[35]
Estey *et al.* (2002)	19	50 (NR)	9 mg/m^2 day 1 or day 5	Untreated APL	ATRA	84/0	[37]
Candoni *et al.* (2008)	30	53 (25–65)	3 mg/m^2 day 6	Untreated AML	Fludarabine, Ara-C, idarubicin	90/0	[78]
Eom *et al.* (2007)	37	64 (55–76)	6 mg/m^2 day 1	Untreated AML	Idarubicin and BH-AC	76/3	[79]

†The subject of this column changes to 'Overall survival' for the Phase III studies.
‡Subset of patients.
§Includes 20 patients with refractory anemia with excess blasts.
¶Not statistically significant.
#Estimated median overall survival, not statistically significant.
††Patients were not considered suitable for conventional therapy.
6-TG: 6-Tioguanine; ADE: Cytarabine, daunorubicin and etoposide; AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; Ara-C: Cytarabine; ATO: Arsenic trioxide; ATRA: All-*trans*-retinoic acid; BH-AC: N4-behenoyl-1-b-arabinofuranosyl cytosine; CAT: Cyclophosphamide with mesna, cytarabine, topotecan; CR: Complete remission; CRp: Complete remission with incomplete platelet recovery; DA: Daunorubicin and cytarabine; FLAG-Ida: Fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin; GO: Gemtuzumab ozogamicin; L: Liposomal; NA: Not applicable; NR: Not reported; OS: Overall survival.

Table 2.2. Relevant gemtuzumab ozogamicin studies in combination therapy in adult patients.

Study (year)	Sample size (n)	Median age, years (range)	GO dose and schedule	AML status	Combined with	CR/CRp (%)[†]	Ref.
Single-arm studies (cont.)							
Tsimberidou *et al.* (2003)	59	57 (27–76)	6 mg/m^2 day 1	Untreated AML[§]	Fludarabine, Ara-C, cyclosporine	46/2	[80]
Kell *et al.* (2003)	64	47 (17–59)	3 or 6 mg/m^2 day 1	Untreated AML	Daunorubicin, Ara-C, 6-TG; or fludarabine, Ara-C, idarubicin	84	[81]
Amadori *et al.* (2004)	57	68 (61–75)	9 mg/m^2 day 1 or day 15	Untreated AML	Mitoxantrone, Ara-C, etoposide	35/19	[82]
Clavio *et al.* (2007)	46	66 (60–80)	3 mg/m^2 at day 4	Untreated AML	Fludarabine, Ara-C, idarubicin	52/0	[83]
Randomized Phase II studies							
Litzow *et al.* (2010)	82	60 (27–75)	6 mg/m^2 day 5	Relapsed, refractory (>50%) AML	Arm A: Ara-C plus GO Arm B: Ara-C plus liposomal daunorubicin Arm C: CAT regimen	8/4	[84]
		52 (27–85)	NA			7/0	
		53 (25–78)	NA			4/0	

[†]The subject of this column changes to 'Overall survival' for the Phase III studies.
[‡]Subset of patients.
[§]Includes 20 patients with refractory anemia with excess blasts.
[¶]Not statistically significant.
[#]Estimated median overall survival, not statistically significant.
[††]Patients were not considered suitable for conventional therapy.
6-TG: 6-Tioguanine; ADE: Cytarabine, daunorubicin and etoposide; AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; Ara-C: Cytarabine; ATO: Arsenic trioxide; ATRA: All-*trans*-retinoic acid; BH-AC: N4-behenoyl-1-b-arabinofuranosyl cytosine; CAT: Cyclophosphamide with mesna, cytarabine, topotecan; CR: Complete remission; CRp: Complete remission with incomplete platelet recovery; DA: Daunorubicin and cytarabine; FLAG-Ida: Fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin; GO: Gemtuzumab ozogamicin; L: Liposomal; NA: Not applicable; NR: Not reported; OS: Overall survival.

Table 2.2. Relevant gemtuzumab ozogamicin studies in combination therapy in adult patients.

Study (year)	Sample size (n)	Median age, years (range)	GO dose and schedule	AML status	Combined with	OS	Ref.
Randomized Phase III studies							
Burnett *et al.* (2011)	1113	49 (0–71)	3 mg/m^2 on day 1 of induction	Untreated AML	Induction: with DA, ADE or FLAG-Ida Control arm: no GO	5-year: 43%¶ 5-year: 41%	[43]
Petersdorf *et al.* (2013)	627	NR (18–60)	6 mg/m^2 on day 4 of induction	Untreated AML	Induction: DA with GO Control arm: no GO	31 months# 35 months	[42]
Löwenberg *et al.* (2010)	232	67 (60–78)	6 mg/m^2 × 3 doses after induction	Untreated AML	GO after induction therapy Control arm: no further therapy	5-year: 28%¶ 5-year: 21 %	[85]
Burnett *et al.* (2013)	495	76 (61–90)	5 mg on day 1	Unsuitable AML††	Low-dose Ara-C with GO Control arm: no GO	1-year: 25% 1-year: 27%	[86]

†The subject of this column changes to 'Overall survival' for the Phase III studies.
‡Subset of patients.
§Includes 20 patients with refractory anemia with excess blasts.
¶Not statistically significant.
#Estimated median overall survival, not statistically significant.
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6-TG: 6-Tioguanine; ADE: Cytarabine, daunorubicin and etoposide; AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; Ara-C: Cytarabine; ATO: Arsenic trioxide; ATRA: All-*trans*-retinoic acid; BH-AC: N4-behenoyl-1-b-arabinofuranosyl cytosine; CAT: Cyclophosphamide with mesna, cytarabine, topotecan; CR: Complete remission; CRp: Complete remission with incomplete platelet recovery; DA: Daunorubicin and cytarabine; FLAG-Ida: Fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin; GO: Gemtuzumab ozogamicin; L: Liposomal; NA: Not applicable; NR: Not reported; OS: Overall survival.

Table 2.2. Relevant gemtuzumab ozogamicin studies in combination therapy in adult patients.

Study (year)	Sample size (n)	Median age, years (range)	GO dose and schedule	AML status	Combined with	OS	Ref.
Randomized Phase III studies (cont.)							
Löwenberg *et al.* (2010)	232	67 (60–78)	6 mg/m^2 × 3 doses after induction	Untreated AML	GO after induction therapy Control arm: no further therapy	5-year: 28%[¶] 5-year: 21 %	[85]
Burnett *et al.* (2013)	495	76 (61–90)	5 mg on day 1	Unsuitable AML[††]	Low-dose Ara-C with GO Control arm: no GO	1-year: 25% 1-year: 27%	[86]
Burnett *et al.* (2012)	1115	67 (51–84)	3 mg/m^2 on day 1 of cycle 1	Untreated AML	DA or daunorubicin plus clofarabine with GO Control arm: no GO	3-year: 25% 3-year: 20%	[45]
Castaigne *et al.* (2012)	280	62 (50–70)	3 mg/m^2 on days 1, 4 and 7 of induction	Untreated AML	Induction: DA with GO Control arm: no GO	2-year: 53% 2-year: 42%	[44]

[†]The subject of this column changes to 'Overall survival' for the Phase III studies.
[‡]Subset of patients.
[§]Includes 20 patients with refractory anemia with excess blasts.
[¶]Not statistically significant.
[#]Estimated median overall survival, not statistically significant.
[††]Patients were not considered suitable for conventional therapy.
6-TG: 6-Tioguanine; ADE: Cytarabine, daunorubicin and etoposide; AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; Ara-C: Cytarabine; ATO: Arsenic trioxide; ATRA: All-*trans*-retinoic acid; BH-AC: N4-behenoyl-1-b-arabinofuranosyl cytosine; CAT: Cyclophosphamide with mesna, cytarabine, topotecan; CR: Complete remission; CRp: Complete remission with incomplete platelet recovery; DA: Daunorubicin and cytarabine; FLAG-Ida: Fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin; GO: Gemtuzumab ozogamicin; L: Liposomal; NA: Not applicable; NR: Not reported; OS: Overall survival.

Aa Maximum tolerable dose (MTD): the maximum dose associated with an acceptable level of dose-limiting toxicity. The MTD is then taken into further testing. In oncology, the MTD is usually the recommended Phase II dose for cytotoxic mechanisms of action.

and Europe in 2010, but it continues to be commercially available in Japan where it has full regulatory approval. Of note, since then, two randomized studies have shown improved survival in patients with AML when low fractionated doses of GO are added to standard front-line chemotherapy [44,45]. This groundbreaking evidence emphasizes the importance of the schedule of administration for an agent with a significant target-mediated drug disposition by the disease under treatment.

Inotuzumab ozogamicin

Inotuzumab ozogamicin is an ADC composed of the derivative of calicheamicin and an IgG_4 isotype that specifically recognizes human CD22. This siglec is expressed in approximately 60 to >90% of B-lymphoid malignancies [46–48]. CD22 has many of the ideal properties for an ADC target **(Box 2.2)**. Similar to GO, the cytotoxic effect of inotuzumab ozogamicin is inversely related to the amount of Pgp, but the effect positively correlates with the surface expression of CD22 ($p = 0.010$) [49]. In xenograft models, inotuzumab ozogamicin showed greater benefit than combination chemotherapy, and had superior antitumor activity when combined with standard chemotherapy [50]. In murine models, it was effective against lymphoma cells resistant to rituximab and its combination with rituximab demonstrated additive effects [51]. PK data indicate that drug disposition is nonlinear with dose or the number of doses. The increase in area under the curve with time is probably due to a reduction in the amount of target (CD22) after the initial dose. PK data also suggest that the linker is considerably stable in plasma [52].

In the first-in-human study, the maximum tolerable dose (MTD) of inotuzumab ozogamicin was 1.8 mg/m^2 every 4 weeks with reversible

Box 2.2. CD22 has many of the ideal properties for an antibody–drug conjugate target.

- The normal function of CD22 is to regulate signal transduction of the surface immunoglobulin receptors on B cells
- CD22 is expressed on the cells of the majority of B-lymphocyte malignancies
- It is not expressed on hematopoietic stem cells or any other nonlymphoid hematopoietic or nonhematopoietic cells
- Memory B cells do not express CD22
- Based on *in vitro* testing of human cell lines, CD22 is one of the better internalizing molecules among several B-lymphoid lineage-specific surface antigens
- CD22 is not shed into the extracellular environment

thrombocytopenia as the main toxicity [52]. It was subsequently tested in single-agent Phase II studies, in patients with indolent relapsed/refractory non-Hodgkin's lymphoma (NHL) and acute lymphoblastic leukemia. An overall response rate (ORR) of 53% (19% complete remission) was observed in patients with CD22-positive indolent NHL, with an ORR of 66% in follicular lymphoma. The most common treatment-related AEs were thrombocytopenia, neutropenia, elevated aspartate aminotransferase, leukopenia, nausea, fatigue, lymphopenia and decreased appetite [53]. A randomized, open-label Phase III trial (INO-VATE NHL study) is evaluating inotuzumab ozogamicin plus rituximab versus a defined investigator's choice of bendamustine plus rituximab or gemcitabine plus rituximab in patients with relapsed/refractory aggressive B-cell lymphoma. Inotuzumab ozogamicin is also being evaluated in a phase III study in adult patients with CD22-positive acute lymphoblastic leukemia (INO-VATE ALL study).

ADCs of auristatin: brentuximab vedotin

Brentuximab vedotin (BV; formerly SGN-35; Adcetris®, Seattle Genetics Inc., WA, USA) is a conjugation of an auristatin (monomethyl auristatin E; MMAE) with an anti-CD30 mAb (a chimeric IgG_1). Approximately four molecules of MMAE (range: 2–8) are linked through a peptide linker to free sulfhydryl groups of cysteine residues of the mAb [54,55]. Auristatins are synthetic analogs of dolastatin 10 and exert their cytotoxic effects by preventing tubulin polymerization, causing cell-cycle arrest at the G2/M phase, and leading to apoptosis. MMAE is attached to the mAb by an enzyme-cleavable linker. CD30 is a member of the TNF receptor superfamily, which comprises more than 25 members. TNF receptor signaling participates in cellular differentiation, proliferation and survival. Different interactions between receptors and ligands can dictate disparate effects: apoptosis or NF-κB activation to prevent cell death [56]. Expression of CD30 is associated with T-cell activation, although both T and B cells may be positive for CD30 expression, mainly in a proliferating state. CD30 has limited expression on normal tissues with highly uniform expression on classical Hodgkin's lymphoma (HL) and anaplastic large cell lymphoma (ALCL), making it a very attractive target for ADC therapy. Internalization of BV is mainly by clathrin-mediated uptake, with subsequent release of MMAE by the lysosomal enzyme cathepsin [56].

In the first Phase I study, BV was administered at a dose of 0.1–3.6 mg/kg every 3 weeks to 45 patients with relapsed or refractory CD30-positive hematologic malignancies, mostly HL and ALCL [5]. Patients were heavily pretreated: they had received a median of three previous systemic treatments (range: 1–7), including autologous stem-cell transplantation

Aa Dose-limiting toxicity: appearance of treatment-related adverse events that are serious or life threatening and prevent further increase in dosage in dose-escalation studies.

(ASCT) in 73% of them. The dose-limiting toxicities were febrile neutropenia, thrombocytopenia and hyperglycemia. The MTD with this schedule of administration was determined to be 1.8 mg/kg. The most common AEs were fatigue, fever, diarrhea, nausea, neutropenia and peripheral neuropathy. The time to peak concentration occurred immediately after infusion for the intact ADC and approximately 2–3 days after infusion for MMAE. Steady-state concentrations occurred by 21 days, consistent with the terminal half-life ($t_{1/2}$) of 4–6 days of the ADC. Increases in exposure were around dose proportionality for both intact ADC and free MMAE. A total of 36 patients out of 42 with evaluable disease had tumor regressions (86%). Seventeen patients had objective responses, including 11 with complete response.

The FDA granted conditional approval to BV under the accelerated approval process in August 2011 for two indications: patients with HL after failure of ASCT or those ineligible for it who have failed at least two chemotherapy regimens; and patients with ALCL after failure of combination chemotherapy. The pivotal Phase II study enrolled 102 patients with relapsed or refractory HL after ASCT who had histologically documented CD30-positive disease by a central pathology review [57]. Patients received BV at 1.8 mg/kg intravenously every 3 weeks for up to 16 cycles. The ORR was 75%, with complete response in 34% of patients, and the median progression-free survival (PFS) was 5.6 months. The safety profile of BV was manageable and similar to the profile observed in the Phase I study.

Another single-arm Phase II trial evaluated the same schedule of BV in 58 patients with relapsed or refractory systemic ALCL after at least one prior therapy [58]. The ORR was 86% with complete response achieved in 57%. The only AE that resulted in treatment discontinuation in more than one patient was peripheral sensory neuropathy (six patients). Neurotoxicity was also the most common AE leading to dose reduction. Grade 3 peripheral neuropathy was reported in 14% of patients. It seemed to be consistent with the neurotoxicity observed with inhibitors of tubulin polymerization, primarily sensory, with a median time to onset of 13.3 weeks for any-grade events. It was largely reversible and could be managed by dose delays and/or dose reduction [3].

Several studies of BV are ongoing: a confirmatory Phase III trial in patients with HL after ASCT, Phase I–II trials evaluating combination with chemotherapy for front-line therapy, and trials in other CD30-positive malignancies [3].

ADCs of maytansinoid: trastuzumab emtansine (T-DM1)

T-DM1 (also known as trastuzumab-DM1) comprises the approved anti-HER2 antibody trastuzumab, a humanized IgG_1, and the microtubule polymerization inhibitor DM1 (derivative of maytansine) [59]. HER2 is a transmembrane oncoprotein encoded by the *HER2/neu* gene and overexpressed in approximately 20–30% of invasive breast cancers [9]. It is a tyrosine kinase receptor and its homo- or hetero-dimerization (with other HER growth factor receptors) results in phosphorylation of the intracellular domain of the protein. This initiates several downstream signaling pathways, including RAS/Raf/MAPK and PI3K/Akt. HER2 activation in cancer cells elicits a number of hallmarks of cancer: increased cell motility, proliferation and resistance to cell death [60,61]. All this translates into an aggressive biological behavior in breast cancer, with shorter overall survival in patients. Maytansine was evaluated by the National Cancer Institute in Phase I and II studies in the 1970s. Despite clinical activity in early-phase trials, further development was discontinued due to its toxicity and the opportunities with other antimicrotubule agents. Severe toxic effects included nausea, vomiting, diarrhea, elevations of liver enzymes, lethargy and weakness. Nevertheless, on the basis of the extremely potent cytotoxic properties, maytansine was believed to be an ideal payload for ADCs [62]. DM1 is a maytansine derivative, which is three- to ten-fold more potent than maytansine, with an IC_{50} in the picomolar range. Trastuzumab and DM1 are linked through an uncleavable crosslinking reagent, nonreducible thioether linkage (SMCC), which couples the thiol of DM1 to lysine residues of the antibody via a thioether bond [63]. The average loading of DM1 on the antibody is 3.5 DM1 molecules per antibody. This conjugate design was selected over reducible disulfide linker candidates based on potent activity in *in vitro* and *in vivo* preclinical models, including HER2-overexpressing models resistant to trastuzumab [63]. Internalization and payload release of T-DM1 follow the general steps of ADCs, although DM1 is a microtubule polymerization inhibitor with a cytoplasmic target **(Figure 2.2)**.

The first-in-human study of T-DM1 was conducted in patients with advanced HER2-positive breast cancer who had progressed on trastuzumab-based therapy, with a median of four prior regimens for metastatic disease [6]. This Phase I study followed an accelerated titration design and enrolled 24 patients. T-DM1 was administered by intravenous infusion over 90 min, once every 3 weeks at a starting dose of 0.3 mg/kg. Premedication was not routinely used for the first infusion. In the absence of infusion-related reactions, subsequent infusions were given over 30 min. Dose-limiting toxicity was transient thrombocytopenia at the 4.8 mg/kg dose level and the MTD was 3.6 mg/kg. The PKs of T-DM1 were nonlinear across

the doses tested in this study. A twofold increase in dose from 1.2 to 2.4 mg/kg resulted in approximately an eightfold increase in area under the curve consistent with a lower clearance at doses >1.2 mg/kg. T-DM1's volume of distribution approximated the physiologic blood volume, similar to human IgG antibodies. C_{max} of free DM1 was >10 ng/ml at all dose levels. Of 22 patients tested, only one had an anti-antibody response, with no impact on PK parameters. Common treated-related AEs included grade ≤2 thrombocytopenia, elevated transaminases, fatigue, nausea and anemia. There were no grade >1 nausea, vomiting, alopecia, neuropathy or cardiac events requiring dose modification. One patient experienced a serious AE considered to be possibly treatment related (grade 3 pulmonary hypertension). The stability of the linker is demonstrated by the 70-fold difference between molar concentrations of DM1 and the whole ADC and the low incidence of neurotoxicity observed in this study. Six patients had a partial response, five of which were confirmed. Five responses occurred at 3.6 mg/kg and one at 2.4 mg/kg. All patients with responses had previously received an antimicrotubule agent (paclitaxel, docetaxel and/or vinorelbine).

A single-agent, proof-of-concept Phase II study was then conducted in 112 patients with HER2-positive metastatic breast cancer, who had received prior trastuzumab therapy [64]. The ORR, assessed by an independent review committee, was 26%. The most common AEs were fatigue, nausea and headache, with severe grade being infrequent (hypokalemia: 9%; thrombocytopenia: 8%; and fatigue: 5%). A second Phase II trial confirmed a high ORR in heavily pretreated patients (33%, as assessed by independent review)[8]. Actually, the extent of previous therapy in the enrolled patients makes this reported ORR striking. All patients previously received a taxane, an anthracycline, capecitabine, lapatinib and trastuzumab. The ORR was 40% and the median PFS was 8.0 months in patients with retrospective central confirmation of HER2-positive status. This study did not detect any new safety signal.

Population PKs for T-DM1 were characterized using data from 273 patients from the first-in-human study and both Phase II studies referenced above [65]. PKs were best described by a linear two-compartment model. Population estimates (interindividual variability) for PK parameters were: clearance: 0.7 l/day (21.0%); central compartment volume: 3.33 l (13.2%); peripheral compartment volume: 0.89 l (50.4%); and intercompartmental clearance: 0.78 l/day. Bodyweight, albumin, tumor burden and aspartate aminotransferase levels were identified as statistically significant covariates accounting for interindividual variability in PKs, with bodyweight having a greater effect on clearance and central compartment volume than

other covariates. T-DM1 exposure was relatively consistent across the weight range following bodyweight-based dosing. Another PK analysis concluded that the PK profile of T-DM1 is predictable and well characterized, and exposure at 3.6 mg/kg every 3 weeks does not correlate with clinical responses [66]. Furthermore, clinical outcomes are not affected by circulating HER2 levels. The analysis could not find a relationship between T-DM1 exposure and the incidence of grade 3 thrombocytopenia or alanine aminotransferase/aspartate aminotransferase elevations.

The $t_{1/2}$ of T-DM1 is approximately 4 days, which probably explains the absence of intact ADC accumulation with repeated dosing. After T-DM1 administration, naked trastuzumab was found to have a slower clearance (approximately 3–6 ml/day/kg) and longer $t_{1/2}$ (~9–11 days) compared with intact ADC. It is hypothesized that intact ADC is mainly cleared by deconjugation, proteolytic degradation and dual mechanisms (i.e., target [HER2] antigen specific and nonspecific [e.g., Fc mediated]), similar to the clearance mechanisms observed with other humanized antibodies [66]. Antigen-mediated disposition may play a significant role in clearance, and that would explain a faster clearance at or below the 1.2 mg/kg dose level in the Phase I study [65].

A randomized Phase II trial in HER2-positive breast cancer patients previously untreated for metastatic disease showed an improvement in PFS, from 9.2 months with standard-of-care trastuzumab plus docetaxel (n = 70) to 14.2 months with T-DM1 (n = 67) [67]. Moreover, the incidence of significant AEs (≥grade 3) was considerably lower in the T-DM1 arm (46 vs 91%) [67]. The conclusive evidence of T-DM1 clinical benefit for patients with HER2-positive advanced breast cancer has been recently provided by a randomized Phase III study (known as the EMILIA study) [68]. Patients, previously treated with trastuzumab and a taxane, were randomly assigned in a 1:1 ratio to T-DM1 or lapatinib plus capecitabine, and stratified by region, number of prior regimens for advanced disease (0 or 1 vs >1) and disease involvement (visceral vs nonvisceral). Inclusion criteria included progression during or after the most recent treatment for advanced disease or within 6 months after treatment for early-stage disease. Consequently, this Phase III study enrolled two different conditions: progressive or refractory disease. Among 991 randomly assigned patients, median PFS as assessed by independent review was 9.6 months with T-DM1 versus 6.4 months with lapatinib plus capecitabine (hazard ratio: 0.65; 95% CI: 0.55–0.77; $p < 0.001$), and median overall survival at the second interim analysis crossed the stopping boundary for efficacy (30.9 vs 25.1 months; hazard ratio: 0.68; 95% CI: 0.55–0.85; $p < 0.001$). The objective response rate was higher with T-DM1 (44 vs 31% with

lapatinib plus capecitabine; $p < 0.001$). Rates of ≥grade 3 AEs were higher with lapatinib plus capecitabine than with T-DM1 (57 vs 41%). The incidences of thrombocytopenia and increased serum aminotransferase levels were higher with T-DM1, whereas the incidences of diarrhea, nausea, vomiting and palmar–plantar erythrodysesthesia were higher with lapatinib plus capecitabine. Of note, reports of hyperbilirubinemia of any grade were more frequent in the lapatinib–capecitabine group than in the T-DM1 group (8 vs 1%), and no patients met Hy's law criteria for drug-induced liver injury. The FDA approved T-DM1 (Kadcyla®; Genentech Inc., CA, USA) for the treatment of patients with HER2-positive metastatic breast cancer (who previously received trastuzumab and a taxane, separately or in combination) in February 2013.

The clinical development of T-DM1 is a large, highly orchestrated program set to change the treatment paradigm for HER2-positive tumors. The drug is being evaluated in further Phase III studies in HER-2 positive advanced breast cancer as well as in a Phase II/III trial in HER2-positive gastric cancer [101].

ADCs in early clinical development

The clinical insights obtained with ADC development within the last few years have translated into a number of new strategies and clinical agents. Several novel ADCs are in late preclinical or early clinical trials, utilizing technology advancements in all of the components: the platform (mAb), the linker and the payload **(Table 2.3)** [3].

Conclusion

If the target has suitable properties, efficient drug delivery to malignant cells through ADCs minimizes drug exposure in normal tissues, increasing the therapeutic index of the attached cytotoxic drug. GO and BV were granted conditional approval, although the New Drug Application for GO was later (voluntarily) withdrawn due to a negative Phase III result. On February 22 2013, the FDA approved T-DM1 for use as a single agent for the treatment of patients with HER2-positive metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination.

Hematologic malignancies localize in areas readily accessible to ADCs and the PK disposition of mAbs is also favorable for their treatment, as distribution into other tissues is slow [10]. Moreover, a significant proportion of hematologic malignancies are inherently sensitive to cytotoxic agents, making ADCs an attractive therapeutic approach even in second- or third-line treatment. Inotuzumab ozogamicin and SAR3419 are in different phases of

Table 2.3. Antibody–drug conjugates in early clinical development.

Description	Target	Indication	Phase
SAR3419 (huB4-DM4). Humanized mAb conjugated to DM4	CD19	B-cell malignancies (ALL and DLBCL)	II
Glembatumumab vedotin (CDX-011). Fully-human mAb conjugated to MMAE	GPNMB	Breast cancer, melanoma	II
Lorvotuzumab mertansine (IMGN901). Humanized mAb conjugated to DM1	CD56	SCLC, Merkel cell carcinoma, multiple myeloma	I/II
BT-062. Chimeric mAb conjugated to DM4	CD138 (syndecan-1)	Multiple myeloma	I/II
PSMA ADC. Fully-human mAb conjugated to MMAE	PSMA	Prostate cancer	I
BAY 94-9343. Fully human mAb conjugated to DM4	Mesothelin	Mesothelin-positive solid tumors	I
MDX-1203. Fully-human mAb conjugated to a prodrug of duocarmycin analog (DNA minor-groove binder and alkylator)	CD70	Renal cell cancer, NHL	I
SGN-75. Humanized mAb conjugated to MMAF	CD70	Renal cell cancer, NHL	I
Anti-AGS-16 ADC (AGS-16M8F). Fully human mAb conjugated to MMAF	AGS-16	Renal cell and liver cancer	I
IMGN529. Humanized mAb conjugated to DM1	CD37	B-cell malignancies	I
IMGN853. Humanized mAb conjugated to DM4	FOLR1	NSCLC (adenocarcinoma) and ovarian cancer	I
ASG-22ME. Fully-human mAb conjugated to MMAE	AGS-22 (nectin-4)	Solid tumors	I
RG7593. Human mAb conjugated to an auristatin	CD22	B-cell lymphomas	I
ASG-5ME. Fully-human mAb conjugated to MMAE	SLC44A4 (AGS-5)	Pancreatic and prostate cancer	I
Milatuzumab–doxorubicin (hLL1-Dox). Humanized mAb conjugated to doxorubicin	CD74	Multiple myeloma	I
SAR566658. Humanized mAb conjugated to DM4	CA6	CA6-positive solid tumors	I

†It is not currently under investigation.
‡There is no disclosure of new studies.
ADC: Antibody–drug conjugate; ALL: Acute lymphoblastic leukemia; DM1: *N*-methyl-*N*-[3-mercapto-1-oxopropyl]-L-alanine ester of maytansinol; DLBCL: Diffuse large B-cell lymphoma; DM4: *N*-methyl-*N*-[4-mercapto-4-methyl-1-oxopentyl]-L-alanine ester of maytansinol; FOLR1: Folate receptor α;
GPNMB: Glycoprotein nonmetastatic melanoma protein B; mAb: Monoclonal antibody; MMAE: Monomethyl auristatin E; MMAF: Monomethyl auristatin phenylalanine; NHL: Non-Hodgkin's lymphoma;
NSCLC: Non-small-cell lung cancer; PSMA: Prostate-specific membrane antigen; SCLC: Small-cell lung cancer.

Table 2.3. Antibody–drug conjugates in early clinical development.

Description	Target	Indication	Phase
MLN2704. Humanized mAb conjugated to DM1†	PSMA	Prostate cancer	I/II
IMGN388. Fully-human mAb conjugated to DM4‡	Integrin	Solid tumors	I
BIIB015. Humanized mAb conjugated to DM4‡	Cripto	Solid tumors	I

†It is not currently under investigation.
‡There is no disclosure of new studies.
ADC: Antibody–drug conjugate; ALL: Acute lymphoblastic leukemia; DM1: *N*-methyl-*N*-[3-mercapto-1-oxopropyl]-L-alanine ester of maytansinol; DLBCL: Diffuse large B-cell lymphoma; DM4: *N*-methyl-*N*-[4-mercapto-4-methyl-1-oxopentyl]-L-alanine ester of maytansinol; FOLR1: Folate receptor α; GPNMB: Glycoprotein nonmetastatic melanoma protein B; mAb: Monoclonal antibody; MMAE: Monomethyl auristatin E; MMAF: Monomethyl auristatin phenylalanine; NHL: Non-Hodgkin's lymphoma; NSCLC: Non-small-cell lung cancer; PSMA: Prostate-specific membrane antigen; SCLC: Small-cell lung cancer.

clinical development, but both are interesting prospects. By contrast, the clinical development of ADCs against solid tumors has been more difficult; however, T-DM1 has impressive activity against HER2-positive advanced breast cancer.

The future of ADCs is bright. The biotechnology available today provides the means for tailoring a mAb against a selected tumor type and several effective payloads are available. Antibody fragments, including single-chain Fvs, diabodies, triabodies and nanobodies (which are a tenth of the size of a mAb), combine the advantages of both small molecules and mAbs, resulting in lower costs, improved efficacy, flexible formatting, low toxicity and the potential for alternative delivery routes. They have not been tested in the clinic as a component of an ADC, but they might be a future opportunity.

The biology and the standard therapeutic options for one particular cancer should be taken into account in the design of ADCs. Reciprocally, the selection of patients and the treatment setting should be guided by the proved technical features of the ADC and the characteristics of the target antigen. Two good examples of this are BV in HL and T-DM1 in HER2-positive advanced breast cancer; both diseases are generally sensitive to antimicrotubule agents. Different paradigms must be adopted in the clinical development process: enriched clinical designs with the level of target expression as a predictive biomarker (the bottom-up approach, as with naked trastuzumab), imaging to help early defining of the targeting characteristics of the mAb, and adaptive designs in Phase II development [1].

Although most mAbs in oncology are administered on the basis of bodyweight or body surface area, it is still controversial how to dose an antibody with a significant target-mediated drug disposition such as GO or inotuzumab ozogamicin [11]. The schedule of administration also seems to be an

important factor. ADCs are molecularly targeted agents that clearly suggest a new paradigm. To really move these agents towards personalized medicine, development should be based on the expression of the specific targets [69]. Besides how to dose ADCs, one question remains to be answered: how to integrate them with conventional chemotherapy to provide the most effective and best-tolerated treatment option?

Financial & competing interests disclosure

AD Ricart has stock ownership at Pfizer Inc. (CA, USA). The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Summary.

- Antibody–drug conjugation can be perceived as a strategy for improving the specificity of cytotoxic chemotherapy, and for enhancing the efficacy of passive immunotherapy, with the ambition of integrating the best characteristics of both therapeutic approaches.
- Hematologic malignancies localize in areas readily accessible to antibody–drug conjugates (ADCs), such as the circulating blood, bone marrow and lymph nodes. They possess antigens with the right grade of specificity, and the pharmacokinetic disposition of monoclonal antibodies (mAbs) is favorable for their treatment, as distribution into other tissues is slow.
- By contrast, significant obstacles prevent an ideal targeting against solid tumors.
- Gemtuzumab ozogamicin (GO; Mylotarg®, Pfizer Inc., NY, USA) contains a semisynthetic derivative of calicheamicin, a potent enediyne DNA-binding antibiotic, linked to a humanized mAb directed against CD33. The US FDA granted marketing approval under the accelerated approval regulations in 2000, with an indication for patients with acute myeloid leukemia. However, the required postapproval study (SWOG S0106), combining GO with standard chemotherapy in first-line acute myeloid leukemia patients, failed to confirm clinical benefit. Pfizer Inc. voluntarily withdrew the New Drug Application for GO (Mylortag®) in the USA and Europe in 2010.
- Brentuximab vedotin (Adcetris®; Seattle Genetics Inc., WA, USA) is a conjugation of an auristatin with an anti-CD30 mAb. The FDA granted conditional approval to brentuximab under the accelerated approval process in August 2011 for two indications: Hodgkin's lymphoma and anaplastic large cell lymphoma.
- Trastuzumab emtansine (also known as trastuzumab-DM1) comprises the approved anti-HER2 antibody trastuzumab, a humanized IgG_1, and the microtubule polymerization inhibitor DM1 (derivative of maytansine). The FDA approved trastuzumab emtansine (Kadcyla®; Genentech Inc., CA, USA) for the treatment of patients with HER2-positive, metastatic breast cancer (who previously received trastuzumab and a taxane) in February 2013.
- The future of ADCs is bright. The biotechnology available today provides the means for tailoring a mAb against a selected tumor type, and several effective payloads are available.

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Chapter 3

Fusion proteins

Samir Dalia & Salvador Bruno

Fusion proteins (FPs) are playing an increasingly important role in targeted therapies for cancer treatment. These FPs usually help deliver cytotoxic therapy directly to malignant cells, while sparing normal cells and tissues. Currently, denileukin diftitoxis is the only approved FP used in the treatment of cutaneous T-cell lymphomas. Multiple other FPs are currently in clinical trials and have shown promise in treating different malignancies. This chapter helps provide information on how FPs work and the current FPs in clinical trials for the treatment of malignancy.

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Aa Targeted therapy: therapy that is targeted to a specific pathway or marker, such as cluster differentiation markers.

Immunotoxin: a fusion protein that replaces the binding domain of a toxin with the Fv portion of an antibody to direct the toxin to a malignant cell.

Immunogenicity: the ability of a substance, such as an antigen or epitope, to provoke an immune response in the body.

Targeted therapy has become the mainstay of drug development in cancer treatment in the 21st century. Monoclonal antibodies, antibody–drug conjugates and toxin–drug conjugates have all come to the forefront in the treatment of cancer. Toxin fusion proteins (FPs) have recently been demonstrated to help deliver cytotoxic therapy directly to malignant cells, while sparing normal cells and tissues. FPs are generally made by combining a targeting moiety with a toxin used to kill the malignant cell, while leaving the normal cells intact [1]. In general, recombinant immunotoxins are produced by replacing the binding domain of the toxin with the Fv portion of an antibody, which directs the toxin to a malignant cell [2]. Human IL-2, antibodies to cluster differentiation markers (CDs) and antibodies to growth factors have been combined with toxins to produce FPs.

Since toxin FPs cause strong immunogenicity of the toxin, humoral immune responses to toxins can be observed after just one treatment course. This can reduce the serum half-life and also inhibits cytotoxic activity, especially when multiple treatment courses are required. Adding immunosuppressive agents or modifications to the toxin lessens the risk of immunogenicity, although this has not been tested in humans to date. Currently, genetic engineering to generate humanized toxins and PEGylation of the toxin are future approaches that may lessen immunogenicity and improve toxin FP efficacy, while reducing toxicity [1,3,4].

When a FP is created, the toxin directly blocks cellular processes that trigger cell death. Current drug development focuses on bacterial toxins such as *Pseudomonas* exotoxin (PE) and diphtheria toxin (DT). Both agents prevent protein synthesis by inactivating elongation factor 2 through ADP ribosylation [3,4]. Other toxins that have been used in the creation of FPs include anthrax, cholera, ricin and pokeweed, but these have not shown as much promise for drug development as PE and DT. The different toxin-FP agents currently being tested in patients are discussed below and are presented in **Table 3.1**.

The domain of the toxin is replaced with the Fv portion of an antibody that directs the toxin to a malignant cell.

Fusion proteins combine a targeting moiety linked to a toxin used to kill a malignant cell.

Denileukin diftitoxis is currently the only approved fusion protein used in the treatment of cutaneous T-cell lymphoma.

Multiple other fusion proteins are currently in clinical trials and show promise in the safe treatment of malignancies.

Denileukin diftitox

Denileukin diftitox (DD; Ontak®, Eisai Inc., NJ, USA) is a US FDA-approved immunotoxin

Table 3.1. Fusion proteins currently in clinical trials.

Fusion protein	Toxin	Targeting moiety	Current clinical trials
Denileukin diftitox	DT	Human IL-2	Cutaneous T-cell lymphoma† Follicular Melanoma Renal cell carcinoma Chronic lymphocytic leukemia
Moxetumomab pasudotox	*Pseudomonas*	CD22	Chronic lymphocytic leukemia Hairy cell leukemia Non-Hodgkin lymphoma Acute lymphoblastic leukemia
Oportuzumab monatox	*Pseudomonas*	Humanized anti-EpCAM	Noninvasive urothelial carcinoma
DT_{388}GM-CSF	DT	GM-CSF	Removed from testing for liver toxicity
L19-IL-2	Human IL-2	EDB domain of fibronectin	Melanoma Renal cell carcinoma Pancreatic cancer
sEPHB4-HAS	Human serum albumin	Extracellular domain EPHB4	Solid tumor malignancies
DTGM	DT	GM-CSF	Relapsed or refractory acute myeloid leukemia
L19TNF-α	TNF-α	Fibronectin	Solid tumor malignancies
DT_{388}IL-3	DT	IL-3	Hematological malignancies
F16-IL-2	IL-2	Human mAb F16 fragment	Breast cancer
Hu14.18-IL-2	IL-2	Human mAb GD2 disialoganglioside	Neuroblastoma Melanoma

†Only US FDA-approved indication for a fusion protein.
DT: Diptheria toxin; EDB: Extra domain B; EpCAM: Epithelial cell adhesion molecule; mAb: Monoclonal antibody.

for the treatment of relapsed cutaneous T-cell lymphoma. DD is a genetically engineered FP combining the cytotoxic and membrane-translocating domains of the DT with the full-length sequence of human IL-2. DT is cytocidal and targets cells expressing the high-affinity IL-2 receptor, inhibiting protein synthesis and leading to cell death [5]. In a Phase III clinical trial of previously treated patients with cutaneous T-cell lymphoma, DD had an overall response rate of 44%, with 10% complete responses, compared with a 15.9% response rate for those who received the placebo. Progression-free survival (PFS) was longer (median: >2 years) for DD compared with the

placebo (median: 124 days). Side effects included capillary leak syndrome, rigors, pyrexia, nausea and hypotension [5].

DD was combined with rituximab in patients with advanced-stage follicular lymphoma. In 23 patients PFS was 55% at 2 years, but 57% of patients developed grade 3 toxicity. The authors concluded that DD added to toxicity without improving the response rate or time to progression [6]. Since IL-2 has been demonstrated to be efficacious in melanoma, renal cell carcinoma and other lymphoid malignancies, DD is also currently being tested in these disorders. Recently, Phase I and II studies have demonstrated that DD may have activity and improve PFS in metastatic melanoma, renal cell carcinoma and indolent lymphomas, including chronic lymphocytic leukemia (CLL), although larger trials are needed to establish if there will be more widespread use [7–9].

Moxetumomab pasudotox

Moxetumomab pasudotox (MP; CAT-8015, AstraZeneca) is a FP combing the bacterial toxin PE with a CD22 antibody. CD22 is absent on normal tissues, such as liver and skin, and is not expressed on B-cell precursors, allowing B cells to be rapidly generated after therapy ceases. CD22 is expressed on many B-cell malignancies including hairy cell leukemia (HCL), CLL, and non-Hodgkin lymphoma [2].

A Phase I clinical trial evaluating MP in patients with relapsed/refractory HCL showed promising results for this new immunotoxin. In the trial 28 patients who received previous treatment with at least a purine analog for HCL, were given five different doses of MP with a maximum dose of 50 µg/kg three-times a day given every other day. The overall response rate was 86%, with median survival time not reached at 26 months. Common toxicities included hypoalbuminemia, abnormalities in liver function tests, edema, nausea and fever. The authors concluded that MP in doses up to 50 µg/kg three-times a day given every other day has activity in relapsed/refractory HCL and a safety profile that supports the futher development of MP in HCL [10]. Currently, MP is in clinical trials for treatment of advanced CLL, non-Hodgkin lymphoma, acute lymphoblastic leukemia and HCL [101].

Oportuzumab monatox

Oportuzumab monatox (OM; VB4–845, Viventia Bio, MB, Canada) is a recombinant FP comprising a humanized antiepithelial cell adhesion molecule single-chain antibody likened to PE. Once OM is bound to the cancer cell it is internalized and the toxin moiety released into the cytosol, leading to apoptosis [11]. OM was developed for locoregional delivery and

is currently being investigated in patients with noninvasive urothelial carcinoma *in situ* who have previously been treated with bacillus Calmette–Guérin. Intravesical administration limits systemic exposure and maximizes local drug concentration.

In a Phase II clinical trial of patients with noninvasive urothelial carcinoma *in situ* previously treated with bacillus Calmette–Guérin, 46 patients received one induction cycle of six or 12 weekly intravesical OM instillations of 30 mg, followed by up to three maintenance cycles of three weekly administrations for up to 3 months. In total, 44% achieved a complete response. Side effects included dysuria, localized pain and potential infection, although there were few moderate adverse events. The authors concluded that this study makes OM a potential second-line therapy for nonmuscle invasive bladder cancer [12].

DT_{388} GM-CSF

DT_{388} GM-CSF was a toxin FP combining DT to GM-CSF. GM-CSF receptors are expressed on the majority of myeloid leukemia cells, but are poorly expressed on early hematopoietic stem cells making it a potential therapy in patients with acute myeloid leukemia. In a Phase I clinical trial in patients with relapsed or refractory acute myeloid leukemia, DT_{388}GM-CSF was given in a dose escalation trial for up to 5 days. One complete remission and two partial remissions were seen in 31 patients who were resistant to chemotherapy. Hepatic toxicity was a major side effect and was dose dependent. Based on the liver injury this FP did not continue in future clinical trials [13].

Fusion proteins currently in cancer clinical trials

L19-IL-2 is a FP that combines an antibody fragment specific to the extra domain B domain of fibronectin, a tumor angiogenesis marker, and human IL-2. L19-IL-2 delivers IL-2 to the tumor site by exploiting the selective expression of extra domain B on newly formed blood vessels. A dose escalation study in patients with metastatic melanoma when combined with decarbazine demonstrated that eight of 29 patients achieved a Response Evaluation Criteria in Solid Tumors-confirmed objective response with one complete response at 21 months after treatment began. Overall survival was 14.1 months [14]. L19-IL-2 was also tested in a Phase I clinical trial of progressive solid tumor malignancies. Promising results were seen in patients with renal cell carcinoma with a median PFS of 8 months [15]. Currently, Phase II clinical trials of L19-IL-2 in patients with metastatic melanoma are ongoing. L19-IL-2 is also being tested in patients with metastatic pancreatic cancer.

sEphB4-HAS is a FP containing the extracellular domain of EphB4 and human serum albumin. EphB4 has been implicated in angiogenesis and is a potential target in solid tumor malignancies. Currently, a Phase I clinical trial is open in solid tumor malignancies to see the efficacy and safety of this FP [102]. Multiple other FPs are currently being tested in early clinical trials including diphtheria toxin–GM-CSF in patients with relapsed or refractory acute myeloid leukemia, L19TNF-α in solid tumor malignancies, DT_{388}IL-3 in hematological malignancies, F16-IL-2 in breast cancer, and hu14.18-IL-2 in neuroblastoma and melanoma [103].

As science advances, delivery of toxins to tumor cells will be more efficient, produce fewer side effects and hopefully improve overall outcomes in patients with malignancy.

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Summary

- Fusion proteins provide a mechanism to direct cytotoxic therapy directly into a malignant cell, while sparing normal cells, thereby lessening side effects.
- Currently, fusion proteins can cause immunogenicity, which can increase the side effect profile of these medications. PEGylation of fusion proteins may lessen this effect in the future.
- Denileukin diftitox fusion protein combining diphtheria toxin and human IL-2 is the only approved fusion protein currently used for the treatment of relapsed cutaneous T-cell lymphoma.
- Multiple other fusions proteins are currently in clinical trials in hematological and solid tumor oncology.

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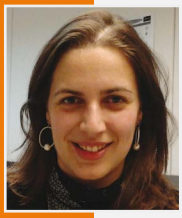

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Chapter 4

Polymer–drug conjugates

Núria Mulet-Margalef, Josep Maria Miquel & Jordi Rodon

During the last few years, the scientific community has witnessed an emerging growth of the number of available anticancer therapies in development, with encouraging results. Despite this, cancer is still one of the main causes of mortality in the general population; so an unmet need of further improvement in targets and pharmacology exists.

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Aa Polymer: a molecule whose structure is composed of multiple repeating units (monomers).

In order to improve anticancer-drug efficacy, two main approaches can be used. On one hand, designing new therapies against tumor-specific molecular targets, and on the other hand, improving the pharmacology with the use of delivery systems for well-known chemotherapeutic agents. This second approach is the basis of 'Polymer therapeutics'. In this chapter, the different approaches that have been explored in combining chemotherapeutic agents with polymers will be described.

The term of 'polymer therapeutics' in cancer refers to the use of water-solubilizing polymers forming part of the drug's structure. These polymers can be either bioactive by themselves (independently of the action of the original drug) or inert. The main goal of using polymers is to improve the delivery of the chemotherapeutic agent by improving the pharmacokinetic profile (by increasing the stability and tumor delivery, and providing an adequate molecular weight). In addition, it is expected that as a secondary effect of this improved delivery they could decrease the side effects of the original drug while not adding important side effects from the polymer itself. Finally, they should be nonimmunogenic and easy to produce.

For this objective, pharmacologists in nanoengineering have designed diverse classes of polymer therapeutics based on different basic structures: polymeric drugs, polymer–protein conjugates, polymer–drug conjugates, polymeric micelles and multicomponent polyplexes [1,2]. **Table 4.1** describes the most frequently used basic polymer structures used in therapeutics, as well as some examples of drugs conjugated with them.

Some of the examples have already been described in other sections of this book (see liposomal doxorubicin and PEGylated liposomal doxorubicin in **Chapter 7**). Although in this chapter, polymeric drug and polymer–protein conjugates will mostly be focused on the different classes of polymer therapeutics will be described in the next section.

Conjugation of anticancer agents with polymers

Some of the anticancer agents used nowadays have limited efficacy due to inadequate pharmacology. Most of the efforts of developing polymer therapeutics have been focused on improving those pharmacological properties and, as a consequence, their safety profile and their efficacy (widening the therapeutic window and narrowing inter-patient variability) of the

Properties of polymers used in polymer therapeutics:

- Low toxicity
- Nonimmunogenic
- Easy to produce
- With an optimal molecular weight

Table 4.1. List of the principal polymers used in therapeutics.

Polymer	Comments	Examples
N-(2-hydroxypropyl) methacrylamide	Also used as a plasma expander	FCE28068
Polyethylene glycol	Also known as polyethylene oxide polyoxyethylene and used as a basis of several laxatives	Pegfilgrastim (Neulasta® [Amgen; Milton, UK]) Pegilatedliposomaldoxorubicin Pegaspargase (Oncaspar® [Enzon Pharmaceuticals, Inc.; NJ, USA]) EZN-2208 Pegamotecan
Polyglutamic acid	Produced by bacterial fermentation Major constituent of the Japanese food natto	
Cyclodextrin	Involved in cholesterol transport out of lysosomes in Niemman-Pick type C disease Other applications: cosmetic, fragrances, chemical engineering	IT 101
Carbocymethyldextran	Also used in glucose biosensors production	DE-310 Delimotecan
Poliglumex	Paclitaxel–poliglumexis the most clinically developed polymer drug conjugate	CT2106 Paclitaxel poliglumex

anticancer drugs. Independently of the basic polymer structure used, polymer therapeutics can be classified in the following classes.

Polymer–drug conjugates

A polymer–drug conjugate is a structure of approximately 5–15 nm, which contains a hydrophilic polymer and a peptide linker that binds to the active anticancer drug, such as a taxane or a camptothecin [1].

There are several polymers used in the production of polymer–drug conjugates, such as *N*-(2-hydroxypropyl) methacrylamide, polyglutamic acid, cyclodextrin and poliglumex. The ideal polymer–drug conjugate should have the basic properties of polymers (e.g., low

Aa Taxanes: agents that stabilize microtubules against depolymerization, blocking the last phases of the cell cycle and leading to cell apoptosis.

toxicity, nonimmunogenic and easy to produce) and the peptide linker should be stable enough during drug transport to the tumor, but at the same time be capable of releasing the drug load within tumor cells [1,2].

The potential advantages of polymer–drug conjugates in comparison with the original drug are described below [1,2]:

- Due to the binding of the drug to the polymer, the level of its renal elimination is decreased. As a consequence, it can recirculate in the blood during a major period of time, enhancing its half-life and potentially its effect;
- In addition, as the drug stably circulate through the bloodstream, the access to normal tissues is initially limited and, as a consequence, the potential toxicity secondary to drug penetration in normal tissues is reduced;
- Due to the increased permeability of the tumor vasculature compared with normal vessels, the polymer drug could achieve higher drug concentration in the tumor microenvironment.

Once in the tumor interstitium, the uptake of the polymer-drug by cancer cells is mediated by pinocytosis instead of passive diffusion (due to the increased molecular weight). Intracellularly, the linker and the drug structure itself are metabolized by endosomes and lysosomes. This mechanism can circumvent some mechanisms of drug resistance (e.g., p-glycoprotein membrane efflux pumps). After drug release, the polymer and the linker are excreted from the cell through exocytosis.

In addition, it seems that the circulation in the bloodstream of the hydrophilic polymer can elicit stimulation of the immune system.

Polymer–protein conjugates

Characteristically peptidic anticancer agents have poor stability, short plasma half-life and frequently induce high immunogenicity [1]. Polymer–protein conjugates are being designed to improve these limitations. A polymer–protein conjugate is a structure of approximately 20 nm, which contains a peptide with anticancer properties (e.g., interferon or granulocytic colonies stimulating factors) covalently bound to a polymer.

Polyethylene glycol (PEG) is one of the most used polymers since it is versatile, easy to manufacture and it offers a good 'protection' of the inner protein of the structure [2]. The potential advantages of PEGylation – the conjugation of PEG to proteins – are several [1,2]:

- Increasing protein stability and solubility
- Reducing protein immunogenicity

- Prolonging plasma half-life through two mechanisms: by preventing rapid renal clearance of proteins and by preventing protein uptake by cells of the reticulo-endothelial system

Main objectives of polymer therapeutics:

- Improve the pharmacological properties of anticancer drugs
- Improve the safety profile of anticancer drugs
- Improve the efficacy of anticancer drugs

Polymeric micelles

Polymeric micelles are spherical structures of 60–100 nm with an active drug inside (e.g., doxorubicin) bound to multiple monomers that have hydrophilic and hydrophobic domains. The main objective for encapsulating a drug in a polymeric micelle is increasing the solubility of the anticancer agent [1].

Improving the pharmacology of anticancer drugs

In order to compare the potential advantages that conjugation with polymers can offer, we have divided the following section by families of anticancer agents that form the basis of the polymer and we have described the available data on each one of the conjugates.

The currently approved and most well-known examples of conjugation with polymers are pegfilgrastim and pegaspargase, both polymer–protein conjugates.

Pegfilgrastim (Neulasta® [Amgen; Milton, UK]) is composed by filgrastim (recombinant human granulocyte colony stimulating factor) covalently bound to PEG. Due to the conjugation with this polymer, the renal clearance of pegfilgrastim is reduced compared with filgrastim and, as a consequence, the half-life is significantly increased from 3.5 h of filgrastim to 25–49 h of pegfilgrastim [101]. The US FDA approved the use of subcutaneous pegfilgrastim for the primary and secondary prophylaxis of febrile neutropenia in 2002 [101].

Pegaspargase (Oncaspar® [Enzon Pharmaceuticals, Inc.; NJ, USA]) was approved by the FDA for the treatment of acute lymphoblastic leukemia in 2006 [3]. It is composed of L-asparaginase covalently bound to PEG. Similar to pegfilgrastim, as a consequence of the PEGylation, the half-life is prolonged (L-asparaginase has a half-life of approximately 20 h, while pegaspargase has a half-life of 5.8 days) [101].

The prolongation of the half-life by conjugating the drug with a polymer is the basis of the benefit observed with pegfilgrastim and pegaspargase, since it allows a less frequent administration and, therefore, a more comfortable treatment for patients. Another potential advantage of PEGylation could be the reduction in the immunogenicity, but this has not been clearly shown in the case of these drugs.

Aa Anthracyclines: agents that damage DNA through intercalation of anthracycline portion and they can also inhibit topoisomerase II, leading to cell death.

Camptothecin analogs: cytotoxic quinolone alkaloids that exert their anticancer effect by inhibiting the DNA enzyme topoisomerase I, an enzyme with an essential role in the preservation of DNA during different cellular processes.

This field is an area of extensive research, and many anticancer compounds are being conjugated with polymers in order to improve their pharmacology. These include camptothecins, platinum compounds, taxanes and anthracyclines. In the following sections, each family of anticancer agents and the efforts of conjugating those with polymers by using some of the basic polymer structures previously described will be reviewed.

Camptothecin analogs

Camptothecin analogs are anticancer drugs that target the DNA enzyme topoisomerase I. DNA topoisomerases have an essential role in the preservation of DNA during different cellular processes such as RNA transcription and DNA replication. The most well-known camptothecin analogs are irinotecan and topotecan, and their use is well-established in advanced colorectal and ovarian cancer, or cervical and small-cell lung cancer, respectively [101].

Despite the undeniable anticancer activity of irinotecan, it has some limitations due to its pharmacokinetic properties. Irinotecan is unstable in aqueous solutions, so once in plasma after intravenous infusion, it is quickly hydrolyzed. It is metabolized to different active metabolites, such as the active metabolite SN38. Enzymatic plasma hydrolysis and metabolism quickly occurs through different enzymes (e.g., carboxylesterase or uridine 5'-diphospho-glucuronosyltransferases) but with a high inter-patient variability. As a consequence, the efficacy and toxicity of irinotecan are highly variable and difficult to predict [101].

For these reasons, polymer–drug conjugates with SN38 (the main active metabolite of irinotecan) and other camptothecin molecules have been developed in order to improve the anticancer effect and their pharmacological properties.

PEG: EZN-2208 & pegamotecan

EZN-2208 is a polymer–drug conjugate linking SN38 with PEG via a glycine linker. EZN-2208 has been tested in different tumor cell lines. Due to the properties derived from polymer conjugation, EZN-2208 showed a prolonged blood circulation compared with irinotecan preclinically and, as a consequence, increased tumor exposure. In particular, the half-life of irinotecan was observed to be 11.7 h, while the half-life of EZN-2208 was of 19.4 ± 3.4 h [4,101]. Based on this and on an observed higher potency of

EZN-2208 compared with irinotecan, in different xenografts, including irinotecan-resistant cell lines [5], clinical development of EZN-2208 was initiated.

The Phase I trial of intravenous EZN-2208 was recently published with encouraging results. In this trial, 41% of patients achieved stable disease, and in some of them, the response lasts more than 4 months, including patients previously treated with irinotecan.

EZN-2208 also seemed to have a better safety profile in comparison with historical data of irinotecan. The side effects observed included diarrhea (33% in patients treated with EZN-2208, compared with the 51–88% of patients in historical data of patients treated with irinotecan), vomiting (21 vs 67%), nausea (33 vs 86%) and fatigue (41 vs 76%). Although, in the case of hematological toxicity, febrile neutropenia seemed to be increased (10% of febrile neutropenia for EZN-2208 vs 2–7% for historical data on irinotecan). In fact, the dose-limiting toxicity of EZN-2208 was febrile neutropenia [4,101]. **Table 4.2** shows clinical trials where EZN-2208 efficacy is being tested and their status.

Pegamotecan is a polymer–drug conjugate that consists of two camptothecin molecules conjugated with PEG through an alaninate ester linkage.

Pegamotecan was clinically tested in a Phase I trial. The authors described a half-life of 46 ± 12.8 h (higher than the previously described half-life of irinotecan of 11.7 h). Regarding toxicity, grade 4 neutropenia was the most common dose-limiting toxicity. A total of 50% of patients treated

Table 4.2. Clinical trials with EZN-2208.

Trial title	Clinical trial identifier	Status
A Phase 2 study of EZN-2208 in patients with metastatic breast cancer (PEG-SN38)	NCT01036113	Ongoing, not recruiting
A Phase I, study to evaluate the safety and tolerability of intravenous EZN-2208 in patients with advanced solid tumors or lymphoma (EZN-2208-01)	NCT00520637	Completed
A study of EZN-2208 administered with or without cetuximab in patients with metastatic colorectal carcinoma (Phase II)	NCT00931840	Unknown status
Study of EZN-2208 pediatric patients with solid tumors (Phase I–II)	NCT01295697	Ongoing, not recruiting
EZN-2208 (pegylated SN-38) in combination with bevacizumab in refractory solid tumors (Phase I)	NCT01251926	Active, not recruiting

Data taken from [102].

at the recommended dose level experienced grade 4 neutropenia. In comparison, historical data show that after treatment with irinotecan, neutropenia is observed in approximately 10% of patients. In addition, other frequent side effects observed upon treatment with pegamotecan at the recommended dose level include grade 3 thrombocytopenia (in up to 50% of patients), prolongation of partial thromboplastin time, and gastrointestinal symptoms. Surprisingly, no significant diarrhea was reported, a frequent side effect observed upon treatment with irinotecan [6,101].

After demonstrating promising results in a Phase I trial, pegamotecan was tested in a Phase II trial. A total of 35 patients with untreated, inoperable or metastatic gastric cancer or gastroesophageal adenocarcinoma were enrolled in a nonrandomized Phase II trial of intravenous pegamotecan every 21 days until progression or unacceptable toxicity. Of those, 14.3% patients had a partial response, indicating that pegamotecan has some antitumor activity. The most frequent grade 3–4 toxicities were hematological, as observed in the previous study. Based on these two trials, the authors suggested that pegamotecan could have a better safety profile than irinotecan, although no formal head-to-head comparison has been explored so far. There are no further registered clinical studies with pegamotecan [7,102].

Carboxymethyldextran: DE-310 & delimotecan

DE-310 is a polymer–drug conjugate composed of exatecan (DX-8951f, a camptothecin analog) and carboxymethyldextran, which are covalently linked via a peptidyl spacer.

In a Phase I trial with intravenous DE-310 in patients with advanced solid tumors, the authors describe neutropenia, thrombocytopenia and liver toxicity as the dose-limiting toxicities [8]. In the recommended dose level, they report a frequency of 25% of grade 4 neutropenia, a 50% of grade 3 thrombocytopenia and a 50% of grade 3 elevation of aspartate aminotransferase serum and alanine aminotransferase serum. Despite the fact that hematological toxicity of DE-310 seems to be more frequent than with irinotecan, authors argued that the gastrointestinal toxicity was lower (no diarrhea was described, and nausea and vomiting were mostly grade 1 or 2) [8,101]. Nevertheless, no further trials with DE-310 have been published, neither are ongoing at the time of this review [102].

Delimotecan is a polymer–drug conjugate composed of an active camptothecin derivative (T-2513) and a carboxymethyldextran polymer, with a triglycine linker. Preclinically, the compound showed promising

preclinical activity in melanoma cell lines that were resistant to dacarbazine [9].

One Phase I trial with delimotecan has been completed and published. The conjugation of the camptothecin with the polymer significantly increased the systemic exposure, since the half-life of delimotecan in this trial was 109 h. The authors state that the treatment was safe at the recommended doses. Regarding the toxicity profile, they describe that the dose-limiting toxicities were grade 4 thrombocytopenia and grade 3 increases in hepatic enzymes with a pattern of cholestasis. Intriguingly, they observed other toxicities that had not been frequently observed with irinotecan or other camptothecin–polymer conjugates (even those related with irinotecan), such as grade 3 stomatitis and rash. However, there was one case of multiorgan failure [10]. After completion of this Phase I trial, development of delimotecan seems to be abandoned, since there are no clinical trials currently ongoing with this drug [102].

Cyclodextrin: CRLX101 or IT 101

CRLX101 or IT 101 is composed by camptothecin molecules that are covalently bound to cyclodextrin–PEG polymers, which self-assemble into nanoparticles.

This polymer–drug conjugate has demonstrated some advantages compared with other 'naked' camptothecin analogs in several preclinical models. In those studies, investigators have described a longer half-life, and a higher antitumor activity, with a good safety profile [11]. There is no available clinical data on CRLX101 yet, but several clinical trials with IT 101 are ongoing, as depicted in **Table 4.3**.

Poly-L-glutamate: CT-2106

CT-2106 is a polymer–drug conjugate composed of camptothecin molecules conjugated with poly-L-glutamate. This is expected to confer a higher aqueous solubility and an increased drug delivery to the tumor. Intravenous CT-2106 has been tested in a Phase I trial [12]. Similar to other polymer–drug conjugates developed with camptothecin analogs, the dose-limiting toxicities in the highest-dose cohort were thrombocytopenia and neutropenia, and fatigue (22% of patients experimented grade 3). CT-2106 is currently being tested in several ongoing clinical trials shown in **Table 4.4**.

Platinum compounds

Oxaliplatin is an alkylating agent that covalently binds to the DNA, and the damage that generates leads cancer cells to apoptosis. When administered intravenously, a high proportion of the drug is rapidly distributed into

Table 4.3. Clinical trials with IT-101 or CRLX101.

Trial name	Clinical trial identifier	Status
Pilot trial of CRLX101 in treatment of patients with advanced or metastatic stomach, gastroesophageal, or esophageal cancer that cannot be removed by surgery	NCT01612546	Recruiting
Study of CRLX101 (formerly named IT-101) in the treatment of advanced solid tumors (Phase I–II)	NCT00333502	Completed
A Phase 2 study of CRLX101 in patients with advanced non-small cell lung cancer	NCT01380769	Ongoing, not recruiting
Efficacy study of maintenance IT-101 therapy for ovarian cancer patients (Phase II)	NCT00753740	Terminated (poor recruitment)
CRLX101 for recurrent ovarian/tubal/peritoneal cancer (Phase II)	NCT01652079	Recruiting
CRLX101 plus bevacizumab in advanced RCC (Phase I)	NCT01625936	Recruiting

Data taken from [102].

tissues or eliminated in the urine. Unlike other platinum agents such as cisplatin and carboplatin, oxalipatin can be also metabolized into an active metabolite, diaminocyclohexane, which is the main contributor to the observed antitumor effect.

To improve the efficacy and pharmacological properties of oxaliplatin, AP5346 (also known as diaminocyclohexane platinum polymer or ProLindac® [Jiangsu Aosaikang Pharmaceutical Co.; Nanjing, China]) was developed by binding diaminocyclohexane to a polymer of N-(2-hydroxypropyl) methacrylamide by means of a special linker. In this way, the active drug would be released more selectively in a low-pH environment (where the linker is more unstable), such as in tumor intersticium. Currently, there is preclinical evidence that AP5346 has improved anticancer activity [13] and

Table 4.4. Clinical trials with CT-2106.

Trial name	Clinical trial identifier	Status
CT-2106 for the second line treatment of ovarian cancer (Phase II)	NCT00291837	Completed
Phase I/II CT 2106 and 5-FU/FA in colorectal cancer	NCT00291785	Completed
Poly-L-glutamate-camptothecin in treating patients with advanced cancer (Phase I)	NCT00059917	Completed

5-FU: 5-fluorouracil; FA: Folinic acid.
Data taken from [102].

a Phase I trial has been completed, but no data has been published so far. Other strategies, such as polymeric micelles with platinum compounds are being explored but these agents are in early preclinical development [14].

Taxanes

Taxanes are anticancer agents derived from the plant *Taxus* whose principal mechanism of action is the disruption of microtubule function, inhibiting the mitotic spindle and forcing cells into apoptosis. Paclitaxel, one of the most active taxanes, is approved for the treatment of many solid tumors. It has poor water solubility so paclitaxel is dissolved in a mixture of ethanol and Cremophor® (Caesar & Loretz GmbH; Hilden, Germany) EL. The latter is the main cause of hypersensitivity reactions observed with paclitaxel treatment. Since these reactions can occur in nearly 40% of cases (and in 2% of cases these reactions can be severe) [101], there is extensive research in delivery engineering in an attempt to avoiding the use of Cremophor EL.

Polymeric micelles: methoxy-PEG-poly (D,L-lactide) taxol

Methoxy-PEG-poly (D,L-lactide) taxol (Genexol-PM® [Samyang Pharma; Seoul, South Korea]) is a polymeric micelle-formulated paclitaxel, free of Cremophor EL. After demonstrating promising preclinical activity in several

Table 4.5. Phase II trials published with methoxy-polyethylene glycol-poly (D,L-lactide) taxol (Genexol-PM® [Samyang Pharma; Seoul, South Korea]).

Description	Patients	RR (%)	TTP (months)	AE (grades 3 and 4)	Ref.
Metastatic breast cancer G-PM 300 mg/m^2/3 weeks	41	58.5 59.5 in first line	9	68% neutropenia 51% peripheral neuropathy 22% thrombocytopenia 4% hypersensitivity reactions	[16]
Advanced urothelial carcinoma after progression to cisplatin/gemcitabine G-PM 300 mg/m^2/3 weeks	37	21	2.7	15% peripheral neuropathy 5.9% infection	[17]
Advanced NSCLC. First-line treatment CDDP 60 mg/m^2 + G-PM 300 mg/m^2/3 weeks	69	37.7	5.8	46% neutropenia 13% peripheral neuropathy 7.3% arthralgia 5.8% hypersensitivity reactions	[18]

AE: Adverse event; CDDP: Cisplatin; G-PM: Genexol-PM® (Samyang Pharma; Seoul, South Korea); NSCLC: Non-small-cell lung cancer; RR: Response rate; TTP: Time to progression.

tumor models, a Phase I trial was initiated. The maximum tolerated dosage of methoxy-PEG-poly (D,L-lactide) taxol was 300 mg/m^2 every 3 weeks, and this was the dose level used for further Phase II trials. Its half-life is approximately 11 h, similar to the paclitaxel (9.9 h) [15,101].

Currently, three Phase II trials with methoxy-PEG-poly (D,L-lactide) taxol have been published, as summarized in **Table 4.5**. Based on these studies, one could argue that the rate of hypersensitivity reactions is dramatically reduced with methoxy-PEG-poly (D,L-lactide) taxol (~5–6%) in comparison with historical data of paclitaxel (~41%). However, peripheral neuropathy and neutropenia, both characteristic toxicities of taxanes too, are not clearly reduced with methoxy-PEG-poly (D,L-lactide) taxol. Peripheral neuropathy is observed in approximately 13–50% of patients, and neutropenia in 46–68% of patients treated with methoxy-PEG-poly (D,L-lactide) taxol, while in historical data of paclitaxel they are observed in 64 and 27–50%, respectively [16–18,101]. In terms of efficacy, the polymeric drug

Table 4.6. Clinical trials with methoxy-polyethylene glycol-poly (D,L-lactide) taxol (Genexol-PM®).

Trial name	Clinical trial identifier	Status
A clinical trial of paclitaxel loaded polymeric micelle in patients with taxane-pretreated recurrent breast cancer (Phase IV)	NCT00912639	Enrolling by invitation
Determine MTD, evaluate efficacy and safety of Genexol-PM plus carboplatin with advanced ovarian cancer (Phase I)	NCT00877253	Completed
Evaluate the efficacy and safety of Genexol-PM compared with Genexol^R in recurrent or metastatic breast cancer (Phase III)	NCT00876486	Recruiting
Efficacy study of Genexol-PM and cisplatin in locally advanced head and neck cancer (Phase II)	NCT01689194	Recruiting
A trial to evaluate efficacy and safety of the combination therapy of Genexol®-PM pus Carboplatin® compared to Genexol® plus Carboplatin® as a firstline treatment in subjects with ovarian cancer	NCT01276548	Unknown
Paclitaxel in treating patients with unresectable locally advanced or metastatic pancreatic cancer (Phase II)	NCT00111904	Completed

Table 4.7. Phase III trials published with paclitaxel poliglumex.

Main inclusion criteria	Intervention	Patients (n)	PE	HR	Conclusion	Ref.
Second-line advanced NSCLC (previous treatment with platinum)	PPX 175 mg/m^2 every 3 weeks vs PPX 210 mg/m^2 every 3 weeks vs DCT 75 mg/m^2 every 3 weeks	849	OS	HR: 1.09 p = 0.257	Similar OS Different toxicity: ▪ PPX: – ↑ neurotoxicity – ↓ alopecia – ↓ febrile neutropenia	[19]
First-line advanced NSCLC in chemotherapy-naive patients with performance status 2	PPX 175 mg/m^2 every 3 weeks vs GEM or VNR every 3 weeks	477	OS	HR: 0.95 p = 0.686	Similar OS Different toxicity: ▪ PPX: – ↑ neurotoxicity – ↓ neutropenia	[20]
First-line advanced NSCLC in chemotherapy-naive patients with performance status 2	CBDP AUC 6+PPX 210 mg/m^2 every 3 weeks vs CBDP AUC 6+PPX 225 mg/m^2 every 3 weeks	400	OS	HR: 0.97 p = 0.769	Similar OS Similar HR	[21]

AUC: Area under the curve; CBDP: Carboplatin; DCT: Docetaxel; GEM: Gemcitabine; HR: Hazard ratio; NSCLC: Non-small-cell lung cancer; OS: Overall survival; PE: Primary end point; PPX: Paclitaxel poliglumex; VNR: Vinorelbine.

seems similarly active to the efficacy of nonpolymeric forms of taxanes compared with historical data in similar disease settings.

Based on these data, several Phase II and III trials comparing polymeric and nonpolymeric formulations of paclitaxel head to head are ongoing, as well as other combination studies **(Table 4.6)** [102].

Poliglumex: paclitaxel poliglumex or CT2103

Paclitaxel poliglumex or CT 2103 (Xyotax® [Cell Therapeutics; WA, USA]) is a polymer–drug conjugate of paclitaxel and polyglutamic acid. Paclitaxel poliglumex is one of the most developed polymer–drug conjugate since several Phase I, II and III studies have already been completed. As is it shown in **Table 4.7**, paclitaxel poliglumex has not shown to be superior to other taxanes in terms of overall survival, but it has a different toxicity profile. Although, based on these results, paclitaxel poliglumex has not been approved by the FDA or the EMA.

Table 4.8. Clinical trials ongoing with paclitaxel poliglumex.

Trial name	Clinical trial identifier	Status
Phase II study of combination of paclitaxel poliglumex and Alimta for advanced non-small cell lung cancer (NSCLC)	NCT00487669	Completed
CT-2103 in combination with gemcitabine in metastatic breast cancer (Phase I)	NCT00270907	Completed
A Phase I/II study of radiation therapy, paclitaxel poliglumex and cetuximab in advanced head and neck cancer	NCT00660218	Recruiting
Paclitaxel poliglumex and capecitabine in treating patients with metastatic breast cancer (Phase II)	NCT00265733	Completed
Paclitaxel or polyglutamate paclitaxel or observation in treating patients with stage III or stage IV ovarian epithelial or peritoneal cancer or fallopian tube cancer (Phase III)	NCT00108745	Recruiting
CT-2103/carboplatin vs. paclitaxel/carboplatin for NSCLC in women with estradiol >25 pg/ml (Phase III)	NCT00576225	Active, not recruiting
A pilot study of PPX in women with metastatic colorectal cancer	NCT00598247	Completed
BrUOG-Brain-223: a study of PPX (CT-2103), temozolomide, and concurrent radiation for newly diagnosed brain tumors (CTI#CT2103) (Phase II)	NCT00763750	Completed
PPX and concurrent radiation for newly diagnosed glioblastoma without MGMT methylation (Phase II)	NCT01402063	Recruiting

Data taken from [102].

Several trials with paclitaxel poliglumex are still ongoing, as summarized in **Table 4.8**. Interestingly, it seems that paclitaxel poliglumex could be especially efficacious in premenopausal women, and this effect could correlate with estrogen levels [102]. Based on this, some trials consider the levels of estradiol as inclusion criteria.

Anthracyclines

Anthracyclines are antibiotics with antitumor activity through the inhibition of topoisomerase II and thus impeding DNA transcription and replication. Although they have high antitumor activity, they also have significant chronic toxicities (e.g., cardiomyopathy) related with drug exposure to normal tissues (e.g., the myocardium). The conjugation of anthracyclines with polymers seeks to increase the tumor exposure while decreasing toxicity. This has been especially explored with liposomal formulations and this has been reviewed in **Chapter 7**. Apart from these liposomal formulations, there is one polymer–drug conjugate,

N-(2-hydroxypropyl)methacrylamide, that has initiated clinical development.

N-(2-hydroxypropyl) methacrylamide: FCE28068 (or PK1)

FCE28068 is a polymer–drug conjugate that consists on a doxorubicin molecule linked to a *N*-(2-hydroxypropyl) methacrylamide through a tetra peptide spacer. Based on preclinical data, one Phase I study with FCE28068 was published in 1999 [22], and a Phase II trial 10 years later [23]. It seems that the development of FCE28068 has been discontinued, since currently there are no ongoing registered clinical trials with this drug.

Conclusion

In conclusion, polymer–drug therapeutics in cancer are being explored as a potential option for improving drug delivery. By conjugating with polymers, several chemotherapeutic agents have been 're-engineered', such as camptothecines, taxanes, platinum compounds and antracyclines. The benefit of this strategy for classical cytotoxicsis still unclear. Preliminary data seem to be encouraging and further investigation in polymer–drug therapeutics in cancer is warranted.

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Summary.

- Polymer therapeutics improve anticancer drugs by improving their pharmacology, especially their drug delivery (widening their therapeutic window), by the use of advanced nanostructures.
- The application of nanomedicine in anticancer pharmacology is mainly focused in re-engineering existing anticancer agents, and polymer–drug conjugates, polymer–protein conjugates and polymeric micelles are all being explored clinically with some promising initial results.
- Since the main objectives of the use of polymer therapeutics are improving the pharmacokinetic, safety and efficacy of anticancer agents, comparative randomized clinical trials are still needed to prove the concept. Since these agents may change the safety profile, quality-of-life measurements will also help in proving the benefit of these drugs.

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Chapter 5

Drug protein-bound particles and polysaccharide–drug conjugates

Karina A Peters, Matías Chacón & Alejandro D Ricart

The susceptibility of cancer cells to antimitotic drugs while undergoing division serves as a critical intervention in clinical oncology, giving a better therapeutic index [1]. This approach includes a prolonged arrest of malignant cells in mitosis, ending in cell death [2,3]. However, microtubule-targeting agents target interphase cells too, as microtubule functions are prevalent throughout the cell cycle [1]. Taxanes stand out among antimitotic agents for the treatment of solid tumors. They are believed to promote the assembly of microtubules and to stabilize them by inhibiting their disassembly. This stability is thought to prevent the usual dynamics of the microtubules that are vital for their functions during mitosis. Therefore, taxanes induce aberrant arrays of microtubules and may increase damage to cells from S phase to mitosis [1].

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Nanoparticle: a particle with one or more dimensions of the order of 100 nm. Nanomaterials are mainly designed to improve the transport of diagnostic agents or drugs through biological, biophysical and biomedical barriers.

Secreted protein, acidic and rich in cysteine; or osteonectin: an albumin-binding protein, noted to be overexpressed in the microenvironment of several tumors. Nab-paclitaxel may, in part, use the albumin receptor (glycoprotein 60)-caveolin-1-caveolae-secreted protein, acidic and rich in cysteine pathway to increase intratumoral drug concentrations.

Therapeutic index: the ratio given by the dose of a drug that produces a defined level of damage to a normal tissue (toxicity) divided by the dose that produces a defined level of effect (antitumor activity). Therefore, the therapeutic index is a measure of the relative efficacy of a drug against a tumor compared with the toxicity caused.

Paclitaxel was the first taxane to be approved and is a fundamental component in the systemic treatment of breast, lung and ovarian cancer, among others [4–6]. Despite its broad clinical activity, paclitaxel is formulated in cremophor EL (Cr-EL), which causes infusion-related reactions, restricts the infusion rate and requires the use of premedication [7,8]. At the beginning of the new century, a solvent-free formulation of paclitaxel (ABI-007) was developed to circumvent the toxicity related to Cr-EL. This formulation utilizes a nanoparticle (130 nm) albumin-bound (nab) technology (nab-paclitaxel, Abraxane®; Abraxis BioScience, CA, USA). Nab-paclitaxel is free of solvents and the active agent paclitaxel exists in the particles in a noncrystalline, amorphous state. Albumin-bound paclitaxel may have preferential transport and a better concentration in tumors. The proposed mechanism involves, in part, glycoprotein 60-mediated endothelial cell transcytosis and accumulation in the tumor by albumin binding to secreted protein, acidic and rich in cysteine; or osteonectin (SPARC) [7,8]. With nab-paclitaxel, the free drug in the serum was 5.4-fold lower compared with concentrations found inside tumor masses and 2.2-fold lower compared with concentrations in normal tissues, suggesting a higher therapeutic index than Cr-EL paclitaxel [9]. Hypothesized improvements of nab-paclitaxel over the standard formulation include higher dose intensity, higher exposure to cells in specific phases of the cell cycle, stronger induction of endothelial cell apoptosis and concomitant antiangiogenic effects [6,10–16]. The mechanisms responsible for antiangiogenic effects may be diverse and may include the induction of inhibitors of angiogenesis, modulation of VEGF and cytokines, endothelial cell apoptosis, and effects on immune cells [6,11–16]. Solvents used in taxane formulations may adversely affect their efficacy due to entrapment of the active drug within micelles in plasma, leading to increased systemic exposure, reduced drug clearance from the central compartment and possible nonlinear pharmacokinetics (PK) [17–20]. Drug entrapment may also affect coadministered drugs [17–19].

Nab-paclitaxel is free of solvents, and the active agent paclitaxel exists in the particles in a noncrystalline, amorphous state. Albumin-bound paclitaxel may have preferential transport and a better concentration in tumors.

Nab-paclitaxel is indicated for the treatment of: metastatic breast cancer, after failure of

combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy, prior therapy should have included an anthracycline, unless clinically contraindicated; locally advanced or metastatic non-small-cell lung cancer (NSCLC), as first-line treatment in combination with carboplatin, in patients who are not candidates for curative surgery or radiation therapy; and metastatic adenocarcinoma of the pancreas as first-line treatment, in combination with gemcitabine [21].

Aa Dose-limiting toxicity: the appearance of treatment-related adverse events that are serious or life threatening, and prevent further increasing of dosage in dose-escalation studies.

Safety & clinical pharmacology

Generally, premedication to prevent infusion-related reactions is not needed prior to the administration of nab-paclitaxel. A Phase I trial reported the safety and PK of nab-paclitaxel administered every 3 weeks in 19 patients with advanced solid tumors [22]. Dose-limiting toxicity, which occurred in three out of six patients at the dose level of 375 mg/m^2, included sensory neuropathy, stomatitis and superficial keratopathy; the maximum-tolerated dose (MTD) was determined to be 300 mg/m^2. No severe hypersensitivity reactions were reported with nab-paclitaxel, despite administration over 30 min without premedication. Hematological toxicity was dose dependent and noncumulative, with only one episode of febrile neutropenia and one platelet transfusion over 96 cycles of treatment. PK analysis revealed linear paclitaxel exposure between 135 mg/m^2 and 300 mg/m^2. Of note, when the highest dose level, of 375 mg/m^2, was included nonlinearity became evident. Neutropenia level correlated with both exposure parameters: maximum concentration ($r = 0.610$, $p = 0.027$) and AUC ($r = 0.614$, $p = 0.025$).

The most common adverse events (≥20%) of single-agent nab-paclitaxel in patients with breast cancer include neutropenia, sensory neuropathy, fatigue, asthenia, myalgia, arthralgia, diarrhea, nausea, infections, electrocardiogram abnormalities, liver-enzyme elevations, anemia and alopecia [5,23,24]. The most common adverse events (≥20%) of nab-paclitaxel in combination with carboplatin are neutropenia, thrombocytopenia, anemia, fatigue, nausea, peripheral neuropathy and alopecia [4]. The most common adverse event leading to withholding or delaying the dose of nab-paclitaxel was myelosuppression. However, neutropenia was dose dependent and reversible in patients with breast cancer, with grade 4 neutropenia in 9% of the patients compared with 22% of the patients receiving Cr-EL paclitaxel [5].

The PK of total paclitaxel following different infusions of nab-paclitaxel at doses between 80 mg/m^2 and 375 mg/m^2 were determined in early

studies [19,22]. Dose levels of mg/m^2 refer to the mg of paclitaxel in the nab-paclitaxel. After nab-paclitaxel administration, plasma concentrations declined in a typical biphasic manner; an α phase representing distribution to peripheral compartments and a β phase representing drug elimination. The PK was dose proportional over the range of 80–375 mg/m^2 and independent of the iv. rate. At a dose of 260 mg/m^2, the mean total clearance was 15 l/h/m^{2}and the mean volume of distribution was 632 l/m^2, indicating extensive tissue binding of paclitaxel. The terminal half-life of nab-paclitaxel was approximately 27 h. The PK of 260 mg/m^2 nab-paclitaxel administered as a 30-min infusion was compared with the PK of 175 mg/m^2 paclitaxel administered over a 3-h infusion. The clearance was higher (43%) and the volume of distribution was also larger (53%) for nab-paclitaxel than for Cr-EL paclitaxel, but there was no difference in terminal half-life [19].

As described in the US Product Insert, "*in vitro* studies with human liver microsomes and tissue slices showed that paclitaxel was metabolized primarily to 6α-hydroxypaclitaxel by CYP2C8; and to two minor metabolites, 3′-p-hydroxypaclitaxel and 6α, 3′-p-dihydroxypaclitaxel, by CYP3A4". *In vitro*, the metabolism of paclitaxel to 6α-hydroxypaclitaxel was inhibited by many drugs (e.g., ketoconazole, verapamil, diazepam, quinidine, dexamethasone, cyclosporin, etoposide and vincristine), but at higher concentrations than those found in the clinic. "Testosterone, 17α-ethinyl estradiol, retinoic acid, and quercetin, a specific inhibitor of CYP2C8, also inhibited the formation of 6α-hydroxypaclitaxel *in vitro*" [21]. Therefore, "caution should be exercised when administering nab-paclitaxel concomitantly with medicines known to inhibit or induce either CYP2C8 or CYP3A4" [21].

Administration of carboplatin immediately after a nab-paclitaxel infusion in patients with NSCLC did not show drug interaction. The concentration versus time curve from time 0 to infinity ($AUC_{0-\infty}$) of carboplatin was approximately 23% higher than the targeted value (6 min × mg/ml), but the observed clearance was consistent with previous reports of single-agent carboplatin [21].

The urinary recovery of nab-paclitaxel is low and indicates extensive nonrenal clearance. Nab-paclitaxel is predominantly eliminated by the liver.The PK profile of nab-paclitaxel was evaluated in 15 patients with solid tumors with mild to severe hepatic impairment. Nab-paclitaxel doses were assigned based on the degree of hepatic impairment. The 260 mg/m^2 dose for mild hepatic impairment and the 200 mg/m^2 dose for moderate hepatic impairment resulted in paclitaxel exposures within the range seen in patients with normal hepatic function (mean $AUC_{0-\infty}$ = 14,789 ± 6703 h × ng/$\mu\lambda$). No dose adjustment is necessary for patients with mild hepatic impairment. As

described in the US Product Insert, "the 130 mg/m^2 dose in patients with severe hepatic impairment resulted in lower paclitaxel exposures than those seen in patients with normal hepatic function". There are no available data for patients with aspartate transaminase greater than ten-times the upper limit of normal or bilirubin greater than five-times the upper limit of normal. The effect of renal impairment on the disposition of nab-paclitaxel has not been studied [21].

Clinical studies

Metastatic breast cancer

There are data from two single arm, open-label studies and from one randomized comparative open-label study to support the use of nab-paclitaxel in metastatic breast cancer [5,23]. In the first study, nab-paclitaxel was given as a 30-min iv. infusion at a dose of 175 mg/m^2 to 43 patients. The second trial tested a higher dose (300 mg/m^2) as a 30-min infusion every 3 weeks in 63 patients [22]. This Phase II trial demonstrated an objective response rate (ORR) of 48% (95% CI: 35.3–60.0%) for all patients. The ORR was 64% for patients who received nab-paclitaxel as a first-line therapy. Time to tumor progression was 26.6 weeks and median overall survival (OS) was 63.6 weeks. No hypersensitivity reactions were reported despite the absence of premedication. These findings suggested that nab-paclitaxel could offer important advantages over Cr-EL-formulated paclitaxel and supported the conduct of the Phase III clinical trial. Other Phase II studies have been performed in advanced breast cancer and the most relevant are listed in **Table 5.1**.

A multicenter, randomized, open-label Phase III trial was conducted in 460 patients with measurable metastatic breast cancer. The patients included in the study had not relapsed within 1 year of adjuvant taxane treatment and had not received previous taxanes for metastatic disease. They were randomly assigned (1:1) to receive treatment every 3 weeks with either 260 mg/m^2 nab-paclitaxel given as a 30-min infusion without premedication or 175 mg/m^2 standard paclitaxel given as a 3-h infusion with premedication and infusion sets. The study was designed to directly compare the efficacy and safety of nab-paclitaxel with standard paclitaxel, the primary end point being ORR with a noninferiority design [5].

Patients in the nab-paclitaxel arm had a statistically significantly higher ORR based on an independent radiologic assessment for the first six cycles of therapy; 21.5% (95% CI: 16.2–26.7%) compared with 11.1% (95% CI: 6.9–15.1%) for patients in the Cr-EL paclitaxel arm [5]. According to the investigators' assessment, nab-paclitaxel also showed higher ORR compared with the standard treatment (33 vs 19%; p = 0.001) and longer time to tumor

Table 5.1. Relevant published Phase II and Phase III studies of nab-paclitaxel in patients with metastatic breast cancer.

Author (year)	Sample size (n)	Dose & schedule	ORR (%)	Median PFS	Median survival	Ref.
Gradishar *et al.* (2005)	460	260 mg/m^2 nab-paclitaxel every 3 weeks 175 mg/m^2 Cr-EL-paclitaxel every 3 weeks	33† 19	TTP: 23.0 weeks† TTP: 16.9 weeks	65.0 weeks 55.7 weeks	[5]
Gradishar *et al.* (2012)	302	150 mg/m^2 nab-paclitaxel for the first 3 of 4 weeks 100 mg/m^2 nab-paclitaxel for the first 3 of 4 weeks 300 mg/m^2 nab-paclitaxel every 3 weeks 100 mg/m^2 docetaxel every 3 weeks	49 45 37 35	12.9 months† 12.8 months 11 months 7.5 months	33. 8 months 22.2 months 27.7 months 26.6 months	[24,34]
Schwartzberg *et al.* (2012)	50	825 mg/m^2 capecitabine twice a day (days 1–15) + 125 mg/m^2 nab-paclitaxel (days 1 and 8) every 3 weeks	61	10.6 months	19.9 months	[35]
Mirtsching *et al.* (2011)	72	125 mg/m^2 nab-paclitaxel +/- trastuzumab weekly	42	14.5 months	2-year rate: 62%	[36]
Conlin *et al.* (2010)	32	100 mg/m^2 nab-paclitaxel (days 1–15) + carboplatin AUC 6 (day 1) every 4 weeks‡ + 2mg/kg trastuzumab weekly	62.5	16.6 months		[37]
Lobo *et al.* (2010)	30	10 mg/kg bevacizumab + 1500 mg/m^2 gemcitabine + 150 mg/m^2 nab-paclitaxel (days 1–15) every 4 weeks	75.9	10.4 months	18-month rate: 77.2%	[38]
Roy *et al.* (2009)	50	125 mg/m^2 nab-paclitaxel + 1000 mg/m^2 gemcitabine (days 1–8) every 3 weeks	50	7.9 months	6-month rate: 92%	[39]
Blum *et al.* (2007)	181§	100 mg/m^2 nab-paclitaxel weekly 125 mg/m^2 nab-paclitaxel weekly	14 16	3.0 months 3.5 months	9.2 months 9.1 months	[40]
Ibrahim *et al.* (2005)	63	300 mg/m^2 nab-paclitaxel every 3 weeks	48	TTP: 26.6 weeks	63.6 weeks	[23]

†Significantly higher or significantly longer.
‡Initially: carboplatin AUC 2 (days 1–8–15) every 4 weeks.
§Previously treated with taxanes.
AUC: Area under the curve; ORR: Objective response rate; PFS: Progression-free survival; TTP: Time to tumor progression.

progression (23.0 vs 16.9 weeks; hazard ratio [HR] = 0.75; p = 0.006). Nevertheless, there was no statistically significant difference in OS between the two study arms. As previously mentioned, the incidence of grade 4 neutropenia was significantly lower for nab-paclitaxel compared with standard paclitaxel (9 vs 22%; p < 0.001) despite there being a 49% higher quantity (mg) of paclitaxel in the nab-paclitaxel dose. However, febrile neutropenia was rare (<2%) in both arms. Grade 3 sensory neuropathy was more frequent with nab-paclitaxel than with Cr-EL paclitaxel (10 vs 2%; p < 0.001); however, severe neuropathy improved with the discontinuation of treatment to grades 2 or 1 in 22 days (median). After this the neuropathy was effectively managed with treatment interruption or dose reduction. The occurrence of hypersensitivity reactions (any grade) was low for both arms (<1% for nab-paclitaxel and 2% for Cr-EL paclitaxel). Severe (grades 3 or 4) hypersensitivity reactions were not reported in the nab-paclitaxel arm, despite the absence of premedication. On the other hand, a few grade 3 hypersensitivity reactions were reported with Cr-EL paclitaxel (chest pain in two patients, and allergic reaction in three patients). Corticosteroids and antihistamines were not routinely administered in the nab-paclitaxel arm; but, premedication was administered for emesis, myalgia/arthralgia or anorexia in 18 patients (8%) in the nab-paclitaxel arm in 2% of the treatment cycles, while 224 patients (>99%) in the Cr-EL paclitaxel arm received premedication in 95% of the cycles.

Clinical studies have shown that nab-paclitaxel significantly increases the objective response rate compared with paclitaxel formulated as cremophor EL. However, there has not been a statistically significant difference in overall survival between both formulations in patients with metastatic breast cancer or in patients with advanced non-small-cell lung cancer.

NSCLC

A multicenter, randomized open-label study was conducted in 1052 untreated patients with Stage IIIb–IV NSCLC to compare nab-paclitaxel in combination with carboplatin to Cr-EL paclitaxel in combination with carboplatin as a first-line treatment [4]. A dose of 100 mg/m^2 nab-paclitaxel was administered weekly and 200 mg/m^2 Cr-EL paclitaxel was administered every 3 weeks after standard premedication. In both treatment arms, carboplatin was administered at AUC 6 (per Calvert formula) on day 1 of each 21-day cycle. Treatment was continued until evidence of disease progression or until unacceptable treatment-related adverse events. The primary end point was ORR using Response Evaluation Criteria in Solid Tumors 1.0, as reviewed by a central radiology independent committee. A total of 49% of the patients had adenocarcinoma and 43% had squamous carcinoma. They received a median of six cycles of chemotherapy in both treatment arms. Patients in the nab-paclitaxel/carboplatin arm had a significantly higher ORR compared

with patients in the Cr-EL paclitaxel/carboplatin arm (33 vs 25%; response rate ratio: 1.313; 95% CI: 1.082–1.593; p = 0.005). Likewise, the investigator's assessment showed a statistically significant improvement in ORR (nab-paclitaxel: 38 vs Cr-EL paclitaxel: 30%; response rate ratio: 1.274; 95% CI: 1.076–1.509; p = 0.005). Interestingly, the independent radiology assessment showed a significant improvement of ORR for nab-paclitaxel/carboplatin in patients with squamous cell histology (41 vs 24%; response rate ratio: 1.680; 95% CI: 1.271–2.221; p < 0.001), while nab-paclitaxel/carboplatin was as effective as Cr-EL paclitaxel/carboplatin in patients with nonsquamous histology (26 vs 25%; response rate ratio: 1.034; 95% CI: 0.788–1.358; p = 0.808). Median progression-free survival (PFS) was 6.3 months (95% CI: 5.6–7.0 months) in the nab-paclitaxel/carboplatin versus 5.8 months (95% CI: 5.6–6.7 months) in the Cr-EL paclitaxel/carboplatin arm. Analysis of noninferiority was conducted according to the EMA methodological considerations with a 15% margin (upper bound of the $HR_{nab\text{-}paclitaxel/Cr\text{-}EL\ paclitaxel}$; 95% CI: < 1.176). The PFS in the nab-paclitaxel/carboplatin arm was noninferior to the PFS in the Cr-EL paclitaxel/carboplatin arm ($HR_{nab\text{-}paclitaxel/\ Cr\text{-}EL\ paclitaxel}$; 95% CI: upper bound, 1.086). Median OS was 12.1 months (95% CI: 10.8–12.9 months) in the nab-paclitaxel/carboplatin arm compared with 11.2 months (95% CI: 10.3–12.6 months) in the Cr-EL paclitaxel/carboplatin arm. The OS in the experimental arm was noninferior ($HR_{nab\text{-}paclitaxel/\ Cr\text{-}EL\ paclitaxel}$; 95% CI: upper bound, 1.066).

There was a clear difference in cumulative dose and dose intensity between nab-paclitaxel and Cr-EL paclitaxel in this study. The study design encouraged at least six cycles of therapy, but treatment could continue in the absence of disease progression and unacceptable toxicity. The median number of cycles was 6.0 in both arms, with 350 patients in the nab-paclitaxel arm and 358 patients in the Cr-EL paclitaxel arm receiving six or fewer cycles of treatment. The median cumulative dose of paclitaxel was 1325 mg/m^2 in the nab-paclitaxel arm versus 1125 mg/m^2 in the Cr-EL paclitaxel arm, with a median paclitaxel dose intensity of 82 mg/m^2 per week versus 65 mg/m^2 per week, respectively. The median cumulative carboplatin dose was 3140 mg in the nab-paclitaxel arm versus 3315 mg in the Cr-EL paclitaxel arm, with the median carboplatin dose intensity of 166 mg per week versus 204 mg per week, respectively. A total of 46% of the patients had a paclitaxel dose reduction in the nab-paclitaxel arm and 23% in the Cr-EL paclitaxel arm, due to: neutropenia (29 and 10%), thrombocytopenia (13 and 4%), anemia (6 and <1%) and sensory neuropathy (2 and 6%). Despite this higher rate of dose reduction in the nab-paclitaxel arm, the paclitaxel dose intensity was 26% greater and the cumulative dose was 18% greater for nab-paclitaxel than for Cr-EL paclitaxel. Dose delays were also more frequent in the

nab-paclitaxel arm (82%) compared with the Cr-EL paclitaxel arm (54%). However, for the analysis of these results it is important to keep in mind that the comparison was a weekly administration of nab-paclitaxel versus a 3-week administration of Cr-EL paclitaxel.

There were less instances of grade ≥3 neuropathy, neutropenia, arthralgia and myalgia in the nab-paclitaxel/carboplatin arm; and less thrombocytopenia and anemia occurred in the Cr-EL paclitaxel/carboplatin arm (all $p < 0.05$). For grade ≥3 sensory neuropathy, the median time to improvement to grade 1 was 38 days in the nab-paclitaxel arm compared with 104 days in the Cr-EL paclitaxel arm. All grades of sensory neuropathy were significantly less with nab-paclitaxel (46%) compared with Cr-EL paclitaxel (62%; $p < 0.001$). Furthermore, as expected, the percentage of patients without neuropathy was significantly higher with nab-paclitaxel (54%) compared with Cr-EL paclitaxel (38%; $p < 0.001$).

Pancreatic cancer

Single-agent gemcitabine has been the standard treatment for advanced pancreatic adenocarcinoma for the last 15 years, with a consistent median OS of approximately 6 months and a 20% 1-year survival [25]. A combination of 5-fluorouracil, oxaliplatin and irinotecan improved the median survival in highly selected patients, but with more toxicity [26]. Pancreatic cancer cells and stroma overexpress SPARC. SPARC regulates the assembly, organization and turnover of the extracellular matrix by binding and modulating the deposition of components and by ameliorating the activity of extracellular proteases. Consequently, SPARC has some role in tumor development, invasion, metastases and angiogenesis, and its expression is related to the aggressive behavior of tumors [27]. Median survival was inferior in patients with pancreatic tumors that expressed SPARC (15 vs 30 months), and when matched with other prognostic factors (tumor size, positive lymph nodes, margin status, tumor grade and age) the HR was significant (HR: 1.89; 95% CI: 1.31–2.74) [28]. There is preliminary evidence that increased SPARC expression in tumors resulted in an improved response to nab-paclitaxel, due to SPARC albumin binding [29].

A Phase I study explored the combination of nab-paclitaxel with gemcitabine as a first-line treatment in patients with metastatic pancreatic adenocarcinoma [30]. The objectives of the study were to: identify the MTD, evaluate safety and report preliminary efficacy of the combination. A total of 67 patients received 100, 125 and 150 mg/m^2 nab-paclitaxel with 1000 mg/m^2 gemcitabine on days 1, 8 and 15 every 28 days. The MTD was 1000 mg/m^2 of gemcitabine plus 125 mg/m^2 of nab-paclitaxel once a week for 3 weeks, every 28 days. The dose-limiting toxicities were sepsis and

neutropenia, and the most common adverse events were anemia, leukopenia, neutropenia, thrombocytopenia, fatigue, sensory neuropathy and nausea. Neuropathy and fatigue were the main reasons for treatment discontinuation. A remarkable ORR of 46% was reported in this study. Improved OS was correlated with complete metabolic response on PET scan, decrease in CA 19–9, and SPARC in the stroma, but not in the tumor. The median PFS was 7.1 months (95% CI: 5.7–8.0), with a 48% 1-year survival.

The Phase III MPACT trial presented by Von Hoff *et al.* randomized patients with metastatic pancreatic adenocarcinoma without previous treatment to standard single-agent gemcitabine or the combination of 125 mg/m^2 nab-paclitaxel followed by 1000 mg/m^2 gemcitabine on days 1, 8, and 15 every 4 weeks. For the primary end point of OS, 608 events from 842 patients provided a power of 0.9 to detect a HR of 0.769 (2-side $\alpha = 0.049$) [31]. Median OS was improved with the addition of nab-paclitaxel to gemcitabine from 6.7 months with the single-agent gemcitabine to 8.5 months with the combination (HR: 0.72; 95% CI: 0.62–0.84; p = 0.000015). The combination treatment showed a remarkable increase in 1-year survival (35 vs 22%; p = 0.0002) and doubled the rate of survival at 2 years (9 vs 4%; p = 0.02) compared with gemcitabine monotherapy. The most common grade ≥3 treatment-related adverse events in the study for nab-paclitaxel plus gemcitabine verus gemcitabine alone were neutropenia (38 vs 27%), fatigue (17 vs 7%), and neuropathy (17 vs 1%). Of note, there was no difference in serious life threatening toxicity, 4% in each arm.

Polysaccharide–drug conjugates

Different polysaccharides are under investigation as potential useful systems for achieving controlled drug release and drug targeting in cancer therapy. Dextran is a family of natural polysaccharides being used for solubilization, long-circulating carriers, nonviral vectors, stabilization of enzymes and functionalization of nanomaterials [32]. Various dextran–drug conjugates enhance the effectiveness of cytotoxic drugs. Chitosan is a biodegradable matrix under development for gene therapy. According to preliminary results chitosan could exhibit anticancer properties in *in vitro* and *in vivo* models [33]. Finally, hyaluronic acid nanogel–drug conjugates seem to preferentially target tumor cells with elevated levels of the CD44 receptor. However, most of these attempts are still in preclinical development and, unlike the other delivery systems described in this book, do not have a clear clinical application.

Conclusion

Clinical studies have shown that nab-paclitaxel significantly increases the ORR compared with paclitaxel formulated as Cr-EL. However, there has not

been a statistically significant difference in OS between both formulations in patients with metastatic breast cancer or patients with advanced NSCLC. The absence of Cr-EL from the formulation has decreased treatment-related adverse events. Nab-paclitaxel can be administered using higher doses of paclitaxel than is achievable with Cr-EL paclitaxel, as a short infusion and without the requirement for premedication to reduce the risk of solvent-mediated hypersensitivity reactions. The recent results of nab-paclitaxel in metastatic pancreatic cancer deserve a special comment. Over the last 15 years, the combination of gemcitabine with other cytotoxic agents has been disappointing. Nab-paclitaxel is the first cytotoxic agent to show a statistically significant improvement in OS when combined with gemcitabine versus standard single-agent gemcitabine. Taking all this into account, nab technology seems to increase the therapeutic index of paclitaxel compared with the conventional, solvent-based formulation.

Financial & competing interests disclosure

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Summary.

- Paclitaxel was the first taxane to be approved, and is a fundamental component in the systemic treatment of breast, lung and ovarian cancer, among others. Despite its broad clinical activity, paclitaxel is formulated in cremophor EL, which causes infusion-related reactions, restricts the infusion rate and requires the use of premedication.
- A solvent-free formulation of paclitaxel utilizes a nanoparticle (130 nm) albumin-bound (nab) technology (nab-paclitaxel; Abraxane®; Abraxis BioScience, CA, USA). Generally, premedication to prevent infusion-related reactions is not needed prior to the administration of nab-paclitaxel.
- Albumin-bound paclitaxel may have preferential transport and better concentration in tumors.
- Nab-paclitaxel is indicated for the treatment of metastatic breast cancer after the failure of combination chemotherapy or relapse within 6 months of adjuvant chemotherapy, and for the first-line treatment of advanced non-small-cell lung cancer, in combination with carboplatin.
- Nab-paclitaxel is the first cytotoxic agent to show a statistically significant improvement in overall survival when combined with gemcitabine versus standard single-agent gemcitabine for the treatment of metastatic pancreatic cancer.
- Different polysaccharides are under investigation as potentially useful systems for achieving controlled drug release and drug targeting in cancer therapy.

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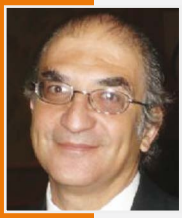

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Chapter 6

Liposomal drug carriers

Daniel S Lewi, Karina A Peters & Pedro M Politi

Liposomal drug carriers provide a unique advantage in that continuous drug delivery to the systemic circulation is achieved with minimized peak drug concentrations and a favorable, less toxic target tissue-to-plasma drug level ratio. As far as anticancer chemotherapy is concerned, these advantages are desirable and are expected to reduce clinical toxicity while improving patient conveniency and, in some cases, antitumor efficacy as well. A limited number of liposomal formulations of cytotoxic drugs have been approved for clinical use, with anthracyclines and vincristine as the main examples, but several other antitumor drug formulations are in clinical development. This chapter briefly describes the clinical pharmacology of commercially available cytotoxic drug formulations, approved indications and toxicity profile and summarizes the outlook for novel liposomal formulations of antitumor drugs and biologic agents.

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Aa Liposomes: drug-delivery systems consisting of small spherical vesicles with one or more lipid bilayers with an aqueous core. They are able to carry a payload of drug entrapped in the vesicles.

Nanoparticle: a small object that behaves as a whole unit and is sized between 1 and a few hundred nanometers.

Optimizing cancer therapy remains a pressing need. Liposomes are interesting drug-delivery systems designed to decrease toxicity to normal tissues while providing some of the therapeutic advantages of continuous infusion schedules [1,2]. Liposomes are a byproduct of nanoparticle research applied to drug therapy. This field has evolved enormously during the last 15 years [3].

Liposomes are small, spherical vesicles composed of one or more lipid bilayers with an aqueous core and the ability to carry a payload of complex molecules. While most other drug-delivery systems are designed to control the input rate into the systemic circulation, liposomes circulate within the central compartment and distribute drug to tissues [4]. Tissue distribution patterns depend on the specific formulation involved.

Commercially available liposomal anticancer drug formulations are shown in **Table 6.1**. Even if liposomal formulations have been developed for most of the cytotoxic drugs in clinical practice, only two anthracycline agents (daunorubicin and doxorubicin) and the tubulin poison, vincristine, have been licensed in a liposomal drug formulation for intravenous administration so far.

Liposomal encapsulation of cytotoxic drugs and biological antitumor agents is an area of active research, with more than 400 ongoing clinical trials and many preclinical studies [5–7]. However, liposomal formulations have not focused exclusively on anticancer drugs: the antifungal agent amphotericin B

Table 6.1. Commercially available liposomal drug formulations of anticancer drugs.

Drug formulation	Trade name
Liposomal daunorubicin	DaunoXome® (Gilead Sciences [CA, USA])
Pegylated liposomal doxorubicin	Doxil®/Caelyx® (Johnson & Johnson [NJ, USA]; Alza [CA, USA])
Liposomal doxorubicin (nonpegylated)	Myocet® (Elan [Dublin, Ireland]) – approved in Europe
Liposomal vincristine	Marqibo® (Talon Therapeutics [CA, USA])
Liposomal cytarabine	DepoCyt® (Sigma-Tau Pharmaceuticals [MD, USA])
Liposomal mifamurtide	Mepact® (Takeda Pharmaceutical [Osaka, Japan])

has been successfully developed as a liposomal formulation and is commercially available (AmBisome®; Gilead Sciences [CA, USA]/Fujisawa Healthcare [IL, USA]).

Liposomes are biodegradable and generally nontoxic vehicles with the ability to encapsulate both hydrophilic and hydrophobic molecules [8]. Whereas conventional intravenous injections (or short infusions) of conventional cytotoxic drugs reach peak plasma levels that may be toxic, most liposomal formulations maintain relatively low free-drug levels in plasma for several days and achieve some degree of differential delivery to target (tumor) versus normal tissue levels, reportedly due to increased permeability of the tumor microvasculature. This often translates into improved safety (reduced cardiac toxicity of liposomal anthracycline formulations compared with conventional anthracyclines). Liposomal uptake by the reticuloendothelial system creates a new depot mechanism with subsequent free-drug release to the central compartment. Adequate delivery of the payload drug to the tumor tissue may be hampered by rapid trapping of liposomes by the reticuloendothelial system. To this end, several modifications of liposome structural properties have been explored, including polyethyleneglycol (PEG) addition to the outer layer of liposomes. Therefore, better local delivery to tumor tissue is achieved. Theoretical benefits of liposomal formulations are listed in **Table 6.2**.

Lipid encapsulation is not completely free from serious systemic adverse effects; however, acute infusion reactions to liposomal antitumor drugs are well documented, and skin toxicity is commonly observed as palmoplantar erythrodysesthesia ('hand–foot syndrome') with liposomal anthracyclines, sometimes severe enough to limit therapy. As it can be easily anticipated, physicochemical drug and liposome properties and payload drug stability are important considerations towards achieving an improved therapeutic index.

Liposomal formulations may be specifically designed to carry a payload of complex molecules, including biologic antitumor agents, and may be fitted with surface molecules to further optimize drug delivery.

Liposomal drug formulations of well-known cytotoxic drugs may decrease peak plasma level-associated drug toxicity, improve drug delivery to target tumor tissue and enhance patient compliance with a less toxic, less demanding schedule.

Characteristics of approved liposomal formulations of cytotoxic drugs

Liposomal daunorubicin

Liposomal daunorubicin citrate (DaunoXome® [NeXstar Pharmaceuticals Inc.; CO, USA]) was first marketed in the 1990s for the systemic treatment of advanced AIDS-related Kaposi's sarcoma (KS) where available conventional combination chemotherapy at the time carried severe,

Table 6.2. Theoretical benefits of liposomal formulations.

Action	Involved mechanism	Benefit
Passive targeting	Liposomal formulations restrict extravasation into normal tissues and facilitate accumulation in tumors	Improved therapeutic index of the encapsulated anticancer agent (also extended drug exposure, see below)
Extended drug exposure	▪ Slow release that influences the pharmacokinetics of the encapsulated drug. It can reduce C_{max}-related toxicity ▪ It can prevent early inactivation of the drug (e.g., for gene therapy). In addition, if the encapsulated drug is cell-cycle specific, the formulation could affect a higher proportion of cancer cells	▪ Reduced adverse events ▪ Increased efficacy
Solubilization	Poorly soluble drugs are entrapped inside a vesicle	Stable formulation for intravenous administration
Active targeting	Immunoliposomes: combination of liposomes with a ligand (monoclonal antibody or antibody fragment)	Targeted therapy: accumulation of the drug in the tumor with overexpression of the antigen
Improved immune response	Through immune modulation	Locoregional therapy for vaccine approaches?

C_{max}: Maximum drug concentration in plasma.

life-threatening toxicity. DaunoXome contains an aqueous solution of the citrate salt of the anthracycline cytotoxic agent, daunorubicin, entrapped in liposomes with a bilayer of distearoylphosphatidylcholine and cholesterol in a 2:1 molar ratio. These particles have a mean diameter of approximately 45 nm. Preclinical data showed preferential drug accumulation in highly vascularized KS lesions [9]. In randomized clinical trials, liposomal daunorubicin (40 mg/m^2 intravenously every 2 weeks) exhibited a response rate and median survival time comparable to that of conventional doxorubicin, bleomycin and vincristine, and was associated with less toxicity, including less alopecia and fewer and milder gastrointestinal and neurologic side effects but more grade 4 neutropenia [10]. No cases of congestive heart failure were reported in either treatment arm. Moreover, hand-foot syndrome was not

Liposomal drug encapsulation has been shown to significantly decrease selected drug-limiting toxicity. For example, chronic cardiotoxicity is less frequent with liposomal doxorubicin than with the classic, nonliposomal formulation of this anthracycline.

frequent with usual doses of liposomal daunorubicin.

Liposomal drug formulation may substantially change the clinical spectrum of therapeutic efficacy of a 'classic' cytotoxic agent. As an example, single agent liposomal doxorubicin has been demonstrated safe and effective against both AIDS-related Kaposi's sarcoma and relapsed epithelial ovarian cancer, whereas nonliposomal doxorubicin has not been approved for these indications as monotherapy.

Liposomal doxorubicin formulations

Commercially available liposomal formulations of the anthracycline agent doxorubicin differ sharply and are not bioequivalent. Myocet® (Elan [Dublin, Ireland]; liposomal doxorubicin, non-PEGylated) displays a large oligolamellar liposomal vesicle, with a particle size of approximately 180 nm. The drug-loading method involves a hydrogen ion gradient (with lower internal pH due to the citric acid buffer). Doxorubicin is added externally. Myocet is supplied as a three-vial system: doxorubicin as lyophilized powder, liposomes in suspension (e.g., phosphatidyl choline/cholesterol in a 1:1 ratio, citric acid and sodium chloride) and buffer. The recommended dosage is 60–75 mg/m^2 intravenously every 3 weeks. Dose-limiting toxicity (DLT) is myelosuppression (mostly neutropenia). Myocet is clinically active against AIDS-related KS, with dose-dependent neutropenia as the main toxicity [11]. Myocet is not currently approved in the USA.

Doxil® (Johnson & Johnson [NJ, USA]; sold under the name Caelyx® outside of the USA) is a PEGylated liposomal doxorubicin formulation with ten to 15 molecules of doxorubicin per liposome. The liposomes are large, unilamellar vesicles, approximately 100 nm in diameter, composed of hydrogenated soy phosphatidyl choline, cholesterol, PEG and phosphatidylethanolamine. Pegylation enables long plasma residence times (~4 days) and doxorubicin accumulates in tumor tissue, reportedly due to highly permeable tumor blood vessels. An ammonium ion gradient is used to load the doxorubicin. The recommended drug schedules are: 20 mg/m^2 intravenously every 3 weeks for KS, 50 mg/m^2 every 4 weeks for ovarian cancer and 30 mg/m^2 when combined with bortezomib for multiple myeloma (see US FDA-approved drug label [101]). In addition, PEGylated liposomal doxorubicin has been approved by the EMA as single-agent chemotherapy for metastatic breast cancer. DLT of Doxil/Caelyx is stomatitis/mucositis (single dose) and hand–foot syndrome (for multiple doses).

As previously shown for liposomal daunorubicin, PEGylated liposomal doxorubicin was more effective and less toxic than doxorubicin, bleomycin and vincristine for first-line chemotherapy of AIDS-related KS in a Phase III clinical trial [12]. PEGylated liposomal doxorubicin (50 mg/m^2 every 4 weeks) showed antitumor efficacy similar to commonly used salvage chemotherapy regimens in taxane-refractory advanced breast cancer patients [13].

Striking pharmacologic differences are noted between conventional doxorubicin and its liposomal formulations: decreased cardiac toxicity and very low potential for tissue damage after extravasation are evident, particularly with PEGylated liposomal doxorubicin. However, these advantages come with a downside: increased skin toxicity under the form of 'hand–foot syndrome'. In addition, severe acute infusion-related reactions have been reported in up to 10% of patients treated with liposomal anthracyclines: flushing, facial edema, chills, back pain, dyspnea, tightness in the chest and hypotension encompass a wide spectrum of severity. In some cases, the reaction resolves upon slowing the infusion rate, but others may require aggressive rescue therapy.

In comparison, Myocet, which lacks PEG coating, results in shorter circulation times than those of Doxil/Caelyx. DaunoXome contains daunorubicin encapsulated in a smaller liposome of a different lipid composition [14]. It has circulation times between those of PEGylated and non-PEGylated liposomal doxorubicin. Liposomal composition of currently approved formulations is presented in both **Table 6.3** and **Figure 6.1**.

Liposomal vincristine

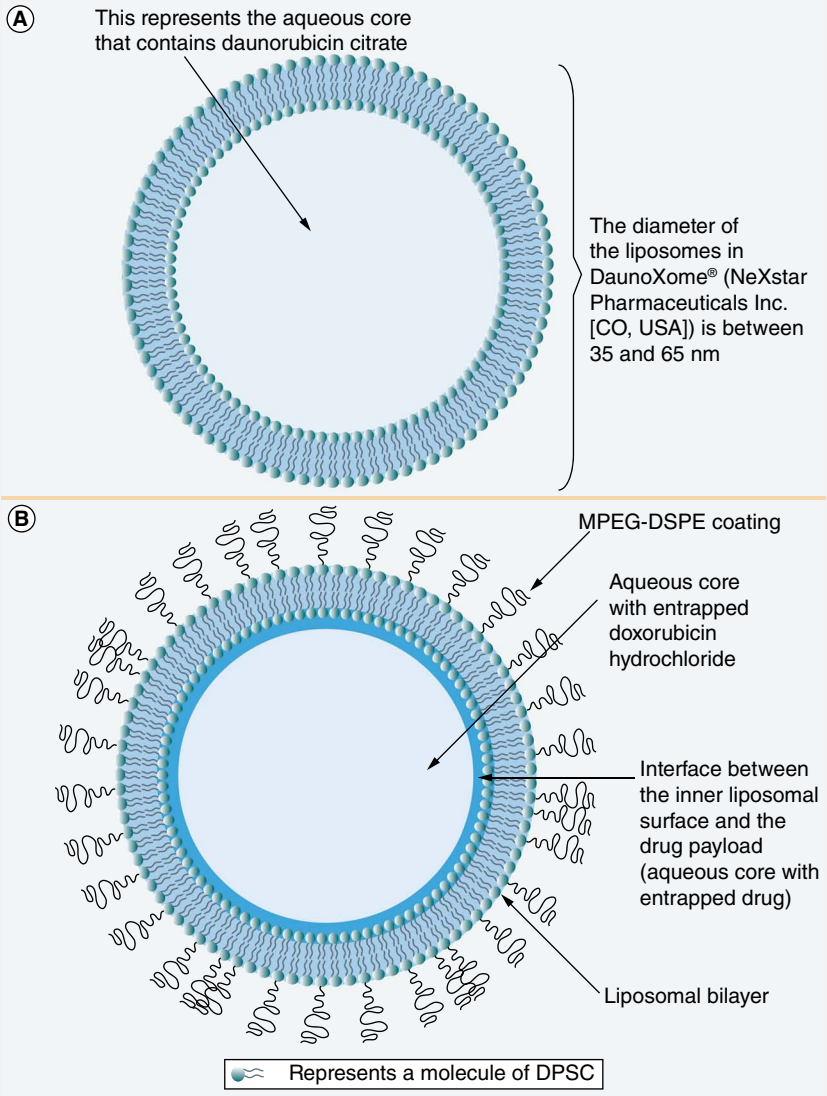
A liposomal formulation of the vinca alkaloid, vincristine sulfate, has been recently approved by the FDA for the treatment of Philadelphia chromosome-negative acute lymphoblastic leukemia in second or later relapse; under the registered name Marqibo® (Talon Pharmaceuticals [CA, USA]) [102]. Vincristine is encapsulated in a sphingomyelin/cholesterol

Table 6.3. Liposomal composition and approved indications of commercially available liposomal anticancer drug products.

Liposomal product	Liposomal composition	Approved indications
Liposomal daunorubicin	Distearoylphosphatidylcholine/cholesterol	Kaposi's sarcoma
PEGylated liposomal doxorubicin	PEG-coated liposomes, phosphatidyl choline, cholesterol, phosphatidyl ethanolamine	Kaposi's sarcoma, multiple myeloma, ovarian cancer and advanced breast cancer†
Liposomal doxorubicin (non-PEGylated)	Phosphatidyl choline/cholesterol	Kaposi's sarcoma†
Liposomal vincristine	Sphingomyelin/cholesterol	Philadelphia-negative ALL
Liposomal cytarabine meningitis	Distearoylphosphatidylcholine/cholesterol	Lymphomatous meningitis
Liposomal mifamurtide	Phosphocoline/phospho-L-serine	Osteosarcoma

†Not licensed in the USA for this indication.
ALL: Acute lymphoblastic leukemia; PEG: Polyethylene glycol.

Figure 6.1. Liposomal structure for DaunoXome® (NeXstar Pharmaceuticals Inc. [CO, USA]) and Doxil®/Caelyx® (Johnson & Johnson [NJ, USA]).



(A) Liposomal daunorubicin (DaunoXome® [NeXstar Pharmaceuticals Inc.; CO, USA) and **(B)** PEGylated liposomal doxorubicin (Doxil®/Caelyx® [Johnson & Johnson; NJ, USA]). DSPC: 1,2-distearoyl-*sn*-glycero-3-phosphocholine; MPEG-DSPE: Methoxy-polyethylene glycol 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine.
(A) Taken from [104] and **(B)** taken from [105].

liposome (100-nm diameter) with lipid components at a 60:40 molar ratio and supplied as a three vial ready-to-mix kit with vincristine sulfate 5 mg/5 ml with manitol, a liposome injection vial (sphingomyelin, cholesterol and citric acid, sodium citrate, and ethanol) and a third vial with dibasic sodium phosphate and sodium chloride.

The main toxicity of liposomal vincristine includes extravasation injury, peripheral motor, sensory and autonomic neuropathy (which can be dose-limiting), myelosuppression (neutropenia, thrombocytopenia and anemia), tumor lysis syndrome, constipation and/or paralytic ileus, fatigue, and hepatic and embryofetal toxicity. In addition, intrathecal injection may be lethal and should be avoided. All of these risks do not seem to differ qualitatively from those described for conventional vincristine sulfate. However, clinical experience with liposomal vincristine is limited to less than 100 patients older than 18 years with heavily pretreated acute lymphoblastic leukemia in a noncontrolled clinical trial.

Liposomal cytarabine

Liposomal cytarabine (DepoCyt® [Sigma-Tau Pharmaceuticals; MD, USA]) is a suspension of this antimetabolite drug encapsulated in multivesicular lipid vesicles approved for intrathecal administration. Lymphomatous meningitis is a devastating complication and its optimal treatment still requires development. However, conventional intrathecal agents maintain cytotoxic concentrations for a short period in the cerebrospinal fluid (CSF) and require frequent lumbar punctures. Liposomal cytarabine, a sustained-release formulation, usually maintains cytotoxic levels of cytarabine (Ara-C) in the CSF for more than 14 days [15]. The FDA granted full approval for liposomal cytarabine for the treatment of patients with lymphomatous meningitis in 2007. This decision was based on results of two randomized clinical trials that enroled over 200 patients with neoplastic meningitis related to solid tumors or hematologic malignancies. The first study demonstrated that a higher percentage of patients treated with liposomal cytarabine were free of neurological worsening. The second study showed an increase in clearing of malignant cells in the CSF (complete cytologic response) among patients treated with liposomal cytarabine [15,16].

Chemical arachnoiditis, a potentially fatal clinical condition usually presenting with fever, headache, nausea and vomiting, was a common adverse event. Concomitant dexamethasone should be given to patients receiving liposomal cytarabine to improve symptomatic control. Dexamethasone, at a dose of 4 mg twice a day, either orally or intravenously for 5 days starting on the day of liposomal cytarabine injection is recommended in order to decrease the incidence and severity of this

complication. Neurotoxicity other than arachnoiditis is uncommon. Nevertheless, concomitant systemic chemotherapy with the ability of crossing the blood–brain barrier could increase neurotoxicity [17].

The usual dose of liposomal cytarabine for adults is 50 mg, administered every 2 weeks during induction and consolidation therapy, and every 4 weeks while on continuation therapy. If neurotoxicity develops, dose reduction to 25 mg is recommended. Treatment discontinuation is in order if neurotoxicity persists.

Mifamurtide

Muramyl tripeptide phosphatidylethanolamine (mifamurtide, Mepact® [Takeda Pharmaceutical; Osaka, Japan]) is a lipophilic analog of muramyl dipeptide, the smallest naturally occurring immune stimulatory component of cell walls from *Mycobacterium* species capable of immune system stimulation. Mifamurtide has similar immunostimulatory effects as natural muramyl dipeptide with a longer half-life in plasma. By binding to NOD2, a pattern recognition receptor mainly in monocytes, macrophages, granulocytes and myeloid dendritic cells, mifamurtide simulates a bacterial infection. In patients, the intravenous infusion of mifamurtide results in an increased production of cytokines, principally TNF-α, IL-6 and neopterin, with a subsequent anticancer effect [18].

As monotherapy in Phase I studies, the most common adverse events were fever, chills, myalgias, fatigue and hypertension. The combination of mifamurtide plus chemotherapy significantly improvement overall survival (OS) in a randomized trial in patients with osteosarcoma. The addition of mifamurtide to backbone chemotherapy improved 6-year OS from 70 to 78% ($p = 0.03$). The hazard ratio (HR) for OS with the addition of mifamurtide was 0.71 (95% CI: 0.52–0.96), a reduction in the risk of death of almost a third [19]. Mifamurtide has been licensed by the EMA since March 2009, for the treatment of children, adolescents and young adults with high-grade resectable nonmetastatic osteosarcoma after macroscopically complete surgical resection. Mifamurtide should be used in combination with standard chemotherapy. However, mifamurtide has missed full approval by the FDA.

Characteristics of experimental liposomal formulations

Liposomal paclitaxel

Paclitaxel has been embedded in cationic liposomes (EndoTAG®-1 [Medigene; Martinsried, Germany]; ET) in an attempt to target endothelial cells in the tumor environment. A randomized Phase II trial has recently evaluated the safety and efficacy of the ET plus gemcitabine doublet as first-line

therapy in advanced pancreatic cancer [20]. Patients were randomly assigned to receive weekly gemcitabine at a standard dose of 1000 mg/m^2 of gemcitabine plus twice-weekly ET at 11, 22 or 44 mg/m^2 for 7 weeks. After a safety run-in of 100 patients, a second cohort was opened. A total of 212 patients were enroled and 200 patients were treated, with 80% of them having metastatic disease and 20% having locally advanced disease. Median OS was 8.1 months in the gemcitabine and ET11 arm, 8.7 months with gemcitabine and ET22, and 9.3 months in the gemcitabine and ET44 arm, compared with 6.8 months in the gemcitabine control arm. HRs for OS relative to gemcitabine control arm were 0.93, 0.69 and 0.66, respectively. Survival rates at 12 months were 21, 35 and 30%, respectively, for ET arms compared with 15% for the gemcitabine control arm. Accordingly, median progression-free survival was longer in the gemcitabine and ET arms (4.1, 4.6 and 4.4 months, respectively) compared with the gemcitabine control arm (2.7 months). The corresponding adjusted HRs for progression-free survival were 0.84, 0.58 and 0.74, respectively, for the gemcitabine and ET arms. Unexpected adverse events were not observed.

During the first cycle of treatment, severe hematological toxic effects (grade 3/4) occurred in 22, 32 and 40% of patients in the different ET-containing arms (11, 22 and 44 mg/m^2, respectively), compared with 24% of the controls. Nevertheless, the combination of ET and gemcitabine resulted in a higher frequency of grade 3/4 thrombocytopenia, up to 16 and 14% at the two higher dose levels. There were no bleeding complications. At the highest ET dose level (44 mg/m^2 twice weekly), increased rates of grade 3/4 neutropenia (22%) and anemia (12%) were observed. Febrile neutropenia was observed (seven patients) with the two higher ET dose levels. Infusion-related reactions, predominantly pyrexia and chills, were more frequent in the ET arms. These results need confirmation in a randomized Phase III trial.

Liposomal vinorelbine

A Phase I study recently evaluated the safety and the pharmacokinetics of a liposomal formulation of vinorelbine (NanoVNB® [Taiwan Liposome Co.; Taipei, Taiwan]) in patients with advanced solid tumors [21]. Liposomal vinorelbine was administered intravenously at doses of 2.2–23 mg/m^2 every 14 days in 22 patients. Skin rash was the DLT and the most frequent nonhematological toxicity. The maximum tolerated dose (MTD) was 18.5 mg/m^2. Grade 3–4 hematological adverse events were infrequent. Compared with intravenous free vinorelbine, the liposomal formulation showed a high maximum concentration and low plasma clearance. Of 11 evaluable patients, five had stable disease as best response. Based on the DLT, the recommended Phase II dose is 18.5 mg/m^2 as a single agent.

Liposomal platinum formulations

Several liposomal formulations of platinum agents have been tested in clinical trials. Cisplatin has been formulated into liposomes (Lipoplatin™) in an attempt to reduce its systemic toxicity, mainly its nephrotoxicity. The liposomal formulation measures 110 nm and is composed of a lipid shell, a central core of cisplatin, and a PEG polymer coat. Lipoplatin has shown significant concentration in primary tumors and metastases in preclinical models. In clinical studies, Lipoplatin has shown activity mainly in non-small-cell lung cancer (NSCLC), but it has also been tested in pancreatic, breast, and head and neck cancers. A Phase I study determined the single agent MTD to be 300 mg/m^2. In this study, Lipoplatin was also combined with paclitaxel, being its recommended dose for combination 200 mg/m^2 [22].

A Phase III study compared Lipoplatin plus paclitaxel with cisplatin plus paclitaxel, as first-line treatment in NSCLC [23]. Lipoplatin 200 mg/m^2 or cisplatin 75 mg/m^2 were combined with paclitaxel at 135 mg/m^2 on day 1 every 14 days. It is worth noting that the dose intensity of the control arm was higher than that of the standard regimen. There was no significant difference in median survival. The response rate was 59% in the Lipoplatin arm compared with 42% in the cisplatin arm, and the difference was statistically significant ($p = 0.036$). Patients in the Lipoplatin arm showed a statistically significant lower incidence of nausea, vomiting, asthenia and nephrotoxicity. However, these results seem to be irrelevant for NSCLC due to the lack of a standard control, and where carboplatin has universally replaced cisplatin in the combination with paclitaxel.

Liposomal DACH platinum or L-NDDP (Aroplatin™ [Agenus Inc.; MA, USA]) is a liposomal formulation of *cis-bis*-neodecanoato-trans-*R*,*R*-1,2-diaminocyclohexane platinum(II), an analogue of oxaliplatin. Myelosuppression was the DLT of this compound in a Phase I trial, with a MTD of 312.5 mg/m^2. A Phase II trial in patients with advanced colorectal cancer refractory to 5-fluorouracil/leucovorin or capecitabine and irinotecan evaluated L-NDDP given intravenously every 28 days [24]. The protocol stipulated a starting dose of 300 mg/m^2 with intrapatient dose escalation allowed, provided drug-related toxicity was no worse than grade 1. Grade 3–4 toxicities included infusion-related reactions (20%), vomiting (15%), fatigue (15%), anemia (10%) and alanine aminotransferase/aspartate aminotransferase elevation (5/15%). Peripheral neuropathy (grade 1/2) was observed in 15% of patients. Only one confirmed partial response was noted in 18 patients (6%). Since oxaliplatin is not active as a single agent in colorectal cancer, the lack of data of L-NDDP in combination with 5-fluorouracil/leucovorin seems odd. There are no active studies of L-NDDP

at present. A liposomal formulation of oxaliplatin (MBP-426) started Phase I evaluation, but its clinical development was discontinued.

Liposomal OSI-7904

OSI-7904, a noncompetitive inhibitor of thymidylate synthase, was encapsulated within a liposome dispersion consisting of small, unilamellar vesicles (ranging in diameter from 20 to 80 nm) of hydrogenated soy phosphatidylcholine and cholesterol. Liposomal OSI-7904 showed preliminary activity in combination with cisplatin, but its clinical development was discontinued after reports of low response rate as a single agent [25–27].

CO-101

CO-101 is a lipid-conjugated form of gemcitabine, not a liposomal formulation. It was designed to enter cancer cells regardless of human

Table 6.4. Experimental liposomal formulations in current development.

Compound	Drug class embedded	Phase
Lyso-thermosensitive liposomal doxorubicin (ThermoDox® [Celsion Corp.; NJ, USA])	Cytotoxic	II
Liposome-encapsulated irinotecan hydrochloride PEP02	Cytotoxic	II
BLP25 liposome	Vaccine	II
Pegylated liposomal mitomycin-C lipid-based prodrug (PROMITIL™ [LipoMedix Pharmaceutical Inc.; Ramat HaSharon, Isreal])	Cytotoxic	I
IHL-305 (irinotecan liposome injection)	Cytotoxic	I
Nanoliposomal CPT-11	Cytotoxic	I
Anti-EGF receptor immunoliposomes loaded with doxorubicin	Cytotoxic	I
ONT-10, liposomal MUC1	Vaccine	I
DPX-0907	Vaccine/adjuvants	I
DOTAP:cholesterol–Fus1 liposome complex	*FUS-1* gene	I
Lipovaxin-MM	Vaccine	I
Bifunctional shRNA-STMN1 (pbi-shRNA™STMN1 [Gradalis, Inc.; TX, USA]) bilamellar invaginated vesicle, lipoplex, pbi-shRNA STMN1 LP	Bifunctional shRNA	I
PNT2258	Oligonucleotide	I
C-VISA BikDD: liposome	Plasmid	Starting I
Octreotide-targeted liposomes loaded with CPT-11	Cytotoxic	Preclinical

equilibrative nucleoside transport 1 expression and targeted at patients with pancreatic cancer low-level expression of human equilibrative nucleoside transport 1; thus, expected to be resistant to standard chemotherapy with gemcitabine. The LEAP study, a randomized Phase III comparison of CO-101 versus gemcitabine in metastatic pancreatic cancer, has been recently reported negative [103]. There was no difference in OS between the two arms. Median survival was approximately 6 months with a HR of 0.99. Toxicities were comparable between the two arms.

Aa Immunoliposomes: combination of liposomes with a ligand (monoclonal antibody or antibody fragment).

Perspectives: the road ahead

Table 6.4 lists experimental liposomal formulations in active development. Expanding the list of liposome encapsulated drugs to include other widely used antitumor agents such as topoisomerase I-inhibiting drugs and biologic agents [28,29] has vastly increased the number of liposomal formulations undergoing preclinical and clinical testing.

Other exciting areas of current research in liposomal anticancer therapy include:

- Targeting more than one drug in a single liposome [30,31]
- Selective targeting of liposome encapsulated cytotoxic drug to estrogen (or any other clinically relevant) receptor-positive tumor cells [32]
- Development of immunoliposomes loaded with antitumor cytokines or monoclonal antibodies [3,4]
- Use of liposome-encapsulated radiolabeled compounds or magnetic contrast agents to assess drug delivery to tumor tissue by means of PET- or MRI-monitored therapy, respectively [34,35]
- Insertion of peptide or other targeting molecules to enable improved drug delivery across the blood–brain barrier or to tumor vasculature [36,37]

Finally, it is expected that accelerated progress in nanoparticle research may enable further refinements in antitumor drug delivery.

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Summary

- Liposomal drug carriers enable continuous delivery of drug to the systemic circulation and minimize peak drug concentrations, thus improving both efficacy and safety.
- Liposomal formulations of anticancer drugs are expected to reduce clinical toxicity, improve patient convenience and possibly increase clinical efficacy by optimizing drug levels in tumor tissue.
- Several well-known cytotoxic drugs (e.g., doxorubicin, daunorubicin, vincristine and cytarabine) have been marketed as drug-loaded liposomal formulations, whereas a wide spectrum of drugs and biologicals are currently in clinical trials.
- Many exciting areas of current research in liposomal anticancer therapy include dual or multiple drug-loaded liposomes, immunoliposomes and a wide variety of biologicals as payload.

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