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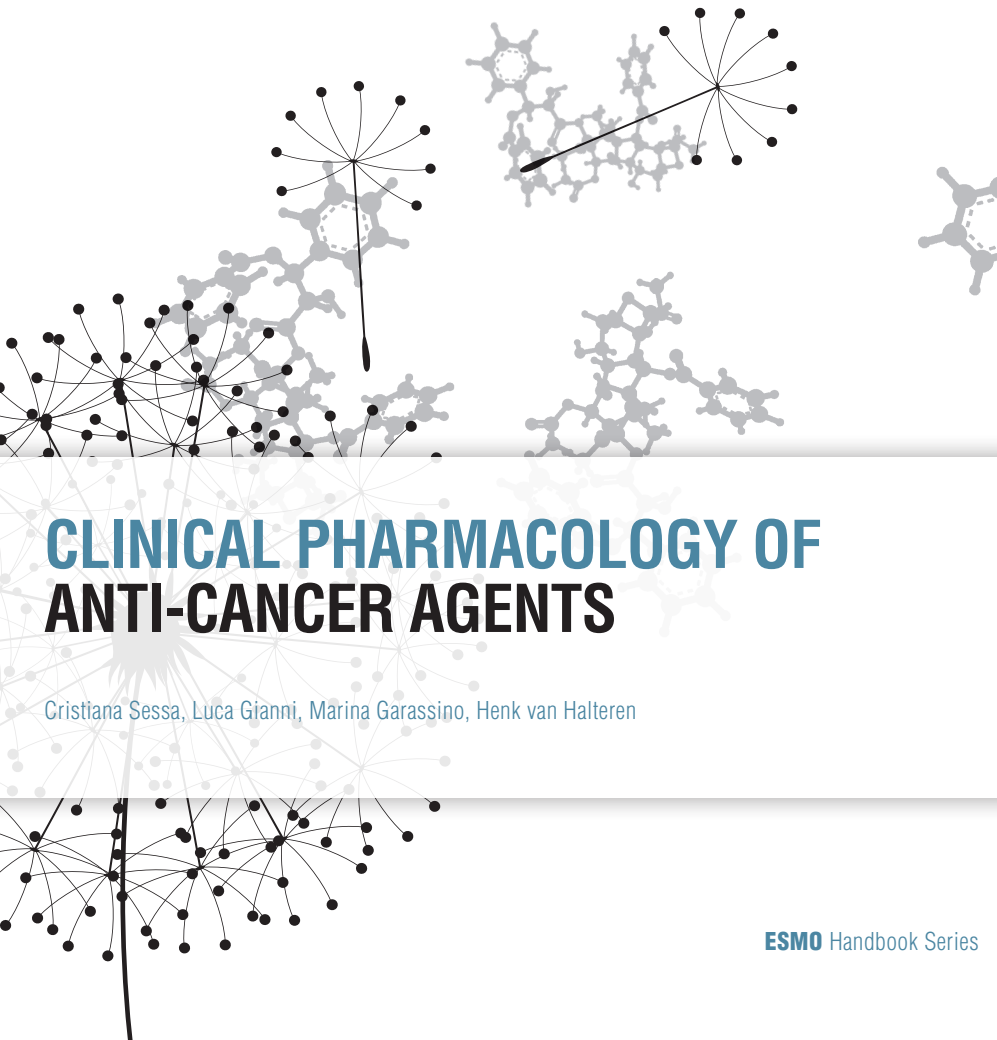

CLINICAL PHARMACOLOGY OF ANTI-CANCER AGENTS

Cristiana Sessa, Luca Gianni, Marina Garassino, Henk van Halteren

ESMO Handbook Series

European Society for Medical Oncology

ESMO HANDBOOK OF **CLINICAL PHARMACOLOGY OF ANTI-CANCER AGENTS**

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ESMO Press

First published in 2012 by ESMO Press

A CIP record for this book is available from the British Library.

ISBN: 978-88-906359-1-5

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6962 Viganello-Lugano
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Printed through s|s|media limited, Rickmansworth, Hertfordshire, UK

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The editors and authors wish to thank the following reviewers.

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Acknowledgments

I would like to thank the ESMO Publishing Working Group for supporting the realization of this book, where each chapter was prepared by a senior and one or more junior oncologists.

I would like to thank Luca Gianni, with whom we shared the idea of the book and the definition of its content, and Josep Tabernero and his team, with whom I discussed the structure of the chapters and who finalized the first one.

Above all, I would like to thank the friends who were authors, reviewers, and tutors and made the book reality.

Professor Cristiana Sessa
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Introduction

Clinical pharmacology is the science of drugs and their clinical use. It should connect the gap between medical practice and laboratory science.

Traditionally, medical oncologists have been skilled and deeply trained in this field, as the drugs they were using had a narrow therapeutic window, making it difficult to gamble between activity and toxicity. In the past, this task was simplified by the fact that the majority of anti-cancer agents were DNA-disrupting agents and had a similar toxicity profile despite different pharmacokinetics. Things became more complicated in the last two decades with the progressive appearance of drugs with completely different mechanisms of action: monoclonal antibodies, antiangiogenic drugs, drugs targeting tyrosine kinases or intracellular pathways, or even other mechanisms involved in cell functioning such as the proteasome. A whole new spectrum of possible side effects, drug interactions, schedules, and combinations appeared.

We therefore considered it timely to produce a book in which all of the important information is included, in a synthetic manner, to allow clinicians to make quick decisions on how to administer drugs to their cancer patients.

This book is small and informative, edited in such a way that it can be carried around and is easily available, and it contains the essential information: only anti-cancer agents are covered (supportive drugs such as antiemetics, growth factors, antibiotics, and so on have been excluded), divided into chapters according to their mechanism of

action. Each chapter has a short introduction which is followed by the most important section: the tables summarizing the pharmacological characteristics of each drug.

This handbook opens a new season in the strategy of the ESMO Publishing Working Group. We intend in the next years to edit a total of nine books (which will be called "Essentials for Clinicians") covering the management of the most common tumors divided by organ of origin, while the general oncology topics will continue to be covered in the annual handbooks. These will be prepared in such a way that the two series will be complementary and will cover all the important domains of oncology for which practicing physicians and juniors preparing their exams will need to have a knowledge.

In the name of the ESMO Publishing Working Group, I want to thank all the authors, the editors, and particularly Cristiana Sessa, who has spent a lot of time and energy making this book a reality and a tool which is at once concise, useful, and precise.

Professor Michele Ghielmini
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Chairman of the ESMO Publishing Working Group

Abbreviations

Drugs

5-FU	5-fluorouracil
5-FUTP	5-fluorouridine triphosphate
5-HT	5-hydroxytryptamine
ACE	angiotensin converting enzyme
AI	aromatase inhibitor
Ara-C	cytarabine
Ara-CDP	Ara-C diphosphate
Ara-CMP	Ara-C monophosphate
Ara-CTP	Ara-C triphosphate
ASA404	vadimezan
ATO	arsenic trioxide
ATRA	tretinoin (all-trans-retinoic acid)
BLM	bleomycin
CA4P	combretastatin A4 phosphate
Cd-AMP	cladribine monophosphate
Cd-ATP	cladribine triphosphate
CPA	cyclophosphamide
CPT	camptothecin
dCK	deoxycytidine kinase
DHAD	mitoxantrone
DNMT	DNA methyltransferase
DNMTi	DNA methyltransferase inhibitor
DNR	daunorubicin
DNR-OL	daunorubicinol
DOX	doxorubicin
d-TMP	deoxythymidine monophosphate
DXM	dexamethasone
E1	estrone
E2	estradiol
EPI	epirubicin
EPI-OL	epirubicinol
EPO	erythropoietin
FAA	flavone acetic acid
FH_2	dihydrofolic acid
FH_4	tetrahydrofolic acid
GM-CSF	granulocyte-macrophage colony-stimulating factor
GnRH	gonadotropin-releasing hormone
HDACi	HDAC inhibitor
HU	hydroxyurea
IDA	idarubicin
IDOL	idarubicinol
IFN	interferon
IGF	insulin growth factor
IL	interleukin
IMiD	immunomodulatory drug
LV	leucovorin
m-AMSA	amsacrine
MMAE	monomethylauristatin E
MTX	methotrexate
NSAID	nonsteroidal anti-inflammatory drug
PDGF	platelet-derived growth factor
PDN	prednisolone

PLD	pegylated liposomal doxorubicin
TGF	transforming growth factor
TNF	tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
VCR	vincristine
VEGF	vascular endothelial growth factor
VM26	teniposide
VP16	etoposide
XL184	cabozantinib

Other

2/day	two per day
ACTH	adrenocorticotropic hormone
ADCC	antibody-dependent cellular cytotoxicity
AFP	alpha-fetoprotein
ALC	absolute lymphocyte count
ALCL	anaplastic large-cell lymphoma
ALDH	aldehyde dehydrogenases
ALK	anaplastic lymphoma kinase
ALL	acute lymphoblastic leukemia
ALT	alanine transaminase
AML	acute myelogenous leukemia
ANC	absolute neutrophil count
AP	acute phase
APL	acute promyelocytic leukemia
AR	androgen receptor
AST	aspartate aminotransferase
ATE	arterial thrombotic event
AUC	area under the curve
BAL	bronchoalveolar lavage
BC	breast cancer
BCRP	breast cancer resistance protein
BM	bone marrow
BP	blood pressure
BUN	blood urea nitrogen
C	concentration
CDC	complement-dependent cytotoxicity
CEA	carcinoembryonic antigen
CEC	circulating endothelial cell
CHF	cardiac heart failure
Cl	clearance
Cl_{TB}	total body clearance
CLL	chronic lymphocytic leukemia
C_{max}	peak concentration
CML	chronic myelogenous leukemia
CMML	chronic myelomonocytic leukemia
CMV	cytomegalovirus
CNS	central nervous system
COMT	catechol-O-methyltransferase
CP	chronic phase
CPK	creatine phosphokinase
Cr	creatinine
CRC	colorectal cancer
CSCC	cutaneous squamous cell carcinoma
CSF	cerebrospinal fluid
C_{ss}	average steady state concentration
CTCAE	Common Terminology Criteria for Adverse events
CTCL	cutaneous T-cell lymphoma
DC	dendritic cell

DCE-MRI	dynamic contrast-enhanced MRI
DHFR	dihydrofolate reductase
DIC	disseminated intravascular coagulation
DL	dose limiting
DLBCL	diffuse large B-cell lymphoma
DL_{CO}	diffusing capacity for carbon monoxide
DLT	dose-limiting toxicity
DPD	dihydropyrimidine dehydrogenase
DSB	double-strand break
DVT	deep vein thrombosis
EC	endothelial cell
EGFR	epidermal growth factor receptor
EIA	enzymo-immunoassay
EMA	European Medicines Agency
EOD-CS	early-onset diarrhea-cholinergic syndrome
ER	estrogen receptor
ERCC	excision repair cross complement
F	bioavailability
FDA	Food and Drug Administration
FGF	fibroblastic growth factor
FISH	fluorescence in-situ hybridization
FL	follicular lymphoma
FSH	follicle stimulating hormone
GFR	glomerular filtration rate
GI	gastrointestinal
GI_{50}	growth inhibition of 50% of cells
GIST	gastrointestinal stromal tumor
GPCRs	G protein-coupled receptors
GRFT	glycinamide ribonucleotide formyl transferase
Gy	Gray
HAT	histone acetyltransferase
HCG	human chorionic gonadotropin
HD	high dose
HDAC	histone deacetylase
HFS	hand–foot syndrome
HGF	hepatocyte growth factor
HIF	hypoxia-dependent transcription factor
HL	Hodgkin lymphoma
HNSCC	head and neck squamous cell carcinoma
hr	hour
HSR	hypersensitivity reaction
HUS	hemolytic–uremic syndrome
IC_{50}	concentration at which growth/activity is inhibited by 50%
IDMS	isotope dilution mass spectroscopy
IFNAR	IFN-alpha receptor
IGF-1R	IGF-1 receptor
IGFBP	IGF-binding protein
IHC	immunohistochemistry
IM	intramuscular
IND	Investigational New Drug
inf	infusion
INR	international normalized ratio
InsR	insulin receptor
IP	intraperitoneal

IPSS	International Prognostic Scoring System
IRS	inflammatory response syndrome
ISG	interferon stimulating genes
IV	intravenous
JAK	Janus kinase
KS	Kaposi's sarcoma
LC_{50}	cytotoxic killing of 50% of cells
LD_{10}	lethal dose to 10% of animals
LFT	liver function test
LH	luteinizing hormone
LLN	lower limit of normal
LOD	late-onset diarrhea
LVEF	left ventricular ejection fraction
mAbs	monoclonal antibodies
MAOI	monoamine oxidase inhibitors
MAP	mitogen-activated protein
MCL	mantle cell lymphoma
mCRC	metastatic colorectal cancer
MDP	myeloproliferative disease
MDR	multidrug resistance
MDS	myelodysplastic syndrome
Mg	magnesium
MHC	major histocompatibility complex
MIU	million international units
MM	multiple myeloma
mRCC	metastatic renal cell cancer
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
mTOR	mammalian target of rapamycin
MW	molecular weight
NCI	National Cancer Institute
ND	not done
NER	nucleotide excision repair
NHL	non-Hodgkin lymphoma
NK cell	natural killer cell
NO	nitric oxide
NOS	nitric oxide synthase
NSCLC	non-small cell lung cancer
NV	normal value
OS	overall survival
P	potassium
PARP	poly ADP-ribose polymerase
PCR	polymerase chain reaction
PDGFR	platelet-derived growth factor receptor
PEG	pegylated
PET	positron emission tomography
PFS	progression-free survival
PgP	P-glycoprotein
PI3K	phosphoinositide 3-kinase
PIF	interstitial fluid pressure
PIP2	phosphatidylinositol-4,5-bisphosphate
PK	pharmacokinetics
PKB	protein kinase
PLT	platelet
PML	promyelocytic leukemia
pNET	pancreatic neuroendocrine tumor
PO	per os
PPE	palmar–plantar erythrodysesthesia
pt	patient
PT	prothrombin time
PTEN	phosphatase and tensin homolog
q	every

RA-APL	retinoic acid–antiphospholipid syndrome
RARs	retinoic acid receptors
RBC	red blood cell
RCC	renal cell carcinoma
RIA	radioimmunoassay
RNR	ribonucleotide reductase
RPLS	reversible posterior leukoencephalopathy syndrome
RR	response rate
RT	radiotherapy
RTK	receptor tyrosine kinase
RXR	retinoid X receptor
SCLC	small cell lung cancer
SERMs	selective estrogen receptor modulators
SIADH	syndrome of inappropriate antidiuretic hormone
SIRS	systemic inflammatory response syndrome
SNPs	single nucleotide polymorphisms
SSB	single-strand break
$T_{1/2}$	half-life
TDL	lowest dose that results in no toxicity
TE	thromboembolism
TEE	thromboembolic event
TGI	total growth inhibition
TKI	tyrosine kinase inhibitor
T_{max}	time to peak concentration
TOP1ccs	topo-I cleavable complex
Topo	topoisomerase
TS	thymidylate synthase
TSC	tuberous sclerosis
ULN	upper limit of normal
UPGT	uridine diphosphate glucuronosyltransferase
UPS	ubiquitin proteasome pathway
US	ultrasound
Vd	volume of distribution
VEGFR	vascular endothelial growth factor receptor
V_{max}	maximum disappearance rate
VOD	veno-occlusive disease
VTA	vascular-targeting agent
VTE	venous thromboembolic event
WBC	white blood cell
wk	week

Preclinical Drug Development: Translating Basic Research into Clinical Work

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Introduction

Recent advances in biomedical research and the expansion of preclinical laboratory investigation have resulted in improved knowledge of the biology and genesis of cancer, and have shed relevant insights on the complex relationship between tumor cells and their microenvironment. Several signaling pathways deregulated in cancer cells have been discovered and components of these pathways that are critical for tumor genesis and progression represent targets for the development of new agents. The identification of a large number of possible targets has resulted in an even larger number of new therapeutic agents that have entered clinical evaluation, giving hope that better treatment strategies could be developed to overcome resistance of cancer cells to standard treatments and to improve patient outcomes. Unfortunately, despite the efforts of the pharmaceutical industry and academic institutions, only few new agents have shown a significant impact on survival and most patients with advanced disease remain incurable.

There are several reasons that could explain why most of the new anticancer agents have failed to improve treatment results. Cancer is a complex disease and several genetic changes accumulate that affect components of

the same or different pathways, and render it difficult to identify a specific target that would be uniquely susceptible to pharmacological inhibition. On the other hand, many agents that have shown significant antitumor activity in preclinical studies have failed to reproduce these results when tested in the clinic. Preclinical studies are important because they represent the first step toward the development of a new therapeutic compound, providing important information on the mechanism of action, antitumor activity, pharmacology, and toxicology that can guide its subsequent clinical development. The selection of appropriate preclinical experimental models, most reflective of the complexities of different cancers, may help to identify the most promising therapeutic agents that would sustain the rigor of clinical evaluation. Moreover, it has now become evident that elucidation of "driver" molecular changes can identify patients most likely to benefit from specific targeted therapy. It is therefore expected that the development of new anti-cancer agents should proceed together with the identification of predictive biomarkers of response, which should be evaluated early in the discovery phase.

In-vitro Evaluation of New Anti-cancer Agents

The development of a new anti-cancer agent begins with the evaluation of its antitumor activity against a panel of malignant cell lines. These tests help to identify compounds that deserve further evaluation in animal models. Several methods exist to detect the antitumor effect of a new agent against tumor cell lines, such as antiproliferative assays using incorporation of radioactive nucleotides like [^{3}H]thymidine, direct cell counting, or colony formation. Others assess viability or growth using colorimetric assessment.

A screening model that has been very useful for the evaluation of hundreds of compounds is represented by the US National Cancer Institute screening model (NCI-60). It is composed of 60 different cell lines derived from the major human tumors and provides a source for rapid evaluation of the in-vitro antitumor activity of new compounds. Every new agent is tested against each of the cell lines in order to evaluate its ability to inhibit growth or cause cell death. Based on parameters such as the concentrations of the drug that cause: growth inhibition of 50% of the cells (GI_{50}), or total growth inhibition (TGI), or cytotoxic killing of 50% of the cells (LC_{50}), a specific

fingerprint is produced for each compound that can be compared with the activity of others with the same or different mechanisms of action.

Although the empirical screening of natural products has discovered anti-cancer drugs such as paclitaxel and trabectedin, the current strategy of drug discovery favors approaches that are rationally and biologically driven to develop agents that inhibit specific molecular targets involved in tumor formation and progression. This can be achieved through high-throughput screening of small-molecule libraries or through a more sophisticated structure-guided discovery approach that leads to the identification of compounds that interfere with specific molecular targets. Lead compounds are subjected to specificity evaluations to test their ability to engage and interact with their putative molecular targets. Studies in vitro are therefore designed not only to show that a new agent has inhibitory or cytotoxic activity against cell lines, but also to demonstrate that it is able to produce target inhibition to support its underlying mechanism of action.

The assessment of target inhibition of a new agent in vitro (in cell-based and non-cell-based assays) is generally based on the concentration of the drug necessary to inhibit the activity of its target. For many new agents that target specific enzymes, this is assessed by measurement of the concentration of the drug needed to produce 50% of enzymatic inhibition (IC_{50}). Drugs that cause inhibition at low doses in vitro (i.e. IC_{50} at low nanomolar range) are preferred, as they more likely result in a favorable therapeutic index in clinical studies. The IC_{50} against other enzymes in the same family must also be determined in order to define the specificity of the agent against its target. If a drug inhibits several enzymes at low IC_{50}, it may act by modulating different targets. This finding must be taken into consideration when studying the mechanism of antitumor activity of a new drug, and it may also raise the possibility of undesirable side effects due to multiple target inhibition.

While in-vitro studies using tumor cell lines represent an initial step in the evaluation of antitumor activity and the elucidation of the mechanism of action of a new anti-cancer agent, they have limitations in predicting positive effects in animal models and, more importantly, in patients with cancer. In fact, cell lines present important biological differences from the tumors they derive from and they do not reflect the intricacy of human cancers and the complex interplay between cancer cells and their microenvironment.

It has now become clear that important mechanisms of resistance to treatment depend on the relationships between cancer cells and the surrounding stromal cells and these conditions are not easy to reproduce in preclinical studies. The use of ex-vivo models (i.e. using cells or tissues taken directly from patients and tested in an external environment with minimal artificial alterations), co-culture of tumor cells together with stroma cells, and the development of in-vitro models with tumor spheroids or multilayered cells represent some of the possibilities to better reproduce in vitro the complexity of human tumors and the relationships with their microenvironment.

Recently, the discovery that specific genetic changes are responsible for the development of particular tumor types has permitted the antitumor activity of some new agents to be evaluated in tumor cell lines expressing these genetic changes. Furthermore, comparison of the behavior of these agents in wild-type cell lines of the same tumor types can be informative. An example is represented by the antitumor activity of PARP inhibitors in the context of BRCA1/2-deficient cell lines in comparison with lack of activity in cell lines with heterozygous or wild-type BRCA1/2. The evidence from such in-vitro studies was the basis for the subsequent clinical development of several PARP inhibitors for tumors bearing homozygous mutations in BRCA1/2 genes. The in-vitro evaluation of a new agent's antitumor activity against tumors with specific genetic changes may therefore provide a strong rationale to support its clinical development in a genetically selected patient population.

Studies in Animal Models

Once studies in cell lines have shown that a new agent has antiproliferative properties and is able to inhibit its target, in-vivo studies in experimental animal models are undertaken to further define the antitumor activity and provide pharmacology and toxicology data needed for the subsequent clinical development. The antitumor effect of a new agent must be evaluated in vivo and, for those agents in which the target is known (or believed to be known), efforts should be made to show that the observed antitumor effect is related to target modulation and to establish if a dose-dependent relationship exists between target inhibition and the observed antitumor effect.

Pharmacodynamic endpoints used to define target inhibition in vivo may vary based on the target and the mechanism of action of the drug (e.g. measurement of substrate phosphorylation for kinase inhibitors, measurement of mRNA and protein levels for small oligonucleotides, etc). More recently, imaging techniques have also been used to detect the effect of some new agents in animal models (e.g. detection of angiogenesis inhibition using dynamic contrast-enhanced magnetic resonance imaging).

In addition to pharmacodynamic measurements, pharmacokinetic studies provide information about drug absorption, metabolism, excretion, and plasma-protein binding. Safety pharmacology and toxicology studies are also performed in animals. The objective is to estimate a safe starting dose for first-in-human phase I studies, assess toxic effects with respect to target organs, and help to select different dosing regimens and dose-escalation schemes for clinical studies.

The choice of starting dose for first-in-human studies is usually based on toxicology evaluation in both rodent and non-rodent (dog or monkey) species and the most sensitive species is chosen for safe starting dose determination. The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) recommend that new anti-cancer agents be evaluated in both rodent and non-rodent species before undergoing human phase I evaluation. One tenth of the lethal dose to 10% of mice (LD_{10}) and one sixth to one third of the lowest dose that results in no toxicity (TDL) in non-rodent species are some of the parameters that have been most frequently used to select a starting dose for many anti-cancer agents. While these methods have been widely used for cytotoxic agents, their ability to predict a safe starting dose for molecularly targeted agents is debated, and thus far it is not clear which animal models could better predict a safe starting dose. In addition, no standard parameter exists and for most new agents a multitude of parameters has been used to determine a safe starting dose. In our recent review of first-in-human studies of molecularly targeted agents, only 3.7% of phase I trials had a starting dose that exceeded the maximum tolerated dose, providing evidence that, with the exception of a very small proportion of new agents, the choice of the starting dose has been generally safe. A better understanding of the target and mechanism of action of a new agent can help to select the animal models that would best predict for toxicities in humans.

The most important question regarding the in-vivo study of a new anti-cancer agent, however, is represented by the likelihood that the antitumor activity observed in animals may translate into a clinically significant efficacy. Substantial controversy exists as to the best animal model that would positively predict for antitumor activity in humans. Generally, there is no single system that is considered the best positive predictor of antitumor activity for human tumors. Xenograft tumors implanted in immunodeficient mice by subcutaneous or orthotopic inoculation of tumors (grown in vitro or obtained from patients' tumor biopsies) have served as a model for the evaluation of a large number of anti-cancer agents. Xenografts have several limitations (e.g. a low tumor establishment rate for many human tumors, low reproducibility of "real" cancer with respect to surrounding tumor environment, and growth rates that do not mimic the ones in human cancer, among others), but they represent a valid model and they have contributed to the identification and development of many new agents. More recently, the possibility to obtain genetically engineered mice that recapitulate a specific cancer genotype has opened new horizons in the preclinical evaluation of new compounds. The discovery that genes with either oncogenic or tumor-suppressor activity may be altered in human cancer, and the possibility to introduce these changes by various techniques into mice, raise the possibility to study the antitumor activity of a new agent against tumors that more closely recapitulate the biology of human cancer.

Companion Diagnostics Development

The high failure rate observed in late clinical trials of many "promising" new anti-cancer agents has motivated efforts to define alternative strategies of drug development and evaluation of antitumor activity of new agents, both in the clinical as well as in the preclinical settings. Up to now, only few predictive biomarkers are used in routine clinical practice and most have been established retrospectively. The emergence of the so-called companion diagnostics would potentially help expedite the drug development process by identifying predictive biomarkers early in the preclinical setting and carrying out analytical and clinical validation during drug development. Companion diagnostics are assays performed starting from the preclinical stage to help elucidate the efficacy and/or safety of a new

drug for a target patient population, based on specific genotype and biological characteristics of the tumor.

Spigel and colleagues reported recently the results of a randomized phase II trial of erlotinib in combination with MetMab (a monoclonal antibody targeting MET) versus erlotinib plus placebo in patients with previously treated non-small cell lung cancer. The study included evaluation of the expression of cMET in tumoral tissue through both fluorescence in-situ hybridization (FISH) and immunohistochemistry (IHC). They showed that the addition of MetMab to erlotinib resulted in significant improvement in progression-free and overall survival only in patients who had high expression levels of cMET and that expression by IHC was the most sensitive predictor of benefit from MetMab. Conversely, patients with low cMET expression did not benefit from MetMab. In fact, those treated with the combination had significantly worse outcomes that those treated with erlotinib alone. This study represents an example of prospective evaluation of a predictive biomarker and underlines the importance of companion diagnostics in the evaluation of experimental treatments.

To support drug development toward a tumor-specific focus after early clinical trials, preclinical studies should be performed to allow the discovery of biomarkers and the development of assays to evaluate them. This will lead to the development of a diagnostic predictive signature to be clinically evaluated in early clinical trials and prospectively validated in randomized phase II and III studies.

Summary

Modern drug development in oncology relies on the identification of molecular changes that drive the malignant transformation and are responsible for the development and progression of cancer. This is now possible through improvements in our knowledge of the biology of cancer. The development of new cancer therapeutics has been, however, slow and inefficient; as such, alternative strategies are needed. Preclinical studies are important by providing information that is necessary for the subsequent clinical development of any new anti-cancer agent. Data generated from relevant cell lines and xenograft models are crucial to

facilitate a better understanding of the agent's target, mechanism of action, antitumor activity against different tumor types, pharmacology, and toxicology, before entering human clinical testing. The use of preclinical models that more closely reflect the biology of human cancer will help to improve the success rate of new anti-cancer compounds. Finally, the discovery of predictive biomarkers of response, their development in preclinical studies, and their subsequent validation in clinical studies will help to define patient populations most likely to benefit from future treatments.

Further Reading

Damia G, D'Incalci M. Contemporary pre-clinical development of anticancer agents - What are the optimal preclinical models? Eur J Cancer 2009; 45:2768–2781.

Garcia VM, Cassier PA, de Bono J. Parallel anticancer drug development and molecular stratification to qualify predictive biomarkers: dealing with obstacles hindering progress. Cancer Discovery 2011; 1:207–212.

Le Tourneau C, Stathis A, Vidal L, et al. Choice of starting dose for molecularly targeted agents evaluated in first-in-human phase I cancer clinical trials. J Clin Oncol 2010; 28:1401–1407.

Ocana A, Pandiella A, Siu LL, et al. Preclinical development of molecular-targeted agents for cancer. Nat Rev Clin Oncol 2010; 8:200–209.

Politi K, Pao W. How genetically engineered mouse tumor models provide insights into human cancers. J Clin Oncol 2011; 29:2273–2281.

Senderowicz AM. Information needed to conduct first-in-human oncology trials in the United States: a view from a former FDA medical reviewer. Clin Cancer Res 2010; 16:1719–1725.

Spigel DR, Ervin TJ, Ramlau R, et al. Final efficacy results from OAM4558g, a randomized phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. J Clin Oncol 2011; 29(Suppl): Abstr 7505.

2 Pharmacokinetics and Pharmacodynamics: Main Concepts and Clinical Applications

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Introduction

Pharmacokinetics is the description of what happens to a drug after it has been administered to the patient. This includes absorption, distribution, metabolism, and excretion. Absorption may be from the gastrointestinal tract if the drug is administered orally, or from an injection site. If, as is the case with many cytotoxic anti-cancer drugs, the drug is given intravenously, then absorption is removed from the equation.

Pharmacodynamics is the description of the pharmaceutical effects that the drug has on the patient. When treating a patient with cancer the objective is usually to reduce the size of the tumor – so tumor response can be regarded as a pharmacodynamic effect. More frequently a surrogate measure is a more useful marker of a pharmacodynamic effect – for example, the effect of an aromatase inhibitor (used for treating hormone-dependent breast cancer) in reducing the plasma estradiol level.

When studying an agent with a broad therapeutic index, such as an agent for reducing blood pressure, it is easy to see the role of pharmacodynamics. The pharmacodynamic response is measured by the reduction in blood pressure, and the dose may be titrated to achieve the desired level of reduction. The pharmacokinetics of the drug may be important in determining the dosing frequency. A drug with a short half-life might have to be administered several times a day, while one with a longer half-life might need only daily or weekly dosage.

When treating cancer we have to deal with drugs that, on the whole, have a poor therapeutic ratio. The dose of the drug that can be expected to have a significant anti-cancer effect will be close to the toxic (and sometimes potentially lethal) dose. In the case of the traditional cytotoxic agents, the dose-limiting toxicity is usually to the bone marrow or other rapidly proliferating tissues. In the case of the targeted agents, the tissue that exhibits the dose-limiting toxicity is determined by the target of the drug. For example the dose-limiting toxicities of the epidermal growth factor receptor (EGFR) inhibitors (erlotinib and gefitinib) are skin rash and gastrointestinal toxicities, since both of these tissues express EGFR, where it has a physiological role in their maintenance. There are only a few cases where the therapeutic ratio of a drug used for treating cancer is high enough that it does not need to be given at a maximum tolerated dose (MTD). Examples of such cases are where the drug targets a mutation of a fusion protein unique to the tumor (for example, imatinib in chronic myeloid leukemia) or where the target is uniquely amplified in the tumor (for example, trastuzumab in *HER2*-amplified breast cancer).

Since we have to use anti-cancer drugs at doses which are very close to the MTD, the variability of individual patients' responses to the toxicities of the drugs becomes of great importance. We are all familiar with the variable response to alcohol. Some individuals display the symptoms of intoxication (which is the pharmacodynamic effect of alcohol) having only consumed a small amount, while others have a much higher tolerance. Similarly, with anti-cancer drugs, it is not uncommon for them to have a four-fold or greater range in the dose which will elicit a toxic effect in an individual patient. The necessity to treat each patient at nearly the MTD to achieve any therapeutic benefit means that some form of dose individualization may be required. The contribution that the study of pharmacokinetics and pharmacodynamics makes to cancer treatment is chiefly to enable such individualization, permitting optimum dosage to the more tolerant patients, while avoiding overly toxic doses to those who are more sensitive. In the research setting of early drug development, pharmacokinetic studies are essential to define dosage intervals and to ensure that therapeutic drug levels are achieved. Pharmacodynamic studies are also essential to establish in-vivo proof of principle that the proposed target of the drug is

being modulated in the desired manner.

In this chapter we shall discuss some examples of how pharmacokinetics and pharmacodynamics affect clinical practice in cancer treatment.

Pharmacokinetics

Basic Principles

Absorption

The study of pharmacokinetics involves documenting absorption, metabolism, distribution, and excretion. Drug absorption from the gastrointestinal tract may be complete or incomplete. If incomplete, only a proportion of the dose administered may find its way into the circulation. The fraction of the dose of a drug that is absorbed is called the bioavailability and this can be assessed by measuring the plasma pharmacokinetics of the drug after oral and intravenous administration. If a drug is given intravenously we know that the whole of the administered dose is in the circulation. A graph of the concentration of the drug in the plasma over time can be plotted, continuing the measurements until the plasma level falls to virtually zero. The area under the curve of this graph (AUC) can then be measured. For many drugs the AUC is the best measure of the overall effect, both toxic and therapeutic. It is also known as "systemic exposure" and "concentration × time" (C × T). Once the AUC obtained by giving a particular dose intravenously is known, the AUC obtained by giving the same dose orally can be measured. The bioavailability is then defined as

$$\textit{Oral bioavailability} = \frac{AUC_{oral}}{AUC_{intravenous}}$$

The oral bioavailability of anti-cancer drugs varies from almost 100% in the case of temozolomide to as low as 20–30% for many other commonly used drugs. A low oral bioavailability may be due to poor absorption through the gastrointestinal mucosa, or due to first-pass metabolism in the liver. The chief problem of a low oral bioavailability is that it is frequently variable between patients, adding to the difficulty in obtaining a consistent exposure in each individual patient.

Distribution

Once a drug is in the plasma, it will be distributed into other body compartments, in particular the extracellular fluid and the intracellular space. Many drugs have an intracellular target (for example, a platinum drug needs to form adducts on DNA) and so intracellular distribution will be essential to their action. Others may act in the extracellular fluid (for example, trastuzumab, which binds to the cell membrane HER2 receptor). In addition to affecting the ability of a drug to reach its target, the nature of the distribution of a drug also affects its half-life (see below) and therefore the duration of action in the body. In particular, drugs that are extremely lipid soluble will be retained in fat depots and may therefore have a long half-life.

Metabolism

Many drugs are metabolized, most commonly in the liver. A large group of enzymes known as the cytochrome P450 superfamily (officially abbreviated as CYP) perform mono-oxygenase reactions of the form:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

where R represents the drug molecule. This introduction of hydroxyl groups usually serves to detoxify the drug and promote its excretion.

Drugs may also be conjugated to hydrophilic groups such as sulfate or glucuronide, and in some cases they are metabolized by enzymes that have a physiological role in normal metabolism. Drugs that are extensively metabolized by the P450 system may be subject to interactions with other drugs that are degraded by the same system. Drug metabolism may also be a source of variability in drug handling between individuals, since several of the drug-metabolizing enzymes exhibit single nucleotide polymorphisms (SNPs) that affect the rate at which the drug is degraded. SNPs are normal variations in a particular gene that can differ between individuals.

Several drugs, in particular some of the antimetabolites, are metabolized by the enzymes normally acting on the normal metabolites that

they mimic. For example, 5-FU is converted to the active form (5-fluorodeoxyuridine monophosphate) by the enzymes that normally act on uracil nucleosides and nucleotides. 5-Fluorouracil is also, importantly, degraded and inactivated initially by dihydrothymine dehydrogenase (see below).

Most drug metabolism reactions take place in the liver. However, metabolism in other tissues may also be important, particularly in the lungs. In addition, intratumoral activation or inactivation of drugs may also play a role.

The critical importance of understanding drug pharmacokinetics and metabolism is illustrated dramatically in the non-cancer arena by the example of the antihistamine, terfenadine. After its introduction into clinical practice, it was noted that cardiac deaths occurred occasionally, and were associated with patients eating grapefruit after taking terfenadine. Examination of the pharmacokinetics revealed that terfenadine itself was cardiotoxic but was normally converted rapidly to fexofenadine (an active antihistamine) by cytochrome P450, CYP3A4. Grapefruit juice contains a natural compound, furancoumarin, that inhibits CYP3A4, thus increasing the exposure to terfenadine (Figure 1).

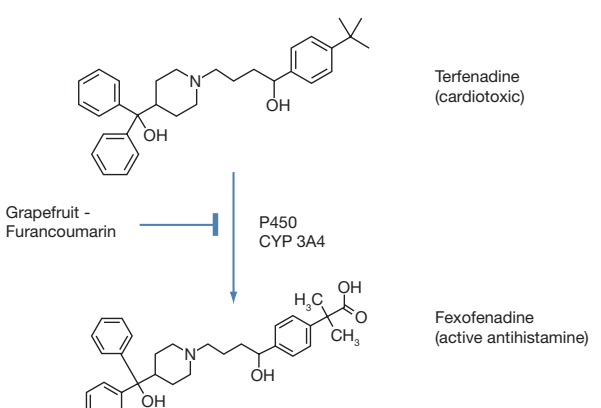

Figure 1 *Interaction of terfenadine with grapefruit juice.*

Excretion

The major routes for the excretion of drugs are renal and biliary. Many anti-cancer drugs, such as carboplatin and methotrexate, are excreted primarily in the urine, while others, such as doxorubicin, are excreted mainly in the bile. Excretion in the urine can be either by glomerular filtration, tubular excretion, or a combination of the two. Excretion in the bile is facilitated by the expression of the MDR1 gene, producing P-glycoprotein in the canaliculi. P-Glycoprotein is one of a superfamily of cell membrane transporters that actively export a wide range of xenobiotics, including doxorubicin and taxanes, from the cell. It can also be responsible for drug resistance due to expression in the tumor. Drugs that block P-glycoprotein can also therefore greatly affect the pharmacokinetics of these agents.

Factors that affect pharmacokinetics

The molecular structure of a drug is the biggest influence on its pharmacokinetics. Drugs that have sites susceptible to hydroxylation by the P450 system will tend to be metabolized rapidly and have a short half-life. Drugs that are highly lipophilic tend to be sequestered in fat depots and have a long half-life. They may also be distributed into the brain, since they can cross the blood–brain barrier. Drugs with a molecular weight of less than about 500 tend to be excreted mainly in the urine, while those with higher molecular weights are excreted in the bile. Drugs that are absorbed from the gastrointestinal tract and then excreted in the bile can be reabsorbed, a phenomenon known as enterohepatic recycling. Many drugs are also bound to plasma proteins, in particular albumin. Drugs that bind tightly to albumin will tend to have a longer half-life, but only the free fraction of the drug (which may be as low as 1% of the total) will be available to effect its action in the tissues. Certain drugs also bind tightly to α-1-acid glycoprotein, and this can greatly diminish their effective systemic bioavailability. The substrate affinity of a drug for P-glycoprotein is also a major factor, since such drugs will be excreted in the bile and will tend not to cross into the brain, because the blood–brain barrier expresses the P-glycoprotein efflux pump.

Patient factors also affect the pharmacokinetics of drugs, in particular renal function and the presence of polymorphisms in drug-metabolizing enzymes.

Pharmacokinetic modeling

Pharmacokinetic modeling is useful both as a descriptive tool of the pharmacokinetics of a drug and also as a method for predicting drug exposure in an individual. Compartmental models attempt to mimic the body compartments and calculate the predicted drug levels from estimates of the rate constants for the transfer between these compartments and drug clearance. Figure 2 illustrates a single-compartment model showing the three major routes of elimination (renal, hepatic, and chemical degradation). The solution of such a model yields a single exponential function of the form:

$$C = Ae^{-\alpha t}$$

where C is the blood concentration, A and α are constants, and t is time, and will allow the derivation of a single half-life (the time for the blood level to halve) for the drug.

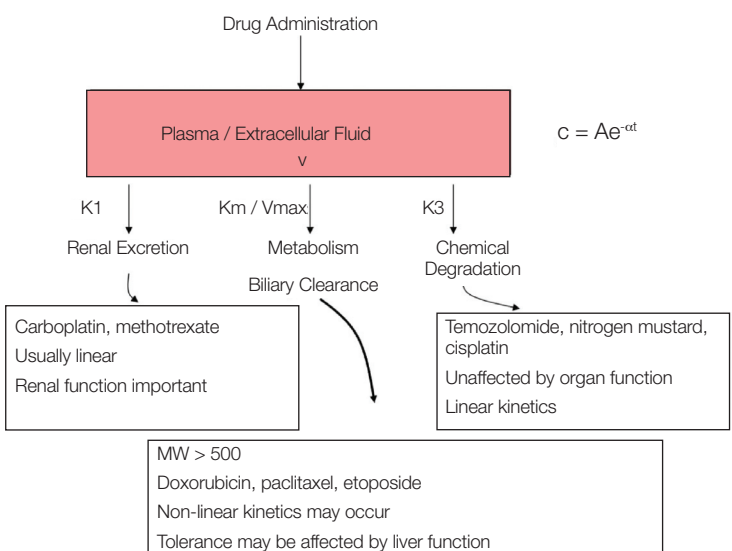

Figure 2 *A single-compartment pharmacokinetic model.*

Note that the elimination rate constants, K1 and K3, are linear: that means that a doubling of the drug level will result in the rate of transfer of the drug also doubling. Drug clearance by glomerular filtration is typically linear. The rate of flux through many cell membrane transporters and enzymes also approximates to linearity in many cases, because the drug levels encountered are much lower than that which would be necessary to saturate the process. When saturation of an enzyme or a carrier occurs then Michaelis–Menten kinetics will operate and the rate of elimination of the drug cannot exceed a maximum level (V_{max}) for the process. This leads to non-linear kinetics, which is important because an increase in the drug level can lead to a greater than expected increase in the AUC, or systemic exposure, of the drug. In practice, most anti-cancer drugs exhibit linear kinetics, although those of paclitaxel display non-linearity at higher doses and shorter exposures.

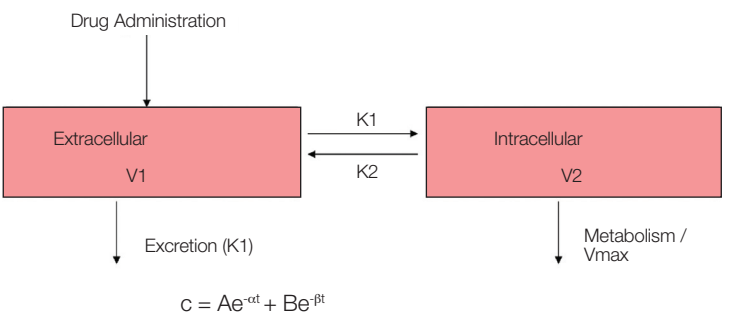

Figure 3 *A two-compartment model.*

Figure 3 shows a two-compartment model where the first compartment represents the plasma plus the extracellular fluid and the second compartment is the intracellular fluid. The solution to such a model will be in the form:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

In this case the model reveals two half-lives for the drug. The first is the distribution half-life, representing the speed with which the drug equili-

brates between the two compartments, and the second is the elimination phase: the major determinant of the time for which the patient is exposed to the drug.

It is, of course, possible to create models with any number of compartments, possibly representing individual organs such as the liver and kidney. These models become increasingly complex, and for the most part one- or two- (and occasionally three-) compartment models are sufficient.

A further important form of pharmacokinetic modeling is population modeling. This is useful if a large number of patients are to be treated and it is necessary to estimate the average exposure that the patients will receive.

Examples of the Application of Pharmacokinetics in Cancer Treatment

Methotrexate – showing a requirement for drug level monitoring

Methotrexate is one of the longest-established anti-cancer drugs, and acts by interfering in folic acid metabolism. It is therefore described as an antifolate, in the class of antimetabolites. The relevant pathways of folate metabolism and the site of action of methotrexate are illustrated in Figure 4. Folic acid is represented by F, and is present in the cell in its reduced form, tetrahydrofolate (FH_4). This acts as a one-carbon carrier and is responsible for inserting carbon atoms into purines (adenine and guanine) and thymidine. Uniquely, the formation of thymidine results in the oxidation of the folate molecule to dihydrofolate (FH_2), which is reduced back to tetrahydrofolate by the enzyme, dihydrofolate reductase (DHFR). Methotrexate blocks DHFR and results in the sequestration of the intracellular folates in the inactive dihydrofolate form, leading to diminution of the tetrahydrofolate pools and the cessation of thymidine and purine synthesis, thus precluding DNA synthesis and cellular proliferation.

Methotrexate is one of the few drugs for which there is a specific antidote. The provision of a source of tetrahydrofolate (folinic acid) permits the continuation of folate metabolism despite the block imposed by methotrexate, and thus reverses all of its toxic effects. This phenomenon has been utilized clinically in the treatment of osteosarcoma and childhood leukemia. Extremely high doses of methotrexate are given, allowing the

drug to penetrate the tumor (osteosarcoma) or to cross the blood–brain barrier (leukemia). These doses would be lethal without the use of folinic acid, which is given a number of hours after the methotrexate. When high-dose methotrexate regimens with folinic acid rescue were first introduced, occasional patients experienced unexpected and severe (sometimes lethal) toxicity despite the administration of folinic acid. This was due to variability in the rate of clearance of methotrexate between patients, with a few patients still having high circulating levels of methotrexate when the folinic acid rescue was stopped. It is now standard practice to monitor the levels of methotrexate and prolong the duration of folinic acid rescue until the plasma level of methotrexate has fallen below the threshold for toxicity.

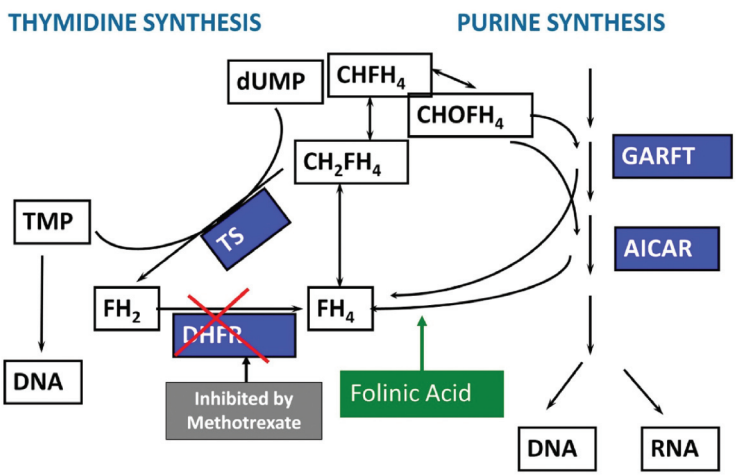

Figure 4 *Pathways of folate metabolism and the action of methotrexate.*

Carboplatin – compensating for pharmacokinetic variability

The discovery of cisplatin was a breakthrough in cancer therapy, but the severe toxicities (nausea, vomiting, renal, neuropathy) rapidly led to a search for less toxic analogs. The first successful one was carboplatin, which had much reduced non-hematological toxicity with dose-limiting thrombocytopenia. Although carboplatin had activity in clinical trials similar to that of cisplatin, its adoption in clinical practice was compromised by extreme

interpatient variability in the level of thrombocytopenia. Doses as high as 550 mg/m^2 were tolerated without toxicity by some patients, while others suffered life-threatening thrombocytopenia at doses of 200 mg/m^2. Carboplatin possesses two properties which made it ideal for some kind of pharmacokinetically based dosing.

Firstly, it is reasonable to expect its toxicity and therapeutic effects to be related to the AUC. Platinum-based drugs are chemically unstable and act by reacting with DNA to form DNA–platinum adducts by losing their "leaving groups", which are the chlorine atoms in the case of cisplatin, or the cyclobutane dicarboxylate moiety in the case of carboplatin. Much of the reduced toxicity of carboplatin may be due to the greater stability of its leaving groups. It is known that the level of cytotoxicity induced by platinum drugs is proportional to the number of DNA–platinum adducts formed and, since these are formed by a straightforward chemical reaction, it follows that their formation will follow the law of mass action – that is, that the number of adducts formed will be determined by concentration × time or AUC. It follows that if the AUC can be predicted in a patient then the level of toxicity can also be predicted.

Secondly, carboplatin has unusually simple and predictable pharmacokinetics, making it practical to predict the AUC that will be experienced by an individual patient. The majority (approximately 80%) of an administered dose of carboplatin is excreted in the urine by glomerular filtration with very little tubular secretion or reabsorption. The remainder binds irreversibly to plasma and tissue proteins, and is effectively inert. Thus, by measuring the glomerular filtration rate (GFR) and adding a small constant to estimate the non-renal clearance due to tissue binding, it is possible to predict the carboplatin clearance. This concept is illustrated with hypothetical data in Figure 5. Once the total clearance is known, a simple one-compartmental model (similar to that shown in Figure 2) can be established and used to calculate the dose of carboplatin that will be required to achieve a predefined AUC in each patient. The formula derived to do this is:

$$DOSE\ (mg) = AUC \times (GFR+25)$$

where the dose is in milligrams, the AUC is the required AUC in mg/ml·min, GFR is the measured GFR in ml/min and 25 is the constant representing the non-renal clearance. Note that this formula

does not utilize any measurement of body size, because this variable is eliminated in the solution of the model. Theoretically the non-renal clearance would vary with body size, but in practice this makes very little difference to the calculated dose except in the case of pediatric patients, where alternative formulae have been derived. This formula has proved to be a reasonably reliable way of dosing carboplatin, providing that the estimate of GFR is accurate. It has been in use for over 20 years and has become eponymously known as the "Calvert formula". This was not the intention of the author, because it was the result of the work of a cohesive team of people rather than one individual (see Further Reading below). Other formulae have been derived which can also be used for calculating the dose of carboplatin, notably by Merrill Egorin and Etienne Chatelut.

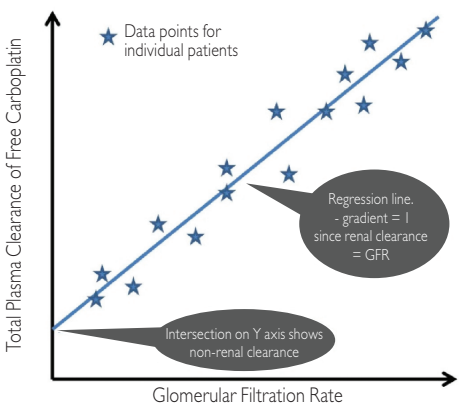

Figure 5 *The relationship between total plasma clearance of carboplatin and glomerular filtration rate.*

Paclitaxel – non-linear pharmacokinetics

When paclitaxel was introduced in the early 1990s, a high level of anti-cancer activity was seen, especially in ovarian and breast cancer. The drug was initially given as a 24 hr infusion because it was felt that this would reduce the frequency of dangerous acute hypersensitivity reactions. However, it became clear that premedication with steroids and

antihistamines largely solved the problem of hypersensitivity, regardless of the length of infusion. Paclitaxel binds to and stabilizes tubulin, thus preventing the formation of mitotic spindles necessary for cell division. It is thus a phase-active drug and might be expected to have some time-dependency in its action. A comparison of a 24 hr infusion with a 3 hr infusion showed that the incidence of myelosuppression was distinctly lower with the shorter infusion. Pharmacokinetic studies showed that the duration of time for which the plasma paclitaxel level was above a threshold level of 0.05 μmol/L was correlated with suppression of the white cell count, while other pharmacokinetic parameters were not. Subsequent studies also showed a correlation between the time above a threshold and clinical response. Paclitaxel is therefore a drug where the AUC (or systemic exposure) is a comparatively unimportant determinant of its therapeutic and toxic effects, while the time above a threshold has clinical significance. Contemporary schedules of paclitaxel are usually 1 or 3 hr infusions, with the dose being adjusted to obtain a sufficient duration of exposure. The practicality of short schedules is probably, at least in part, due to the fact that paclitaxel pharmacokinetics are non-linear. This means that an increase in dose leads to a disproportionately high increase in the plasma level, so that the dose required for a 3 hr infusion to achieve the required time above a threshold is not markedly higher than the dose that would be needed in a 24 hr infusion.

Irinotecan – the importance of pharmacogenetics

Irinotecan is a topoisomerase-I inhibitor which is used primarily in colorectal cancer treatment. Its metabolism is complex. Although it is active as a topoisomerase-I inhibitor as the parent drug, it is also metabolized by carboxylesterases to SN-38, which has 100–1000-fold higher activity as an anti-cancer agent. SN-38 is excreted in the bile and undergoes extensive enterohepatic recycling, which is thought to be the cause of diarrhea, one of the principal side effects of irinotecan. SN-38 is metabolized by glucuronidation. This is accomplished by hepatic uridine diphosphate-glucuronosyltransferases, in particular by the isoform, UGT1A1. The glucuronide of SN-38 is significantly less toxic, both to the gastrointestinal tract and to the bone marrow. Certain polymorphisms of UGT1A1 are associated with increased toxicity from irinotecan and dose reduc-

tions are recommended for patients homozygous for the UGT1A1*28 allele. UGT1A1*6 polymorphisms have also been associated with severe myelosuppression and these patients have also been shown to require significant dose reductions.

5-Fluorouracil

5-Fluorouracil is a very widely used drug that acts mainly as an inhibitor of thymidylate synthase. It is metabolically activated to 5-fluorodeoxyuridine monophosphate, which is the active inhibitor, by the enzymes normally responsible for de-novo pyrimidine synthesis. Similarly it is inactivated by the enzymes that normally degrade naturally occurring pyrimidines. The rate-limiting step is the enzyme dihydrothymine dehydrogenase (DPD), which is thought to be responsible for the degradation of about 80% of an administered dose of 5-fluorouracil. The importance of this enzyme is illustrated by the trials done using specific inhibitors of DPD, where the MTD of 5-fluorouracil was reduced by about 100-fold. DPD activity is highly variable (8–21-fold) between different cancer patients and these variations in activity are reflected by different tolerances to treatment with 5-fluorouracil, or to the related drug, capecitabine. In very rare patients who completely lack DPD activity, a normal therapeutic dose of 5-fluorouracil is potentially lethal.

The "third-space" hypothesis – beware the methodology

In the early 1970s, studies of the pharmacokinetics of methotrexate were performed with the hope of accounting for some of the variability in individual patents' tolerance to this drug. Some of the patients studied had effusions (pleural or ascitic). It was noted that they seemed to suffer more side effects and also that there were high levels of methotrexate which persisted for a long time in the fluid taken from the effusions. It was hypothesized that the effusion effectively formed an additional compartment for drug distribution (the "third space") and that this formed a depot causing prolonged exposure to the drug and increased toxicity. This idea has been widely adopted in oncology, and it is common practice to reduce doses of all cytotoxics in patients who have effusions. However, there are no systematic studies of the pharmacokinetics to establish that

there is a clinically significant third-space effect. If a third space with a volume of 1–5 L is added to a two-compartment model, such as that shown in Figure 3, it has very little effect on the predicted plasma levels. Also a systematic study of topotecan, a drug that has broadly similar pharmacokinetics to methotrexate, has been done in patients with effusions. The pharmacokinetics were measured in the same patients both before and after the effusion was drained. No significant differences in the pharmacokinetic parameters were seen, as would be expected from pharmacokinetic modeling. Subsequent data cast doubt on the observations that led to the third-space hypothesis. In the original study, tritiated methotrexate was used and the drug assayed simply by counting the radioactivity in the samples. The subsequent studies showed that much of the tritium was substituted at the 7-position on the methotrexate molecule and that hydroxylation at this position releases tritiated water, which has a half-life of several weeks (Figure 6).

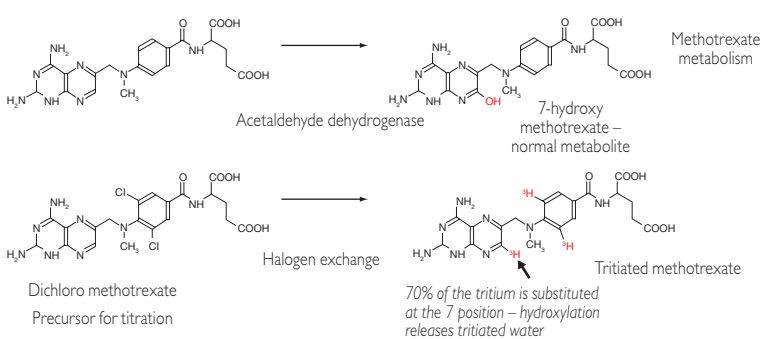

Figure 6 *The normal metabolism of methotrexate (above) and the synthesis of tritiated methotrexate (below).*

The "third-space" hypothesis is therefore an artefact of the methodology used and has no relevance to the use of anti-cancer drugs. Any unexpected toxicities seen in patients with effusions are most likely due to other features in patients with advanced cancer.

Pharmacodynamics

Markers of Drug Efficacy

Pharmacodynamics is the study of the effects that the drugs have on the recipient and includes the desired therapeutic outcome. In the treatment of leukemia, the reduction in the blast count is a pharmacodynamic measure of the efficacy of the treatment regimen being used. The drugs and dosages can be tailored to achieve the desired reduction. However, it is unusual in cancer medicine to be able to assess directly the effect of the treatment on the tumor in real time. In most solid tumors, responses are apparent only several weeks after the initiation of therapy. For this reason it is common to measure something that reflects the therapeutic action of the drug, but is not in itself the therapeutic event. In general medicine it is common to use such surrogates; antihypertensives and lipid-lowering drugs are used to reduce the risk of cardiovascular disease over a long period, but their dosage is adjusted to achieve surrogate endpoints. These endpoints are a reduction in blood pressure and a reduction in the plasma level of lipids, respectively.

Perhaps one of the first examples of the use of a pharmacodynamic surrogate marker in cancer therapy was devised during the development of aromatase inhibitors. Aromatase inhibitors are designed to reduce plasma estrogen levels and thereby inhibit the growth of estrogen-dependent tumors. Measurement of plasma estradiol levels showed that the dose of drug being given to the patient was sufficient to reduce the levels within a few hours of administration, although the clinical reductions in tumor size took many weeks to become apparent (Figure 7).

For most of the traditional cytotoxic agents, the most obvious pharmacodynamic effect is the suppression of the blood count. Since this reflects the antiproliferative action of these drugs, and is also the mechanism by which they eliminate tumor cells, it is reasonable to use the maximum dose tolerated by the bone marrow in order to maximize the probability of the response of the tumor.

It is clearly very useful to have a pharmacodynamic marker for antitumor activity, rather than for toxicity. A number of tumors secrete specific proteins, known as tumor markers, which can be measured in the plasma

and used as a surrogate for the tumor response. Some of these are summarized in Table 1.

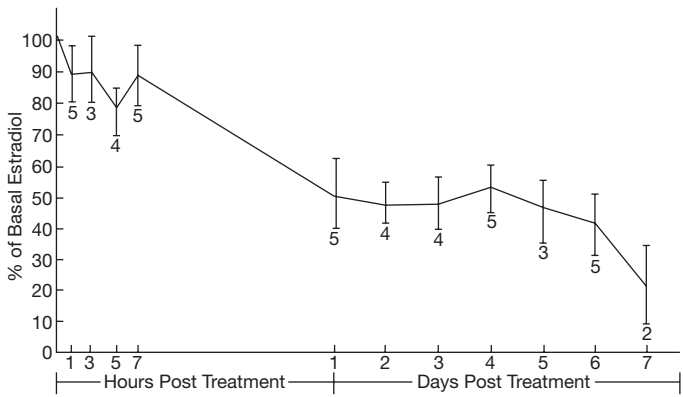

Figure 7 *Plasma estradiol levels in a patient who received 500 mg 4-hydroxyandrostenedione, one of the first aromatase inhibitors. From: Coombes RC, et al. 4-Hydroxyandrostenedione in treatment of postmenopausal patients with advanced breast cancer. Lancet 1984; 2:1237–1239 with permission.*

Table 1 *Tumors that secrete tumor markers, which can be measured in plasma and used as a surrogate for tumor response*

Tumor types	Marker
Germ cell tumors, hepatocellular carcinoma	AFP (α-fetoprotein)
Ovarian cancer	CA125
Breast cancer	CA153
Pancreatic cancer	CA199
Colon cancer, rectal cancer, other GI cancers	CEA (carcinoembryonic antigen)
Choriocarcinoma, germ cell tumors	HCG (human chorionic gonadotropin)

HCG is closely related to the tumor load in the case of choriocarcinoma and is also very useful in monitoring the treatment of germ cell tumors. CA125 has become accepted as a surrogate for tumor response in ovarian cancer, although it is not expressed in all cases. In general these markers, when expressed by the tumor concerned, tend to increase with tumor progression and reduce with tumor response. However, the correlation with tumor burden is far from precise and tumors may change their expression profile over time (becoming "marker negative").

In the case of HIV treatment, using the polymerase chain reaction (PCR) to identify the viral RNA provides a quantitative measure of the viral load. This, coupled with the availability of 30 or so antiviral drugs, has revolutionized outcomes in this disease. It is possible for the treating physician to obtain a rapid readout of the efficacy of the chosen combination of drugs and then to modify the combination accordingly until a reduction in viral load is achieved. In the case of chronic myeloid leukemia, PCR can also be used to estimate the tumor burden, and the specific kinase inhibitors for the Bcr-Abl protein can be used to treat the disease with very good results. However, currently there are relatively few specific and quantitative markers of tumor burden for the common solid tumors. If good markers are developed in the future, they may permit this kind of interactive management of cancer therapy. The burgeoning number of specific targeted agents would provide an "armory" from which suitable rational combinations could be chosen. A rapid assessment of their efficacy could then be made and the drugs changed as required to provide a reduction in tumor burden. Such an approach would require changes to the regulatory environment and the support of health care providers.

Pharmacodynamic Endpoints in the Use of Targeted Anti-cancer Agents

A number of factors have combined over the last decade or so which have led to an unprecedented number of new drugs being available for evaluation in cancer treatment. Advances in molecular biology and genomics have permitted the sequencing of the many cancer genomes and have identified numerous potential drug targets. Advances in medicinal chemistry, in silico and robotic drug screening, and in crystallography have resulted in a large number of selective targeted agents being made. These developments have

required a parallel change in the methods for the early clinical evaluation of such drugs.

The biological target for each of these drugs is almost always known. It is therefore desirable to develop methods to measure whether the target is being modulated during the early development of the drug. Clinical proof of mechanism establishes that a drug that was shown to act on a particular target in laboratory experiments does indeed modulate the same target in the clinical setting.

Drugs that are designed to act on the tumor vasculature can have their effect measured by suitable scanning techniques, utilizing MRI or PET scanning. Scans can be done before and after the administration of the drug and the changes in blood flow quantified. An example of this technique is the studies of PTK787/ZK-222584 (vatalanib). This inhibitor of vascular endothelial growth factor showed dramatic changes in tumor blood flow as assessed by MRI techniques in phase I and II trials, clearly providing a clinical proof of mechanism. However, a phase III trial in colorectal cancer was negative. Similarly, during the early clinical development of the EGFR inhibitor, gefitinib, skin biopsies were taken and examined by immunohistochemistry (IHC). Marked changes were seen in activated EGFR and MAP kinase (a downstream marker of the signaling pathway), showing clearly that the drug was inhibiting EGFR as intended. Nevertheless phase III studies of gefitinib in combination with cancer chemotherapy in lung cancer were negative. (Parenthetically, gefitinib was subsequently shown to be very active in a subset of lung cancer patients whose tumors possessed an activating mutation in EGFR, making them particularly sensitive to EGFR inhibition.) While the pharmacodynamic studies described above demonstrate and confirm that the mechanism of the drug is achieved in the clinical setting, they also show that care is needed in interpreting these data in a quantitative way. In particular, we often do not know what level and duration of inhibition is required for antitumor activity. Inhibition of a target by 80% will produce marked changes in perfusion scan or an IHC slide, but the remaining 20% may be more than sufficient for tumor survival. Further, pharmacodynamic tests are conducted at a particular time after dosage. It may be that substantial modulation of the target for a long period after the test has been conducted is necessary for successful therapy.

Considerations such as these have led many investigators to believe that the dose of most targeted agents should be escalated until some level of toxicity is apparent, providing that the accompanying pharmacodynamic studies show a clinical proof of mechanism. The exception to this principle is in the case of agents where the target itself is clearly tumor-selective. A few examples of such drugs are the Bcr-Abl kinase inhibitors, selective BRAF inhibitors for patients with melanomas possessing a BRAF mutation, and, more recently, the ALK inhibitor, crizotinib, in the subset of patients with non-small cell lung cancer possessing an EML4-ALK fusion.

Phase 0 Clinical Trials

The importance of the pharmacokinetic and pharmacodynamic properties of a new drug in determining its success as a new cancer therapeutic agent has led to the phase 0 trial design. A phase 0 trial is specifically designed to answer questions about these properties of the drug, but is not designed to assess an MTD based on toxicity or to assess therapeutic efficacy. The US FDA has introduced an exploratory Investigational New Drug (IND) license that requires less stringent preclinical toxicology studies than are needed for a full phase I trial. This supports phase 0 studies, which are defined as first-in-human trials with no therapeutic or diagnostic intent, a limited number of patients (less than 15), and with limited drug exposure (meaning low doses administered for a limited period). Possible objectives of a phase 0 trial are listed below.

- Determine whether a mechanism of action defined in preclinical models is achieved in the clinical setting
- Seek relationships between the pharmacokinetics and pharmacodynamics prior to phase I
- Determine a dose range and sequence of a biomodulator to be used in combination with a traditional agent
- Evaluate the pharmacokinetics and pharmacodynamics of two or more analogs to allow an informed choice for further development
- Develop a novel imaging probe
- Refine a biomarker assay using human material

The potential advantages of phase 0 studies are that they can reduce the risk of failure in developing new targeted anti-cancer agents, allowing those with poor pharmacokinetic properties to be excluded at an early stage. In countries where the toxicology requirements are reduced, it can also reduce the cost of the first introduction of the drug into man. The disadvantages are that it may slow the overall course of drug development, by introducing an extra stage in the process. There is also the question of whether phase 0 trials will be acceptable to patients. In a phase I trial there is the possibility of therapeutic benefit. Indeed, most contemporary phase I trials are designed to maximize that possibility. A phase 0 trial, with limited dose and duration of exposure, cannot offer the possibility of therapeutic benefit.

Summary and Conclusions

The drug therapy of cancer remains challenging. The relatively small biological differences between cancer cells and their normal counterparts make the development of truly selective drugs with a wide therapeutic margin difficult. An understanding of the pharmacokinetics of the commonly used anti-cancer drugs is essential for the practicing oncologist to administer optimal doses to his or her cancer patients. Pharmacodynamic endpoints, such as tumor response and myelosuppression, are used every day in medical oncology practice, although rarely formalized as such. However, surrogate endpoints, in particular clinical proof of mechanism, are essential tools in the development of new targeted agents.

Further Reading

Booth CM, Calvert AH, Giaccone G, et al. Endpoints and other considerations in phase I studies of targeted anticancer therapy: Recommendations from the task force on Methodology for the Development of Innovative Cancer Therapies (MDICT). Eur J Cancer 2008; 44:19–24.

Brunton L, Chabner BA, Knollman B. Goodman and Gilman's The Pharmacological Basis of Therapeutics, Twelfth Edition. New York: McGraw-Hill; 2011.

Calvert AH, Newell DR, Gumbrell LA, et al. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol 1989; 7:1748–1756.

Collie-Duguid ESR, Etienne MC, Milano G, et al. Known variant DYPD alleles do not explain DPD deficiency in cancer patients. Pharmacogenetics 2000; 10:217–223.

Gelderblom H, Loos WJ, Verweij J, et al. Topotecan lacks third space sequestration. Clin Cancer Res 2000; 6:1288–1292.

Gianni L, Kearns CM, Giani A, et al. Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans. J Clin Oncol 1995; 13:180–190.

Iyer L, King CD, Whitington PF, et al. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest 1998; 101:847–854.

Kummar S, Doroshow JH, Tomaszewski JE, et al. Task Force on Methodology for the Development of Innovative Cancer Therapies (MDICT). Phase 0 clinical trials: Recommendations from the Task Force on Methodology for the Development of Innovative Cancer Therapies. Eur J Cancer 2009; 45:741–746.

Stoller RG, Hande KR, Jacobs SA, et al. Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. N Engl J Med 1977; 297:630–634.

Wan SH, Huffman DH, Azarnoff DL, et al. Effect of route of administration and effusions on methotrexate pharmacokinetics. Cancer Res 1974; 34:3487–3491.

Alkylating Agents

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Introduction

The antitumor alkylating agents represent the oldest class of anti-cancer agents. All these agents are active or latent nitrogen mustards: some are direct acting and some are prodrugs; some require enzymatic activation and some undergo chemical breakdown to active species. The active alkylating moiety includes an ethylene immonium ion (-N-CH_3CH_2-)+. These highly reactive intermediates covalently bind to electron-rich nucleophilic moieties on all large and small biological molecules. The favored atoms to be alkylated are sulfur, nitrogen, oxygen, and phosphorus. They have no specificity, but the chance reaction with DNA forms the basis for their antitumor effects. The most frequent site of DNA alkylation is the N7 position of guanine; however, adducts at the O6 and N1 position of guanine, the N7, N8, and N1 position of adenine, the N3 position of cytosine, and the O4 position of thymidine are also formed.

Bifunctional alkylating agents form covalent bonds at two nucleophilic sites on different DNA bases to induce interstrand (between two opposite strands) and/or intrastrand (on same strand) crosslinks. Monofunctional agents have only one alkylating group and cannot form crosslinks. Irrespective of the specific mechanisms involved in the formation of adducts, the end effect of these DNA-interactive agents is to inhibit DNA replication, which in turn may affect the production of RNA and proteins both in normal and in tumor cells. The capacity to interfere with DNA integrity and function leads to cell death by not yet defined mechanisms. Specific cellular responses include cell-cycle arrest and attempt to repair

the DNA damage; recognition of extensive DNA damage by p53 can trigger apoptosis and mutation of p53 has been shown to be associated with resistance to alkylating agents in some cases.

Although covalent adduct formation on DNA is the mechanism of cytotoxicity common to all the alkylating agents, these drugs have widely different potency, toxicity, and disease selectivity. It is the difference in the non-alkylating part of the molecules that leads to the differences in the pharmacokinetics, biodistribution, and normal tissue toxicities of these drugs.

At present five major types of alkylating agents are used in the chemotherapy of neoplastic diseases, as described below.

Alkyl Sulfonates (Busulfan)

Busulfan is the best known of the alkyl sulfonates, and has a linear symmetrical chemical structure that facilitates crosslink formation. However, the mechanism of alkylation of this molecule is different. Unlike the mustards and the aziridines, which must first generate reactive species, busulfan interacts directly with the N7 position of guanine and leads to the formation of DNA mono- and then biadducts, with release of methyl sulfonate groups.

Nitrogen Mustards (Melphalan, Chlorambucil, Cyclophosphamide, Ifosfamide)

These were the first molecules specifically developed for potential clinical application as anti-cancer agents, starting from the clinical observation of the side effects of the sulfur mustard gas (a biological warfare agent used in World War I) which involved skin, eyes, and respiratory tract, but also bone marrow with delayed myelosuppression and lymphoid aplasia. This group includes nitrogen mustard (mechlorethamine), chlorambucil, melphalan, cyclophosphamide and its activated prodrug form 4-hydroperoxycyclophosphamide, and ifosfamide. The common structural feature is the bischloroethyl group, which is the precursor for the activated function that predominantly alkylates the N7 of guanine.

The chloroethyl group of the biotransformed mustard cyclizes to the immonium (aziridinium) ion and interacts with the DNA molecule. Since the two chloroethyl groups in the nitrogen mustard drugs are retained in phosphoramide and nitrogen molecules, a bifunctional reaction with macromolecules ensues through a sequential alkylation process; that is, a monofunctional adduct is formed first and this is followed by the second adduct in the opposite strand of the DNA. This bifunctional reaction generates an interstrand crosslink between the two strands of DNA in the helix which is critical in preventing the two opposing strands from separating during replication, leading to inhibition of DNA synthesis.

Ethylenimines/Methylmelamines (Thio-TEPA, Altretamine)

The aziridines, also known as ethylenimines, are a family of alkylating agents that contain three-membered aziridine rings. The aziridine ring is structurally similar to that present in the reactive immonium ion formed by nitrogen mustards. However, since the aziridine ring does not carry a charge, these drugs are much less reactive than the mustards. The aziridines are activated spontaneously or by an enzymatic oxidative reaction. Following activation, alkylation can occur at a number of nucleophilic sites in DNA, RNA, protein, and other molecules such as glutathione.

Nitrosoureas (Carmustine [BCNU], Streptozotocin, CCNU)

When they were first developed, these molecules represented a new class of chemical structure. Mechanisms of action studies demonstrated that they could rapidly decompose in aqueous solution and that they could both alkylate DNA and carbamoylate proteins via isocyanate formed during decomposition. In contrast to other DNA-reactive agents, the nitrosoureas also alkylate the O6 site of guanine to a large extent. The significance of O6 alkylation can be recognized from the knowledge that cytotoxicity correlates inversely with cellular activity of the DNA-repair enzyme O6-alkylguanine–DNA alkyltransferase, which removes the monofunctional O6 adduct from the DNA. Thus, when the O6-alkyltransferase is overexpressed, sensitivity of tumor cells to nitrosoureas diminishes. Indeed, the preclinical data with these compounds showed a striking antitumor activity that was not reproduced in human tumors.

Subsequent studies showed that an enzyme responsible for the repair of O6-alkylguanine (O6-methylguanine–DNA alkyltransferase, MGMT), was expressed at low levels in mice, but at high levels in humans, and this was correlated with the lower clinical efficacy.

Triazenes (Dacarbazine, Temozolomide)

The triazenes are chemically synthesized small molecules that release during chemical drug decomposition a highly reactive diazonium ion intermediate, which alkylates DNA. Also in this case, the O6-alkylguanine DNA lesion is highly cytotoxic and correlates inversely with the activity of the DNA-repair enzyme MGMT, which removes the monofunctional O6 adduct from the DNA, limiting response to these cytotoxics.

Clinical Pharmacokinetics

Table 1 reports the main pharmacokinetic parameters for the more clinically used alkylating agents.

Even though the pharmacokinetics of the different alkylating agents is different, they are all characterized by high reactivity and short half-lives. Among the nitrogen mustards, mechlorethamine is unstable and has to be administered rapidly in a running intravenous infusion to avoid its rapid breakdown to inactive metabolites. On the contrary, chlorambucil and cyclophosphamide are sufficiently stable to be administered orally and are rapidly and completely absorbed from the gastrointestinal tract; on the other hand, melphalan has a poor and variable oral absorption. All of them require activation: either non-enzymatic, like nitrosoureas, or enzymatic by the P450 liver microsomes, like cyclophosphamide, ifosfamide, and dacarbazine.

The nitrosoureas form a class of alkylating agents with distinct metabolism and pharmacology. Because of their lipophilicity and capacity to cross the blood–brain barrier, the chloroethylnitrosoureas were found to be effective against intracranially transplanted murine tumors.

Therapeutic Uses

Alkylating agents are used alone or in combination in a large variety

of solid tumors and hematological malignancies. The most versatile agent is cyclophosphamide, while the other alkylating agents have more restricted clinical use. Many tumors are managed with drug combinations using several alkylating agents (Table 1).

Toxicities

Qualitative and quantitative differences in the sites and severities of toxicities have been reported, including nausea and vomiting, bone marrow depression, renal, bladder, and lung toxicity, teratogenesis and carcinogenesis, alopecia, allergic reaction, and immunosuppression (Table 1).

Nausea and Vomiting

This is a common side effect with a frequency and extent variable among patients and doses; it usually occurs when patients are treated with high doses.

Bone Marrow Toxicity

This toxicity generally involves all blood elements. The extent and the duration of suppression have marked interindividual fluctuations and class effects. For example, the depth of leukocyte nadir produced by cyclophosphamide is similar to the one produced by nitrogen mustard, but the recovery is faster. Relative platelet sparing is a characteristic of cyclophosphamides; even at high doses of cyclophosphamide, some recovery of hematopoietic cells occurs within 21 to 28 days. This stem cell-sparing property is also reflected by the fact that cumulative damage to the bone marrow is rarely seen when the cyclophosphamide is given as single agent and repeated, high drug doses can be administered without cumulative myelosuppression. On the contrary, busulfan is quite toxic to the bone marrow stem cells and treatment can lead to prolonged hypoplasia. Again, recovery after melphalan treatment is lower than after cyclophosphamide and cumulative marrow depression has been reported after repeated administrations. The bone marrow toxicity caused by nitrosoureas is delayed, with leukopenia and thrombocytopenia occurring 3 to 4 weeks after drug administration and lasting for an additional 2 to 3 weeks. Thrombocytopenia usually occurs earlier and is more severe than leukopenia.

Table I *Pharmacological aspects of alkylating agents*

Agent	Dosing	Pharmacokinetics	Metabolism
1. **Alkyl sulfonate** Busulfan	PO: 4–8mg (daily) HD: 4 mg/kg on days 1–4 (MTD 640 mg/m^2) with prophylaxis against seizures	Bioavailability 80% Protein bound 33% $T_{1/2}$ 2–2.5 hrs Extensively transformed with only 1% of the dose excreted unchanged in urine over 24 hrs	The drug is extensively metabolized with 10–50% of a dose excreted in the urine as metabolites within 24 hrs
2. **Nitrogen Mustards** Melphalan	PO (daily dose at one time and fasting): 1 mg/kg total dose over 5 days q 4–5 wks IV: 8 mg/m^2 q 4–5 wks IV HD: 40–200 mg/m^2	Bioavailability variable: 25–90%; lower after food ingestion $T_{1/2}$ 1.5 hrs Urinary excretion at 24 hrs 50%; inactivation by non-enzymatic hydrolysis	
3. Chlorambucil	PO: 0.1–0.2 mg/kg (4–10 mg total) daily for 3–5 wks	Extensive oral absorption $T_{1/2}$ 1–2 hrs and its major metabolite 3 hrs	Extensively metabolized, primarily by the liver and excreted by the kidney
4. Cyclophosphamide	PO: 50–100 mg/m^2 daily IV: 40–50 mg/kg (in divided doses over 2–5 days); 10–15 mg/kg (q 7–10 days); 3–5 mg/kg (2/wk) HD: 200 mg/kg (IV, in high-dose regimen in bone marrow transplantation) plus hydration plus mesna	Bioavailability >75% Low protein binding $T_{1/2}$ 3–12 hrs Urinary excretion at 24 hrs <15%	The drug is activated by CYP2B to the active species 4-hydroxy-cyclophosphamide, which requires further activation to phosphoramide mustard (cytotoxic species) and acrolein (responsible for bladder toxicity)
5. Ifosfamide	Adequate hydration before, up to 72 hrs after to avoid hemorrhagic cystitis IV short (3 hr) or cont inf: 1.2–1.5 mg/m^2 for 3 days or days 1–5 q 3–4 wks; 24 hr cont inf: 5 g/m^2 IV HD (cont inf): 3–4 g/m^2 on days 1–4 Add mesna PO/IV	$T_{1/2}$ varies from 4 to 15 hrs according to whether a high dose, low dose, or fractionated regimen is employed; excretion is mainly by the kidney with a low clearance; induction of its metabolism after 3 days of IV bolus or cont inf treatment	The drug is metabolized by P450 system in the liver

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
	Immunization with live attenuated vaccines can result in life-threatening infection Inhibitors of CYPs (imidazoles and metronidazole) increase VOD and hepatotoxicity	Selective neutropenia after 11–30 days. Thrombocytopenia, pulmonary fibrosis, a syndrome resembling Addison's disease may develop characterized by anorexia, weight loss, weakness, severe fatigue and hyperpigmentation High-dose busulfan causes hepatotoxicity with increased LFT and VOD (10%)	Stop for WBC between 10 000–20 000/mm^3, restart when WBC count rises Treatment discontinuation in case of sharp fall in PLT count
In pts with moderate to severe renal impairment, the initial IV dose should be reduced to 50% with subsequent doses according to the myelosuppression	With ciclosporin, increased risk of nephrotoxicity	Nausea and vomiting; neutro/thrombocytopenia delayed (after 2–3 wks, recovery up to 6 wks) More leukemogenic than other alkylators	
		Myelosuppression; CNS; skin reaction; hepatotoxicity; interstitial pulmonary fibrosis reported in CLL on long-term treatment High incidence of secondary leukemia after prolonged treatment	
Renal impairment: Cl <20 ml/min, decrease the dose to 50–75%	Inducers of microsomal activity (barbiturates)	Dose-limiting neutropenia after 8–14 days, recovering within 10 days; nausea and vomiting; diarrhea; alopecia; hemorrhagic cystitis, prevented by adequate pre- and post-hydration Cardiotoxicity: higher incidence in case of prior anthracyclines, large single infusion, glutathione-depleting agents At high dose, the drug can produce tachyarrhythmias and intractable heart failure; SIADH	
Renal impairment: Cl <20 ml/min, decrease the dose to 50–75%	Modulators of the P450 system, can interfere with activity	As for cyclophosphamide Severe encephalopathy with confusion, lethargy, seizures, hallucinations; ↑ CNS toxicity in elderly pts with renal impairment and concomitant CNS-active agents Methylene blue to reverse and prevent CNS toxicities	

Table I Pharmacological aspects of alkylating agents (Continued)

Agent	Dosing	Pharmacokinetics	Metabolism
6. **Ethylenimines** Thio-TEPA	IV bolus: 0.3–0.4 mg/kg q 1–4 wks IV HD: 500–1125 mg/m^2 Intravesical: 30–60 mg/wk × 4 Intrapleural, intrapericardial: 60 mg at 1 wk interval	$T_{1/2}$ 5 hrs Protein binding 40%; 24% of the dose excreted in 24 hr urine	Activation by CYP450 to main metabolite TEPA
7. **Nitrosoureas** BCNU (Carmustine)	IV: 150–200 mg/m^2 q 6 wks IV HD: 1200 mg/m^2 (single agent) split in two fractions at 12 hr intervals; 300–600 mg/m^2 (combination)	Protein bound 80% $T_{1/2}$ 30 mins; partial metabolization in liver to inactive species; 30% of the dose in 24 hr urine Rapidly crosses the blood–brain barrier	Undergoes spontaneous decomposition and significant hepatic metabolism mainly by the P450 cytochrome
8. CCNU	PO: 100–150 mg/m^2 q 6 wks on empty stomach	Rapidly metabolized after oral dose to active metabolites with $T_{1/2}$ 16–48 hrs Metabolites cross the blood–brain barrier	Rapidly 4-hydroxylated on its first passage through the liver
9. **Triazenes** DTIC (Dacarbazine)	IV: single agent 200–250 mg/m^2 on days 1–5 q 3–4 wks Avoid direct sun exposure for 24 hrs after drug administration, decreased vein irritation and pain if dacarbazine is given protected from light	Low oral bioavailability; initial and terminal $T_{1/2}$ of 20 mins and 5 hrs Protein bound 5–20%	Spontaneous photodegradation in light Extensively metabolized by liver P450 cytochrome
10. Temozolomide	PO: 150-200 mg/m^2 (according to the extent of pre-treatment) for 5 days in a 4-wk cycle	100% oral bioavailability, reduced by food Protein bound >15% $T_{1/2}$ 1.8 hrs No accumulation with daily dosing Crosses blood–brain barrier. Cl not affected by anticonvulsants (with exception of valproic acid)	

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Liver impairment: no guideline available, but use caution	Inhibition of pseudocholinesterase activity with increased effect of succinylcholine	Dose-related and cumulative myelosuppression with short WBC and longer PLT nadir; mucositis; hyperpigmentation of skin; hepatotoxicity	
	Drugs inducing microsomal activity (phenobarbitone and other barbiturates) antagonize carmustine antitumor effect. Cimetidine enhances both toxicity and antitumor effect of the drug	Delayed (after 3–6 wks) potentially cumulative myelosuppression; acute severe nausea and vomiting; burning pain in the vein; flushing; mild reversible hepatotoxicity Pulmonary toxicity (interstitial pneumonitis and fibrosis), VOD are a function of duration and cumulative dose Possible delayed onset (>10 years) in cured children having received cranial RT	Dose reduction when given with other myelotoxic agents. Previous administration of CTX. MTX, busulfan, bleomycin, and previous thoracic irradiation increase carmustine pulmonary toxicity
	Cimetidine enhances both toxicity and antitumor effect of the drug	Delayed (after 3–6 wks) potentially cumulative myelosuppression; acute severe nausea and vomiting; burning; mild reversible hepatic toxicity; increased risk of second malignancy after long-term therapy Rare: cumulative (after 110 mg/m^2) pulmonary fibrosis, possibly delayed onset (>10 years after) in cured children having received cranial RT	
Liver and renal impairment: no guideline available, but prepare to lower the dose	Pts receiving dacarbazine should not receive immunization with live vaccines	Acute, severe nausea and vomiting; myelosuppression; occasional influenza-like syndrome after 1 wk; sunlight reaction with facial flushing, pain on the head and hands; hepatotoxicity	
Liver and renal impairment: no guideline available, but caution in pts with severe impairment	Possible synergism with antitumor drugs with similar mechanism of action to deplete MGMT	Nausea and vomiting; fatigue and malaise; myelosuppression (mainly neutropenia and thrombocytopenia) with delayed nadir >20 days and recovery in 7–14 days	Dose reduction by 50 mg/m^2 daily according to ANC/PLT nadirs; do not reduce below 100 mg/m^2

Renal and Bladder Toxicity

Hemorrhagic cystitis is unique to cyclophosphamide and in particular to ifosfamide and may range from mild to severe. This toxicity is caused by the excretion of toxic metabolites (mainly acrolein) in the urine and a direct irritation of the bladder mucosa. Adequate hydration and irrigation of the bladder with a solution containing mesna (sodium-2-mercaptoethanesulfonate) have reduced its incidence and severity. Mesna is a thiol compound that functions as a regional detoxificant of urotoxic ifosfamide and cyclophosphamide metabolites such as acrolein, 4-hydroxyifosfamide, and chloroacetaldehyde. After entering the circulation, mesna is oxidized to dimesna, which is then excreted by the kidneys. Between 30% and 50% of glomerularly filtered dimesna is reduced back to mesna in the renal tubular epithelium by glutathione reductase. The resulting free sulfhydryl groups of mesna can combine directly with the double bond of acrolein, or with other toxic oxazaphosphorine metabolites in the bladder to form stable and nontoxic compounds. Mesna is highly water soluble and has very little tissue penetration. It is readily excreted by the kidneys, with the result that it is concentrated in close apposition to the urothelium. The nonurinary toxic effects and, more importantly, the systemic cytotoxic activity of oxazaphosphorine are not attenuated by concomitant mesna. Mesna has a short plasma half-life, necessitating repeated administrations to provide continuous adequate prophylactic protection of the bladder. Mesna can be administered via oral or intravenous routes. Mesna is administered at doses at least equal to 60% of the ifosfamide dose, given in three divided doses concurrent with or 15 mins before and then 4 hrs and 8 hrs after ifosfamide. At high cumulative doses, all nitrosoureas cause a dose-related renal toxicity that can result in renal failure and death.

Lung Toxicity

Long-term busulfan therapy can lead to a gradual onset of fever, nonproductive cough and dyspnea, followed by tachypnea and cyanosis, progressing to severe pulmonary insufficiency. Similar effects have been described after a cumulative dose >1 g/m^2 of cyclophosphamide and BCNU. Two distinct patterns of lung toxicity have been reported

after cyclophosphamide treatment: an early-onset pneumonitis, which is reversible and may respond to corticosteroids, and a late-onset pneumonitis, frequently associated with pleural thickening, that has a chronically progressive course and is unresponsive to corticosteroids.

Gonadal Toxicity, Teratogenesis, and Carcinogenesis

Alkylating agents have profound toxic effects on reproductive tissues (aspermia in men and amenorrhea with ovarian atrophy in women). Due to their action on DNA, all alkylating agents are to some degree teratogenic and carcinogenic. In particular, development of second cancer (acute leukemia) has been reported in patients treated with melphalan, cyclophosphamide, chlorambucil, and nitrosoureas.

Allergic Reaction and Immunosuppression

As alkylating agents bind covalently to protein, they can act as an aptamer and cause allergic reactions. This could be the reason for the skin eruption, angioneurotic edema, urticaria, and anaphylactic reactions reported after systemic administration of different alkylating agents. More importantly, alkylating agents have been reported to suppress both humoral and cellular immunity, due to a direct cytotoxic effect on lymphocytes and to interference on lymphocyte functions. The most immunosuppressive alkylating agent is cyclophosphamide. Clinically, the immunosuppression can lead to increased susceptibility to infections (viral, fungal, and protozoal).

Drug Resistance

Intrinsic and acquired resistance to alkylating agents occurs and limits their therapeutic utility. Several preclinical studies have highlighted the mechanisms as the basis of the resistance, which can be briefly summarized as follows (Table 2):

- Alteration in drug uptake or transport at the level of tumor cell
- Failure to activate alkylating agent prodrug
- Increased scavenging of reactive moieties by non-essential cellular nucleophiles
- Increased deactivation of agents by deactivating enzymes such as aldehyde dehydrogenases (ALDHs)

- Increased repair of the induced DNA damage
- Altered cellular apoptotic response to DNA repair, e.g. deficient apoptosis due to lack of cellular mechanisms to result in cell death following DNA damage

Table 2 *Mechanisms of resistance to alkylating agents*

Pre-target mechanisms (mechanisms that limit the formation of lethal DNA adducts)	Reduced drug accumulation Increased detoxification or activation systems (reductase, hydrolase, aldehyde dehydrogenase, metallothionein, glutathione/glutathione S transferase system)
Post-target mechanisms (mechanisms that enable a cell to repair or tolerate DNA damage)	Tolerance to the damage DNA-repair mechanisms (increased levels of enzymes involved in the repair of DNA lesions) Alterations in genes involved in cell-cycle arrest and/or apoptotic response

The results of these studies have led to the development of strategies aimed at interfering with the above-mentioned mechanisms. Efforts have been made to sensitize tumor cells with specific inhibitors of glutathione (GSH) biosynthetic enzyme (buthionine sulfoximine) to decrease intracellular GSH, which has a well-known detoxifying function. There is well-documented evidence that a major mechanism of resistance to chloroethylating agents is related to an increased expression of MGMT. MGMT is a protein that is able to transfer the methyl/alkyl adducts from the O6 position of guanine to the cysteine residue within its active site by a direct repair process. It repairs with a stoichiometric and auto-inactivating reaction, rendering this repair system saturable. Many alkylating agents, including temozolomide, streptozotocin, procarbazine, dacarbazine, and nitrosoureas, cause O6 adducts that are repaired by MGMT. This mechanism of resistance is the rationale for the development of methods to deplete MGMT as a strategy to overcome drug resistance, through an indirect inhibition of MGMT by the use of methylating agents and a direct inhibition by using analogs of guanine. In both cases, an increased toxicity (bone marrow and lung toxicity) has been reported.

Further Reading

Damia G, D'Incalci M. Mechanisms of resistance to alkylating agents. Cytotechnology 1998; 27:165–173.

de Jonge ME, Huitema AD, Rodenhuis S, et al. Clinical pharmacokinetics of cyclophosphamide. Clin Pharmacokinet 2005; 44:1135–1164.

Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. Nat Rev Cancer 2004; 4:296–307.

Giraud B, Hebert G, Deroussent A, et al. Oxazaphosphorines: new therapeutic strategies for an old class of drugs. Expert Opin Drug Metab Toxicol 2010; 6:919–938.

Hall AG, Tilby MJ. Mechanisms of action of, and modes of resistance to, alkylating agents used in the treatment of haematological malignancies. Blood Rev 1992; 6:163–173.

Harrison JC, Haber JE. Surviving the breakup: the DNA damage checkpoint. Annu Rev Genet 2006; 40:209–235.

Klastersky J. Side effects of ifosfamide. Oncology 2003; 65(Suppl 2):7–10.

Kummar S, Gutierrez M, Doroshow JH, et al. Drug development in oncology: classical cytotoxics and molecularly targeted agents. Br J Clin Pharmacol 2006; 62:15–26.

Marchesi F, Turriziani M, Tortorelli G, et al. Triazene compounds: mechanism of action and related DNA repair systems. Pharmacol Res 2007; 56:275–287.

Martinez GR, Loureiro AP, Marques SA, et al. Oxidative and alkylating damage in DNA. Mutat Res 2003; 544:115–127.

Pinto N, Ludeman SM, Dolan ME. Drug focus: Pharmacogenetic studies related to cyclophosphamide-based therapy. Pharmacogenomics 2009; 10:1897–1903.

Rabik CA, Njoku MC, Dolan ME. Inactivation of O6-alkylguanine DNA alkyltransferase as a means to enhance chemotherapy. Cancer Treat Rev 2006; 32:261–276.

Tentori L, Graziani G. Recent approaches to improve the antitumor efficacy of temozolomide. Curr Med Chem 2009; 16:245–257.

Platinum Agents

4

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Introduction

Platinum compounds are commonly used as systemic agents in a wide variety of cancers. There have been a number of platinum compounds developed and investigated over the years, but the three commonly used platinum agents are cisplatin, carboplatin, and oxaliplatin. Platinum compounds are often combined with other cytotoxics, targeted agents, and/or radiotherapy and they exert anti-cancer effects over a wide range of tumors.

Cisplatin (cis-diammine-dicholoroplatinum[II]) was the earliest platinum compound to enter mainstream clinical use. It has a broad range of anti-cancer activities with substantial toxicities. Structurally, this agent consists of a platinum core with two amine and two opposing chloride-leaving groups in cis configurations. Carboplatin (cis-diammine-cyclobutanedicarboxylato-platinum[II]) was developed during a systematic search for platinum compounds with a more favorable toxicity profile. It has fewer side effects and possesses a similar clinical activity spectrum to that of cisplatin. Structurally, carboplatin is different from cisplatin, as the leaving group consists of cyclobutane dicarboxylate, instead of chloride. Oxaliplatin (1,2-diamminocyclohexaneoxalato-platinum[II]), on the other hand, has a different spectrum of antitumor activity compared to cis- or carboplatin, as this agent had been shown to be active in colorectal cancer. The reason for this is not fully understood, but is thought to be associated with the 1,2-diamminocyclohexane carrier ligand, which inhibits cellular resistance against platinum agents such

as by the replicative bypass mechanism (see Drug Resistance section). The platinum core of this compound is not dissimilar to its sister platinum compounds, although the leaving group consists of an oxalato moiety.

Platinum compounds exert their cell-killing effect via the development of covalent adducts with cellular DNA, which is thought not to be cell-cycle specific. Despite platinum binding to other cellular components, it is widely understood that the primary mode of cell death is damage exerted to the cellular DNA.

Clinical Pharmacology

The pharmacokinetic of platinum complexes is usually studied by measuring the total and ultrafilterable platinum, the latter being the active form of the drug. The initial clearance of free cisplatin is rapid (i.e. short distribution phase), but, due to covalent binding to plasma proteins, the terminal half-life of the total platinum in the plasma is prolonged (>5 days). Free cisplatin in aqueous solution is also unstable since the chlorine leaving groups become aquated – the rate of loss of the chlorine leaving groups is reduced by the presence of chloride ions in the solution. A large proportion of an administered dose of cisplatin remains bound to tissues for very long periods, but is inert. A smaller proportion is excreted in the urine and the clearance of total and ultrafilterable compounds exceeds the glomerular filtration rate (GFR) of the kidneys, indicating active secretion of platinum by the renal tubules. The involvement of renal tubules may also, in part, account for the nephrotoxicity of cisplatin. The renal clearance of free platinum is non-linear and variable and is dependent on dose, urine flow rate, and individual variability in the extent of active secretion and possible tubular reabsorption. Small amounts of platinum are present in the bile and large intestine and the fecal excretion of platinum is minimal.

In contrast, the cyclobutane dicarboxylate leaving group of carboplatin is much more stable and this is reflected in the pharmacokinetics. Intact carboplatin remains in the circulation with a half-life of a few hours. Covalent binding to proteins occurs but much more slowly. The terminal half-life of protein-bound carboplatin is similar to that of cisplatin, approximately

5.8 days. Also, carboplatin is eliminated intact in the urine through glomerular filtration, with little contribution from tubular excretion or reabsorption. There are insufficient data to determine whether biliary excretion occurs. Administration of carboplatin using body surface area methods results in 2–3-fold change in interpatient drug level variability. This observation, coupled with the fact that carboplatin excretion is defined by GFR, has led to the development of area under the curve (AUC)-based dosing formulae. The most widely used formula was described by Calvert:

$$DOSE\ (mg) = AUC \times (GFR+25)$$

This formula was developed and has been prospectively validated using creatinine clearance measured with the ^{51}Cr-EDTA method. In routine clinical use, though, many institutions use estimated GFR calculations (e.g. the Cockroft–Gault formula), which may lead to erroneous carboplatin dosing. Both over- and underestimation of GFR using Cockroft–Gault as well as the Jelliffe formulae have been described. Moreover, serum creatinine estimation in the United States has also been recently changed from a colorimetric method utilizing the Jaffé reaction to the isotope dilution mass spectroscopy (IDMS) method. The colorimetric method overestimates the creatinine levels due to interfering compounds in the plasma. IDMS provides a more accurate, but lower, estimate of the serum creatinine level. Since this could result in overestimation of the GFR and carboplatin dose, the US FDA currently recommends that, when GFR is estimated based on serum creatinine using the IDMS method, the GFR estimate is capped to no more than 125 ml/min. However, this policy runs the risk of under-dosing people who genuinely have a high GFR.

The pharmacokinetics of oxaliplatin are intermediate between those of cisplatin and carboplatin. There is low interpatient and intrapatient variability in ultrafilterable platinum exposure. Plasma protein binding of platinum is irreversible and ranges from 70–95%. The major route of elimination is renal excretion and oxaliplatin is cleared at a level that is similar to or exceeds the average GFR.

Table 1 summarizes the clinical pharmacology properties, usual dosing levels, toxicities, and dose reductions for cisplatin, carboplatin, and oxaliplatin.

Therapeutic Uses

Cisplatin and Carboplatin

The use of cisplatin and carboplatin has been integral to the systemic therapies of many cancers. Cis- or carboplatin combination regimens are currently used as mainstay chemotherapy regimens for ovarian and lung cancers, as these have been shown to produce superior overall survival outcomes to other regimens or best supportive care alone. In the management of these cancers, cis/carboplatin have been combined with paclitaxel, docetaxel, gemcitabine, vinorelbine, pemetrexed, and etoposide. Recent data also support the use of platinum doublet regimens together with bevacizumab, a vascular endothelial growth factor inhibitor in ovarian and non-small cell lung cancers. Cisplatin, in combination with either other chemotherapies or radiation, is also principally used for squamous cancers of the head and neck, cervix, urothelial, germ cell, biliary, and upper gastrointestinal cancers.

Comparative trials have demonstrated equivalent activity between cisplatin and carboplatin in the management of ovarian cancer. In advanced non-small cell lung cancer (or extensive stage small cell lung cancer), although the use of cisplatin doublet seems to produce higher rates of response, there has been no convincing evidence that survival is superior with the use of cisplatin. The equivalence between cisplatin and carboplatin doublets in adjuvant therapy for non-small cell lung or limited stage small cell lung cancer remains unclear.

There are disease sites where the use of cisplatin is commonly favored over carboplatin. Studies that substituted carboplatin over cisplatin in combination with etoposide with/without bleomycin for the treatment of good-risk germ cell tumors demonstrated that cisplatin is superior for relapse-free survival. Comparative trials of cisplatin or carboplatin with 5-fluorouracil (5-FU) in the management of squamous cell head and neck cancer also tend to support the use of cisplatin with superior rates of response over carboplatin. It is worthy to note that many of the studies that suggest the superiority of cisplatin are either underpowered or suffer from under-dosing issues of carboplatin.

Table 1 *Pharmacological aspects of cisplatin, carboplatin, and oxaliplatin*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism	Dosing when organ dysfunction
1. Cisplatin	DNA inter- / intrastrand crosslinks	IV 75–100 mg/m^2, in combination or monotherapy q 3–4 wks; IV 30–40 mg/m^2/wk Pre/post hydration	High tissue distribution >90% protein bound >90% renal excretion, with 25% in the first 24 hrs, mainly by glomerular filtration and renal secretion $T_{1/2}$ ~130 hrs	Undergoes non-enzymatic conversion to several inactive metabolites, highly bound to plasma proteins	Not to be used in pts with pre-existing renal or hearing impairment Substitution with carboplatin may be appropriate
2. Carboplatin	Same as cisplatin, slower reactivity with DNA and lower potency than cisplatin	IV AUC 4–6 mg/ml/min q 3–4 wks, as single agent or combination	Minimal protein binding 70% renal excretion in the first 24 hrs by glomerular filtration $T_{1/2}$ 139 ± 38 hrs; 30% plasma levels in CSF after IV treatment	Undergoes intracellular hydrolysis to form reactive platinum complexes	Liver impairment: no dose adjustment needed Doses based on AUC method in mild to moderate renal impairment
3. Oxaliplatin	Same as cisplatin with bulkier DNA adducts	IV 85–100 mg/m^2 q 2 wks; 130 mg/m^2 q 3 wks, in combination regimen	Low inter- and intrasubject variability 70–95% plasma protein binding Renal excretion 50% within 72 hrs $T_{1/2}$ 273 ± 19 hrs	Rapid non-enzymatic biotransformation to reactive platinum complexes	Renal impairment: consider omitting drug if Cr Cl is <30 ml/min No dosage change necessary for mild-moderate liver dysfunction

Oxaliplatin

The use of oxaliplatin, together with 5-FU/capecitabine, has greatly impacted the outcome of colon cancer patients. The use of FOLFOX (oxaliplatin and infusional 5-FU) has been shown to increase survival in the adjuvant and metastatic disease setting in colon cancers. In the metastatic setting, the combination of 5-FU/capecitabine with oxaliplatin has shown clinical efficacy in both first- and second-line therapy settings. Addition-

Drug interactions	Toxicity	Dose adjustment for toxicity
Aminoglycosides/ amphotericin B: ↑ nephrotoxicity; phenytoin: ↓ serum levels	70–100% early delayed nausea/vomiting; 30% dose-dependent ototoxicity; 25–30% anemia; electrolyte disturbance (mild to moderate): 20–40% hypomagnesemia, hypokalemia, and hypocalcemia; 28–36% (8% long term) renal dysfunction; cumulative slowly reversible peripheral sensory neuropathy	If long-term complications are of concern, such as nephro/ototoxicities, consider switching to carboplatin
Phenytoin: ↓ serum levels; aminoglycosides: ↑ risk of oto- and nephrotoxicity (rare)	18% severe neutropenia; 25% severe thrombocytopenia after 2–3 wks; mild electrolyte disturbance: 20–30% hypomagnesemia, hypokalemia, and hypocalcemia; 2–3% HSR. Increased after repeated treatments, no cross-reactivity with cisplatin	Dose reduction by one AUC level in case of prolonged and significant myelosuppression
May potentiate warfarin effects	70–85% cumulative peripheral (hands, feet) neuropathy (significant in 5%); type I acute early reversible in 14 days, type II persistent >14 days; 60% fatigue; 30–60% nausea and vomiting, 10% neutropenia; 0.5–2% HSR	Consider dose reduction (20–25%) for persistent (>7 days) G2 or functional neuropathy, discontinuing drug for G3 neuropathy

ally, in the first-line setting, combination with bevacizumab is considered as one of the standard treatments of metastatic colon cancers. Oxaliplatin, 5-FU, and irinotecan have also been successfully combined together. The use of these agents, in a regimen called FOLFIRINOX, resulted in a superior survival outcome in advanced pancreatic cancer patients with, however, extensive gastrointestinal and bone marrow toxicity.

Toxicities

The use of cisplatin is commonly associated with nausea and vomiting. The use of 5-hydroxytryptamine type 3 ($5\text{-}HT_3$) antagonists and, of late, aprepitant (substance P antagonist) has made these side effects more manageable. The administration of saline around cisplatin administration (especially in the case of high-dose cisplatin use) has also allowed repeated doses to be administered, albeit dose-accumulated nephrotoxicities are often observed. It is estimated that up to 8% of patients who have received cisplatin have long-term renal dysfunction. The other complications of cisplatin, such as neuropathy, tinnitus, and sensorineural hearing loss, have proved much more difficult to anticipate and manage. A number of agents (mostly thiol compounds) have been assessed with the hope of preventing cisplatin-associated toxicities such as nephrotoxicity. One such compound is amifostine, which was initially developed as a radioprotective agent. This agent is thought also to protect against the side effects of cisplatin by improving tissue selectivity. A randomized trial demonstrated that use of amifostine results in equivalent antitumor activity with reduced cumulative toxicity rates.

Carboplatin has a vastly superior toxicity profile for nausea, vomiting, neuropathy, and renal dysfunction when compared to cisplatin. The principal toxicity for this agent is thrombocytopenia. Interestingly, when combined with paclitaxel, the thrombocytopenic effects of this combination regimen are less than carboplatin therapy alone with no apparent compromise on anti-cancer effects.

The use of oxaliplatin is associated with peripheral neuropathy. Peculiarly, the use of this agent can also produce an unusual type of neuropathy in the form of cold-induced dysesthesia affecting the face and extremities. At present, there are several studies supporting the use of calcium/magnesium, glutamine, glutathione, N-acetylcysteine, or xaliproden as primary prophylaxis to reduce the incidence and severity of oxaliplatin-induced neuropathy. However, it is worth noting that there is no proven treatment for platinum-based neuropathy once it has occurred.

Drug Resistance

The mechanisms of platinum drug resistance are best summarized into two broad classifications: (a) failure to accumulate adequate drug in order to produce significant DNA damage, and (b) failure of cell death post-platinum binding to DNA. Bodies of work have demonstrated that decreased drug transport and increased cytosolic drug inactivation, the latter by means of increased levels of thiols such as glutathione or metallothioneins, represent major causes of failure to accumulate adequate drug concentration to produce meaningful levels of cell kill. Failure of cell death post-platinum–DNA binding may be explained by relevant mechanisms that are involved in DNA repair. One such mechanism is the nucleotide excision repair (NER) pathway. Upregulation of the NER pathway results in increased platinum–DNA adduct repair, contributing to platinum resistance. Several markers exist to indicate the activity of the NER pathway, such as the excision repair cross complement 1 (ERCC1). Protein or mRNA expression of ERCC1, or polymorphisms affecting ERCC1 gene, can possibly be used to indicate NER activity. Presence of ERCC1 protein expression in non-small cell lung cancer has been shown to inversely affect outcome in patients treated with platinum-based regimens. Patients with familial ovarian cancer as a result of a BRCA1 or BRCA2 mutation are characteristically highly sensitive to cisplatin treatment. These patients develop tumors which lack the capability for homologous recombination repair (HR). This observation, coupled with the documentation of reactivating mutations in patients who have become resistant to platinum, has led to the suggestion that HR may also be a mechanism of resistance to platinum compounds. Another pathway that may be relevant in failure of cell death post-cisplatin binding is enchanced replicative bypass. This resistance mechanism is best defined as the ability of the replicative complex in a cell to synthesize DNA post the site of damage. Multiple in-vitro studies have demonstrated this mechanism to be essential in platinum resistance across different cell line models.

The need to overcome platinum resistance and the quest of lower toxicity profiles have led to the development of novel platinum compounds such as satraplatin, picoplatin, and a micellar formulation of cisplatin. However, to date, these agents are either in early phase drug development or have not yet found a niche in mainstream oncology.

Further Reading

Calvert AH. Dose optimisation of carboplatin in adults. Anticancer Res 1994; 14:2273–2278.

Culy CR, Clemett D, Wiseman LR. Oxaliplatin. A review of its pharmacological properties and clinical efficacy in metastatic colorectal cancer and its potential in other malignancies. Drugs 2000; 60:895–924.

Cvitkovic E, Bekradda M. Oxaliplatin: a new therapeutic option in colorectal cancer. Semin Oncol 1999; 26:647–662.

Go R, Adjei A. Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. J Clin Oncol 1999; 17:409–422.

Meyer KB, Madias NE. Cisplatin nephrotoxicity. Miner Electrolyte Metab 1994; 20:201–213.

Murry DJ. Comparative clinical pharmacology of cisplatin and carboplatin. Pharmacotherapy 1997; 17:140S–145S.

Antimetabolites

5

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Antimetabolites interfere with the synthesis of DNA and RNA. Most antimetabolites act during the S phase of the cell cycle, when cell proliferation is more prominent, but, depending on their mechanism of action, they may act in the G1 or G2/M phase. Most antimetabolites are structural analogs of molecules that are normally involved in the process of cell growth and division and are incorporated into DNA and/or RNA, conveying from there false messages. Other antimetabolites inhibit specific enzymes needed for the synthesis of essential compounds.

Antifolates (Table 1)

1. Methotrexate (MTX)

MTX is the N10 methylated derivative of the natural folate, folic acid. MTX is a potent inhibitor of dihydrofolate reductase (DHFR). This enzyme reduces folic acid to dihydrofolic acid (FH_2) and FH_2 to tetrahydrofolic acid (FH_4). Deoxythymidine monophosphate (d-TMP) is an essential component of DNA. Inhibition of DHFR leads to depletion of reduced critical folates and inhibits DNA, RNA, d-TMP, and purine synthesis (Figure 1). MTX can be polyglutamylated by the addition of one to five glutamate residues to the molecule. The more polar nature of these polyglutamates prevents extrusion out of the cell.

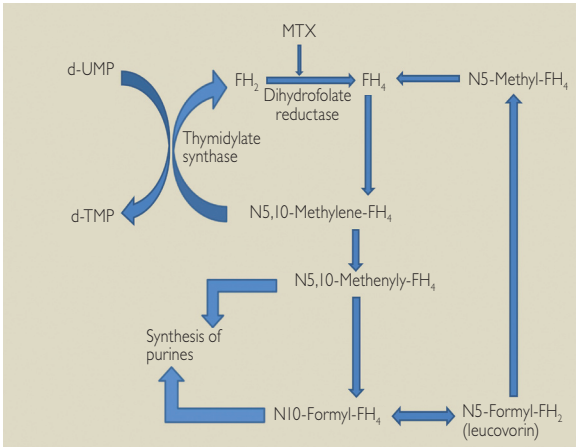

Figure 1 *Transformation and activation of methotrexate.*

Several mechanisms of resistance have been described:

- Decreased DHFR affinity to the drug due to mutations
- Decreased active MTX transport through the cell membrane
- Increased amount of DHFR, due to amplification
- Decreased polyglutamylation of MTX, decreasing its retention in the cell
- Increased degradation to 7-OH-MTX

With conventional MTX doses of 25–100 mg/m^2, the plasma concentrations achieved are 1–10 $\times 10^{-6}$ mol/L. A rapid infusion of 1.5 g/m^2 or more (so-called "high-dose" MTX [HD-MTX]) produces peak levels of 1–10 $\times 10^{-4}$ mol/L. The major metabolite of MTX is 7-OH-MTX and its concentration can exceed that of MTX, especially at HD-MTX. At low dose, the majority of MTX is excreted unchanged, but at HD-MTX more 7-OH-MTX is formed and both MTX and 7-OH-MTX are eliminated in the urine, both by filtration and by active secretion. Urinary alkalinization facilitates MTX renal excretion by increasing solubility and avoiding precipitation in the renal tubules. Sodium bicarbonate is taken to keep the urinary pH >7.0. Monitoring of plasma levels is essential when using the HD regimen to control the late phase of distribution, which is the

most important in terms of toxicity. Special emphasis measures include IV hydration, urinary alkalinization, monitoring of plasma levels, and leucovorin (LV, calcium folinate) rescue.

Aggressive hydration with IV normal saline (2.5 to 3.5 L of fluid per m^2 per 24 hrs, beginning 12 hrs before MTX infusion and continuing for 24 to 48 hrs), with alkalinization of the urine (45 to 50 mEq sodium bicarbonate per liter of IV fluids) to keep the urinary pH >7.0 at the time of drug infusion, is recommended before and in the first 48–72 hrs following HD-MTX (>1 g/m^2). LV may prevent the damage caused by MTX when elimination is prolonged. LV rescue should be initiated 24 hrs after high-dose infusion. MTX drug levels above 5×10^{-7} mol/L at 48 hrs after the start of MTX infusion require a prolongation of LV rescue. LV rescue should be maintained until MTX plasma levels decrease below 8×10^{-8} mmol/L. When LV is given too late, the damage cannot be reversed.

A general guideline to leucovorin rescue dependent on MTX plasma levels could be a follows:

MTX level at 48 hrs	Leucovorin dosage
5×10^{-7} mol/L	15 mg/m^2 q 6 hrs × 8
1×10^{-6} mol/L	100 mg/m^2 q 6 hrs × 8
2×10^{-6} mol/L	200 mg/m^2 q 6 hrs × 8

HD-MTX is used in the treatment of lymphomas, acute leukemias, and high-grade osteogenic sarcomas. Intrathecal (IT) administration is used to prevent meningeal infiltration from leukemia and lymphoma, with a maximum cumulative dose of 12 mg for children older than 3 years.

The main toxic effects of MTX are mucositis, myelosuppression, and hepatitis. Mucositis usually appears 3 to 7 days from the administration and precedes myelosuppression, mainly neutropenia, but also anemia and cumulative thrombocytopenia. MTX should not be administered to patients with ascites or pleural effusions, because of the toxic accumulation of the drug. Toxicity may also be dependent on the dosing or schedule by which MTX is administered (Table 1). In case of slowly decreasing plasma levels of methotrexate, despite leucovorin rescue and hyperhydration, the use of carboxypeptidase is highly recommended.

Table 1 *Pharmacological aspects of antifolates*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
1. Methotrexate (MTX)	DHFR inhibitor	Moderate dose: IV 100–500 mg/m^2 q 2–3 wks HD: IV 1–12 g/m^2 as 3 to 24 hrs q 1–3 wks IT: 10–15 mg 2/wk until CSF is clear, then weekly for 2–6 wks, then monthly IM: 25 mg/m^2 q 3 wks	Oral bioavailability is saturable and erratic at doses > 25 mg/m^2 T_{max} 1–2 hrs after PO and peak plasma 30–60 mins after IM Excretion: 80–90% in urine and 10–20% in bile; major metabolite 7OH-MTX	Hepatic
2. Pemetrexed	Inhibits three folate-dependent enzymes: DHFR, TS, and GRFT	Single agent: 500 mg/m^2 IV q 3 wks	$T_{1/2\beta}$ 3 hrs, renal excretion with 90% excreted unchanged in the first 24 hrs	Hepatic

2. Pemetrexed

Pemetrexed is a pyrrolopyrimidine analog with activity in the S phase of the cell cycle. The drug inhibits three folate-dependent enzymes: DHFR, thymidylate synthase (TS), and glycinamide ribonucleotide formyl transferase (GRFT), involved in the "de novo" synthesis of thymidine and purine nucleotides. Therefore, DNA and RNA synthesis and function are inhibited. Inhibition of TS by the parent compound is relatively poor, but is 10–100-fold enhanced by the polyglutamates of pemetrexed. Pemetrexed is used for the treatment of non-squamous

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal: dose should be reduced according to Cr Cl Hepatic: no adjustment necessary Plasma monitoring is essential for HD; ↓ dose in elderly due to ↓ renal function	Penicillins, probenecid, cephalosporins, sulphamides, phenytoin, aspirin, and NSAIDs inhibit renal excretion of MTX Warfarin: MTX may ↑ the anticoagulant effects of warfarin Omeprazole: ↑ MTX levels and toxicity	Mucositis; myelosuppression; and hepatitis Toxicity is dependent on dosing of MTX HD: ↑ transaminases; renal failure; vomiting; exfoliative dermatitis Chronic administration: cirrhosis; interstitial pneumonitis; portal fibrosis; osteoporosis; immunosuppression MTX simultaneously with brain RT: necrotizing leukoencephalopathy IT and HD: fever, headache and meningism	
Renal: dose to be reduced according to Cr Cl Hepatic: no adjustment necessary	5-FU: pemetrexed may ↑ the antitumor activity of 5-FU Leucovorin: may ↓ the antitumor activity of pemetrexed NSAIDs and aspirin: may inhibit the renal excretion of pemetrexed	Myelosuppression; skin rash (hand–foot syndrome); mucositis; diarrhea, nausea and vomiting; ↑ transaminases and bilirubin	Dietary folate status of pt is an important risk factor for toxicity. All pts should receive vitamin supplementation with 350 μg/day of folic acid PO and 1000 μg of vitamin B12 SC q three cycles (begin 1 wk prior to the start of pemetrexed). Use of steroids may ameliorate the development of skin rash. (Dexamethasone 4 mg PO 2/day for 3 days beginning the day before therapy)

carcinoma of the lung and mesothelioma, usually in combination with cisplatin or carboplatin.

Resistance to pemetrexed could be attributed to:

- Increased expression of the target enzyme, TS
- Alterations of the binding affinity of TS
- Decreased transport of drug into cells
- Decreased polyglutamylation

Pyrimidine Analogs (Table 2)

3. 5-Fluorouracil

5-Fluorouracil (5-FU) damages cancer cells by three mechanisms:

- Inhibition of TS with induction of parental DNA fragmentation
- Incorporation into RNA as the ribonucleotide, 5-fluorouridine triphosphate (5-FUTP) (Figure 2)
- Incorporation into DNA as the deoxyribonucleotide, 5-FdUTP, with inhibition of DNA synthesis and function

Increase of the target enzyme, TS, is the most commonly described mechanism of resistance. Cell lines and tumors with higher levels of TS, usually due to gene amplification, are more resistant to 5-FU.

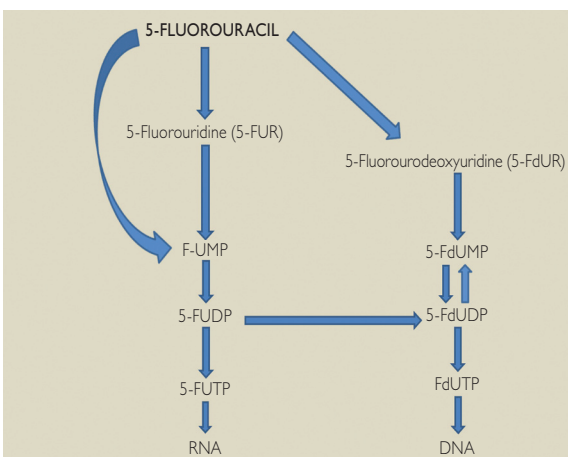

Figure 2 *Transformation and activation of 5-fluorouracil.*

In the case of IV bolus injection, the peak concentration achieved in plasma and bone marrow is 100–1000-fold higher than with continuous infusion. Over 85% of the administered dose is inactivated by dihydropyrimidine dehydrogenase (DPD), expressed mainly in the liver.

Attention should be given to the occurrence of homozygous (2%) and heterozygous (5%) deletions/point mutations in the DPD gene in the gen-

eral population. Some mutations are related to serious DPD deficiency and life-threatening toxicity after treatment with 5-FU or capecitabine, but are less likely for UFT and S-1, which are formulations containing a prodrug of 5-FU, ftorafur, and an inhibitor of DPD in order to increase the bioavailability of 5-FU. Doses of ftorafur are adapted to the inhibition of DPD.

5-FU is usually given in combination with leucovorin, which enhances the antitumor activity of 5-FU. This combination is extended with oxaliplatin in the FOLFOX regimens or with irinotecan in the FOLFIRI regimen for the treatment of colon cancer.

4. Capecitabine

Capecitabine is a prodrug of 5-FU and its activation to cytotoxic forms involves three enzymatic steps: first, in the liver to 5'-deoxy-5-fluorocytidine (5'-DFCR) by carboxylesterase, then to 5'-deoxy-5-fluorouridine (5'-DFUR) by cytidine deaminase (in the liver and in tumor tissues), and then to 5-FU by thymidine phosphorylase or uridine phosphorylase, which are expressed at higher levels in tumor than in the corresponding normal tissues.

After ingestion, peak plasma levels are reached in 1.5 hrs. Due to the specific tumor conversion of 5'-DFUR to 5-FU, peak levels of plasma 5-FU are very low and occur 2 hrs later. Capecitabine and its metabolites are primarily excreted by the kidney. Capecitabine is now often given instead of 5-FU, especially in the treatment of colon cancer.

5. Cytarabine (Ara-C)

Cytarabine is an analog of deoxycytidine, with the arabinose sugar instead of the deoxyribose. Cytarabine enters the cell by the equilibrative nucleoside transporter, which also transports deoxycytidine into the cell. The first metabolic step is the conversion of Ara-C to Ara-C monophosphate (Ara-CMP) by deoxycytidine kinase (dCK). Ara-CMP is then phosphorylated to Ara-C diphosphate (Ara-CDP) and Ara-C triphosphate (Ara-CTP). The incorporation of Ara-CTP into DNA results in inhibition of DNA synthesis and function. Ara-CTP also inhibits several DNA polymerases: α, β, and γ.

Table 2 *Pharmacological aspects of pyrimidine analogs*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
3. 5-Fluorouracil (5-FU)	Inhibition of TS Incorporation into RNA Incorporation into DNA	Bolus monthly: 425–450 mg/m^2 IV on days 1–5 q 4 wks Bolus weekly: 500–600 mg/m^2 IV/wk for 6 wks q 8 wks 24 hr inf: 2400–2600 mg/m^2 IV/wk 48 hr inf: 3500 mg/m^2 IV q 2 wks 96 hr inf: 800–1000 mg/m^2/day IV 120 hr inf: 1000 mg/m^2/day IV on days 1–5 q 3-4 wks Protracted cont inf: 200–400 mg/m^2/day IV	$T_{1/2\beta}$ 20 mins Excretion: urine (5%) and lung (90%)	Inactivated by DPD Hepatic and extrahepatic tissues (GI mucosa, WBCs, and kidney)
4. Capecitabine	Fluoropyrimidine carbamate prodrug form of FU	Single agent: 1250 mg/m^2 2/day Combination with oxaliplatin/irinotecan: 1000 mg/m^2 2/day Combination with gemcitabine: 830 mg/m^2 2/day Combination with docetaxel: 1250 mg/m^2 2/day Combination with RT: 825 mg/m^2 2/day	T_{max} 1.5 hrs Excretion: mainly renal The rate and extent of absorption are reduced by food Post 5-FU plasma levels after 2 hrs	Hepatic
5. Cytarabine (Ara-C)	Antitumor activity determined by the formation of the cytotoxic Ara-CTP metabolite	Standard dose: 100 mg/m^2/day IV on days 1–7 as cont IV inf, with anthracycline (induction for AML) HD: 1.5–3.0 g/m^2 IV q 12 hrs for 3 days (intensification regimen for AML) SC: 20 mg/m^2 for 10 days q 4 wks for 6 months, with IFN-alpha (for CML) IT: 10–30 mg up to +/- 3 times/wk (for leptomeningeal/carcinomatosis secondary to leukemia or lymphoma)	T_{max} 2–6 hrs $T_{1/2\alpha}$ 12 mins $T_{1/2\beta}$ 2–11 hrs 80% of dose in urine as Ara-U	Hepatic

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
No dose adjustments in pts with mild to moderate liver or renal dysfunction Relatively contraindicated in pts with active ischemic heart disease, or history of MI within previous 6 months	Leucovorin: enhances the antitumor activity and toxicity of 5-FU Thymidine and uridine rescue against toxic effects	Most common: myelosuppression; mucositis; and/or diarrhea HFS for cont inf Rare: cardiac symptoms of chest pain; ECG changes; and ↑ serum enzyme; conjunctivitis; and acute neurological symptoms (paresthesia, somnolence, and cerebellar ataxia)	Pts with early G3 or 4 myelosuppression, GI toxicity, and/or neurological toxicity may have an underlying deficiency in DPD. Therapy must be discontinued immediately If symptoms compatible with acute coronary syndrome, suspend 5-FU
Moderate renal dysfunction (baseline Cr Cl 30-50 ml/min): ↓ 25% dose Contraindicated in pts with Cr Cl <30 ml/min	Warfarin: coagulation parameters (PLT and INR) monitored Phenytoin: capecitabin may ↑ phenytoin blood levels Leucovorin: enhances the toxicity of capecitabine	Most common: diarrhea and HFS Rare: ↑ bilirubin (transient and asymptomatic); myelosuppression; neutropenic fever; mucositis; nausea and vomiting	Moderate to severe diarrhea: interrupt therapy; therapy to be stopped for G2 to 4 hyperbilirubinemia or G >2 adverse events until complete resolution or to G1. Treatment should continue at 75% of the initial starting dose If symptoms compatible with acute coronary syndrome, suspend
Caution in pts with abnormal liver and/ or renal function Monitor hepatic and renal function during therapy	Digoxin: Ara-C decreases its efficacy. Monitor digoxin levels MTX: the combination increases cytotoxicity Fludarabine, hydroxyurea: potentiate the cytotoxicity of Ara-C L-Asparaginase: ↑ risk of pancreatitis when Ara-C is given before asparaginase	Dose-limiting: myelosuppression, in the first wk lasts 7–14 days Most common: diarrhea, nausea, and vomiting Rare: neurological toxicity with clinical signs of cerebellar dysfunction (cerebellar ataxia), lethargy, and confusion HD can cause non-cardiogenic pulmonary edema, acute respiratory distress, and pneumonitis Ara-C syndrome (in pediatric pts with hematological malignancies, 12 hrs after start of drug infusion): fever; myalgia; bone pain; maculopapular rash; conjunctivitis; malaise; and occasional chest pain	Alkalinization of the urine (pH >7.0), rasburicase (0.2 mg/kg IV) or allopurinol (300 mg/24 hrs PO) and vigorous IV hydration are recommended to prevent tumor lysis syndrome in AML pts

Table 2 *Pharmacological aspects of pyrimidine analogs (Continued)*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
6. Gemcitabine	Intracellular activation to the cytotoxic triphosphate nucleotide metabolite dFdCTP, which inhibits DNA polymerases α, β, and δ; dFdCDP is a potent inhibitor of ribonucleotide reductase	Pancreatic cancer: 1000 mg/m^2 IV for 3 wks followed by 1 wk off Bladder cancer: 1000 mg/m^2 IV on days 1, 8, and 15 q 4 wks Non-small cell lung cancer: 1250 mg/m^2 IV on days 1 and 8 q 3 wks	With short infusions (<70 mins), $T_{1/2}$ 10–20 mins Excretion: 80% of dose as dFdU	Hepatic

Several mechanisms of resistance have been described:

- Deficiency of dCK, often by deletion of the gene encoding dCK
- Decreased intracellular half-life of Ara-CTP

Ara-C is used for the treatment of acute myeloid leukemia and is administered by rapid IV injection or continuous infusion. Ara-C can be given IT in cases of leptomeningeal involvement.

6. Gemcitabine

Gemcitabine is another deoxycytidine analog, with two fluorine atoms at the 2' position of the sugar. Gemcitabine is intracellularly activated by dCK to the monophosphate and triphosphate metabolite (dFdCTP). Incorporation of dFdCTP into DNA results in masked chain termination and inhibition of DNA synthesis and function. dFdCTP inhibits DNA polymerases α, β, and δ, which, in turn, interfere with DNA synthesis, repair, and chain elongation. Gemcitabine can also be incorporated into RNA.

The dFdCDP metabolite inhibits the enzyme ribonucleotide reductase, resulting in decreased levels of essential deoxyribonucleotides for DNA synthesis and function.

Gemcitabine is being used for the treatment of non-small cell lung cancer, usually in combination with cisplatin. It is also used for the treatment of pancreatic, bladder, and ovarian cancer.

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Caution in pts with abnormal liver and/or renal function	RT: gemcitabine is a potent radiosensitizer	Dose-limiting: myelosuppression, mainly thrombocytopenia Most common: diarrhea, nausea and vomiting, and transient ↑ AST. Fever in 40% of pts within the first 6–12 hrs after treatment Rare: hemolytic-uremic syndrome and skin rash. Severe pulmonary effects including edema, interstitial pneumonitis	

The known mechanisms of resistance are:

- Decreased expression of dCK
- Decreased nucleoside transport of drug into cells
- Increased expression of ribonucleotide reductase

Purine Analogs (Table 3)

7. Thiopurines

6-Mercaptopurine (6-MP) and 6-thioguanine (6-TG) are sulfur analogs of hypoxanthine and guanine, respectively. 6-MP and 6-TG are activated in their respective monophosphate forms in a reaction catalyzed by hypoxanthine–guanine phosphoribosyltransferase (HGPRT). Both drugs inhibit the enzymes involved in de-novo purine synthesis and purine interconversion reactions. The triphosphate forms can be directly incorporated into cellular RNA, with alterations in RNA processing and/or translation. The deoxynucleotide forms are also incorporated into DNA, with inhibition of DNA synthesis and function. Accumulation of thioguanine nucleotides (TGN), both ribo- and deoxyribonucleotides, in red blood cells is used for monitoring and relates to the efficacy of the thiopurines.

6-MP and particularly its nucleoside are excellent substrates for the enzyme thiopurine methyltransferase (TPMT), with formation of 6-methylmercaptopurine nucleotides which are less active than 6-MP nucleotides. Interin-

Table 3 *Pharmacological characteristics of purine analogs*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
7. 6-Thiopurines: 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG)	Both drugs inhibit enzymes involved in de-novo purine synthesis and purine interconversion reactions; accumulation as TGN, which can be incorporated into RNA and DNA	6-MP: In ALL: Induction: 2.5 mg/kg PO daily Maintenance: 1.5–2.5 mg/kg PO daily 6-TG: In leukemia: Induction: 100 mg/m^2 PO 2/day on days 1–5, usually in combination with cytarabine Maintenance: 100 mg/m^2 PO 2/day on days 1–5, q 4 wks (usually in combination) Single-agent: 1–3 mg/kg PO daily	6-MP: $T_{1/2}$ 50 mins Excretion: urine (50% of parent drug and metabolites) 6-TG: $T_{1/2}$ 80–90 mins Excretion: metabolites are eliminated in both feces and urine $T_{1/2}$ of 6-MP increased by allopurinol	6-MP: hepatic 6-TG: hepatic
8. Fludarabine	Rapidly dephosphorylated to 2-fluoro-ara-adenosine (F-ara-A)	IV: 25 mg/m^2 on days 1–5 q 4 wks PO: recommended 40 mg/m^2 on days 1–5 q 4 wks	$T_{1/2}$ 6.9–19.7 hrs Renal as major route of elimination Absorption not affected by food	Hepatic
9. Cladribine	Intracellularly activated to the cytotoxic Cd-ATP to be incorporated into DNA	0.09 mg/kg/day IV cont inf for 7 days	$T_{1/2}$ 5–7 hrs 20% bound to plasma protein Cleared by the kidneys	

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
6-MP: caution in pts with abnormal liver and/or renal function. Consider dose reduction In the presence of other hepatotoxic drugs, administer on an empty stomach and take at bedtime 6-TG: caution in the presence of other hepatotoxic drugs administer on an empty stomach 6-MP: must be reduced by 50–75% when given concurrently with allopurinol and with abnormal liver and/or renal function	6MP: Coumadin: anticoagulant effects of coumadin are inhibited by 6-MP Allopurinol: ↑ toxicity of 6-MP	6-MP and 6-TG: dose-limiting: myelosuppression Most common: nausea, vomiting, diarrhea; stomatitis and anorexia	
Use with caution in pts with abnormal renal function (dose ↓ according to Cr Cl) Pts are at increased risk for opportunistic infections (including herpes, fungus, and *Pneumocystis carinii*). Pts should be empirically placed on trimethoprim/sulfamethoxazole prophylaxis, 1 DS tablet 2/day 3 times/wk) Allopurinol prior to initiation of therapy to prevent hyperuricemia	Cytarabine: fludarabine may enhance the antitumor activity of cytarabine Cyclophosphamide, cisplatin, mitoxantrone: fludarabine may enhance antitumor activity Pentostatin: ↑ incidence of fatal pulmonary toxicity when fludarabine is used in combination with pentostatin. **Use of this combination is absolutely contraindicated**	Dose-limiting: myelosuppression; leukocyte nadir occurs in 10–13 days; autoimmune hemolytic anemia and drug-induced aplastic anemia Rare: opportunistic infections (fungus, herpes, and *Pneumocystis carinii*); tumor lysis syndrome; rash; vomiting; stomatitis; diarrhea; anorexia; renal dysfunction; transient ↑ transaminases; and pneumonitis	
Caution in pts with abnormal renal function ↑ risk for opportunistic infections (herpes, fungus, and *Pneumocystis carinii*) ↑ risk of tumor lysis syndrome in pts with a high tumor cell burden Allopurinol (before initiation of therapy	None known	Dose-limiting: 70% neutropenia after 1–2 wks with recovery in 4 wks; immunosuppression (↓ in CD4+ and CD8+ cells), 28% documented infections (fungus, herpes, and *Pneumocystis carinii*); 27% cutaneous rash and tumor lysis syndrome	

Table 3 *Pharmacological characteristics of purine analogs (Continued)*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
10. Clofarabine	Intracellularly activated to the cytotoxic cl-ATP to be incorporated into DNA	52 mg/m^2 IV over 2 hrs daily x 5 days q 2–6 wks	$T_{1/2}$ 5 hrs 50–60% of dose eliminated by renal clearance	

dividual TPMT activity is controlled by a common genetic polymorphism. Patients with low TPMT are more susceptible to 6-MP and 6-TG-induced myelosuppression.

The known mechanisms of resistance to 6-MP and 6-TG are:

- Decreased expression of the activating enzyme, HGPRT
- Decreased expression of mismatch repair enzymes (e.g. hMLH1, hMSH2)
- Increased expression of TPMT

6-MP is incompletely absorbed orally. The plasma half-life is approximately 50 mins; when allopurinol is given concomitantly, the dose of 6-MP should be reduced by 50% to 75%.

8. Fludarabine

Fludarabine is an analog of adenosine, but its sugar moiety is replaced by an arabinose sugar similar to that in cytarabine. Fludarabine has a fluorine atom at the 2' position in the base part of the molecule. Fludarabine is rapidly dephosphorylated to 2-fluoro-ara-adenosine (F-ara-A), which enters cells where it is re-phosphorylated by dCK, first to F-ara-A monophosphate and then to the active 5'-triphosphate metabolite (F-ara-ATP).

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Caution in pts with abnormal liver and/or renal function; avoid drugs with renal toxicity or liver toxicity	None known	Dose-limiting: myelosuppression Rare: capillary leak syndrome with tachypnea, tachycardia, pulmonary edema and hypotension; pericardial effusion; nausea/vomiting and diarrhea; ↑ serum transaminases and bilirubin; renal and cardiac toxicity	Pts to be closely monitored for evidence of tumor lysis syndrome and systemic inflammatory response syndrome (SIRS)/capillary leak syndrome Hydrocortisone 100 mg/m^2 IV on days 1–3 may prevent the development of SIRS or capillary leak syndrome

F-ara-ATP is then incorporated into DNA, with inhibition of DNA chain extension, ribonucleotide reductase, and DNA polymerase α and β. F-Ara-ATP is also incorporated into RNA, with inhibition of function, processing, and mRNA translation.

Fludarabine is used for the treatment of acute myeloid leukemia in combination with Ara-C and granulocyte–macrophage colony-stimulating factor (GM-CSF; the FLAG regimen) and for chronic lymphoblastic leukemia.

The known mechanisms of resistance are:

- Decreased expression of dCK, resulting in diminished intracellular formation of F-Ara- AMP
- Decreased nucleoside transport of drug for entry into cells

9. Cladribine

Cladribine is also an adenosine analog, but has a chlorine atom at the 2' position in the base and a normal sugar moiety. This modification makes it a poor substrate for degradation by adenosine deaminase. On entry into the cell, cladribine first undergoes conversion to cladribine monophosphate (Cd-AMP) by a reaction catalyzed by dCK, and is then metabolized to the active metabolite, cladribine triphosphate (Cd-ATP).

Cd-ATP is incorporated into DNA with inhibition of DNA chain extension and inhibition of DNA synthesis and function. Cd-ATP is a potent inhibitor of ribonucleotide reductase, which further facilitates inhibition of DNA biosynthesis. Cladribine is used for the treatment of hairy cell leukemia.

The major mechanism of resistance is a decreased expression by dCK, while increased dephosphorylation by nucleotidases and phosphatases also decreases its activity.

10. Clofarabine

Clofarabine is an adenosine analog with a chlorine atom at the 2' base position and a fluorine at the 2' sugar position and is also resistant to degradation by adenosine deaminase. Clofarabine requires intracellular activation by dCK to the monophosphate and to the cytotoxic triphosphate nucleotide: which (a) is incorporated into DNA with chain termination and inhibition of DNA synthesis and function; (b) inhibits DNA polymerases α and ß, interfering in turn with DNA chain elongation, DNA synthesis, and DNA repair; (c) inhibits ribonucleotide reductase, with decreased levels of essential deoxyribonucleotides for DNA synthesis and function.

The known mechanisms of resistance are a decreased expression of dCK and decreased intracellular transport. Clofarabine is used for the treatment of acute lymphoblastic leukemia.

Further Reading

Ackland SP, Clarke SJ, Beale P, et al. Thymidylate synthase inhibitors. Update Cancer Ther 2006; 1:403–427.

Peters GJ, Ackland S. New antimetabolites in preclinical and clinical development. Exp Opin Invest Drugs 1996; 5:637–679.

Peters GJ, Jansen G. Resistance to antimetabolites. Chapter 25 in: Principles of Antineoplastic Drug Development and Pharmacology (Eds: Schilsky RL, Milano GA, Ratain MJ). New York: Marcel Dekker Inc., 1996; 543–585.

Peters GJ, Jansen G. Antimetabolites. Chapter 4.16 in: Oxford Textbook of Oncology; 2nd edition (Eds: Souhami RL, Tannock I, Hohenberger P, Horiot J-C). Oxford, UK: Oxford University Press. Vol. 1, 2001; 663–713.

Topoisomerase-I and -II Inhibitors

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Topo-I Inhibitors (Table 1)

DNA topoisomerases (topos) are essential enzymes that regulate the topological state of DNA during cellular processes such as replication, transcription, recombination, and chromatin remodeling. Topoisomerase-I (Topo-I) is a ubiquitous nuclear enzyme highly conserved, which catalyzes the relaxation of superhelical DNA, generating a transient single-strand break (SSB) in DNA duplex, through cycles of cleavage and religation.

The function of Topo-I is to maintain the three-dimensional conformation of DNA by removing torsional stress generated during DNA replication and transcription, causing a transient enzyme bridge with DNA SSB (Topo-I cleavable complex, TOP1ccs), through which the intact strand can pass. Next, Topo-I reseals the cleaved strand and dissociates from the DNA so that the replication process can proceed. Inhibition of Topo-I activities is lethal and leads to cell death.

Deficiencies in the cell-cycle checkpoints and DNA-repair pathways determine cellular sensitivity to Topo-I inhibitors. Topo-I inhibitors exhibit S-phase cytotoxicity and G2/M cell-cycle arrest. An advancing replication fork and the inhibitor-trapped TOP1ccs, triggering replication fork arrest and breakage to generate DNA double-strand breaks (DSBs) and a covalent Topo-I–DNA complex, have been proposed to explain the S-phase cytotoxicity. In the presence of inhibitors, Topo-I is downregulated and targeted to the two-step ubiquitin/proteasome pathway.

The prescription of Topo-I inhibitors is currently based on the cancer histology and tissue of origin, rather than by the molecular characteristics of the tumors. However, current studies indicate that: (1) tumor expression levels of TOP1 protein or mRNA, (2) chromosome 20q amplification status, (3) sensitive biomarkers of Topo-I activity and strand breaks such as γ-H2AX, CHK2 phosphorylation, and ATM activation by autophosphorylation could be of interest in identification of tumor sensitivity.

The main mechanisms of resistance to Topo-I inhibitors include: (1) decrease of cellular uptake of drug due to changes in overexpression of MDR1 (P-glycoprotein), breast cancer resistance protein (BCRP), and/or other transporters, (2) alteration of the catalytic activity (point mutations of the Topo-I gene), or (3) decrease of Topo-I levels in tumor cells, as could happen after continuous exposure to Topo-I inhibitors. Other mechanisms of resistance are: increased drug inactivation by glucuronidation by UGTs, or oxidative metabolism by cytochrome P450 isozymes.

Camptothecins

Many Topo-I inhibitors are analogs of camptothecin (CPT), from the Chinese tree *Camptotheca acuminata*. Camptothecins interact with the enzyme Topo-I and bind covalently to and stabilize the TOP1ccs. The specific cytotoxicity can be attributed to the arrest of the replication fork by the stabilized drug–DNA–enzyme complex with DSBs and eventually cessation of RNA synthesis. Camptothecins are cell-cycle specific drugs for the S phase. The mechanism of action and cell-cycle specificity indicate that their activity should be highly schedule-dependent.

Water-soluble derivatives of camptothecin, topotecan and irinotecan (CPT-11), were successfully developed. A large number of CPT derivatives and non-CPT derivatives have been evaluated to overcome the limitations of known CPTs, such as lactone instability, reversibility of the drug interaction in the cleavable complex, and drug resistance. The peculiar features of the camptothecin structure (i.e. opening of the lactone ring) and of the mechanism of action (i.e. conversion of the SSBs into irreversible DSBs during S phase), indicate that prolonged drug exposure is critical to overcome the reversibility of the cleavable complex. Many of these compounds, evaluated in clinical trials, have shown an

improved pharmacological profile. Recent investigations have indicated that CPT-11 and topotecan are also inhibitors of angiogenesis, a property that might contribute to their antitumor activity.

1. Irinotecan (CPT-11)

Irinotecan (CPT-11) is a semisynthetic derivative of CPT, a Topo-I inhibitor. In preclinical studies, CPT-11 demonstrated significant antitumor activity in a broad spectrum of experimental human tumor xenografts and murine tumor models. The metabolic conversion of CPT-11 to the active metabolite, SN-38, is mediated by carboxylesterase enzymes and primarily occurs in the liver. The formed SN-38 is approximately 1000-fold more potent an inhibitor of Topo-I than CPT-11. Both irinotecan and SN-38 exist in an active lactone form (closed ring) and an inactive hydroxy acid anion form. A pH-dependent equilibrium exists between the two forms. An acid pH promotes the formation of the lactone, while a more basic pH favors the hydroxy acid anion form. Glucuronide conjugation by the hepatic uridine diphosphate glucuronosyltransferase (UPGT) is the major detoxification pathway of SN-38.

SN-38 is conjugated by the enzyme UDP-glucuronosyltransferase 1A1 (UGT1A1) to a glucuronide metabolite; UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. Plasma concentrations of SN-38 glucuronide (SN-38G) are usually about six-fold higher than those of SN-38.

CPT-11 is most active against gastrointestinal (GI) tumors and has predominantly GI and hematological toxicities. Significant pharmacokinetic variability occurs in patients with hepatic dysfunction, particularly those with glucuronidation pathway deficiencies. CPT-11 has been studied in clinical trials as a single agent in a variety of dosing schedules: weekly or once-every-3-weeks schedules of administration. The optimal administration schedule still remains uncertain.

The high interpatient variability in toxicity could be due to pharmacogenetic variations in drug metabolism.

Table I *Pharmacological aspects of topoisomerase-I inhibitors*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
1. Irinotecan (CPT-11) Semisynthetic derivative of camptothecin Water-soluble precursor of the lipophilic metabolite SN-38	Topoisomerase-I inhibition with production of double-strand DNA breaks	All schedules: IV inf 90 mins Single agent: weekly: 125 mg/m^2 x 4 q 6 wks q 3 wks: 300–350 mg/m^2 Combination: q 2 wks 180 mg/m^2	Linear PK not influenced by age, race, or gender Over the recommended dose range: 50–350 mg/m^2 AUC increases linearly with dose; AUC of SN-38 increases less than proportionally with dose $T_{1/2}$ 6–12 hrs $T_{1/2}$ of SN-38 10–20 hrs $T_{1/2}$ of lactone (active) forms of irinotecan and SN-38 similar to those of total irinotecan and SN-38, as the lactone and hydroxy acid forms are in equilibrium Excretion: 28% urinary and 24% fecal Plasma protein binding 30–68%. SN-38: highly bound 95% High interpt PK variability in pts with hepatic dysfunction, glucuronidation pathway deficiencies Pharmacogenetic variations in drug metabolism	Converted in liver to active/inactive metabolites by two pathways: (1) Carboxylesterase: to SN-38 (further inactivated to SN-38G (glucuronidation) (2) CYP3A: to oxidative metabolites: APC (500-fold less potent) and NPC (excreted in bile) Pharmacogenetic variations: lacking UGT1A1 ↑ SN-38 related toxicities Gilbert's syndrome with mutation UGTA1, ↓ SN-38 glucuronidation ↑ risk toxicity
2. Topotecan Semisynthetic water-soluble derivative of camptothecin	Topoisomerase-I inhibition with double-strand DNA damage	IV inf 30 mins Single agent: 1.5 mg/m^2 Schedule 1: days 1–5 q 3 wks Schedule 2: days 1–21, low-dose inf q 4 wks Combination: 0.75 mg/m^2 days 1–5 q 3 wks If G-CSF is used, start at least 24 hrs from last dose	PK: linear Large interpt variability Multiexponential decay two-compartment and one-compartment for topotecan and metabolite N-desmethyl-topotecan $T_{1/2}$ 2–3 hrs $T_{1/2}$ of metabolite much longer (8.8 and 2.8 hrs, respectively) Excretion: after IV inf: 50–70% urine and 18% feces Lactone distribution $T_{1/2}$ 6 mins Bioavailability: ~42% after PO	Active metabolite: N-desmethyl produced by CYP3A in plasma Binding plasma proteins: 7–35%

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Special population Guidelines for 3-wk schedule only Liver impairment: Bilirubin >1 mg/dL: keep dose <145 mg/m^2 Bilirubin NV but AST>3 x NV: start dose 225 mg/m^2 then ↑ if no toxicity Gilbert's syndrome: ↓ dose to 200 mg/m^2 q 3 wks Renal impairment: Cr Cl >1.6–3.5 mg/dL: start dose 225 mg/m^2 then ↑ if no toxicity	Hepatic enzyme inducers or inhibitors should be avoided With antiemetics: ↓ CYP3A metabolism With EIAs: ↑ Cl (phenytoin, carbamazepine, phenobarbital, pyrimidone, felbamate) With valproate: ↓ SN-38 glucuronidation With ciclosporin A: ↓ biliary excretion of CPT-11 and metabolites **Warning:** Concomitant anticonvulsant therapy Allowed: gabapentin, lamotrigine Not allowed: phenytoin, carbamazepine, phenobarbial, pyrimidone, felbamate	Major dose-limiting toxicities: myelosuppression, dual-phase diarrhea: Type I: early-onset diarrhea–cholinergic syndrome (EOD-CS) (rhinitis, ↑ salivation, miosis, lacrimation, diaphoresis, preventable by atropine [IV or SC, 0.25–1 mg]) Type 2: late-onset diarrhea (LOD) lasting for 5–7 days Schedule of administration to influence toxicity: Single agent intermittent: 22% LOD; 22% neutropenia; 15% asthenia; 14% nausea/vomiting; 12% EOD-CS; 12% CNS symptoms; 5% anorexia Weekly: 7% EOD-CS; 31% LOD; 31% neutropenia; 16% nausea; 14% asthenia; 12% vomiting; 7% anorexia; 2% CNS symptoms	Dosage adjustment follows different schedules of administration and grade of toxicity
Liver impairment: Bilirubin 1.7–15.0 mg/dL NO need of ↓ dose Renal impairment: mild, Cr Cl 40–60 ml/min: topotecan Cl_{TB} ↓ to about 67% of the value Moderate, Cr Cl 20–39 ml/min: topotecan Cl_{TB} ↓ to about 34% of value, ↑ $T_{1/2}$ estimated 5.0 hrs, ↓ dose by 50%	With platinum agents: sequence-dependent interaction: myelosuppression ↑ without apparent kinetic interactions and/or sequence dependent effects With DDP: ↑ neutropenia if topotecan given after DDP With EIAs: coadministration phenytoin: 30% ↑ Cl topotecan With PgP and BCRP inhibitors: possible ↑ AUC topotecan	Hematological toxicity: predictable, of short duration, non-cumulative Myelosuppression, primarily neutropenia, dose- and schedule-dependent; thrombocytopenia, anemia frequent Non-hematological: GI system, nausea, vomiting, and diarrhea; ↑ liver enzymes Other: alopecia, fatigue, asthenia and mucositis Extravasation: not cause of serious complications DL neutropenia 78% G4 after 10–12 days; 37% severe anemia after 15 days; 27% severe thrombocytopenia after 15 days; 32% diarrhea; 54% cumulative fatigue; 60% mild-moderate nausea/vomiting; 49% dose-related cumulative alopecia	Coadministration with platinum agent requires lower doses of each agent

Patients lacking an adequate UGT1A1 function are at greater risk for SN-38 related toxicities, especially diarrhea. Patients with Gilbert's syndrome, with deficient UGT1A1 activity, may be at increased risk of toxicity because of decreased SN-38 glucuronidation.

In-vitro resistance to CPT-11 has been shown to be due to mutations and reduced expression of the DNA Topo-1 gene.

2. Topotecan

Topotecan is a semisynthetic water-soluble derivative of CPT with the same mechanism of action. It is used to treat mostly ovarian and cervical cancer, small cell lung cancer (SCLC), neuroblastoma, and glioma. Topotecan has also demonstrated significant activity as second-line therapy in recurrent SCLC. The efficacy of the drug has been demonstrated to be dependent on the schedule of administration. A d × 5 infusion schedule was the best in ovarian cancer, even if no change in pharmacokinetic parameters was observed after administration on 5 consecutive days or as continuous infusion over 21 days.

Topotecan undergoes a reversible pH-dependent hydrolysis of its lactone moiety; the lactone form is pharmacologically active. At pH ≤4, the lactone is the only form present, whereas the ring-opened hydroxy acid form predominates at physiological pH. Topotecan is metabolized to an N-desmethylated metabolite by the CYP3A enzymatic system.

Topotecan lactone distribution is extremely rapid, with a distribution half-life of about 6 mins and volume of distribution at steady-state of approximately 75 L/m^2, suggesting that the lactone form is extensively bound to tissue components. Detectable plasma levels were found for an extended period post-infusion, suggesting slow diffusion of topotecan out of the tissue. Topotecan significantly penetrates into the cerebrospinal fluid.

Alterations in plasma clearance (Cl_{TB}) and mean half-life are present in patients with renal and hepatic impairment and dosage adjustments are recommended.

Topo-II Inhibitors

Topoisomerase-II (Topo-II) enzyme mediates the ATP-dependent induction of nicks in both strands of the DNA duplex, allowing relaxation of superhelical DNA during DNA replication and transcription. Topo-II exists as two highly homologous isoforms, alpha and beta, which differ in their production during the cell cycle. The expression of alpha isoform increases two- to three-fold during G2/M, and is quantitatively higher in rapidly proliferating than in quiescent cells.

The common mechanism of action of Topo-II inhibitors is the "poisoning" of the alpha isoform of the enzyme. These agents interfere with enzyme functions by stabilizing a reaction intermediate, in which DNA strands are cut and covalently linked to tyrosine residues of the protein forming a cleavable complex. The stabilization of this complex poisons the enzyme, transforming it into a DNA-damaging agent that leads to the formation of DSBs. The resultant DNA damage is a critical signal for NF-κB activation and induction of apoptosis.

Most of the Topo-II targeted drugs develop cellular resistance through: (1) mutation in Topo-II gene, (2) decreased Topo-II expression, and (3) overexpression of P-glycoprotein (PgP) and other drug transporters conferring multidrug resistance (MDR).

Different classes of compounds have been demonstrated to target DNA-Topo-II: (1) agents that likely bind directly to the enzyme, such as epipodophyllotoxins, and (2) agents that intercalate in DNA, such as anthracyclines, aminoacridines, and anthracenediones.

Anthracyclines (Table 2)

Anthracycline antibiotics are commonly used antineoplastic agents with activity against breast cancer, leukemias, lymphomas, and sarcomas. These chemotherapeutic agents produce a wide range of biological reactions. The most important are four: (1) Topo-II inhibition, (2) DNA intercalation, (3) helicase inhibition, and (4) one- and two-electron reduction of the anthracycline molecule with production of reactive oxygen species (ROS; O_2^-, H_2O_2, and OH^-), which damage macromolecules such as DNA and lipid membranes.

Table 2 *Pharmacological aspects of anthracyclines and anthracenediones*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
3. Doxorubicin (DOX)	1. Inhibition of topoisomerase-II 2. DNA intercalation 3. Inhibition of helicase 4. One- and two-electron reduction producing reactive compounds ($O_2^{\cdot}$, H_2O_2 and $OH^{\cdot}$), also induced by Fe^{2+} and Cu^{2+} Cardiomyopathy related to 4 Involved in MDR	IV (10–15 mins) Single agent: 60–75 mg/m^2 q 3 wks; 20 mg/m^2 once weekly (↓ cardiotoxicity); 30 mg/m^2 daily on days 1–3 q 4 wks In combination: 40–60 mg/m^2 q 3–4 wks Recommended maximum cumulative dose: ≤450 mg/m^2; 300–400mg/m^2 if cardiac risk factors For weekly bolus: ≤700 mg/m^2; 550 mg/m^2 if cardiac risk factors	Rapid and extensive distribution Triphasic plasma elimination $T_{1/2\gamma}$ 30–35 hrs for both DOX and DOX-OL 74–76% protein bound Excretion: feces (40%) and urine (5-12%) in 7 days Doxorubicin does not cross the blood–brain barrier	1. Reduction to 13(S)-dihydro derivative, doxorubicinol (DOX-OL), in liver and other tissues mainly by cytoplasmic NADPH-dependent aldo-keto reductases 2. Loss of the amino sugar moiety through a hydrolytic process with the formation of aglycones of DOX and DOX-OL 3. Loss of the amino sugar moiety through a redox process with the formation of 7-deoxyaglycones
4. Epirubicin (EPI)	Same as DOX Mechanism 4 is less important due to metabolic inactivation by glucuronidation Involved in MDR	IV (10-15 mins) Single agent: 100–120 mg/m^2 q 3 wks In combination: 90–100 mg/m^2 EPI dose can be divided between days 1 and 8 Recommended maximum cumulative dose : ≤900 mg/m^2	Rapid and extensive distribution Triphasic plasma elimination $T_{1/2\gamma}$ 20–25 hrs for both EPI and EPI-OL Linear pharmacokinetics 77% protein bound Excretion: feces (50%) and urine (<20%)	1. Reduction to 13(S)-dihydro derivative, epirubicinol (EPI-OL) 2. Conjugation of EPI and EPI-OL with glucuronic acid (in liver) 3. Loss of the amino sugar moiety through a hydrolytic process with the formation of aglycones 4. Loss of the amino sugar moiety through a redox process with the formation of 7-deoxyaglycones EPI-OL in vitro cytotoxicity = 1/10 that of EPI
5. Daunorubicin (DNR)	Same as DOX	IV bolus (10–15 mins) Single agent: 60 mg/m^2 on days 1–3 q 4 wks In combination: 30–45 mg/m^2 on days 1–3 q 4 wks Recommended maximum cumulative dose: ≤550 mg/m^2; 300–400 mg/m^2 if cardiac risk factors	Rapidly and widely distributed in tissues $T_{1/2\gamma}$ DNR 19 hrs $T_{1/2\gamma}$ DNR-OL 27 hrs DNR-OL has longer $T_{1/2}$ and higher AUC than DNR Excretion: feces (40%) and urine (25%) It does not cross the blood–brain barrier, but it apparently crosses the placenta	1. Reduction to 13(S)-dihydro derivative, daunorubicinol (DNR-OL) 2. 4'-O demethylation, reduction, cleavage of the glycosidic bond 3. Conjugation with both sulfate and glucuronic acid in liver DNR-OL has high antineoplastic activity

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal: NE Hepatic: if bilirubin >1.25–2 x ULN, ↓ dose by 50%. If bilirubin >2–4 x ULN, ↓ dose by 75%	Hepatic enzyme inducers or inhibitors should be avoided PK interaction with: Paclitaxel: ↓ DOX Cl_{TB} (sequence dependent) Ciclosporin (MDR modulator): ↓ DOX Cl_{TB} and metabolism Phenobarbital: ↑ DOX C_{ITB} Phenytoin: ↓ phenytoin serum concentration Calcium-channel blocking agents (e.g. verapamil): ↑ cardiotoxicity Dexrazoxane, as cardioprotectant, only after cumulative dose of 300 mg/m^2 to avoid lower tumor response	Hematological: neutropenia (after 10–14 days, recovering in 3 wks) Gastrointestinal: nausea/vomiting; mucositis Skin: alopecia; infusion-related reaction Endocrine: amenorrhea Cardiotoxicity: acute or delayed cardiomyopathy (5–8% at a dose of 450 mg/m^2 and 6–20% at a dose of 500 mg/m^2 given as IV bolus once q 3 wks) Risk of secondary AML or MDS: in combination with cyclophosphamide and radiotherapy (0.21% at 5 yrs and 1.5% at 8 yrs)	If nadir PLT counts <50 000/mm^3, ANCs <250/mm^3, neutropenic fevers, or G3 or 4 nonhematological toxicity, ↓ dose in subsequent cycles by 25% Delay subsequent cycles until PLT counts are ≥100 000/mm^3, ANCs are ≥1500/mm^3, and nonhematological toxicities have recovered to G1 or better Discontinue treatment if LVEF >10% ↓ of baseline
Hepatic: if bilirubin 1.2–3 mg/dL or AST 2–4 x NV, ↓ dose by 50% If bilirubin >3 mg/dL or AST >4 x NV, ↓ dose by 75% Bone marrow: consider decreasing dosage for the initial cycle to 75 mg/m^2	Hepatic enzyme inducers or inhibitors should be avoided PK interactions with: Taxanes: prior to EPI ↑ AUC of EPI and metabolites, ↑ myelosuppression Trastuzumab: can ↑ the risk of developing heart dysfunction	Hematological: 80% neutropenia; 72% anemia; 48% thombocytopenia Gastrointestinal: 92% nausea/vomiting; 58% mucositis Skin: 95% alopecia Endocrine: 72% amenorrhea Cardiotoxicity: same as DOX; 0.9% at a cumulative dose of 550 mg/m^2, 1.6% at 700 mg/m^2, and 3.3% at 900 mg/m^2 Secondary AML/MDS: 0.27% at 3 yrs, 0.46% at 5 yrs, and 0.55% at 8 yrs	Same as DOX If EPI dose is divided between days 1 and 8, ↓ day 8 dose by 25% if PLT counts and ANCs are 75 000–100 000 and 1000–1499/mm^3, respectively. If day 8 PLT counts or ANCs are <75 000 or 1000/mm^3, respectively, or G3 or 4 nonhematological toxicity has occurred, omit day 8 dose
Renal: if serum creatinine >3 mg/dL, ↓ dose by 50% Hepatic: if bilirubin 1.2–3 mg/dL, ↓ dose by 25% If bilirubin >3 mg/dL, ↓ dose by 50%	Ciclosporin: ↓ DNR Cl_{TB} Hepatotoxic drugs (high-dose MTX) may ↑ the risk of toxicity	Same as DOX Myelosuppression (after 10–14 days, recovering in 3 wks) Nausea and vomiting; mucositis Cardiotoxicity: acute and cumulative as for DOX; children are more susceptible	

Table 2 *Pharmacological aspects of anthracyclines and anthracenediones (Continued)*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
6. Idarubicin (IDA)	Same as DOX: mechanism 4 is less important	IV bolus (10–15 mins) Single agent or in combination with cytarabine: 12 mg/m^2 on days 1–3 q 4 wks Oral formulation: single agent: leukemia: 25 mg/m^2 on days 1–3 q 4 wks Recommended maximum cumulative dose: IV ≤150 mg/m^2 OS not defined (400 still safe); ↓ dose if cardiac risk factors	Rapid distributive phase, ↑ volume of distribution Bi- or triphasic plasma elimination $T_{1/2\gamma}$ 22 hrs single agent $T_{1/2\gamma}$ 20 hrs in combination with cytarabine $T_{1/2\gamma}$ of IDOL 45–72 hrs Plasma level sustained greater than 8 days 97% protein bound for IDA 94% protein bound for IDOL Excretion: feces and lesser extent urine Oral formulation: F 30–50%. First-pass effect: ↑ AUC ratio IDOL/IDA	Reduction to 13(S)-dihydro derivative, idarubicinol (IDOL) It has cytotoxic activity and presumably contributes to the effects of IDA
7. Liposomal doxorubicin Doxil, Caelyx	Same as DOX	IV inf (60 mins) Single agent: 50 mg/m^2 q 4 wks First inf at 1 mg/min to minimize risk of reaction AIDS-KS: 20 mg/m^2 q 3 wks (IV inf 30 mins) Recommended maximum cumulative dose: not defined Monitor cardiac function after 600 mg/m^2 in naive or 450 mg/m^2 in DOX-pretreated pts	Longer $T_{1/2}$ and tumor tissue accumulation than DOX Biphasic plasma elimination $T_{1/2\beta}$ mean 74 hrs Low distribution volume Vd: 1.9–2.8 L/m^2 Cl_{TB}: 0.030 L/h/m^2 Linear PK at 10–20 mg/m^2 Non-linear PK at 20–60 mg/m^2	Reduction to 13(S)-dihydro derivative, doxorubicinol (DOX-OL)

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal: if serum Cr >1.5 x ULN, ↓ dose by 50% Liver: LFTs >1.25–2 x ULN, ↓ dose by 50%; LFTs >2–5 x ULN, ↓ dose by 75%. Bilirubin >5 mg/dL, avoid administration	Hepatic enzyme inducers or inhibitors should be avoided Ciclosporin: ↓ IDA Cl_{TB}	Same as DOX Severe myelosuppression (after 10 days; recovering in 2 wks); 82% nausea and vomiting; 50% mucositis; 75% alopecia and dermatological reaction Rare: severe enterocolitis Cardiotoxicity: cumulative dose associated to risk Oral formulation: 60% nausea and vomiting; 15% diarrhea; 40% alopecia	Same of DOX Severe mucositis: ↓ dose by 25%
Renal: no modification if Cr Cl 30–156 ml/min Hepatic: at cycle 1: if bilirubin 1.2–3 mg/dL, ↓ dose by 25%. If bilirubin >3 mg/dL, ↓ dose by 50% At second cycle, if first well tolerated: ↑ dose by 25%	Be careful in using concomitant medications known to interact with standard DOX It could potentiate the toxicity of other anti-cancer therapies	Hematological: thrombocytopenia; anemia; neutropenia Gastrointestinal: 46% nausea; 33% vomiting; 41% stomatitis; 40% asthenia Skin: 45% dose-dependent cumulative skin toxicity with palmar–plantar erythrodysesthesia (hand–food syndrome); 28% rash; 20% alopecia Cardiotoxicity: <10%; lower risk compared to DOX	Adverse reactions, such as HFS, hematological toxicities, and stomatitis may be managed by dose delays and adjustments

Table 2 *Pharmacological aspects of anthracyclines and anthracenediones (Continued)*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
7. Liposomal doxorubicin Myocet	Same as DOX	IV inf (60–90 mins) In combination with CPA: 60–75 mg/m^2 q 3 wks Recommended maximum cumulative dose: in combination with CPA >1260 mg/m^2	Longer $T_{1/2}$, ↓ Cl_{TB} and ↓ Vd than DOX $T_{1/2}$ 56.2 hrs Cl_{TB} 5.6 L/h/m^2 Vd 82 L/m^2 Excretion: mainly by feces	Reduction to 13(S)-dihydro derivative, doxorubicinol (DOX-OL), by aldo-keto reductase
8. Mitoxantrone (DHAD)	1. Inhibition of Topo-II 2. DNA intercalation 3. Free radical production, less important than DOX Involved in MDR phenomenon	IV bolus (5–15 mins) Single agent: 12–14 mg/m^2 q 3 wks In combination: ↓ dose of single agent by 2–4 mg/m^2 Recommended maximum cumulative dose: ≤140 mg/m^2; 120 mg/m^2 if cardiac risk factors	Extensive distribution to tissues: Vd >1000 L/m^2 ↑ tissue concentrations during the terminal elimination phase Triphasic plasma elimination $T_{1/2\gamma}$ 75 hrs Linear PK in the range 15 to 90 mg/m^2 78% protein bound Excretion: feces (25%) and urine (11%) in 5 days (65% unchanged, 35% as metabolite)	Hepatic (CYP2E1)

The anthracyclines have similar pharmacokinetic properties. They show triphasic plasma distribution and elimination occurs through hepatic metabolism and biliary excretion. Urinary excretion of intact drug accounts for less than 10% of anthracycline clearance. Anthracyclines are mainly metabolized in liver to 13-dihydro (alcohol) derivatives and, through hydrolytic or redox loss of the amino sugar moiety, to aglycones.

The acute, dose-limiting toxicity of anthracyclines is myelosuppression, more frequent with infusion than with bolus. Other acute toxicities include nausea, vomiting, alopecia, mucositis, and severe local tissue reactions if extravasation occurs during infusion. The most serious toxicity associated with anthracyclines is cardiotoxicity, manifested by early (acute) or late (delayed) events. Early cardiotoxicity consists mainly

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Hepatic: if bilirubin <ULN and ↑ AST, ↓ dose by 25%; if bilirubin > ULN but < 50 µmol/L, ↓ dose by 50%; if bilirubin >50 µmol/L, ↓ dose by 75%	As for standard DOX Concomitant therapy with other liposomal or lipid-complexed substances could interfere with PK	Hematological: 46% neutropenia; 31% thrombocytopenia; 30% anemia, neutropenic fever Gastrointestinal: 73% nausea/vomiting; stomatitis; 42% mucositis Cardiotoxicity: CHF 2% vs 8% for standard DOX	Same as DOX for hematological toxicity If painful erythema, edema, or ulcers and cannot eat: wait 1 wk and ↓ dose by 25% If parenteral or enteral support is required: wait 1 wk and ↓ dose by 50%
Renal: unknown Hepatic: if bilirubin >3.4 mg/dL, ↓ dose Bone marrow: initial dose <12 mg/m^2	Ciclosporin: Cl_{TB} of DHAD ↓ by 42% Hydantoins: plasma concentrations may be reduced ↓ by DHAD	Hematological: 100% neutropenia (after 12 days, recovery in 1 wk); 39% thrombocytopenia Gastrointestinal: 72% nausea/vomiting; 47% diarrhea; 29% mucositis Skin: 61% alopecia Endocrine: 53% amenorrhea Cardiotoxicity: 18% arrhythmia; 11% abnormal ECG; cardiac ischemia, CHF; 5% decreased LVEF; 4% hypertension Secondary leukemia: 1.1% and 1.6% at 5 and 10 yrs when used with other DNA-damaging cytotoxics and RT	If nadir PLT <25 000/mm^3, ANCs <1500/mm^3 suspend the treatment If PLT <50 000/mm^3, ANCs <1500/mm^3 after recovery ↓ dose by 2 mg/m^2 If PLT <25 000/mm^3, ANCs <1000/mm^3 after recovery ↓ dose by 4 mg/m^2 Discontinue therapy in pts with LVEF <50% or a clinically important reduction in LVEF

of sinus tachycardia and/or electrocardiogram (ECG) abnormalities. Late-onset cardiomyopathy appears months to years after treatment is completed and is manifested by a reduction in left ventricular ejection fraction (LVEF) and/or signs and symptoms of congestive heart failure. Cardiac damage is directly related to the cumulative total dose administered (different for each anthracycline) and to the administration schedule, with bolus administration causing greater risk than continuous infusions. Risk factors are active or dormant cardiovascular disease, prior or concomitant radiotherapy to the mediastinal/pericardial area, previous therapy with other anthracyclines or anthracenediones, and concomitant use of other cardiotoxic drugs.

Multiple mechanisms of anthracycline-induced cardiotoxic effects have been proposed. The principal one is the generation of oxygen free radicals (O_2^-, H_2O_2, and OH^-) involving iron–anthracycline complexes that damage cellular membranes of cardiomyocytes. Free radicals also enhance endothelial nitric oxide synthase, leading to nitric oxide and peroxynitrite formation. This mechanism has been linked to nitration and inactivation of key enzymes for heart functionality, including myofibrillar creatine kinase. In addition, secondary alcohol metabolites of anthracyclines (DOX-OL, EPI-OL, etc), which accumulate in the heart, aggravate anthracycline's toxicity, dysregulating calcium and iron homeostasis. Antioxidant agents apparently do not show cardioprotective effects, apart from the iron chelator dexrazoxane (Zinecard). Secondary acute myeloid leukemia (AML) has been reported in patients with breast cancer treated with anthracyclines. These leukemias can have a 1- to 3-year latency period and are more common when anthracyclines are given in combination with DNA-damaging antineoplastic agents or with radiotherapy and when patients have been heavily pretreated with cytotoxic drugs. Anthracycline resistance is mainly related to MDR with the overexpression of the active ABC transporter PgP.

3. Doxorubicin (DOX)

DOX is the most commonly used anthracycline in the treatment of lymphomas, breast cancer, and soft tissue sarcomas. Its use is limited by the cardiotoxic effects. The probability of developing impaired myocardial function, based on a combined index of signs and symptoms, is estimated to be low (1% to 2%) at a total cumulative dose of 300 mg/m^2 of DOX, but rapidly increases over 400 mg/m^2 reaching an incidence of 6% to 20% at 500 mg/m^2. Cardiotoxicity may occur at lower cumulative doses if DOX is given in combination with agents like taxanes, trastuzumab, or cyclooxygenase-2 inhibitors (coxibs). The incidence of cardiac dysfunction was 27% in patients receiving DOX–cyclophosphamide–trastuzumab. It is assumed that trastuzumab blocks the protective role of the ErB/neuregulin system on myocardium; HER2 function is required for the repair of oxidative damage caused by DOX, and its inactivation increases heart vulnerability to these compounds.

4. Epirubicin (EPI)

EPI differs from DOX only in the spatial orientation of the 4' OH on daunosamine. EPI is similar to DOX in its antitumor activity, but less potent: the dose for equivalent antitumor effect is 1.2–1.5 greater than that of DOX. EPI causes slightly less cardiac toxicity than DOX, probably in relation to its different metabolic pathway. The unchanged drug and the 13-dihydro metabolite, epirubicinol (EPI-OL), undergo conjugation with glucuronic acid and this metabolic transformation represents an additional detoxification way. In relation to this additional metabolic pathway, the terminal half-life and volume of distribution of EPI appeared to be smaller, whereas plasma clearance and cumulative urinary excretion were larger in comparison to DOX.

Given the relative risk of cardiomyopathy, a cumulative dose of 900 mg/m^2 of EPI should be exceeded only with extreme caution.

5. Daunorubicin (DNR)

DNR is less commonly used than DOX. It has minimal activity in solid tumors but it is the drug of choice for initial therapy of AML and is an important drug in the treatment of acute lymphoblastic leukemia (ALL) of children and adults. A combination of 3 days of intravenously administered DNR and 7 days of Ara-C produced complete responses in 40–80% of patients with AML.

DNR is more rapidly metabolized to its alcohol metabolite than DOX and EPI. Daunorubicinol (DNR-OL) is six times more cardiotoxic than the parent compound: DNR is responsible for about 25% of cardiac damage while DNR-OL causes the remaining 75%. Total DNR doses associated with cardiac toxicity are slightly higher (650 mg/m^2) than for DOX (450 mg/m^2).

6. Idarubicin (IDA)

IDA is a DNR analog (demethoxy-DNR) that is 6–8-fold more potent than the parent drug in animal models. The absence of a methoxy group at position 4' of the anthracycline structure gives the compound a high lipophilicity that results in an increased rate of cellular uptake compared

with other anthracyclines. The peak of IDA concentrations in nucleated blood and bone marrow cells in leukemia patients is reached a few minutes after injection and concentrations of IDA and its metabolite IDOL are more than a hundred times higher than plasma concentrations. IDOL derivative has a longer half-life than IDA and comparable activity. The high lipophilicity of IDA led to the development of an orally administered anthracycline. This formulation demonstrated a significant interpatient variability in oral absorption, limiting its clinical use.

IDA is used only in the treatment of AML. Despite the preclinical demonstration of greater antitumor activity, IDA has proved to be only slightly more active than DNR in AML treatment, without significant differences in toxicity.

7. Liposomal anthracyclines

Two liposomal formulations of DOX (Doxil/Caelyx and Myocet) and one of DNR (DaunoXone) are currently available. By encapsulating in liposomes, anthracyclines have a longer half-life and may preferentially accumulate in tumor tissue, thereby providing selectivity. The nature and extent of these alterations depends on the lipids used in the liposome formulation. Myocet carries DOX in phosphatidylcholine and cholesterol, while Doxil (Caelyx in Europe and Canada) utilizes pegylated lipids. Myocet releases half of its DOX within 1 hr and 90% within 24 hrs. In contrast, Doxil releases less than 10% of DOX within 24 hrs (half-life of 45–90 hrs). The change in drug release alters the toxicity profile. Myocet causes myelosuppression and mucositis while Doxil therapy produces palmar–plantar erythrodysesthesia (PPE) and mucositis. DaunoXone has a half-life of 5.3 hrs with low concentration of DNR and DNR-OL persisting in plasma for 72 hrs following administration. It demonstrates dose-limiting toxicity of febrile neutropenia.

Studies have suggested that pegylated liposomal doxorubicin (PLD) causes less cardiomyopathy than free DOX. PLD appears to provide comparable antineoplastic efficacy against breast cancer compared to DOX with less nausea, vomiting, and alopecia, but has a significantly increased risk of PPE (hand–foot syndrome) and mucositis. Four types of skin toxicity have been reported with PLD: hand–foot syndrome (40%

of patients), diffuse follicular rash (10%), impetigo-like eruption (8%), and formation of new melanotic macules (5%).

Pharmacokinetic and tissue distribution data demonstrated that DOX encapsulated in liposomes, in contrast to free DOX, exhibited comparable plasma elimination and tissue distribution in the presence or absence of ciclosporin, a P-glycoprotein inhibitor. This suggests that liposomal formulations could overcome the excretion pathways dependent on P-glycoprotein and gives a new opportunity to treat MDR-resistant tumors.

PLD has activity against breast cancer, Kaposi's sarcoma, and ovarian cancer.

Anthracenediones (Table 2)

Anthracenediones are Topo-II inhibitors, derivatives of 9,10-anthraquinone developed to reduce heart damage related to anthracyclines.

8. Mitoxantrone (dihydroanthracenedione, DHAD)

DHAD lacks the ability to form the quinone-type free radicals responsible for anthracycline cardiotoxicity. DHAD is primarily used for the treatment of breast cancer, prostate cancer, leukemia (myelocytic), and lymphoma. It has replaced DOX in selected chemotherapy regimens in which it demonstrated lower toxicity but also lower antitumor activity. The primary toxicities of DHAD are myelosuppression, nausea, vomiting, and cardiac toxicity. A minor, but sometimes worrisome, side effect of DHAD is a bluish discoloration of the sclera, fingernails, and urine. At equivalent myelotoxic doses (75 mg/m^2 DOX versus15 mg/m^2 DHAD), nausea, vomiting, and hair loss are less frequent with DHAD than with DOX. The cumulative dose that can be administered before the development of heart failure is double compared to DOX.

Epipodophyllotoxins (Table 3)

Epipodophyllotoxins are alkaloids naturally occurring in the root of the American mayapple plant (*Podophyllum peltatum*). The two analogs with the best antitumor activity are etoposide (VP16) and teniposide

Table 3 *Pharmacological aspects of epipodophyllotoxins and aminoacridines*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
9. Etoposide (VP16) Vepesid	Inhibition of Topo-II by direct binding to the enzyme Involved in MDR phenomenon	IV inf (30–60 mins) Single agent: 60–120 mg/m^2/day on days 1–5 or on day 1, 3, 5 q 3–4 wks HD (500 mg/hr Single agent: 60 mg/kg; 3000 mg/m^2 (MTD) In combination: 400–800 mg/m^2 on days 1–3 Oral formulation: 100 mg (50 x 2) daily. Untreated pts: days 1–14. Pretreated pts: days 1–10 q 4 wks	Biphasic plasma distribution $T_{1/2b}$ 4–11 hrs Cl_{TB} 16–26 Linear PK also at high doses No accumulation following daily doses of 100 mg/m^2 for 4–5 days 97% protein bound Excretion: feces (44%) and urine (56%) in 120 hrs (45% unchanged) Enters the CSF poorly F dose-dependent: 40–80% up to 200 mg total dose, lower at >200 mg	1. Hydroxy acid metabolite is found in urine 2. Glucuronide and/or sulfate conjugates also excreted in urine 3. 0-demethylation of the dimethoxyphenol ring occurs through the CYP450 3A4 isozyme pathway to produce the corresponding catechol (1–2%) with cytotoxic effect
9. Etoposide Phosphate Etopophos	Same as etoposide	IV inf (5 mins – 2 hrs) Single agent: 50–100 mg/m^2/day on days 1–5 or 100 mg/m^2/day on days 1, 3, 5 q 3–4 wks In combination: 35–50 mg/m^2/day for 4 or 5 days HD (2 hr inf): 1000 mg/m^2 on days 1 and 2	Same as etoposide Molar equivalent doses of Etopophos and Vepesid produce similar AUC and C_{max} Bioavailability is 100%	Same as etoposide
10. Teniposide (VM26)	Same as etoposide	IV inf (30–60 mins) Single agent: 60 mg/m^2 on days 1–5 q 3–4 wks In combination: ALL children: 165 mg/m^2 x 2/wk x 4 (with Ara-C) Pts who failed induction therapy: 250 mg/m^2 weekly x 4–8 wks (with vincristine and prednisone)	Triphasic plasma distribution $T_{1/2\gamma}$ 20 hrs 99% protein bound Excretion: urine (44%) in 120 hrs; feces (0–10%) in 72 hrs	86% of drug is metabolized by liver (metabolites mostly unknown)

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal: Cr Cl <50 ml/min, ↓ dose by 25%; Cr Cl <15 ml/min, further ↓ dose Hepatic: mild to moderate dysfunction does not require ↓ dose Low serum albumin may ↑ risk for associated toxicities	Ciclosporin: ↓ Cl_{TB} Ketoconazole: ↑ VP16 plasma concentrations Phenylbutazone, sodium salicylate, and aspirin: displaced protein-bound VP16 at concentrations achieved in vivo	Hematological: leukopenia (after 5–15 days, recovery 3–4 wks; WBC/mm^3 <4000) Gastrointestinal: 43% nausea/vomiting; 13% diarrhea; 6% mucositis Skin: 66% alopecia Hypersensitivity: 2% allergic reaction including anaphylaxis-like symptoms Risk of acute non-lymphocytic leukemia with 11q23 chromosomal translocation, short latency period (mean 32 months), ↑ risk for cumulative doses > 2000 mg/m^2	In case of serious and potentially fatal hypersensitivity reactions: epinephrine, with or without corticosteroids and antihistamines If hypotensive or bronchospasm reactions occur, use slower rate of infusion and monitor carefully
Same as etoposide	Same as etoposide Caution should be exercised when administering with drugs that are known to inhibit phosphatase activities (e.g. levamisole hydrochloride)	Same as etoposide	Same as etoposide
Hepatic: if bilirubin 1–2.5 mg/dL, ↓ dose by 50%. If bilirubin >2.5 mg/dL, ↓ dose by 75%	Barbiturates and phenytoin: ↑ VM26 Cl_{TB}	Hematological: 95% neutropenia (after 7–10 days, recovering within 1 wk); 98% leukopenia; 85% thrombocytopenia; 88% anemia Gastrointestinal: 29% nausea/vomiting; 33% diarrhea; 76% mucositis Skin: 9% alopecia Hypersensitivity: bronchospasm; chills; dyspnea; fever; flushing; hypertension or hypotension; 5% tachycardia 12% infections Secondary leukemias: as for etoposide	Same as etoposide

Table 3 *Pharmacological aspects of epipodophyllotoxins and aminoacridines (Continued)*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
11. Amsacrine (m-AMSA)	1. Inhibition of Topo-II 2. DNA intercalation 3. One- and two-electron transfer to DNA	IV (60–120 mins) Induction: 75–125 mg/m^2 on days 1–5 q 3–4 wks In combination: 70–90 mg/m^2 on days 1–5 q 3–4 wks	Biphasic plasma distribution $T_{1/2\ \beta}$ 5–9 hrs 96–98% protein bound Excretion: urine (35%) in 72 hrs (12–20% intact drug); feces (8–36%) Does not significantly penetrate into the CNS	No studies of the metabolism of m-AMSA in humans published In animals: hepatic and extensive with production of alkylthiol derivative which is still cytotoxic. Much of m-AMSA was excreted in urine and bile as metabolites

(VM26). While many podophyllotoxin derivatives are inhibitors of tubulin polymerization, and therefore of mitotic spindle production, VP16 and VM26 are Topo-II alpha inhibitors. Both drugs damage DNA by direct interaction (without intercalation) with Topo-II to form cleavable complexes that prevent religation of DNA, leading to DSBs. It has been proposed that the Topo-II–DNA covalent complex triggers 26S proteasome-mediated degradation of Topo-II beta. Resistance to epipodophyllotoxins arises through multiple mechanisms: (1) mutations at Ser-1106 in the Topo-II molecule, (2) rapid repair of DNA breaks through the single-strand invasion pathway of homologous recombination or by non-homologous DNA end-joining, and (3) MDR phenotype.

9. Etoposide (VP16) and etoposide phosphate

VP16 is poorly soluble in water. For IV use, VP16 is dissolved in a solubilizer composed of polysorbate 80, polyethylene glycol, and alcohol; these additives are responsible for the hypersensitivity reactions occasionally seen with VP16 infusion.

The antitumor activity is highly dependent on the schedule of drug administration. A dose of 100 mg/m^2 of VP16 given to SCLC patients daily for 5 days had a significantly greater response rate compared to a 24 hr infusion of 500 mg/m^2 (89% versus 10%), despite producing similar AUCs.

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal: BUN >20 mg%, ↓ dose. Creatinine >1.2 mg%, ↓ dose Hepatic: bilirubin 1–2.5 x ULN or AST 2–5 x ULN, ↓ dose by 50%. Bilirubin >2.5 x ULN or AST >5 x ULN, ↓ dose further or discontinue	Incompatible with chloride ion	Hematological: neutropenia (after 11–13 days, recovering in 7–10 days); thrombocytopenia; anemia Gastrointestinal: 30% nausea/vomiting; 10% stomatitis; 10% diarrhea Hepatotoxicity: transient increases in serum bilirubin and/or hepatic enzymes Skin: alopecia; perirectal abscess Cardiotoxicity: 1% (acute arrhythmias, longer QT). Possible risk factor: hypokalemia	

VP16 is used for cancers such as Ewing's sarcoma, SCLC, testicular cancer, lymphoma, non-lymphocytic leukemia, and glioblastoma multiforme. It is also sometimes used in a conditioning regimen prior to a bone marrow or blood stem cell transplant.

Etoposide phosphate (Etopophos) is a water-soluble ester of etoposide. Its water solubility lessens the potential for precipitation following dilution and during IV administration. Following administration, Etopophos is rapidly and completely converted to etoposide in plasma by dephosphorylation. Two different studies demonstrated no statistically significant differences in the AUC and C_{max} parameters for etoposide when administered as Etopophos or Vepesid. In addition there were no differences in the pharmacodynamic behavior (hematological toxicity).

10. Teniposide (VM26)

VM26 is an analog of VP16. In vitro, VM26 is about 10-fold more potent than VP16. Since both agents have relatively similar abilities to inhibit Topo-II, the greater in-vitro cytotoxicity of VM26 is likely due to better cellular uptake. Equitoxic VM26 doses are approximately one-third lower than those of VP16.

VM26 has less water solubility, lower renal clearance (10%), and is more tightly bound to plasma proteins than VP16. VM26 also has a

longer drug half-life and greater biliary clearance than VP16. Anticonvulsants, such as phenobarbital and phenytoin, increase VM26 clearance, presumably by increasing hepatic metabolism. VM26 toxicities are like those of VP16. Hypersensitivity reactions appear more frequently with VM26 than VP16 infusions and are related to the drug vehicle Cremophor EL (polyoxyethylated castor oil).

Aminoacridines (Table 3)

11. Amsacrine

The synthetic 9-aminoacridine derivative, amsacrine (m-AMSA), is a Topo-II inhibitor. Its strong activity is due to the ability of the acridine nucleus to intercalate into DNA base pairs, stabilizing the DNA–Topo-II cleavable complex, producing lethal DSBs and leading to cell-cycle arrest and apoptosis. The intercalative property has been related to the planar aromatic system of the acridine moiety. Acridine-based anticancer drugs may either donate electrons to, or accept electrons from, the double helix, thus actively participating in long-range electron transfer reactions associated with ROS production. Resistance to aminoacridines arises mainly through mutation of Topo-II molecules. Despite the initial encouraging reports from experimental models, m-AMSA has been shown to be effective only in the treatment of acute leukemias and malignant lymphomas.

Further Reading

Chikamori K, Grozav AG, Kozuki T, et al. DNA topoisomerase II enzymes as molecular targets for cancer chemotherapy. Curr Cancer Drug Targets 2010; 10:758–771.

Ewesuedo RB, Ratain MJ. Topoisomerase I inhibitors. Oncologist 1997; 2:359–364.

Fujita K, Sparreboom A. Pharmacogenetics of irinotecan disposition and toxicity: a review. Curr Clin Pharmacol 2010; 5:209–217.

Hande KR. Topoisomerase II inhibitors. Update Cancer Ther 2006; 1:3–15.

Hande KR. Clinical applications of anticancer drugs targeted to topoisomerase II. Biochim Biophys Acta 1998; 1400:173–184.

Herben VM, Ten Bokkel Huinink WW, Schellens JH, et al. Clinical pharmacokinetics of camptothecin topoisomerase I inhibitors. Pharm World Sci 1998; 20:161–172.

Mathijssen RH, Loos WJ, Verweij J, et al. Pharmacology of topo-isomerase I inhibitors irinotecan (CPT-11) and topotecan. Curr Cancer Drug Targets 2002; 2:103–123.

Mross K, Maessen P, van der Vijgh WJ, et al. Pharmacokinetics and metabolism of epidoxorubicin and doxorubicin in humans. J Clin Oncol 1988; 6: 517–526.

Nitiss JL. Targeting DNA topoisomerase II in cancer chemotherapy. Nat Rev Cancer 2009; 9:338–350.

Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer 2006; 6:789–802.

Pommier Y, Leo E, Zhang H, Marchand C. DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem Biol 2010; 17: 421–433.

Seiter K. Toxicity of the topoisomerase I inhibitors. Expert Opin Drug Saf 2005; 4:45–53.

Smith LA, Cornelius VR, Plummer CJ, et al. Cardiotoxicity of anthracycline agents for the treatment of cancer: Systematic review and meta-analysis of randomised controlled trials. BMC Cancer 2010; 10:337.

Teicher BA. Next generation topoisomerase I inhibitors: Rationale and biomarker strategies. Biochem Pharmacol 2008; 75:1262–1271.

Van Dalen EC, Caron HN, Dickinson HO, et al. Cardioprotective interventions for cancer patients receiving anthracyclines. Cochrane Database Syst Rev 2011; 6: CD003917.

Tubulin-active Agents

7

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Introduction

Microtubules are polymeric filaments composed of α-tubulin and β-tubulin heterodimers. Microtubules have crucial roles in cell division and growth. They are a core component of the mitotic spindle that separates chromosomes during eukaryotic cell division. Several families of compounds that disrupt the complex polymerization dynamics of microtubules such as the taxanes and the vinca alkaloids have potent anti-cancer activity.

To overcome some of the limitations of the existing microtubule-targeted agents, such as resistance and neurotoxicity, several novel classes of agents including ixabepilone and eribulin have been investigated.

Vinca Alkaloids (Table 1)

The vinca alkaloids are structurally similar compounds composed of two multiringed units, vindoline and catharanthine. Vinca alkaloids, at the lowest effective concentrations, suppress microtubule dynamics and microtubule treadmilling and cause cell-cycle arrest at G2/M phase. These events lead to an accumulation of cells in mitosis with cell death by apoptosis. Vinca alkaloids also prevent the assembly of microtubules without affecting their disassembly at higher concentrations, and inhibit mitotic spindle formation. Vinblastine and vincristine were first developed and are still used for hematological malignancies. Vinorelbine and vinflunine are newer agents indicated for the treatment of some solid tumors.

1.Vinblastine

Vinblastine, first isolated from the Madagascar periwinkle plant *Catharanthus roseus,* has a methyl side chain on vindoline.

2.Vincristine

Vincristine, also isolated from the Madagascar periwinkle plant, has the same structure as vinblastine with a formyl side chain on vindoline.

3.Vinorelbine

Unlike other vinca alkaloids, the structural modification of vinorelbine is in the catharanthine unit. The antitumor activity of vinorelbine is thought to be due primarily to inhibition of mitosis at metaphase through its interaction with tubulin.

4.Vinflunine

Vinflunine is the first fluorinated microtubule inhibitor belonging to the vinca alkaloid family. Vinflunine inhibits treadmilling less powerfully than vinblastine and vinorelbine, and does not suppress the rate of microtubule shortening, whereas vinblastine does. Compared with other vinca alkaloids, vinflunine exhibits the weakest overall affinity for tubulin, with formation of fewer and smaller spiral filaments, effects that might theoretically result in reduced neurotoxicity. Vinflunine is approved for the treatment of second-line metastatic transitional cell carcinoma of the urothelium.

Taxanes and New Taxane Formulations (Table 2)

Taxanes act by binding to microtubules with binding sites distinct from the ones of the vinca alkaloids. The binding to tubulin promotes the assembly into microtubules and simultaneously inhibits disassembly, stabilizing microtubule dynamics.

Table I *Pharmacological aspects of vinca alkaloids*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
1. Vinblastine	Binding to a specific site on tubulin with prevention of polymerization, inhibition of microtubule assembly and mitotic spindle formation	IV bolus: 4 mg/m² to begin with, increased to 6 mg/m²/wk	$T_{1/2}$ 19–25 hrs	Hepatic
2. Vincristine	Binding to a specific site on tubulin with prevention of polymerization, inhibition of microtubule assembly and mitotic spindle formation	IV bolus: 0.4–1.4 mg/m² (maximum 2 mg total dose) per wk IV cont inf: 0.5 mg/m²on days 1–5 q 3 wks	$T_{1/2}$ 85 hrs	Hepatic
3. Vinorelbine	Binding to a specific site on tubulin with prevention of polymerization, inhibition of microtubule assembly and mitotic spindle formation	IV (5–10 min inf): 30 mg/m²/wk PO: 60 mg/m² per wk x 3 wks, then ↑ to 80 mg/m²/wk if no severe neutropenia	$T_{1/2}$ 27–43 hrs Cl 0.97-1.26 L/hr/m²	Hepatic
4. Vinflunine	Binding to a specific site on tubulin with prevention of polymerization, inhibition of microtubule assembly and mitotic spindle formation	IV (20 min inf): 320 mg/m² q 3 wks	$T_{1/2}$ 39 hrs	Hepatic

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
↓ dose if obstructive liver disease		Neutropenia, after 5–10 days, recovering within 7–14 days; constipation; abdominal pain; cumulative peripheral neuropathy; mild alopecia; local vesicant on extravasation	
↓ 50% of dose for bilirubin up to 3 mg/mL	Methotrexate	Cumulative sensory peripheral neuropathy; dose-limiting constipation; abdominal pain; mild alopecia; local vesicant on extravasation	
↓ 50% of dose if bilirubin >2 x ULN, ↓ 75% of dose if bilirubin >3 x ULN	Potential interactions with inducers/inhibitors of CYP3A	Neutropenia (DLT), after 7–10 days, recovering in 1 wk; 35% constipation; 25% abdominal pain; cumulative peripheral sensory neuropathy; 12% mild alopecia; local vesicant on extravasation; 50% diarrhea; 50% nausea, with PO formulation	
		Neutropenia; constipation (prophylaxis); abdominal pain; cumulative peripheral neuropathy; mild alopecia; local vesicant on extravasation	

Table 2 *Pharmacological aspects of taxanes*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
5. Paclitaxel	Promotes microtubule assembly of tubulin dimers and stabilizes microtubule dynamics with inhibition of cell proliferation, blockade of mitosis and induction of apoptosis	Premedication with steroids and histamine H_1- and H_2-antagonists to prevent HSR IV (3 hr inf): 175 mg/m² q 3 wks or 80–100 mg/m² (1 hr inf)/wk IP: 82.5–125 mg/m² q 3 wks	$T_{1/2}$ 15-20 hrs; after IP treatment slow peritoneal Cl, prolonged significant IP and plasma concentrations	Extensive hepatic metabolism through CYP2C8 and CYP3A4; high biliary excretion Paclitaxel is also metabolized by cytochrome P450 2C8
6. Docetaxel	Promotes microtubule assembly of tubulin dimers and stabilizes microtubule dynamics	Premedication with steroids to prevent fluid retention IV (1 hr inf): 75–100 mg/m² q 3 wks or 36 mg/m²/wk x 3 q 4 wks	$T_{1/2}$ 11.2 hrs	Hepatic metabolism, through CYP3A4; 74% excreted in feces
7. Nab-paclitaxel	Promotes microtubule assembly of tubulin dimers and stabilizes microtubule dynamics	No premedication needed IV (30 min inf): 260 mg/m² q 3 wks	$T_{1/2}$ 27 hrs	Extensive hepatic metabolism
8. Cabazitaxel	Promotes assembly of tubulin dimers and stabilizes microtubule dynamics	Premedication with steroids and histamine H_1- and H_2-antagonists at least 30 mins before treatment IV (1 hr inf): 25 mg/m² q 3 wks Antiemetic prophylaxis recommended	$T_{1/2}$ 77 hrs	Hepatic

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
↓ dose to 90 mg/m² if transaminases 2–10 x ULN or bilirubin 2–5 x ULN	Concomitant CYP3A4 inhibitors / inducers	60% neutropenia; cumulative dose-dependent sensory peripheral neuropathy; total alopecia; myalgia; dose-dependent HSR (<2% severe); asthenia; transaminases elevation Abdominal pain with IP treatment; mucositis; nail problems with weekly dosing	
↓ dose if transaminases 1.5–3.5 x ULN; discontinue if transaminases >3.5 x ULN or bilirubin >6 x ULN	Concomitant CYP3A4 inhibitors / inducers	80% neutropenia after 7–10 days, recovering in 1 wk; cumulative peripheral sensory neuropathy; total alopecia; myalgia; 47% skin reaction; onycholysis; 15% HSR; 64% fluid retention; ischemic colitis (rare); 62% asthenia; transaminases elevation	
Bilirubin 1.26–2 x ULN and AST >ULN (and <10 x ULN): 200 mg/m² Bilirubin 2–5 x ULN and AST >ULN (and <10 x ULN): 130 mg/m²	Potential interaction with CYP2C8 and CYP3A4 substrates	87% neutropenia (9% G3); 7% cumulative peripheral sensory neuropathy (10% G3); total alopecia; 44% myalgia; 47% asthenia; 59% transaminases elevation	
No treatment if bilirubin >1 x ULN or transaminases >1.5 x ULN	Concomitant CYP3A inhibitors / inducers	90% neutropenia (82% G3); 13% cumulative peripheral neuropathy; 10% total alopecia; 7% myalgia; 20% asthenia; HSR; skin reaction; 47% diarrhea, nausea, vomiting; constipation	

5. Paclitaxel

Paclitaxel, first isolated from the bark of the yew tree, *Taxus brevifolia,* in the 1960s, is a natural product with antitumor activity. Paclitaxel was then obtained via a semisynthetic process from *Taxus baccata.* It promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network, which is essential for vital interphase and mitotic cellular functions. In addition, paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

Because of its poor water solubility, the paclitaxel formulation for clinical use includes 50% polyoxyethylated castor oil (Cremophor EL) and 50% ethanol as vehicle. Cremophor is the main constituent responsible for hypersensitivity reactions, which requires premedications.

6. Docetaxel

Docetaxel is a Cremophor-free, more water-soluble semisynthetic analog of paclitaxel, derived from the needles of *Taxus baccata*; the formulation contains Tween 80 and ethanol and can also lead to toxicity. Docetaxel has a mechanism of action similar to paclitaxel.

7. Nab-paclitaxel

Nab-paclitaxel is an albumin-bound paclitaxel which eliminates the need for Cremophor EL, allowing the delivery of a higher-dose solvent-based paclitaxel. Nab-paclitaxel is indicated for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy including anthracyclines.

8. Cabazitaxel

Cabazitaxel is a semisynthetic taxane selected for clinical development because of its poor affinity for P-glycoprotein and greater penetration of the blood–brain barrier compared with docetaxel and paclitaxel. Cabazitaxel showed activity in both docetaxel-sensitive and docetaxel-resistant cancers in preclinical testing. Cabazitaxel is approved in combination with prednisone for the treatment of patients with hormone-refractory metastatic prostate cancer who had been previously treated with a docetaxel-containing regimen.

Epothilones (Table 3)

9. Ixabepilone

Ixabepilone is a microtubule inhibitor belonging to the class of epothilones and their analogs, isolated from the myxobacterium *Sorangium cellulosum*. Ixabepilone is a semisynthetic analog of epothilone B which binds directly to β-tubulin subunits, leading to suppression of microtubule dynamics. Ixabepilone is indicated in combination with capecitabine for the treatment of patients with metastatic or locally advanced breast cancer resistant to treatment with an anthracycline and a taxane. Ixabepilone is also indicated as monotherapy for the treatment of metastatic or locally advanced breast cancer in patients resistant or refractory to anthracyclines, taxanes, and capecitabine.

10. Estramustine Phosphate

Estramustine phosphate is a molecule combining estradiol and nor-nitrogen mustard by a carbamate link. The antitumor effect is caused by the binding to tubulin and to microtubule-associated proteins. Estramustine phosphate taken orally is readily dephosphorylated during absorption. The major metabolites in plasma are estramustine, the estrone analog, estradiol, and estrone. Estramustine is selectively accumulated in the prostate by binding to specific proteins and is indicated for palliative treatment of advanced carcinoma of the prostate.

Table 3 *Pharmacological aspects of epothilones*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
9. Ixabepilone	Binding β-tubulin subunits with suppression of microtubule dynamics	Premedication with H_1- and H_2-antagonists 1 hr before treatment IV (3 hr inf): 40 mg/m² q 3 wks	$T_{1/2}$ 52 hrs	Extensively in liver via CYP3A4
10. Estramustine phosphate	Derivative of estradiol, directly binding to tubulin and microtubule-associated proteins, leading to inhibition of microtubule dynamics and to anaphase	PO: 14 mg/kg divided in 3 or 4 doses 1 hr before or 2 hrs after meals Lower doses at start	$T_{1/2}$ estramustine 20 hrs	Hepatic
11. Eribulin	Inhibits the growth phase of microtubules and sequesters tubulin into nonproductive aggregates. Exerts its effects via a tubulin-based antimitotic mechanism with G2/M cell-cycle block, disruption of mitotic spindles and apoptotic cell death	IV (2–5 min inf): 1.4 mg/m² on day 1 and 8 q 3 wks	$T_{1/2}$ 37 hrs	Hepatic

11. Eribulin

Eribulin is a synthetic analog of halichondrin B, a product isolated from the marine sponge *Halichondria okadai,* which inhibits the growth phase of microtubules and sequesters tubulin into non-productive aggregates. This leads to a tubulin-based antimitotic mechanism with G2/M cell-cycle block, disruption of mitotic spindles, and apoptotic cell death after

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
↓ dose if transaminases 2.5 x ULN or bilirubin >1 x ULN Discontinue if transaminases >10 ULN or bilirubin >3 x ULN	Concomitant CYP3A4 substrates	50% neutropenia; 65% cumulative peripheral sensory neuropathy; 48% alopecia; 49% myalgia; 5% HSR; 56% asthenia; 42% nausea; 29% vomiting	Add corticosteroids to premedication in case of HSR Combination with capecitabine contraindicated in case of transaminases >2.5 x ULN or bilirubin >1 x ULN
↓ dose if liver impairment	Calcium-containing food	↑ risk of TEE, glucose intolerance; 19% elevated blood pressure; fluid retention; 70% gynecomastia; impotence; asthenia; 31% ↑ transaminases	
↓ dose in case of hepatic impairment: 1.1 mg/m² if mild and 0.7 mg/m² if moderate ↓ dose in case of renal impairment: 1.1 mg/m² if clearance 30–50 ml/min	Potential interactions with inhibitors of CYP3A4 and PgP	82% neutropenia (60% G3–4); 35% cumulative peripheral neuropathy (8% G3); 54% asthenia; alopecia; myalgia; onycholysis; HSR rare; QTc prolongation; asthenia	

prolonged mitotic blockage. Eribulin is indicated for the treatment of patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease. Prior therapy should have included an anthracycline and a taxane in the adjuvant or in the metastatic setting.

Further Reading

Gradishar WJ. Albumin-bound paclitaxel: a next-generation taxane. Expert Opin Pharmacother 2006; 7:1041–1053.

Jain S, Vahdat LT. Eribulin mesylate. Clin Cancer Res 2011; 17:6615–6622.

Rivera E, Lee J, Davies A. Clinical development of ixabepilone and other epothilones in patients with advanced solid tumors. Oncologist 2008; 13: 1207–1223.

van Tellingen O, Sips JH, Beijnen JH, et al. Pharmacology, bio-analysis and pharmacokinetics of the vinca alkaloids and semi-synthetic derivatives (review). Anticancer Res 1992; 12:1699–1715.

Villanueva C, Bazan F, Kim S, et al. Cabazitaxel: a novel microtubule inhibitor. Drugs 2011; 71:1251–1258.

Woodward EJ, Twelves C. Scheduling of taxanes: a review. Curr Clin Pharmacol 2010; 5:226–231.

Miscellaneous

8

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Bleomycin, Mitomycin C, and Actinomycin D (Table 1)

1. Bleomycin

Bleomycin is a mixture of cytotoxic glycopeptide antibiotics isolated from the fungus *Streptomyces verticillus* that possess both antitumor and antimicrobial activity. The exact mechanism of action is unknown; bleomycin binds to DNA to produce single-strand (SSB) and double-strand breaks (DSB) and inhibits further DNA, RNA, and protein synthesis. Bleomycin is used, either as a single agent or in combination, for the treatment of head and neck, testicular, and penile cancers, Hodgkin and non-Hodgkin lymphomas. It may also be injected into the pleural or pericardial space to control malignant effusions.

Most bleomycin is excreted unchanged in the urine and the dose must be decreased in patients with renal failure.

Symptoms of bleomycin-induced lung toxicity include non-productive cough, dyspnea, pleuritic chest pain, and fever. Patients usually present tachypnea, crackles, lung restriction, and hypoxemia. The classic pattern on chest radiography includes bibasilar subpleural opacification with volume loss and blunting of the costophrenic angles. Fine nodular densities may also be present and these early abnormalities may evolve to consolidation and honeycombing. Computed tomography is more sensitive than chest radiography. Impairment of pulmonary function tests (decreased diffusing capacity for carbon monoxide [DL_{CO}] and decrease of lung volumes) could argue for a diagnosis of bleomycin-induced pulmonary toxicity, based on the combination of a compatible clinical pattern and exclusion of infection or pulmonary involvement from the underlying malignancy, to be verified by a bronchoalveolar lavage (BAL). Symptomatic patients may benefit from

Table 1 *Pharmacological aspects of bleomycin, mitomycin C, and actinomycin D*

Agent	Mechanism of action	Dosing	Pharmacokinetics
1. Bleomycin (BLM)	DNA binding with production of SSB and DSB; DNA damage affected by specific repair enzymes, glutathione, ionizing radiation. BLM inactivated by BLM hydrolase; pulmonary toxicity due to low enzyme concentration and high O_2 pressure. When used intrapleurally acts as sclerosing agent	IV bolus: 10–20 mg/m^2/wk IM, SC: same dose as IV, with antipyretics/steroids to prevent fever IV cont inf: 5–10 mg/m^2 on days 1–4 q 3 wks Intrapleural: 60–120 U (50% of dose in the systemic circulation). Avoid NSAID for chest pain mg = unit	10% protein bound; $T_{1/2}$ 2–3 hrs; C_{max} with IM administration after 30–60 mins, 1/3 of that after IV; 45% of systemic absorption after intrapleural administration
2. Mitomycin C (Mitomycin)	Conversion to bifunctional alkylating agent with formation of DNA interstrand crosslinks and oxygen free radicals. Activation by chemical reducing agents, enzymatic reduction, exposure to acidic pH: possible preferential activation in hypoxic environment	IV bolus : single agent, 20 mg/m^2 q 6–8 wks Combination, 10 mg/m^2 q 6–8 wks Intravesical: 20 mg x 3/wk Local vesicant on extravasation	Rapid plasma disappearance due to tissue distribution and liver metabolism; $T_{1/2\beta}$ 25–90 mins; <10% of dose in 24 hr urine, 23% hepatic extraction with HAI administration
3. Actinomycin D (Dactinomycin)	DNA intercalation of the planar multiring phenoxazone between guanine–cytosine base pairs with inhibition of RNA synthesis Radiosensitizer	IV (bolus): single agent, 500 µg (maximum 15 µg/kg) on days 1–5 q 4 wks; Combination, 500 µg on days 1–2 Local vesicant on extravasation	5% protein bound; by RIA, biphasic disappearance with $T_{1/2}$ of 35 hrs, longer in case of liver impairment

glucocorticoid therapy such as prednisone at 0.75 to 1.0 mg/kg (based on clinical experience, case series), tapered gradually after 4 to 8 weeks over an additional 4 to 6 months.

2. Mitomycin C

Mitomycin C is an antibiotic isolated from *Streptomyces caespitosus*, which interferes with the structure and function of DNA. Metabolic activation by

Metabolism	Dosing when organ dysfunction	Drug interactions	Toxicity
Rapidly eliminated, primarily by the kidney 50% of dose in 24 hr urine, mainly as inactive species Not removable by hemodialysis or peritoneal dialysis	Renal failure: Cr Cl 10–50 ml/min: ↓ dose by 25% Cr Cl <10 ml/min: ↓ dose by 50%	↑ Risk of pulmonary toxicity with hyperoxia, concomitant RT, nephrotoxic drugs with ↓ excretion of BLM	Acute DL stomatitis; 50% fever and chills; 50% cumulative skin hyperpigmentation; mild to moderate alopecia Rare: HSR (1% of lymphoma pts) and Raynaud's phenomenon. Hypersensitivity pneumonitis responsive to steroids. 10% late chronic pneumonitis potentially worsening to irreversible interstitial fibrosis (dry cough, dyspnea, rales, basilar infiltrates), ↑ incidence for cumulative dose >250 U, age >70 yr; COPD, thoracic RT; hyperoxia during surgical anesthesia
Metabolic activation by reduction occurs in all tissues	PK unchanged if liver/ renal impairment	With concomitant DOX: ↑ risk of cardiotoxicity	Delayed (after 3–8 wks) cumulative leuko- and thrombocytopenia; cumulative anemia; partial alopecia Rare: HUS with thrombocytopenia; renal and cardiac failure: ↑ risk for cumulative dose >50 mg, exacerbated by RBC transfusions, rarely reversible, steroids ineffective (52% lethal) Rare: severe interstitial pneumonitis with lung infiltrates
Minimally metabolized; 30% of dose in urine and feces as intact drug within 1 wk	No adjustment is necessary for renal failure	Unexpected hepatic toxicity after hepatotoxic agents (halothane, enflurane)	DL myelotoxicity with leuko-/ thrombocytopenia in 1 wk and nadirs up to 3 wks. Severe, prolonged (up to 24 hr) nausea and vomiting; 30% stomatitis and diarrhea; alopecia; late radiation recall toxicity (mainly skin, but also GI, liver, lung). Immunosuppression

reduction occurs in all tissues and implies a rapid clearance of the drug.

Mitomycin has been approved as a single agent for use in bladder (intravesical), esophagus, breast, and cervical cancer.

The major dose-limiting toxicity is myelosuppression, which is cumulative. Nadir is reached usually by 4 weeks. Hemolytic–uremic syndrome (HUS) is a rare adverse effect but poorly responsive to plasmapheresis and other therapies. The overall incidence is related to cumulative dose.

3. Actinomycin D

Actinomycin D (Dactinomycin) is another antibiotic that was first isolated from *Streptomyces* species; it is the principal component of the mixture of actinomycins produced by *Streptomyces parvullus*. This drug exerts its cytotoxic effects by intercalation between guanine–cytosine base pairs with inhibition of the synthesis of messenger RNA.

Dactinomycin is used for the treatment of Wilms' tumor, childhood rhabdomyosarcoma, Ewing's sarcoma, and gestational trophoblastic neoplasms. Dactinomycin potentiates the effects of radiotherapy.

L-Asparaginase, Anagrelide, and Arsenic Trioxide

(Table 2)

4. L-Asparaginase

Asparaginase contains the enzyme L-asparaginase (amidohydrolase), originally isolated from *Escherichia coli*. The antitumor effect results from the rapid and complete depletion of asparagine in the bloodstream and extracellular space, which deprives tumor cells of the required amino acid and inhibits protein synthesis. Asparaginase is indicated for the induction treatment of patients with acute lymphoblastic leukemia (ALL).

Hypersensitivity reactions, including anaphylaxis, occur in 20% to 35% of patients. These reactions are more common with the IV route and are less likely to occur in the first days of treatment, but anaphylaxis is possible after each administration. In case of hypersensitivity to enzymes prepared from *E. coli*, *Erwinia carotovora*-derived asparaginase is a safe alternative without cross-reactivity. Other major adverse effects are related to inhibition of protein synthesis and include hypoalbuminemia, hyperglycemia, and decreased concentrations of fibrinogen, prothrombin, antithrombin III, and factors V and VIII, which could lead to CNS thrombosis and coagulopathy. Glucose, liver, and pancreatic function tests may be performed regularly to exclude asparaginase-related hyperglycemia or pancreatitis.

5. Anagrelide

Anagrelide is an alternative to hydroxyurea for patients with essential thrombocythemia. It was developed as an inhibitor of platelet aggregation but was also demonstrated to inhibit the maturation of platelets from megakaryocytes at doses below those exhibiting an anti-aggregating effect.

Major side effects of anagrelide are consistent with its mechanism of action as a phosphodiesterase inhibitor and include headache, palpitations, fluid retention, and gastrointestinal intolerance. Anagrelide is recommended in case of failure of or intolerance to hydroxyurea, although it was approved by the US FDA and by European authorities for essential thrombocytopenia and thrombocytoses associated with polycythemia vera.

6. Arsenic Trioxide

Arsenic trioxide (ATO) induces morphological changes and DNA fragmentation. It is an effective agent in relapsing or refractory acute promyelocytic leukemia (APL), which induced 85% hematological and 79% molecular complete response rates in a pivotal US study. ATO may act on APL cells through several modes of action, including induction of differentiation and/or apoptosis, growth inhibition, and angiogenesis inhibition and finally triggers the degradation of the characteristic t(15;17) or PML-RARalpha fusion protein.

The potentially fatal APL differentiation syndrome similar to that seen in patients treated with retinoic acid may be seen in some patients and is characterized by fever, dyspnea, weight gain, pulmonary infiltrates, and pleural or pericardial effusions (with or without leukocytosis). High-dose steroids (e.g. dexamethasone 10 mg 2/day) should be used at the first suspicion of the APL differentiation syndrome and appears to mitigate signs and symptoms. ATO can cause QT interval prolongation, torsade de pointes-type ventricular arrhythmia and complete atrioventricular block; baseline 12-lead ECG, serum electrolytes (potassium, calcium, magnesium), and creatinine should be obtained prior to treatment.

Table 2 *Pharmacological aspects of L-asparaginase, anagrelide, and arsenic trioxide*

Agent	Mechanism of action	Dosing	Pharmacokinetics
4. L-Asparaginase	Hydrolyzes L-asparagine with inhibition of protein synthesis, delayed DNA and RNA synthesis in tumor cells dependent on exogenous asparagine. Resistance due to high intrinsic asparagine synthetase activity	Corticosteroids, epinephrine, and O_2 available during / after infusion IV (30 min inf): Induction: 5000 IU/m^2 on days 1–14; 6000 IU/m^2 x 3/wk IM maximum volume: 2 ml	Systemically degraded. Minimal tissue distribution due to large size/highly ionized state, production of binding antibodies; plasma levels proportional to dose; slow variable Cl with $T_{1/2\beta}$ of 8–30 hrs Plasma levels after IM 50% of those after IV with longer $T_{1/2\beta}$ ~ 40 hrs
5. Anagrelide	Dose-dependent ↓ in PLT production possibly due to specific interference in the megakaryocyte post-mitotic phase; inhibition of PLT aggregation at higher doses	PO: 0.5 mg/day or 1 mg 2/day for at least 1 wk, subsequently modified to maintain a PLT count <600 /mm^3; dosage to be ↑ by 0.5 mg daily per wk up to a maximum of 10 mg daily or 2.5 mg single dose, usual dose 1.5–3 mg Response starts within 7–14 days of proper dosage Monitor pt every other day week 1, then weekly	T_{max} 1 hr; $T_{1/2}$ 3 days, does not accumulate in plasma after repeated administrations; ↑ F with fasting
6. Arsenic trioxide (ATO)	Mechanism of action not fully elucidated In vitro: apoptosis in human PML cells, damage or degradation of the fusion protein PML-RARalpha Used in pts with t(15;17) translocation or PML-RARalpha gene expression	Adult and children >5 yrs IV inf (1–2 hr): 0.15 mg/kg daily Induction: until BM remission or for 60 days maximum Maintenance: maximum 25 doses in 5 wks, starting 3–6 wks from completion of induction Acute vasomotor reaction: ↑ inf length to 4 hrs APL syndrome: DXM IV 10 mg 2/day for at least 3 days **Warning:** before treatment check ECG and electrolytes	PK of the active trivalent arsenic species not characterized Complex metabolism Arsenic is stored mainly in liver, kidney, heart, lung, hair, and nails

Metabolism	Dosing when organ dysfunction	Drug interactions	Toxicity
Minimal urinary/biliary excretion; cumulation with daily dosing	No adjustment is necessary for renal failure	With MTX: ↓ MTX toxicity through inhibition of protein synthesis, causing prevention of cell entry into S phase and ↓ polyglutamation With concomitant steroids: ↑ hyperglycemia With VCR and PDN: ↑ toxicity of IV schedule	35% HSR (skin rash, arthralgia, fever, chills, anaphylaxis); ↑ incidence with repeated doses, doses > 6000 IU/m^2, IV route. GI toxicity (mild nausea and vomiting, anorexia, cramps) and early hepatic toxicity with ↑ enzymes and ↓ protein synthesis rapidly reversible; pancreatitis with normal lipase and amylase; hyperglycemia; hypercalcemia
Extensive metabolism with 70% of dose excreted in urine with <1% parent compound	Renal impairment: Cr Cl ≥2 mg/dL: serial monitoring of Cr Severe hepatic impairment (Child-Pugh C): contraindicated	With sucralfate: potential interference with absorption	Dose related: 44% headache; 26% diarrhea; 23% asthenia; 20% edema; 17% abdominal pain Vasodilating and positive inotropic properties: 27% palpitation; 8% tachycardia; CHF; migraine; syncope; thrombosis Caution in pts with known / suspected heart disease Pretreatment cardiovascular assessment
Eliminated through the kidney	Renal failure: no data available, but caution because arsenic is eliminated through the kidney. Monitor closely for toxicity Severe hepatic impairment (Child-Pugh C): monitor closely for toxicity	None known Caution: In pts receiving other drugs known to prolong QT intervals	Most severe: APL differentiation syndrome: fever, dyspnea, ↑ weight, pulmonary infiltrates, pleural or pericardial effusions, leukocytosis; ECG abnormalities: 38% QT prolongation within 1–5 wks with normalization by 8th wk; 13% G3-4. ↓ P and Mg Rare: complete AV block; torsades de pointes, potentially fatal; 5% HSR, 3% severe Most common: reaction at injection site; 55% tachycardia; 58–75% nausea/vomiting; 43% diarrhea; 58% abdominal pain; 60% headache; 63% fatigue; 63% fever; 43% dermatitis; 33% pruritus; 40% edema; 38% rigors; 25% chest pain; 25–30% arthralgia; hematological: 50% leukocytosis

Hydroxyurea, Procarbazine, and Trabectedin (Table 3)

7. Hydroxyurea

Hydroxyurea (or hydroxycarbamide) induces an immediate inhibition of DNA synthesis by inhibiting the enzymatic conversion of ribonucleotides to deoxyribonucleotides (acting as a ribonucleotide reductase inhibitor). Hydroxyurea is used for chronic myeloproliferative disorders, as a cytoreductive agent for essential thrombocythemia. Its potential for leukemic transformation is still a matter of controversy.

8. Procarbazine

Procarbazine is a prodrug, which undergoes microsomal transformation into active metabolites that damage DNA as an alkylating agent. Procarbazine was rapidly identified as an active drug against lymphoid neoplasms and integrated in combination regimens used to treat Hodgkin lymphomas. Procarbazine inhibition of monoamine oxidase (MOA) induces specific toxicities such as mental status changes and hypertensive crisis with concomitant tyramine-rich foods (wine, bananas, yogurt, ripe cheese). The ingestion of alcohol by patients receiving procarbazine may also have a disulfiram (Antabuse)-like action (nausea, vomiting, headache, sweating, flushing).

9. Trabectedin

Trabectedin is an antineoplastic agent derived from the marine tunicate *Ecteinascidia turbinata*, now produced by a semisynthetic process. It binds to the minor groove of DNA, bending the helix toward the major groove, which leads to interference with the intracellular transcription pathways and DNA-repair proteins. Trabectedin has shown objective responses in phase II trials of patients with advanced soft tissue sarcomas and in relapsed ovarian cancer.

Pharmacokinetic parameters demonstrated large interpatient variability. Trabectedin is extensively metabolized in the liver, predominantly by the cytochrome P450 isozyme 3A4. Liver toxicity is frequent, but not cumulative and rapidly reversible; dexamethasone premedication has been shown to reduce the incidence of trabectedin-related adverse events

(reduced frequency of neutropenia and alanine transaminase [ALT] or aspartate transaminase [AST] elevation). No evidence of cumulative toxicities was described.

Tretinoin and Bexarotene (Table 4)

10. Tretinoin

Tretinoin (all-trans retinoic acid, ATRA) can differentiate APL blasts in vitro and in vivo. APL is a specific type of acute myeloid leukemia (AML) characterized by its morphology (M3 or M3v in the FAB classification), t(15;17) translocation leading to PML-RARalpha fusion gene, and by a specific coagulopathy combining disseminated intravascular coagulation, fibrinolysis, and non-specific proteolysis. With ATRA treatment, about 90% of patients with newly diagnosed or relapsed APL can obtain complete response, through differentiation of APL blasts into mature granulocytes.

11. Bexarotene

Bexarotene is a retinoid that binds and selectively activates retinoid X receptor subtypes (RXRs), which act as transcription factors of genes which control cellular differentiation and proliferation. The approved indication is topical treatment in patients with refractory cutaneous T-cell lymphoma (stage IA and IB) or intolerant to other therapies. Skin reactions at the application site are common and may include redness, dryness, pruritus, and pain. Systemic treatment is only for patients with advanced mycosis fungoides (stages IIB to IVB) refractory to at least one prior systemic therapy.

Many toxicities of systemic retinoids are reversible; they include arthralgias, myalgia, headache, photosensitivity, and impaired night vision. Patients are at increased risk for hypertriglyceridemia, which requires monitoring and sometimes temporary antilipidemic treatment.

Table 3 *Pharmacological aspects of hydroxyurea, procarbazine, and trabectedin*

Agent	Mechanism of action	Dosing	Pharmacokinetics
7. Hydroxyurea (HU, Hydroxycarbamide)	Enters cells by passive diffusion; inhibits RNR with depletion of ribonucleotides and inhibition of DNA synthesis and repair Radiation sensitizer	CML PO: 20–30 mg/kg daily; discontinue if WBC <2500/mm^3 or PLT <100/mm^3 Radiosensitizer: 80 mg/kg as a single dose, q 3 days from at least 7 days before radiation	Well absorbed; T_{max} 1 hr, 50% of dose transformed in liver and excreted in urine and as respiratory CO. $T_{1/2}$ 3.5–4 hrs Crosses blood–brain barrier and third space fluids with peaks in 3 hrs
8. Procarbazine	Prodrug; generates several reactive free radicals, with direct damage to DNA through auto-oxidation, chemical decomposition, and CYP450-mediated metabolism	PO: 100 mg/m^2 daily on days 1–14 q 4 wks (MOPP, BEACOPP regimen) **Warning:** start at low dose and then escalate daily to minimize GI toxicity	Completely absorbed with peak concentrations in plasma and in CSF in 1 hr
9. Trabectedin	Binds to the minor groove of DNA, bending the helix toward major groove Affects functions of transcription factors, DNA-binding proteins, and DNA-repair pathways	IV (24 hr inf): 1.5 mg/m^2 q 3 wks Premedication: IV 20 mg DXM 30 mins before the therapy to prevent toxicity Ovarian cancer: 1.1 mg/m^2 3 hr inf q 3 wks **Warning:** central venous line is recommended Alcohol intake should be avoided Higher risk of rhabdomyolysis in case of CPK >2.5 ULN Do not use in pts with ↑ bilirubin	Multiple-compartment disposition model with extensive tissue distribution. 94–98% protein bound

Metabolism	Dosing when organ dysfunction	Drug interactions	Toxicity
Degraded by urease of intestinal bacteria; metabolism unknown; 55% excreted by renal route	Renal impairment: GFR 10–50 ml/min: ↓ dose by 50%; GFR <10 ml/min: discontinue Hepatic impairment: closely monitor for bone marrow toxicity	With RT: radiation recall reactions independent from timing of RT With didanosine: ↑ incidence of pancreatitis and neurotoxicity	DL leukopenia, after a median of 10 days, recovering at discontinuation; maculopapular rash and facial erythema; LFT abnormalities; drowsiness; transient renal function abnormalities
Rapidly concentrated and metabolized ($T_{1/2\beta}$ 10 mins) in liver and kidney with 75% of dose excreted as metabolites in 24 hr urine	Renal and liver impairment: no guidelines available but ↓ dose	Weak MAOI: avoid concomitant use of sympathomimetic drugs (isoproterenol, ephedrine), tricyclic antidepressants, gingseng, tyramine-rich foods (dark beer, cheese, red wine, bananas), MOA and COMT inhibitors (↑ effect with headache, hypertensive crisis, tremor, palpitations) With alcohol: disulfiram-like reaction (severe GI toxicity, headache)	DL delayed myelosuppression (mainly thrombocytopenia after up to 4 wks); acute GI toxicity (nausea and vomiting, diarrhea), with tolerance after continued administration; flu-like syndrome at the beginning of treatment; allergic reactions with skin rash and pulmonary infiltrates (controlled with low-dose cortisone); CNS disturbances (paresthesia, headache, insomnia) Late toxicities: azoospermia, anovulation, ↑ incidence of second tumors after MOPP + RT
Metabolized by CYP3A4; renal elimination <1%, 58% excreted in feces. $T_{1/2}$ 180 hrs. 51% interpt variability	No effect of renal impairment Hepatic impairment: potential higher systemic exposure → close monitoring. Not recommended if AST/ALT >2.5 ULN	Avoid concomitant treatment with strong CYP3A4 inhibitors	Most common: 77% early reversible neutropenia (50% severe); 13% severe thrombocytopenia; 13% severe anemia; 45% hepatic toxicity (↑ ALT/AST peak values on day 5 and recovery by day 15); 23% hyperbilirubinemia (1% severe); 26% CPK elevations. <1% CPK ↑ in association with rhabdomyolysis with severe liver and/or renal impairment. In case of rhabdomyolysis: start immediately parenteral hydration, urine alkalinization, and dialysis. 63% nausea; 38.5% vomiting; 10% severe fatigue/asthenia

Table 4 *Pharmacological aspects of tretinoin and bexarotene*

Agent	Mechanism of action	Dosing	Pharmacokinetics
10. Tretinoin (all-trans retinoic acid, ATRA)	Differentiating effect through binding to cytosolic and nuclear receptors (RARs) with induction of transcription of genes involved in growth inhibition and differentiation. ATRA most active among natural retinoids in reversing changes of epithelial-derived malignancies	For maintenance regimen only Intermittent PO schedule (to overcome metabolic induction): 45 mg/m^2 daily, in two divided doses, on days 1–14 q 3 months Administer in fed conditions Monitor LFTs: temporary withdrawal if >5 x ULN RA-APL syndrome: HD IV steroids at first suspicion	>95% protein bound; T_{max} 1–2 hrs, F 50% affected by biliary pH and high-fat meal; high interpt variability in absorption and plasma levels. $T_{1/2}$ <1 hr, undetectable after 10 hrs
11. Bexarotene	Mechanism of action on CTCL unknown. Binds selectively RXR subtypes (RXRα, RXRβ, RXRγ), forms heterodimers with other RARs, vitamin D receptor, and PPARs Activated receptors function as transcription factors for cellular differentiation and proliferation Induces growth inhibition in vitro and tumor regression in vivo in some animal models	Topical gel: 1st wk: one application every other day, then ↑ frequency, at weekly intervals, to 1/day, 2/day, and 3/day daily. ↑ response with ≥ 2/day Duration: optimal unknown, use until benefit, maximum experienced length 172 wks Wait at least 20 mins before bathing Avoid use of occlusive dressing and contact with healthy skin and mucosa Oral form: PO daily: initial 300 mg/m^2; if not tolerated, decrease by 100 mg/m^2 decrement until tolerated, then re-escalate to 300 mg/m^2. If response not achieved after 8 wks and well tolerated, escalate to 400 mg/m^2 Duration: optimal unknown, use until benefit, maximum experienced length 97 wks	>99% protein bound; after topical application plasma concentration usually low (<5 ng/ml). Tissue uptake not studied After PO, metabolized by CYP3A4 to two active oxidative metabolites then glucuronidated; <1% renal excretion

Metabolism	Dosing when organ dysfunction	Drug interactions	Toxicity
Metabolized by CYP450 to 4-OXO-ATRA then glucuronidated; 60% excretion in urine, 30% in feces Induces its own metabolism: ↑ Cl after 2 wks chronic dosing due to ↑ catabolism and ↑ tissue sequestration		Tetracycline: pts must not be treated by ATRA and tetracycline at the same time (↑ intracranial hypertension)	86% headache, in 50% due to ↑ intracranial pressure (pseudotumor cerebri, especially in children), early signs: papilledema, nausea and vomiting, visual disturbances; 77% skin and mucosal toxicity (dryness, itching, peeling, cheilitis); 70% bone pain, arthralgia; 50% ↑ LFTs slowly reversible; 25% edema, fatigue, fever, and rigors; 17% ocular disorders; 25% RA-APL syndrome (usually during 1st month) with leukocytosis, fever, hypotension, dyspnea, RX lung infiltrates, fluid retention, CHF, DIC-like syndrome (differential diagnosis with APL) Teratogenic: avoid pregnancy
CYP3A4	Renal impairment: Caution: PK might be altered because of high protein binding Hepatic impairment: not studied		72% rash (56% at application site); 36% pruritus; 30% pain, 6% paresthesia; 14% contact dermatitis Common: 70% reversible hypercholesterolemia and hyperlipidemia (30–40% G3-4); 45% asthenia; 41% headache (10% G3-4); 41% diarrhea (5% G3-4); 28% exfoliative dermatitis (8% G3-4); 22% infections (4% G3-4); 11% alopecia; hematological: 47% leukopenia (10% G4), 38% anemia (including 13% hypochromic) Less frequent: ↑ LFTs; pancreatitis; hypothyroidism Severity and incidence ↓ with <300 mg/m^2

Further Reading

Aronoff GM, Bennett WM, Berns JS, et al. Drug Prescribing in Renal Failure: Dosing Guidelines for Adults and Children, Fifth Edition. Philadelphia: American College of Physicians, 2007.

Carter NJ, Keam SJ. Trabectedin: a review of its use in soft tissue sarcoma and ovarian cancer. Drugs 2010; 70:355–376.

Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. N Engl J Med 1995; 332:1132–1136.

Floyd J, Mirza I, Sachs B, et al. Hepatotoxicity of chemotherapy. Semin Oncol 2006; 33:50–67.

Sleijfer S. Bleomycin-induced pneumonitis. Chest 2001; 120:617–624.

Soignet SL, Frankel SR, Douer D, et al. United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol 2001; 19:3852–3860.

Warrell RP Jr, Frankel SR, Miller WH Jr, et al. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid). N Engl J Med 1991; 324:1385–1393.

Hormonal Pathways Modulators of the Reproductive System

9

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Antiestrogens (Table 1)

Selective Estrogen Receptor Modulators (SERMs)

Tamoxifen is a prodrug metabolized by CYP3A4 and CYP2D6 into two active hydroxylated metabolites, 4-hydroxytamoxifen and 4-OH-N-desmethyltamoxifen (endoxifen). The latter has an affinity for the estradiol receptor about 100 times greater than tamoxifen. Tamoxifen and its metabolites commonly exert antagonistic effects on breast tumor cells by inhibition of both translocation and nuclear binding of the estrogen receptor (ER).

More than 80 variants of the CYP2D6 gene have been identified and four different enzymatic phenotypes influencing tamoxifen metabolism have been characterized: ultra-rapid metabolizers (2% of Caucasian population), extensive metabolizers (70% of Caucasian population), intermediate metabolizers (20% of Caucasian population; 40–70% of Asian population), and poor metabolizers (7% of Caucasian population). To date, a clear link between polymorphisms of the CYP2D6 gene and tamoxifen efficacy has not been demonstrated.

Toremifene is an antiestrogen pharmacologically similar to tamoxifen. Data from randomized studies suggest equivalent efficacy of toremifene and tamoxifen, with major cross-resistance in women with metastatic breast cancer and similar rates of secondary endometrial cancer.

Table 1 *Pharmacological aspects of antiestrogens*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
1. Tamoxifen	ER antagonist / partial agonist	PO (with or without food) 20 mg/day	T_{max} 5 hrs $T_{1/2}$ 5–7 days C_{SS} after 4 wks Extensive enterohepatic circulation with 26–65% of dose excreted into bile and 9–13% in urine No food effect	Liver metabolism by CYP2D6, 3A4, and 2C9 to endoxifen
2. Toremifene	ER antagonist / partial agonist	PO (with or without food) 60 mg/day	T_{max} 3–4 hrs $T_{1/2}$ 5 days C_{SS} after 2 wks Excretion 70% in feces, 10% in urine Food effect: none	Liver metabolism by CYP3A4
3. Fulvestrant	ER antagonist	500 mg (two 250 mg IM injections) on days 0, 14, 28, and q 28 days thereafter	T_{max} 7–9 days $T_{1/2}$ 40–50 days Excretion: mainly in feces	Liver metabolism by CYP3A4

Tamoxifen is used for treating ER-positive breast cancer in the adjuvant setting as well as in the metastatic setting. Thromboembolic events and adenocarcinomas of the endometrium are rare. Tamoxifen should be used with caution in patients with a history of thromboembolic events.

Fulvestrant is an ER antagonist that competitively binds to the ER with a much greater affinity than tamoxifen. The downregulation of cellular levels of the ER protein results in complete abrogation of estrogen-sensitive gene transcription. This distinct mechanism of action ensures a lack of absolute cross-resistance with other hormonal agents. In contrast to tamoxifen, fulvestrant has no known estrogen-agonist effects. In second line, fulvestrant and aromatase inhibitors have shown comparable efficacy. A randomized, double-blind phase III study which compared a fulvestrant 500 mg regimen with loading dose at day 14 to the originally approved dose of 250 mg showed a statistically significant increase in progression-free survival for the higher dose. The most common toxicities are injection-site reactions and hot flashes.

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal failure: no adjustment required Hepatic failure: adjustment required, no details found	Avoid strong inhibitors of CYP2D6 such as paroxetine and fluoxetine Avoid grapefruit juice May increase anticoagulant effect of warfarin	25–81% hot flashes; 2–5% thromboembolic events; 0.8% endometrial cancer, endometrial changes (hyperplasia, polyps); 2–23% vaginal bleeding; 4–29% arthralgia/myalgia. Regular Gy control, vaginal US and endometrial sampling if bleeding Monitor PLT in pts on coumarins	NA
Renal failure: no adjustment required Hepatic failure: consider dose reduction of 50%	May increase oral anticoagulant effect Should not be used with drugs that prolong the QT interval	See tamoxifen Arrhythmias, QT/QTc interval prolongation	NA
Renal/hepatic failure: no adjustment required	None	28% GI disturbances; 13% hot flashes; 14% joint disorders; 16.7% injection site pain	NA

Aromatase Inhibitors (AIs) (Table 2)

AIs are agents blocking aromatase, a microsomal enzyme of the cytochrome P450 family, encoded by the *CYP19* gene. Aromatase catalyzes the final step in estrone (E1) and estradiol (E2) biosynthesis. Aromatase is expressed primarily in the ovary and to a lesser extent in brain, adipose tissue, liver, bone, muscle, and breast. In postmenopausal women, as ovarian estrogen production declines, the process of peripheral aromatization becomes the main source of endogenous estrogens and can maintain the growth of hormone-dependent tumors. Aromatase expression in breast cancer cells contributes to tumor intracrine growth stimulation.

AIs can be classified as nonsteroidal or steroidal according to their structure, and reversible or irreversible according to the type (ionic or covalent) of target binding.

Nonsteroidal AIs, anastrozole and letrozole, exert their antagonistic effect by binding the heme iron atom of aromatase. Their antagonistic effect is reversible allowing androgens to competitively displace these inhibitors from the

Table 2 *Pharmacological aspects of aromatase inhibitors (AIs)*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
4. Anastrozole	Reversible, nonsteroidal aromatase inhibitor	PO (with or without food) 1 mg/day	T_{max} 2 hrs $T_{1/2}$ 50 hrs (85%) Excretion: biliary (85%) and renal (10%) Food effect: C_{max} decreased by 16% and median T_{max} delayed from 2 to 5 hrs	Hepatic N-dealkylation, hydroxylation, glucuronidation
5. Letrozole	Reversible, nonsteroidal aromatase inhibitor	PO (with or without food) 25 mg/day	T_{max} 1 hr $T_{1/2}$ 48 hrs Excretion: urine 90%	Hepatic N-dealkylation, hydroxylation, glucuronidation
6. Exemestane	Irreversible, steroidal aromatase Inhibitor	PO (with food) 25 mg/day	T_{max} 1.2 hrs $T_{1/2}$ 24 hrs Less than 1% excreted unchanged in urine Food effect: high-fat food increases plasma levels	Metabolized in the liver to 17-hydroexemestane by multiple P450 isoforms Oxidation and aldo-keto reductases reduction primarily by CYP3A4

active site of the enzyme. Exemestane, a steroidal AI, binds irreversibly to the active site of the enzyme inactivating it and inducing its rapid degradation. It is structurally related to androstenedione, a natural substrate of aromatase, but the compound is resistant to the activity of aromatase. None of the three drugs induce meaningful clinical alterations in adrenal steroidogenesis, and show comparable in-vitro IC_{50} for placenta aromatase, with letrozole only marginally more potent than the other two. In premenopausal women AIs induce an increase in gonadotropin secretion, secondary to the reduced negative feedback of estrogen to the pituitary, with consequent ovarian stimulation.

AIs are used in hormone-responsive postmenopausal breast cancer patients in both the metastatic and adjuvant setting. To date, there are no data suggesting any major differences in clinical efficacy among next-generation AIs. An interesting clinical observation is the lack of complete cross-resistance between nonsteroidal and steroidal AIs in the metastatic setting.

AIs are well tolerated and the rate of discontinuation was <3% in clinical studies. The main side effects of AIs are related to hypoestrogenism.

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal failure: no adjustment required No adjustment required in mild to moderate hepatic dysfunction, with severe hepatic dysfunction information not available	No significant interactions	13–35% hot flashes; 7% hypercholesterolemia; 7% osteoporosis; 6–11% fractures; 5–36% arthralgia/myalgia; 11–19% nausea; 9–14% headache; 8–10% peripheral edema; 19% fatigue; 13% weight gain; 3% vaginal dryness	NA
Renal failure, mild to moderate hepatic dysfunction: no adjustment required	Inhibits CYP3A4 and 2A6	6–47% hot flashes; 4–12% hypercholesterolemia; 6% osteoporosis; 42% fractures; 12–21% arthralgia/myalgia; 11% nausea; 13–18% headache; 9–17% peripheral edema; 11–30% fatigue; 6% weight gain; 8–10% constipation	NA
Renal failure: no adjustment required Hepatic failure: no adjustment required	No significant interactions	13–42% hot flashes; 2–5% peripheral edema; 22–24% fatigue; 11–18% nausea; 7% osteoporosis; 2–5% fractures; 2–33% arthralgia/myalgia; 8–19% headache; 6% gynecological symptoms; 11–20% insomnia	NA

A significant bone mineral loss risk of bone fracture has been demonstrated with these agents. Other common side effects are arthralgias and myalgias.

Gonadotropin-Releasing Hormone (GnRH) Analogs

(Table 3)

GnRH analogs have been obtained by substitution of L-glycine in position 6 and modification of the C-terminal, and show an increased affinity for the GnRH receptor and a reduced susceptibility to enzymatic degradation. They act on the GnRH receptor on the pituitary gland and induce, during the first days of treatment, a stimulation of gonadotropin release, followed by an inhibition of gonadotropin secretion due to receptor downregulation. In metastatic prostate cancer patients, the initial, transient elevation in gonadal steroidogenesis can result in a tumor flare, which can be prevented by administering an antiandrogen for the first 2-4 weeks of treatment by a GnRH analog. No significant differences among available GnRH analogs were shown in preclinical models or in the clinical setting.

In men, treatment by GnRH analogs provides a reduction in serum androgen concentration to castrate levels within 3 to 4 weeks after the initiation of therapy. In women, serum estradiol production is suppressed after about 3 weeks.

In prostate cancer patients, GnRH analogs are used, alone or in combination with an antiandrogen, as adjuvant to curative radiotherapy and in the metastatic setting. In premenopausal breast cancer patients, these drugs are used, alone or in combination with tamoxifen or an AI, in both the adju-

Table 3 *Pharmacological aspects of gonadotropin-releasing hormone (GnRH) analogs / antagonists*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
7. Buserelin, goserelin, leuprolide (leuprorelin acetate), triptorelin	GnRH analogs	Buserelin: 3.3 mg SC/2 months Goserelin: 3.6 mg SC/4 wks, 10.8 mg SC/12 wks Leuprolide (Enantone®): 3.75 mg SC or IM/4 wks, 11.25 mg SC or IM/12 wks, 30 mg SC/6 months Leuprolide (Eligard®): 7.5 mg SC/4 wks, 22.5 mg SC/12 wks, 45 mg SC/6 months Triptorelin: 3 mg IM/4 wks, 11.25 mg IM/12 wks, 22.5 mg IM/6 months	T_{max} 1–12 hrs Renal and biliary excretion	Enzymatic degradation to smaller peptides
8. Degarelix	GnRH antagonist	Initial dose: 240 mg, administered as 2 injections of 120 mg each SC, then monthly maintenance doses of 80 mg	T_{max} <2 days Terminal $T_{1/2}$ 53 days Excretion: 70–80% in feces and 20–30% in urine	Peptide hydrolysis in hepatobiliary system, peptide fragments excreted in feces

vant and the metastatic setting. Their role in the adjuvant setting combined with other hormonal agents will be defined in ongoing clinical trials.

Gonadotropin-Releasing Hormone Antagonists

(Table 3)

GnRH antagonists were developed by modifying the structure of GnRH and showed clinical efficacy comparable to GnRH agonists with a more rapid suppression of testosterone. In contrast to GnRH agonists, they do

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal / hepatic failure: no adjustment required	No significant interactions	Weight gain; headache; 50% hot flashes; drug-induced disease flare; loss of muscle mass; fracture; decreased bone mineral density; 50% depression and memory loss; 60% decreased libido, impotence (very frequent); nausea; 100% amenorrhea; myalgia; arthralgia; vaginal bleeding	NA
Renal failure: use with caution in pts with moderate to severe impairment Hepatic failure: use with caution in pts with moderate to severe impairment	Use with caution with drugs known to prolong the QTc interval or able to induce torsades de pointes	1% myocardial infarction; A-V first degree block; 6–7% hypertension; 5–10% ↑ AST/ALT (severe <1%); 20% QT/QTc interval prolongation; 25–26% hot flashes; 35–44% injection site reactions (<2% severe); 90% impotence; 100% libido decrease	NA

not cause a temporary testosterone increase and concomitant use of an antiandrogen at the beginning of treatment is not necessary. Degarelix is a synthetic linear decapeptide amide containing seven artificial amino acids. It is a reversible competitive antagonist of pituitary GnRH receptors and suppresses the release of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and consequently testosterone. Testosterone suppression occurs rapidly after administration in 96% of patients.

Progestins (Table 4)

Medroxyprogesterone and megestrol are 17-OH-progesterone derivatives, with actions similar to those of the parental compound. These drugs act by reducing LH secretion and ER levels. Medroxyprogesterone and megestrol are used in previously treated metastatic breast cancer or in advanced endometrial carcinoma. Because of the significant risk of thrombosis, they should be used with caution in patients with a history of thromboembolic events.

Antiandrogens (Table 5)

Bicalutamide, Nilutamide, Flutamide

Antiandrogens are competitive antagonists of the androgen receptor (AR); they inhibit androgen binding and prevent translocation of the androgen receptor complex from cytoplasm to nucleus. They do not inhibit pituitary

Table 4 Pharmacological aspects of progestins

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
9. Medroxy-progesterone	Progestin	PO: 200–400 mg daily in divided doses (without food) IM: 500 mg 2/wk	T_{max} 2–4 hrs $T_{1/2}$ 2 days Excretion: 10% in feces, 40% in urine Food increases C_{max} and AUC	Promptly metabolized in liver by conjugation
10. Megestrol	Progestin	160 mg/day (with or without food)	T_{max} 2–5 hrs $T_{1/2}$ 15–20 hrs Excretion: 20% in feces, 66% in urine Food effect: no detail found	Inactivated in intestine and liver to free steroids and glucuronide conjugates

LH secretion, so that during antiandrogen treatment the testosterone levels are normal or increased, and libido and potency often preserved.

In advanced prostate cancer patients, antiandrogens are currently indicated to prevent the initial LH flare-up reaction and in association with medical or surgical castration. In patients resistant to antiandrogen treatment, withdrawing the drug can obtain tumor responses.

Nonsteroidal antiandrogens may be associated with lower overall survival when compared to castration. The steroidal antiandrogen cyproterone is inferior to GnRH agonists in delaying progression and should not be used. Antiandrogens should not be proposed as initial monotherapy, but can be used in patients willing to preserve potency. No difference in efficacy was detected between different nonsteroidal antiandrogens; the toxicity profile of nilutamide is the least favorable, mostly due to visual disturbances and interstitial pneumonitis, whereas hepatic toxicity, albeit rare, occurs more often with flutamide and nilutamide treatment.

MDV3100

MDV3100 (enzalutamide), which is a small-molecule antagonist of the AR with a higher affinity for the receptor than bicalutamide, impairs nuclear translocation, DNA binding, and coactivator recruitment, leading to cellular apoptosis. In a recent phase III trial, MDV3100 showed encouraging antitumor activity in patients with castration-resistant prostate cancer. Further clinical development is ongoing.

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal failure: no adjustment required Hepatic failure: adjustment required, no details found	NA	Hot flashes; vaginal bleeding; weight gain; headache; nausea; depression; adrenal insufficiency; hypertension; thrombophlebitis; fatigue; insomnia; nervousness; amenorrhea; ↑ LFTS; decreased glucose tolerance	NA
NA	NA	Thrombophlebitis; 10% headache; 6% asthenia; 15% diarrhea; 6% vomiting; 5% nausea; 6% hyperglycemia, glucose intolerance	NA

Table 5 *Pharmacological aspects of antiandrogens*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
11. Bicalutamide	AR antagonist	PO (with or without food) 50 mg/day	T_{max} up to 48 hrs $T_{1/2}$ 5–7 days Excretion: urine 16%, feces 43% Food effect: none	Hepatic (oxidation and further glucuronidation of active isomer)
12. Nilutamide	AR antagonist	PO (with or without food) 300 mg/day for 30 days, then 150 mg/day	Rapidly absorbed $T_{1/2}$ 56 hrs Excretion: urine ≤78%, feces 1–7% No food effect	Hepatic (oxidation)
13. Flutamide	AR antagonist	PO 250 mg 3/day	Data refer to hydroxyflutamide, the active metabolite T_{max} 2 hrs $T_{1/2}$ 6 hrs Excretion: primarily in urine, feces 4% No food effect	Hepatic (oxidation)
14. MDV3100	AR antagonist	PO 160 mg/day	T_{max} between 30 min and 4 hrs $T_{1/2}$ about 7 days	NA
15. Abiraterone	CYP17 inhibitor	PO (without food) 1 g/day concomitantly with prednisone 5 mg 2/day	T_{max} 2 hrs $T_{1/2}$ 12 ± 5 hrs Excretion: 88% in feces and 5% in urine Food may increase absorption	Hepatic (via CYP3A4 and SULT2A1)

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal failure: no adjustment required Hepatic failure: caution in pts with moderate to severe impairment Cardiac failure: caution, can cause fluid retention	Warfarin: Check INR at the beginning and at the end of treatment Use with caution concomitantly with CYP3A4 substrates	51% hot flashes; 17% constipation; 15% back pain; 15% fatigue; 4% breast tenderness; 6% gynecomastia; 1–5% increased bilirubin levels; 6% ↑ AST / ALT	Monitor serum transaminase levels at regular intervals for the first 4 months of treatment, and periodically thereafter
Renal failure: no adjustment required Hepatic failure: not recommended in pts with severe hepatic impairment or with AST / ALT >2–3 times the upper limit of normal Respiratory failure: contraindicated	Alcohol: Infrequent disulfiram-like reactions	4–11% gynecomastia; 14–67% hot flashes; 1–13% elevated serum transaminases; 22% visual disturbances; 1–2% interstitial pneumonitis; loss of libido, impotence	Pneumonitis: resolves after stopping therapy, evaluate treating by corticosteroids, fatalities have been described Visual disturbances: may disappear after dose reduction Pts of Japanese origin have an increased risk of hepatic and pulmonary toxicity
Renal failure: no adjustment required Hepatic failure: see nilutamide Cardiac failure: use with caution, can cause fluid retention	Warfarin: check INR at the start and the end of treatment Grapefruit juice may inhibit CYP3A4 metabolism of flutamide in the intestinal wall	12% diarrhea (5% severe); 9% gynecomastia; 61% hot flashes; 11% nausea and vomiting; hepatic dysfunction including hepatic necrosis; <1% interstitial pneumonitis; loss of libido; 7% impotence	Measure LFTs regularly during the first 3 months of therapy
NA	NA	Fatigue (frequent); 5% seizures	NA
Renal failure: no adjustment required Hepatic failure: not recommended in pts with moderate to severe hepatic impairment (Child-Pugh Class B or C) Cardiac failure: caution, can cause fluid retention	Abiraterone is an inhibitor of CYP2D6: avoid coadministration of substrates of CYP2D6 with a narrow therapeutic index, caution with concomitant CYP2D6 substrate drugs Abiraterone is a substrate and a moderate inhibitor of CYP3A4: caution with concomitant strong inhibitors and inducers of CYP3A4	19% hot flashes; 10% hypertension; 13% arrhythmia; 18% diarrhea; 31% high transaminase; 17% low potassium; 10% high total bilirubin; 31% edema; 44% fatigue; 30% nausea	Monitor monthly for arterial hypertension, hypokalemia, and clinical signs of mineralocorticoid excess. Measure LFTs q 14 days for 3 months, then monthly. For G3 hepatic toxicity, withhold treatment until return to < G1, then reinitiate at 500 mg/day. If hepatotoxicity recurs discontinue treatment

Inhibitor of Androgen Biosynthesis

Abiraterone is a potent, selective, and irreversible inhibitor of CYP17-hydroxylase and C-17,20-lyase activities, both essential steps in androgen biosynthesis by adrenal glands, testes, and within prostate tumors. Continuous CYP17 inhibition results in raised levels of ACTH, which increases steroid levels upstream of CYP17 and induces a syndrome of secondary mineralocorticoid excess. These adverse effects are best avoided by the coadministration of steroids. Concomitant castration is necessary to prevent a compensatory LH surge that can overcome CYP17 blockade.

A phase III study of abiraterone versus placebo in patients previously treated by docetaxel showed a significant improvement in overall survival in favor of abiraterone from 10.9 to 14.8 months.

Further Reading

Ali S, Buluwela L, Coombes RC. Antiestrogens and their therapeutic applications in breast cancer and other diseases. Annu Rev Med 2011; 62:217–232.

Attard G, de Bono JS. Translating scientific advancement into clinical benefit for castration-resistant prostate cancer patients. Clin Cancer Res 2011; 17:3867–3875.

Chumsri S, Howes T, Bao T, et al. Aromatase, aromatase inhibitors, and breast cancer. J Steroid Biochem Mol Biol 2011; 125:13–-22.

Croxtall JD, McKeage K. Fulvestrant: a review of its use in the management of hormone receptor-positive metastatic breast cancer in postmenopausal women. Drugs 2011; 71:363–380.

Dent SF, Gaspo R, Kissner M, et al. Aromatase inhibitor therapy: toxicities and management strategies in the treatment of postmenopausal women with hormone-sensitive early breast cancer. Breast Cancer Res Treat 2011; 126:295–310.

Seruga B, Ocana A, Tannock IF. Drug resistance in metastatic castration-resistant prostate cancer. Nat Rev Clin Oncol 2011; 8:12–23.

Tan SH, Wolff AC. Luteinizing hormone-releasing hormone agonists in premenopausal hormone receptor-positive breast cancer. Clin Breast Cancer 2007; 7:455–464.

Growth Factor Receptor Targeting: Anti-HER and Anti-IGF-1R

10

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Introduction

Growth factor receptor targeting is a validated therapeutic strategy in human malignancies. Monoclonal antibodies (mAbs) are directed to the extracellular domains of growth factor receptors, show high specificity, have the potential to engage and activate the immune system, and require intravenous administration. Small-molecule tyrosine kinase inhibitors (TKIs) are orally available drugs developed to block intracellular signaling transduction pathways, thereby leading to deregulation of key cell functions, including proliferation, angiogenesis, invasion, and metastasis.

Anti-HER Agents (Table 1)

Members of the epidermal growth factor family of transmembrane receptors (ERBB/HER family) are potent mediators of cell growth and development. The HER family consists of four closely related tyrosine kinase receptors: epidermal growth factor receptor (EGFR; also known as HER1), ERBB2 (HER2), ERBB3 (HER3), and ERBB4 (HER4). Binding of ligands/growth factors to the extracellular domain of the ERBB receptors (except for ERBB2) stabilizes them in a conformation that allows dimerization (pairing),

Table 1 *Pharmacological aspects of anti-EGFR agents*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
1. Erlotinib	Reversible TKI of EGFR	PO (empty stomach) Single agent: 150 mg daily Combination with gemcitabine (pancreatic cancer): 100 mg daily	T_{max} 3–4 hrs $T_{1/2}$ 24–36 hrs Excretion: feces (83%) and urine (8%) primarily as metabolites Food effect: bioavailability almost 100% with food and 60% without food. Avoid grapefruit juice (increase erlotinib levels) and St John's wort (decrease erlotinib levels)	Hepatic (mainly via CYP3A4)
2. Gefitinib	Reversible TKI of EGFR	PO (can be administered with or without food) Single agent: 250 mg daily	T_{max} 3–6 hrs $T_{1/2}$ 41 hrs Excretion: feces (86%) Food effect: no change in bioavailability with food. Avoid grapefruit juice (increase gefitinib levels) and St John's wort (decrease gefitinib levels)	Hepatic (mainly via CYP3A4)
3. Cetuximab	Anti-EGFR IgG1 chimeric mAb	IV Loading dose: 400 mg/m^2 over 2 hrs Maintenance dose: 250 mg/m^2 over 1 hr Concomitant with radiotherapy: Administer loading dose 1 wk prior to radiation course and then weekly 1 hr before radiation dose (6–7 wks) Biweekly regimen*: 500 mg/m^2 (initial dose over 2 hrs and thereafter over 1 hr) Premedicate with antihistamines (may be discontinued if no reaction in first infs)	$T_{1/2}$ 5 days	Most likely removed by opsonization via the reticuloendothelial system

*Bi-weekly cetuximab approved in colorectal cancer

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal: interrupt treatment if risk of dehydration Hepatic: for G2 transaminase or bilirubin elevation, 50% dose reduction is recommended, with gradual dose escalation if tolerated	Antacids, H_2-antagonists, and proton pump inhibitors may decrease serum concentration. Dose reduction likely to be needed with concomitant strong CYP3A4 inhibitors and dose increase is possible with CYP3A4 inducers. Concomitant smoking may decrease erlotinib exposure	Rash/skin toxicity (up to 75%, G3 in 10%, onset in first 2 wks); diarrhea (up to 55%, G3 in 6%, onset in first 2 wks); 50% fatigue (10% G3); 30% nausea/vomiting; anorexia (up to 50%); stomatitis/mucositis (up to 20%); 10% conjunctivitis; hepatic (transaminitis, increase in bilirubin ≥ G2 in 5% – reaches 20% when combined with gemcitabine); dyspnea/cough (up to 40%, interstitial pneumonitis in 3%); microangiopathic hemolytic anemia with thrombocytopenia when combined with gemcitabine (rare)	Liver toxicity: in pts with normal baseline liver function if transaminase >5 x ULN and/or bilirubin >3 x ULN, interrupt treatment until recovery to baseline. Consider dose reduction of 50% Diarrhea or skin toxicity > G2: interrupt temporarily and consider dose reduction if repeated toxicity (of 30% to 50% – decrements of 50 mg) Pulmonary symptoms: interrupt if suspicious interstitial lung disease. Discontinue if confirmed lung toxicity
Renal: no adjustment necessary Hepatic: no adjustment necessary for moderate liver dysfunction, but gefitinib exposure can be higher	H_2-antagonists and proton pump inhibitors may decrease serum concentration. Dose reduction likely to be needed with concomitant strong CYP3A4 inhibitors and dose increase is possible with CYP3A4 inducers	65% diarrhea (1% G3); 55% rash/skin toxicity; 10% anorexia; interstitial lung disease (up to 2%); hepatotoxicity (rare); conjunctivitis (rare)	Liver toxicity: if severe, discontinue therapy Diarrhea or skin toxicity > G2: interrupt temporarily Pulmonary symptoms: interrupt if suspicious interstitial lung disease. Discontinue if confirmed lung toxicity
Renal: no expected change in disposition with mild/moderate renal dysfunction Hepatic: few data, but no need for adjustment with mild/moderate liver dysfunction	There are no known significant interactions	20% infusion reaction (5% G3, mostly first infusion); 90% rash/skin toxicity (15% G3, onset in first 2 wks); 80% fatigue; 55% hypomagnesemia (15% G3); 40% diarrhea; 40% nausea/vomiting; 30% headache; 25% stomatitis (mild); 10% transaminase increase; interstitial pneumonitis (rare); risk of cardiopulmonary arrest (related to infusion reaction or electrolyte abnormalities)	Infusion reaction: mild to moderate (chills, fever, dyspnea) are managed with slowing infusion rate (50%), antihistamines and eventually steroids. If severe, discontinue therapy Acneiform rash > G2: delay cetuximab infusion for 1–2 wks. If repeated episodes, reduce dose by 50 mg/m^2 Pulmonary symptoms: hold if suspicious interstitial lung disease. Discontinue if confirmed lung toxicity Monitor magnesium levels and supplement accordingly
*Bi-weekly cetuximab approved in colorectal cancer			

Table 1 *Pharmacological aspects of anti-EGFR agents (Continued)*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
4. Panitumumab	Anti-EGFR IgG2 fully humanized mAb	IV 6 mg/kg q 2 wks or 9 mg/kg q 3 wks. Infuse over 1 hr (for doses >1000 mg, over 90 mins). Consider inf over 30 mins if no reaction on first inf	$T_{1/2}$ 7.5 days	Most likely removed by opsonization via the reticuloendothelial system

an essential requirement for transactivation of the tyrosine kinase portion of the dimer moiety, leading to phosphorylation and downstream signaling. Dimerization can occur between two different ERBB receptors (heterodimerization) or between two molecules of the same receptor (homodimerization). Homodimers weakly perpetuate signals as compared to heterodimers. EGFR, HER2, and HER3 are all implicated in the development and progression of cancer. The most important downstream pathways activated in solid tumors are Ras-Raf-MEK-ERK and PI3K-Akt-mTOR.

Targeting EGFR

Gain of function genetic alterations in EGFR are observed in many epithelial tumors and certain neoplasms are clearly dependent on the pathway – "oncogene-addicted" – such as lung cancer cells presenting mutations in the EGFR kinase domain. Small-molecule TKIs of EGFR, such as erlotinib and gefitinib, have impressive clinical activity in this scenario. On the other hand, the genetic background of some tumors simply precludes a function for EGFR inhibition, what is called inherited resistance. For instance, the presence of mutant KRAS is a strong predictor of failure of response to anti-EGFR mAbs, such as cetuximab and panitumumab, in colorectal cancer.

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Renal: no expected change in disposition with mild/moderate renal dysfunction Hepatic: few data, but no need for adjustment with mild/moderate liver dysfunction. Not studied in severe renal/hepatic impairment	There are no known significant interactions	90% rash/skin toxicity (15% G3); 40% hypomagnesemia (4% G2); 20% diarrhea (usually mild); 7% stomatitis; 4% conjunctivitis; 3% infusion reaction (<1% G3); interstitial lung disease (rare)	Infusion reaction: mild to moderate (chills, fever, dyspnea) are managed with slowing infusion rate (50%), antihistamines and eventually steroids. If severe, discontinue therapy Acneiform rash > G2: delay panitumumab infusion for up to 4 wks, then reduce dose by 25% to 50% Pulmonary symptoms: hold if suspicious interstitial lung disease. Discontinue if confirmed lung toxicity Monitor magnesium levels and supplement accordingly

Erlotinib and gefitinib

These agents bind in a reversible fashion to the adenosine triphosphate (ATP) binding site of EGFR, thereby hampering autophosphorylation and downstream cascade signaling. In non-small cell lung cancer (NSCLC), EGFR mutations – particularly deletions in exon 19 and exon 21 point mutations (L858R) – confer tumor cell dependency on EGFR signaling and are predictive of response to the first-generation reversible EGFR TKIs. These mutations occur in 10–20% of NSCLC patients, mainly non-smokers with adenocarcinoma, and are rare in other tumor types. The predictive value of the molecular selection of patients has been confirmed in the first-line treatment of advanced NSCLC. Single-agent erlotinib has also shown efficacy in chemotherapy-refractory NSCLC patients, irrespective of *EGFR* mutation status.

Common toxicities with erlotinib and gefitinib include acneiform rash, diarrhea, and fatigue. Development of rash is associated with improved survival outcomes, which suggests that a mechanism-based toxicity can be a biomarker of efficacy. Interstitial lung disease has also been described with EGFR TKIs and worsening pulmonary symptoms should prompt specific diagnostic tests. Periodic liver function tests are also recommended due to the risk of hepatotoxicity. There is an important

potential interaction with H_2-antagonists and proton pump inhibitors; when given concomitantly, erlotinib exposure can be decreased by 50%, and separate drug administrations by several hours should be considered.

Cetuximab and panitumumab

Cetuximab is a chimeric IgG1 mAb that binds to the extracellular domain of EGFR, thereby blocking signal transduction to the downstream pathways. It also potently induces antibody-dependent cellular cytotoxicity (ADCC). Cetuximab is approved for use in irinotecan-resistant metastatic colorectal cancer (CRC) as well as in the first-line setting in combination with either irinotecan- or oxaliplatin-based chemotherapy, and the benefit is restricted to patients whose tumor had *KRAS* wild-type status. In head and neck squamous cell carcinoma (HNSCC), cetuximab is approved as curative treatment of locally advanced disease in combination with radiotherapy as an alternative to chemoradiotherapy and in recurrent disease with or without cisplatin-based chemotherapy.

Panitumumab is a fully human IgG2 mAb with higher affinity for EGFR than cetuximab and fewer hypersensitivity reactions since it contains no murine sequences. It does not mediate potent ADCC. Large randomized trials confirmed the progression-free survival (PFS) benefit of adding this agent to first-line and second-line chemotherapy in CRC KRAS wild-type patients. Panitumumab represents an alternative to cetuximab in patients who developed severe infusion reactions to cetuximab.

As with EGFR TKIs, common adverse events with mAbs targeting EGFR include skin toxicity, diarrhea, and stomatitis. Acneiform rash can be severe in up to 15% of the patients and specific treatment guidelines have been published (see Further Reading). Those who present rash have improved outcomes, which highlights the importance of proper toxicity management. Magnesium levels should be monitored during treatment with cetuximab and panitumumab.

Targeting HER2 (Table 2)

The HER2 gene is overexpressed in about 20% of breast and 15% of gastric and gastroesophageal tumors, as measured by immunohistochemistry or gene amplification. HER2 positivity is associated with a more aggres-

sive phenotype, higher recurrence rates, and reduced survival. It is a prognostic and a very strong predictive marker for efficacy of HER2 targeting agents, such as trastuzumab and lapatinib.

Trastuzumab

Trastuzumab is an IgG1 humanized mAb that binds to the extracellular juxtamembrane domain of HER2 and inhibits the proliferation and survival of HER2-dependent tumors by preventing the activation of its intracellular tyrosine kinase and inducing ADCC. Initially evaluated in the metastatic setting of HER2-overexpressing breast cancer (BC), it led to significant improvement in response rate (RR), PFS, and overall survival (OS) when added to first-line chemotherapy with paclitaxel. It is active when added to multiple different chemotherapeutic agents.

Alternatives to overcome intrinsic or acquired resistance are the focus of extensive investigation – the TKI, lapatinib, is one such example. In the adjuvant setting, recurrence and mortality rates were reduced in the order of 50% and 30%, respectively, with one year of adjuvant trastuzumab therapy. In HER2-overexpressing gastric and gastroesophageal junction cancer, the addition of trastuzumab to platinum/fluoropyrimidine-based chemotherapy significantly increased RR, PFS, and OS.

The main toxicity observed with this agent is cardiac failure, especially when combined with chemotherapies with overlapping cardiotoxicity, though it occurs only in a small percentage of patients, is often asymptomatic and tends to be reversible; monitoring of left ventricular function in all patients before and during trastuzumab treatment is now recommended.

Lapatinib

The dual EGFR/HER2 TKI, lapatinib, is active as first-line monotherapy and when combined with paclitaxel in HER2+ BC. In patients progressing to trastuzumab, lapatinib added to capecitabine improved PFS compared with chemotherapy alone. Another potential advantage of lapatinib is its ability to inhibit the catalytic activity of a truncated form of HER2 ($p95^{HER2}$, overexpressed in about 25% of HER2+ tumors).

Table 2 *Pharmacological aspects of anti-HER2 agents*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism	Dosing when organ dysfunction
5. Trastuzumab	Anti-HER2 IgG1 humanized mAb	IV Loading: 4 mg/kg for weekly or 8 mg/kg for q 3 wks regimen Maintenance: 2 mg/kg for weekly or 6 mg/kg for q 3 wks regimen**	$T_{1/2}$ mean 6 days for weekly and 21 days for q 3 wks regimen	Most likely removed by opsonization via the reticuloendothelial system	Cardiac: contraindicated if LEVF <50% Renal: disposition is not altered based on serum creatinine (up to 2 mg/dL) Hepatic: few data, but no need for adjustment with mild/moderate dysfunction
6. Lapatinib	Dual reversible TKI of EGFR and HER2	PO (fasting/ empty stomach) With capecitabine: 1250 mg daily With letrozole: 1500 mg daily With trastuzumab: 1000 mg daily	T_{max} 4 hrs $T_{1/2}$ 24 hrs Excretion: feces (27% as unchanged drug) Food effect: systemic exposure is increased when administered with food. Avoid grapefruit juice (increase lapatinib levels) and St John's wort (decrease lapatinib levels)	Hepatic (via CYP3A4 and 3A5, and to a lesser extent via CYP2C19 and 2C8)	Cardiac: contraindicated if LEVF <50% Renal: dose adjustment may not be necessary Hepatic: for severe liver dysfunction (Child-Pugh C), reduce dose by 30% to 40%

**Gastric cancer: q 3 wks regimen (only approved in combination with chemotherapy)

Second-generation Anti-HER Agents (Table 3)

Second-generation anti-HER agents have been developed to reduce toxicity and increase efficacy by prolonging the inhibition of EGFR/HER2 signaling; they are currently undergoing clinical testing.

Tyrosine kinase inhibitors

Potent irreversible pan-HER inhibitors, such as afatinib (targeting EGFR and HER2 kinases), may prevent and overcome primary and acquired resistance to first-generation reversible EGFR TKIs. Afatinib showed activity in tumor xenograft models resistant to erlotinib and gefitinib, including those harboring *EGFR* T790M mutations, which has been

Drug interactions	Toxicity	Dose adjustment for toxicity
May enhance the cardiotoxic effect of chemotherapies	Cardiotoxicity (decrease in LVEF in up to 25%, severe in less than 4%); 40% infusion reactions (usually mild); 25% diarrhea; 15% rash; may increase risk of hematological toxicities of chemotherapy; interstitial pneumonitis (rare); glomerulopathy (rare)	Cardiotoxicity: LVEF ≥16% decrease from baseline (or ≥10% if LVEF below normal limits) – stop treatment for at least 4 wks and repeat LVEF. If LVEF returns to normal limits <8 wks, restart treatment. Discontinue permanently for persistent LVEF or ≥3 treatment interruptions for cardiomyopathy Infusion reaction: mild to moderate (chills, fever, dyspnea) is managed with slowing infusion rate (50%), antihistamines and eventually steroids. If severe, discontinue therapy
Dose reduction needed with concomitant strong CYP3A4 inhibitors; dose increase needed with CYP3A4 inducers. Concurrent use with other drugs that may prolong QTc interval may increase the risk of severe arrhythmias	65% diarrhea (10% G3); 40% nausea/vomiting; 15% mucositis; 20% fatigue; 40% rash/skin toxicity; 50% hepatic toxicity (5% G3 transaminase and bilirubin elevation); 4% cardiotoxicity (decrease in LVEF); may increase risk of hematological toxicities of chemotherapy; interstitial pneumonitis (rare)	Cardiotoxicity: discontinue treatment for decreased LVEF ≥ G2. After recovery and at least 2 wks break, lapatinib may be restarted with 25% dose reduction. Withhold for any other toxicity ≥ G2 until recovery to ≤ G1, then restart with 25% dose reduction
**Gastric cancer: q 3 wks regimen (only approved in combination with chemotherapy)		

found in 50% of the tissue samples from NSCLC patients with acquired gefitinib resistance.

Another potent irreversible pan-HER inhibitor is neratinib. Like lapatinib, neratinib has shown clinical activity in patients with HER2+ advanced BC who have progressed on trastuzumab.

Monoclonal antibodies

Nimotuzumab is an anti-EGFR mAb undergoing phase III clinical development. In preclinical studies, it has demonstrated antitumor activity comparable or superior to that observed with cetuximab and panitumumab. As IgG1 constructs, it has been shown to induce ADCC against

Table 3 *Pharmacological aspects of second-generation anti-HER agents*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Toxicity
7. Afatinib	Dual irreversible EGFR and HER2 TKI (including T790M *EGFR* mutants)	PO 50 mg/day fasting	T_{max} 3 hrs $T_{1/2}$ 24 hrs Metabolism: hepatic	Rash (DLT); fatigue; diarrhea; nausea; vomiting; stomatitis; anorexia
8. Neratinib	Irreversible pan-HER TKI (EGFR, HER2, HER4)	PO 240 mg/day with food	T_{max} 3–6 hrs $T_{1/2}$ 14 hrs Metabolism: hepatic (CYP3A4)	Diarrhea (DLT); fatigue; nausea; vomiting; anorexia; rash
9. Nimotuzumab	Anti-EGFR IgG1 humanized mAb	IV 200 mg (flat dose) weekly	$T_{1/2}$ 5 days Metabolism: reticuloendothelial system	Fatigue (DLT); rash; fever; chills; nausea; hypocalcemia/ hyponatrema; infusion reactions
10. Pertuzumab	Anti-HER2 IgG1 humanized mAb (distinct epitope of trastuzumab, blocks dimerization)	IV 840 mg loading dose (flat) followed by 420 mg q 3 wks	$T_{1/2}$ 20 days Metabolism: reticuloendothelial system	Fatigue; nausea/vomiting; diarrhea; rash; cardiotoxicity when combined with trastuzumab (decrease LVEF)

tumor cells. In addition, as humanized mAb, it has the potential benefit of lower risk of hypersensitivity reactions as compared to cetuximab.

Pertuzumab is an anti-HER2 IgG1 humanized mAb that binds a distinct epitope of the extracellular domain of HER2, thereby preventing ligand-driven dimerization of HER2 with other HER receptors, such as HER3, the most active HER signaling dimer. The combination of pertuzumab and trastuzumab provides a more comprehensive HER signaling blockade and it is under evaluation in HER2+ BC.

Anti-IGF-1R Agents

The insulin growth factor (IGF) axis consists of three cell membrane receptors (IGF-1 receptor [IGF-1R], IGF-2R, and insulin receptor [InsR]), two ligands (IGF-1 and IGF-2), and six high-affinity IGF-binding proteins (IGFBP-1 to -6). IGF-1R is a tyrosine kinase receptor composed of two dimers. IGF-1R has a 60% amino acid homology with InsR and it can be stimulated by insulin, but with 500- to 1000-fold less potency than when it is activated by its cognate ligands IGF-1 and IGF-2. Activation of IGF-1R

results in activation of an intracellular signaling cascade that stimulates cell proliferation and survival, and is implicated in the development and progression of multiple malignancies. As with the epidermal growth factor family of receptors, the most important downstream pathways activated in solid tumors are PI3K-Akt-mTOR and Ras-Raf-MEK-ERK.

Different strategies for the specific inhibition of the IGF pathway have been developed, including mAbs and small-molecule TKIs. The favorable initial clinical data observed with the mAbs targeting IGF-1R in patients with NSCLC have triggered several advanced trials with these drugs. However, two large phase III trials evaluating figitumumab combined with paclitaxel–carboplatin or erlotinib in patients with advanced NSCLC have been suspended after planned interim analyses reported a lack of efficacy. The current data do appear to show, however, that some advanced solid tumors respond to monotherapy with these agents, such as sarcomas of the Ewing's family and adrenocortical carcinomas, due to hyperactivation of the IGF-1R pathway. In detail, EWS-FLI1 fusion protein, the hallmark of Ewing's sarcoma, downregulates IGFBP-3 and upregulates IGF-1 expression, resulting in enhanced IGF-1R activation. In adrenocortical carcinoma, in-vitro data suggest that overexpressed IGF-2 acting via IGF-1R is relevant, and the interaction of IGF-1 and IGF-2 with IGF-1R plays a crucial role in tumorigenesis, proliferation, and metastasis.

Importantly, the next generation of trials is also focusing on combination approaches (both with chemotherapies and other signal transduction inhibitors) in multiple different tumor types, including breast, ovarian, gastric, pancreatic, and hepatocellular carcinomas, small-cell lung cancer, and neuroendocrine tumors.

Because of the ubiquitous expression of the different players of the IGF pathway and the multiple molecular mechanisms by which the IGF pathway can be altered in tumors, it is difficult to develop predictive markers of response to select patients. It is possible that tumor IGF-1R expression has little or no predictive value for response to these therapies, like expression of EGFR in metastatic colorectal cancer and tumor response to anti-EGFR agents. To better understand the role of the IGF pathway in each scenario, rational combinations in selected populations based on stringent preclinical data and smarter trial designs are necessary. In addition, the therapeutic

effect of inhibiting InsR could be an added benefit for small molecules. This is supported by the observation that InsR can substitute for IGF-1R in activating pathways for cancer progression as a compensatory mechanism and that, in some cases, InsR may have a direct role in cancer progression.

Linsitinib (OSI-906) is a potent dual IGF-1R/InsR TKI, which has demonstrated in-vitro antiproliferative effects in a variety of tumor cell lines and has showed robust in-vivo antitumor efficacy in IGF-1R-driven xenograft models.

These agents share a common side effect of hyperglycemia, seen in about 20% of patients. It is generally mild to moderate, reversible, and can be managed with oral hypoglycemic drugs. In addition, fatigue, rash, and nausea/vomiting have been consistently observed. Close monitoring of platelet levels and transaminases is also recommended.

Further Reading

Baselga J, Swain SM. Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. Nat Rev Cancer 2009; 9:463–475.

Buck E, Mulvihill M. Small molecule inhibitors of the IGF-1R/IR axis for the treatment of cancer. Expert Opin Investig Drugs 2011; 20:605–621.

de Azambuja E, Bedard PL, Suter T, et al. Cardiac toxicity with anti-HER-2 therapies: what have we learned so far? Target Oncol 2009; 4:77–88.

Hudis CA. Trastuzumab – mechanism of action and use in clinical practice. N Engl J Med 2007; 357:39–51.

Lacouture ME. Mechanisms of cutaneous toxicities to EGFR inhibitors. Nat Rev Cancer 2006; 6:803–812.

Olmos D, Basu B, de Bono JS. Targeting insulin-like growth factor signaling: rational combination strategies. Mol Cancer Ther 2010; 9:2447–2449.

Potthoff K, Hofheinz R, Hassel JC, et al. Interdisciplinary management of EGFR-inhibitor-induced skin reactions: a German expert opinion. Ann Oncol 2011; 22:524–535.

Scaltriti M, Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res 2006; 12:5268–5272.

Tumor Vessel Targeting

11

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Angiogenesis

The regulation of angiogenesis is a complex process and the result of a delicate balance of stimulating peptides (vascular endothelial growth factor [VEGF], fibroblastic growth factor [FGF], interleukin [IL]-4, IL-8, and others) and endogenous inhibitory factors (thrombospondin, angiostatin, endostatin, and others).

Most human solid tumors are characterized by an initial avascular phase; when the mass exceeds a maximum of 1–2 mm in diameter, tumor hypoxia becomes the principal trigger of angiogenesis, mainly driven by VEGF, with a consequent second phase characterized by rapid growth and invasion. However, inflammation and activation of oncogenes or loss of tumor suppressors may induce the angiogenic switch at pre-hypoxic and pre-invasive stages.

Triggers of angiogenic switch are:

1. Hypoxia (e.g. hypoxia-dependent transcription factor (HIF)-1α upregulates VEGF, platelet-derived growth factor [PDGF], nitric oxide synthase [NOS])
2. Tumor-recruited bone marrow-derived and inflammatory cells
3. Activated/overexpressed receptors (e.g. EGFR, HER2, IGF-1R)
4. Oncogene activation and loss of tumor suppressor genes (e.g. *ras* inhibits thrombospondin 1 and increases VEGF; p53 induces thrombospondin 1)

Any normal vasculature is composed of tightly associated luminal endothelial cells (ECs) and well-layered mural cells (smooth muscle

cells in large vessels and pericytes in capillaries). Tumor ECs are abnormal, they lose their polarity, cell–cell adhesion, and their link to the basal membrane and they stack upon each other. Tumor vessels have wide gaps, are leaky, and cancer cells become exposed to the blood. The entire vessel wall of tumors is so abnormal and so peculiar that we can consider it a real specific target.

Tumor vessel-targeting drugs are divided into two subclasses: antiangiogenic agents and vascular-targeting agents (VTAs), with differences in target, treatment schedule, indication based on the extent of disease, toxicity profile, and biomarkers.

Antiangiogenic Agents (Table 1)

The primary objectives of an antiangiogenic therapy are the prevention of tumor angiogenesis with inhibition of tumor growth and metastatization, inducing a "dormancy" status (mainly in the adjuvant setting) and the induction of vascular regression.

Currently two classes of drug with antiangiogenic activity are available: monoclonal antibodies against growth factors and their receptors and small-molecule tyrosine kinase inhibitors (TKIs) inhibiting multiple proangiogenic growth factor receptors (receptor tyrosine kinases, RTKs).

The VEGF binds to and activates tyrosine kinase receptors (VEGFR) composed of an extracellular portion consisting of multiple immunoglobulin-like domains, a transmembrane region, and an intracellular portion with TK activity.

Receptor TKs are membrane-anchored enzymes that transfer a phosphate group from adenosine triphosphate to intracellular target proteins upon dimerization induced by extracellular ligands. They transduce extracellular signals vehiculated by cognate ligands to the cytoplasm.

There are three VEGFR isoforms: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4), but most of the known biological effects of VEGF-A are mediated by activation of VEGFR-2. Their activation triggers a sequence of downstream events (including activation of the

Ras/Raf mitogen-activated protein kinase pathway and the PI3K-Akt pathway), resulting in endothelial cell proliferation, survival, and migration, thereby contributing to new vessel growth.

Other interesting pathways controlling angiogenesis include PDGF and platelet-derived growth factor receptor (PDGFR) family members, transforming growth factor-beta (TGF-β) and ALK receptors, FGF superfamily members, the ANG and TIE signaling system, the NOTCH and WNT signaling pathway, integrins, and proteases.

A hypersecretion of VEGF, as seen in cancer, causes an endothelial migration and proliferation, vascular architecture distortion, increase of vascular permeability and of interstitial fluid pressure (PIF), and a modulation of immune response typically seen in tumors.

The redundancy of the angiogenesis stimulating pathways makes it difficult to study antiangiogenics in the metastatic setting. Adjuvant or maintenance therapies in conditions of absent or minimal disease could be a more favorable set of conditions for antiangiogenics.

The most relevant toxicity of antiangiogenic therapy is hypertension, which is dose dependent and of still unclear pathogenesis. There are some theories involving nitric oxide (NO) release, a renal impairment contribution, and a new pre-eclampsia-like theory. Other known adverse events of antiangiogenic therapy are thromboembolic events (primarily arterial), hemorrhage, bowel perforation, fatigue, anorexia, diarrhea, rash, hand–foot syndrome, mucositis, and increased risk of congestive heart failure.

There is a lack of validated biomarkers to predict benefit from antiangiogenics and to evaluate antitumoral response. For bevacizumab, the expression levels of VEGF and thrombospondin 2, microvessel density, and K-ras, b-raf, and p53 expression were investigated as predictors of response, but were found to be negative. Circulating endothelial cells (CEC) and bone marrow-derived angiogenic cells are currently under investigation as potential biomarkers.

Table 1 *Pharmacological aspects of antiangiogenic agents*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism	Dosing when organ dysfunction
1. Bevacizumab Recombinant humanized IgG1 mAb anti-VEGF	Binds to VEGF inhibiting interaction with receptor Flt-1 (VEGFR-1) and KDR (VEGFR-2)	IV (1st inf 90 mins, 2nd inf 60 mins, subsequent infs 30 mins): mCRC: 5 mg/kg or 10 mg/kg/2 wks 7.5 mg/kg or 15 mg/kg/3 wks NSCLC: 7.5 mg/kg or 15 mg/kg/3 wks mRCC: 10 mg/kg/2 wks Ovary: 15 mg/kg/3wks	Linear PK from 1 to 10 mg/kg $T_{1/2}$ 18–20 days	Similar to endogenous IgG i.e. via proteolytic catabolism	No information available
2. Sorafenib Tosylate salt of sorafenib	TKI of w-BRAF, m-BRAF, CRAF, KIT, Flt-3, RET, VEGFR, and PDGFR-ß	PO daily 400 mg 2/day without food	Linear PK up to 400 mg 2/day F 38–49% (30% ↓ with a high-fat meal) T_{max} 3 hrs 99.5% protein bound $T_{1/2}$ 25–48 hrs Excretion: 77% in feces (51% unchanged), 19% in urine as glucuronidated metabolites	Metabolized primarily in the liver by CYP3A4, glucuronidated by UGT1A9. 70–85% unchanged in plasma, 5 metabolites identified in plasma	No change for Child-Pugh A-B, no data for Child-Pugh C No change for renal impairment

Drug interactions	Toxicity	Dose adjustment for toxicity
No clinically relevant PK interaction	Most common: (refers to the combination with IFL, 102 pts): 61% abdominal pain; 36% proteinuria; 26% headache; 35% epistaxis; 26% dyspnea; 24% GI hemorrhage; 23% hypertension Most serious: GI perforation (potentially fatal); wound healing complications (15% if surgery after bevacizumab); hemorrhage: 4.7% severe, usually massive hemoptysis (in NSCLC pts), rare GI, subarachnoid and stroke; TE; hypertension crisis; nephrotic syndrome; CHF, risk with concomitant DOX (14%); RPLS (reversible posterior leukoencephalopathy syndrome) with neurological disorders, lethargy, confusion, visual disturbances **Special warnings:** higher risk of TEE in >65 yr old pts; GI perforation in pts with intestinal subocclusion; hemorrhage in pts with squamous NSCLC, recent history of hemoptosis, ongoing hemorrhage	Discontinue permanently in case of: GI perforation, wound dehiscence, serious bleeding, nephrotic syndrome, hypertensive crisis, HSR Temporary suspension in case of severe proteinuria
With CYP3A4 inducers: 37% ↓ AUC With docetaxel: 36–80% ↑ in DOX AUC and 16–32% ↑ C_{max}	Most common: 43% diarrhea (2% G3); 40% rash; 40% increased lipase-amylase (9% G3); 37% fatigue (5% G3); 45% hypophosphatemia (13% G3); 30% HFS (6% G3); 27% alopecia; 23% nausea; 17% hypertension (3% G3); 15% hemorrhage (2% G3) Most serious (uncommon <1%): hypertensive crisis; myocardial ischemia and/or infarction; CHF **Warning:** temporary interruption is recommended in pts undergoing major surgical procedures	Dose may be reduced to 400 mg/day or to 400 mg every other day

Table I *Pharmacological aspects of antiangiogenic agents (Continued)*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism	Dosing when organ dysfunction
3. Sunitinib malate Malate salt of sunitinib	TKI of multiple RTKs: PDGFR-α and β, VEGFR-1, 2, 3, KIT, RET	For GIST and RCC: PO 50 mg daily, 4 wks on/ 2 wks off (schedule 4/2) For pNET: PO 37.5 mg/day	Linear PK between 25–100 mg T_{max} 6–12 hrs No food effect 95% protein bound Excretion: in feces (61%) and in urine (16%)	By CYP3A4 to desethyl S (active metabolite)	No change for Child-Pugh A-B, no data for Child-Pugh C. No change for renal impairment
4. Pazopanib	TKI of VEGFR-1, 2, 3, PDGFR-α and β, KIT	PO 800 mg/day without food	Non-linear PK, 40% inter-pt variability of AUC and C_{max} $T_{1/2}$ ~30–35 hrs >99% protein bound T_{max} 19.4 (range 1.0–11.9) hrs Mainly excreted in the feces: 67% unchanged	85% to 95% unchanged in plasma. The 4 main metabolites account for 6% of the exposure	Renal impairment: Cr Cl >30 ml/min: no dose adjustment No data <30 ml/min Moderate hepatic impairment: ↑ 2-fold C_{max} and AUC (dosage should be reduced to 200 mg/day, contraindicated in pts with severe hepatic impairment. Dose decreased by 200 mg steps Monitor LFT at least q 4 wks
5. Axitinib	TKI of VEGFR-1, 2, 3	PO 5 mg 2/day, no food effect	T_{max} 2.5–4.1 hrs F 58% >99% protein bound $T_{1/2}$ 2.5–6.1 hrs Excreted in feces (41%) and urine (23%)	Primarily in the liver, in N-glucuronide and sulfoxide metabolite (less in vitro potency than parent compound)	Moderate hepatic impairment: ↓ dose by 50%

Drug interactions	Toxicity	Dose adjustment for toxicity
With CYP3A4 inhibitors and inducers: ↑ or ↓ concentrations	In RCC studies: most common: 62% fatigue (15% G3); 66% diarrhea (10% G3); 58% nausea (4% G3); 47% stomatitis (3% G3); 37% bleeding (4% G3); 34% anorexia; 39% vomiting (3% G3); 25% skin discoloration; 34% hypertension (13% G3); 29% HFS (8% G3); 29% mucositis; 29% rash; 23% dry skin; 20% hair color change Most serious: 16% left ventricular dysfunction (3% G3)	Dose modifications by 12.5 mg steps Discontinue permanently if CHF Monitoring for BP and thyroid function, baseline LVEF, and thyroid function required
With CYP3A4 inhibitors: 120% ↑ in AUC and 50% ↑ in C_{max}: ↓ dose to 400 mg With paclitaxel: pazopanib increases paclitaxel (substrate for CYP2C8, CYP3A4, and PgP) AUC_{inf} and C_{max}	Most common (>10%): 52% diarrhea (3% G3, <1% G4); 38% hair color change; 40% hypertension (4% G3); 26% nausea (<1% G3); 22% anorexia; 53% AST/ALT (12% G3); 27% hypothyroidism Most serious (<1%): transient ischemic attack; ischemic stroke; myocardial ischemia; cardiac dysfunction; GI perforation and fistula; QT prolongation; pulmonary, GI, cerebral hemorrhage	
With CYP3A4 inhibitors: ↓ dose by 50%	Most common (>10%): 55% diarrhea (11% G3); 40% hypertension (16% G3); 39% fatigue (11% G3); 32% nausea; 31% dysphonia; 27% HFS (5% G3); 19% hypothyroidism; 3% TEE. Monitor thyroid function	

Vascular regression is an important mechanism of action but also a mechanism of resistance. Intratumoral vascular regression enhances hypoxia that makes cancer cells more invasive. The regression phase is anticipated by a normalization of tumor vessels, and normalization of tumor vascular permeability and interstitial pressure. It has been proposed that these modifications of the tumor vasculature might allow a better delivery of chemotherapeutics and render cancer cells less invasive and metastatic. Normalization, however, is a very transient event followed by a vascular regression and hypoxia. The contribution of vascular normalization to increased chemotherapy delivery has been recently challenged by a study demonstrating that VEGF inhibition actually decreases chemotherapy delivery. The prevention of this vicious circle is an important objective for future studies.

Vascular-targeting Agents (VTAs)

These are agents that selectively and acutely disrupt preformed tumor vessels. There are two main classes of VTA: tubulin polymerization inhibitors (such as combretastatin A4 phosphate [CA4P] and derivatives [OXi-4503, AVE-8062]) and flavonoids (such as flavone acetic acid [FAA] and ASA404 [vadimezan]).

The pharmacological effects of VTAs consist primarily of a rapid disruption of the endothelial cell layer, followed by a blood flow shutdown and acute tumor necrosis.

VTAs may be given intermittently and in the metastatic setting also in the presence of bulky disease. Many ongoing trials are evaluating VTAs in combination with chemotherapy, because, in experimental settings, selective killing of the tumor neovasculature was shown to improve chemotherapy delivery to tumors.

Although VTAs are quite selective for tumor vessels, there are intrinsic side effects on the cardiovascular system, even though these are relatively rare and mild. The most reported in clinical trials are: acute coronary syndromes, thrombophlebitic syndromes, alterations in blood pressure, alterations in heart rate and ventricular conduction (prolongation of QT, transient ECG changes, left ventricular ejection fraction reduc-

tions), flush and hot flashes, ataxia, vasovagal syncope, tumor pain, and abdominal pain due to alterations of splanchnic circulation.

Dynamic contrast-enhanced MRI (DCE-MRI) is used to study the effects of VTAs by changes in functional kinetic parameters. VTA activity consists of a rapid shutdown of the vasculature and a rapid reversibility of effects. The potential clinical value of this approach and the best clinical use of VTAS are still to be defined.

Lenalidomide and Thalidomide (Table 2)

Lenalidomide and thalidomide have many biological effects besides the antiangiogenic one. They can be classified as immunomodulatory drugs (IMiDs) because they enhance the immune system by inhibiting the secretion of pro-inflammatory cytokines (tumor necrosis factor [TNF]-α, IL-1, IL-6, and IL-12) and promoting the secretion of anti-inflammatory cytokine IL-10 by monocytes; by inducing T-cell proliferation and IL-2 and interferon production, and enhancing NK-cell activation; and by inhibiting the proliferation of various hematopoietic tumor cell lines, particularly multiple myeloma. They inhibit some processes of angiogenesis, in particular endothelial cell migration and tube formation, and reduce the growth of solid tumors in vivo.

Table 2 *Pharmacological aspects of lenalidomide and thalidomide*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism	Dosing when organ dysfunction
6. Thalidomide α-(N-phthalimido) glutarimide	Immunomodulatory with anti-inflammatory activities related to suppression of TNF-α production, down-modulation of selected cell-surface adhesion molecules involved in leukocyte migration and antiangiogenic activity	Multiple myeloma: PO: 200 mg/day at bedtime at least 1 hr after meal to reduce somnolence A maximum number of 12 cycles of 6 wks should be used	90% bioavailability C_{max} 1–5 hrs 55–65% protein binding of the (+)-(R) and (-)-(S) enantiomers T ½ 5.5–7.3 hrs >90% renal excretion (<3% unchanged), minor fecal excretion	Minimal liver cytochrome P450 metabolism, non-enzymatic hydrolysis to multiple metabolic species In plasma, 80% unchanged hydrolytic products	No specific dose recommendations
7. Lenalidomide Synthetic derivative of glutamic acid	Same as thalidomide	MDS: PO 10 mg daily Multiple myeloma: PO 25 mg daily days 1 to 21 q 4 wks Teratogen, as thalidomide	Linear PK F 68% T_{max} 1.5 hrs $T_{1/2}$ 3 hrs High volume of distribution 23–29% protein bound 90% eliminated in urine (82% unchanged), 4% in feces No accumulation after repeated doses	Metabolites formed by hydrolysis	Mild renal impairment: 15% ↑ AUC Cr Cl 50–30: 10 mg daily Cr Cl <30: 15 mg eod Cr Cl <30 dialysis: 5 mg daily No studies for hepatic impairment

Drug interactions	Toxicity	Dose adjustment for toxicity
Sedative properties, avoid concomitant sedatives (anxiolytics, hypnotics, antipsychotics, H_1-antihistamines, opiate derivatives, barbiturates and alcohol)	In combination with dexamethasone: most common: 55% constipation; 57% peripheral edema; 20% tremor; 24% asthenia; 28% dizziness; 21% fatigue; 15% hyperglycemia; 12% paresthesia Most severe: 9% DVT/PE: 7% G ≥3 peripheral neuropathy; 3% severe skin reaction ↑ risk of TEE: thromboprophylaxis should be administered for at least the first 5 months, especially in pts with additional thrombotic risk factors. If the pt experiences any TEE, treatment must be discontinued and standard anticoagulation therapy started **Warning:** can cause severe defects in humans, male and female contraception must be implemented	If G2 neuropathy: ↓ dose or interrupt treatment. If no improvement or worsening, discontinue treatment If the neuropathy resolves to G1, treatment may be restarted, if the benefit/risk is favorable G ≥3 neuropathy: discontinue treatment
No known interactions Avoid pro-thrombotic drugs (e.g. hormonal therapy or EPO)	MM pts in combination with dexamethasone: Most common:39% neutropenia (5% G4); 27% fatigue; 24% constipation; 20% cramps; 18% thrombocytopenia (9.9% G3); 18% asthenia; 17% anemia; 14% diarrhea; 10% rash Most serious: 22% G3/4 infections; 15% DVT, antithrombotic prophylaxis needed; 2% G3/4 peripheral neuropathy **Warning:** increase of second primary malignancy in pts treated in all clinical trials with lenalidomide vs controls (3.98 vs 1.38 per 100 yrs-pt) **Warning:** monitor renal function Females of childbearing potential should be advised to avoid pregnancy; they must use at least one effective contraceptive method	For G3-4 neutropenia–thrombocytopenia or any G3-4 toxicity, reduce to 15→10→5 mg

Further Reading

Cai SX. Small molecule vascular disrupting agents: potential new drugs for cancer treatment. Recent Pat Anticancer Drug Discov 2007; 2:79–101.

Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011; 473:298–307.

Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov 2011; 10:417–427.

Escorcia FE, Henke E, McDevitt MR, et al. Selective killing of tumor neovasculature paradoxically improves chemotherapy delivery to tumors. Cancer Res 2010; 70:9277–9286.

Sato Y. Persistent vascular normalization as an alternative goal of anti-angiogenic cancer therapy. Cancer Sci 2011; 102:1253–1256.

Sessa C, Guibal A, Del Conte G, et al. Biomarkers of angiogenesis for the development of antiangiogenic therapies in oncology: tools or decorations? Nat Clin Pract Oncol 2008; 5:378–391.

Syrigos KN, Karapanagiotou E, Boura P, et al. Bevacizumab-induced hypertension: pathogenesis and management. Biodrugs 2011; 25:159–169.

Van der Veldt AA, Lubberink M, Bahce I, et al. Rapid decrease in delivery of chemotherapy to tumors after anti-VEGF therapy: implications for scheduling of anti-angiogenic drugs. Cancer Cell 2012; 21:82–91.

Zweifel M, Padhani AR. Perfusion MRI in the early clinical development of antivascular drugs: decorations or decision making tools? Eur J Nucl Med Mol Imaging 2010; 37(Suppl 1):S164–S182.

Downstream Receptor Targeting: PI3K, mTOR, KRAS, BRAF, MEK

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PI3K-AKT-mTOR Signaling Pathway (Table 1)

Phosphatidylinositol-3-kinases (PI3Ks) are key regulators of many processes of the neoplastic cell, including cell proliferation, survival, growth, and motility. Upstream receptor tyrosine kinases that feed into the PI3K pathway include members of the human epidermal growth factor receptor family (EGFR and HER2), platelet-derived growth factor receptor (PDGFR), and the insulin and insulin-like growth factor 1 (IGF-1) receptors, among others.

PI3Ks are divided into three classes according to structural characteristics and substrate specificity; the most commonly studied are class I enzymes that are activated directly by cell-surface receptors and are related to glucose homeostasis, metabolism, growth, proliferation, and survival. Class I PI3Ks are further divided into class IA enzymes, which are activated by receptor tyrosine kinases (RTKs), G protein-coupled receptors (GPCRs), and certain oncogenes such as G protein Ras, and class IB enzymes, which are regulated exclusively by GPCRs (Figure 1).

Class IA PI3Ks are heterodimers consisting of a p85 regulatory subunit, which mediates receptor binding and activation, and p110α, β, or δ catalytic subunits involved in the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) into PIP3. Following the activation, PIP3 recruits other downstream molecules, particularly the serine–threonine kinases AKT (also called protein kinase B, PKB). Akt stimulates protein synthesis and cell growth by activating mTOR (mammalian target of rapamycin)

through effects on the intermediary tuberous sclerosis (TSC) 1/2 complex. It influences cellular proliferation by inactivating cell-cycle inhibitors (p27 and p21), promoting cell-cycle proteins (c-Myc and cyclin D1), and also regulating a wide range of target proteins relevant for apoptosis (FOXO1, FOXO4). The levels of PIP3 are strictly regulated by several phosphatases, the most relevant of which is PTEN (phosphatase and tensin homolog on chromosome 10), which converts PIP3 back to PIP2.

Class IB PI3Ks are heterodimers consisting of a p101 regulatory subunit and a p110γ catalytic subunit which is activated directly by GPCRs. p110α and β are ubiquitously expressed and influence cellular proliferation and insulin signaling, whereas p110γ and δ, primarily expressed in leukocytes, are involved in immune function and inflammation.

Evidence of PI3K-signaling deregulation is described for many human tumors: PTEN deregulation in glioblastoma, ovarian, breast, endometrial, and prostate tumors; *PIK3CA* mutations (in particular, p110α isoform) in ovarian, breast, and colorectal carcinomas; and PIK3CA amplification in non-small lung cell lung, gastric, and cervical cancer.

PI3K inhibitors can be divided into (a) pan-specific inhibitors, which show little or no selectivity for individual PI3K isoforms (such as GDC0941, BKM120, XL147, GSK1059615), thus reducing the possibility of resistance but with some toxicity in animals; (b) isoform-specific PI3K inhibitors, isoform α-specific (BYL719), isoform β-specific (GS-1101 or AMG 319); and (c) dual PI3K-mTOR inhibitors (GDC0980, BEZ235, XL765, PF04691502), also reducing the possibility of resistance. In addition, specific AKT inhibitors (such as GDC0068, MK2206) are being investigated in early clinical trials.

CAL101, an isoform-specific agent targeting p110δ, is under evaluation in phase III in combination with bendamustine or rituximab in patients with CLL, and in indolent non-Hodgkin lymphoma (NHL), characterized by the constitutive activation of the PI3Kδ (see Table 1).

mTOR is frequently deregulated in cancer cells, where it has a central role in the control of growth, division, survival, and angiogenesis.

The mTOR inhibitors currently in clinical development are derived from the macrolide antibiotic rapamycin (sirolimus), which is a product of the bacterium *Streptomyces hygroscopicus*. After the discovery of rapamycin, two other derivatives (known as rapamycins or rapalogs) have been developed, demonstrating potent anti-cancer activity in numerous preclinical and clinical studies: temsirolimus (CCI-779) and everolimus (RAD001).

The catalytic subunit of mTOR has two biochemically distinct complexes called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is composed of mTOR, raptor, mLST8, and PRAS40. Akt activates mTORC1, which then phosphorylates and inactivates 4E-BP1 with activation of eI-F4E. Activation of mTORC1 also phosphorylates and activates S6K1 (ribosomal S6 kinase 1). Both activated eI-F4E and S6K1 promote translation of mRNA and protein synthesis (Figure 1). mTORC2 is composed by mTOR, mLST8, rector, and mSin1. mTORC2 directly phosphorylates and activates Akt and other kinases, controlling cytoskeletal organization and cell survival.

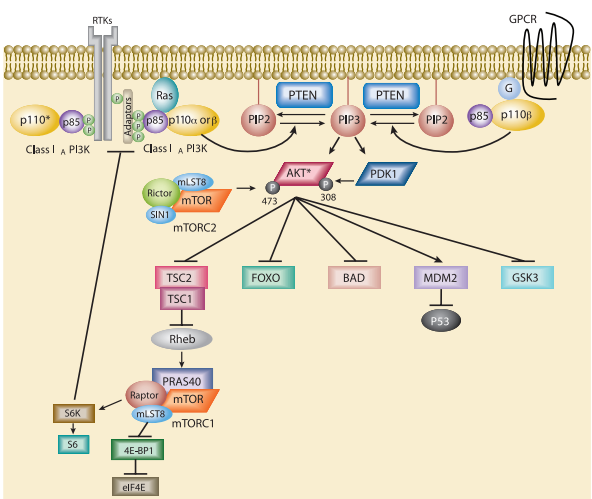

Figure 1 *mTORC1, mTORC2, and PI3K-AKT pathway. From: Courtney KD, et al. The PI3K pathway as drug target in human cancer. J Clin Oncol 2010; 28: 1075–1083 with permission.*

Table I *Pharmacological aspects of PI3K-AKT-mTOR signaling inhibitors*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism	Dosing when organ dysfunction
1. Temsirolimus	Inhibits mTORC1 by binding FKBP12	IV (inf 30–60 mins) 25 mg weekly Premedication: IV antihistamines 30 mins before the administration	$T_{1/2}$ 17.3 hrs Excretion: feces (82%) as metabolites (mainly sirolimus)	Hepatic (mainly via CYP3A4)	Renal: no adjustment necessary Hepatic: if severe, reduce the dose to 10 mg weekly
2. Everolimus	Inhibits mTORC1 by binding FKBP12	PO (can be administered with or without food) 10 mg/day	T_{max} 1–2 hrs Excretion: bile Food effect: no change in bioavailability with food. Avoid grapefruit juice (increase levels)	Hepatic (mainly via CYP3A4)	Renal: no adjustment necessary Hepatic: dose to be reduced to half the normal
3. CAL101	Isoform-specific PI3K inhibitor targeting p110δ	PO (can be administered with or without food) 150 mg 2/day	T_{max} 1–1.5 hrs $T_{1/2}$ 6.5–9.8 hrs Excretion: bile and urine	Hepatic (mainly via CYP3A4) and glucuronidation	Renal: no adjustment necessary Hepatic: if severe, CAL101 to be withheld

Rapamycin and its analogs form an inhibitory complex with the intracellular receptor FKBP12, which binds to mTORC1. Differently from mTORC1, mTORC2 is not sensitive to rapalogs; however, preliminary data suggest that prolonged exposure to rapamycin also inhibits mTORC2 in a minority of cell lines (derived from the hematological system and from cervical and prostatic cancer).

mTOR inhibitors are generally well tolerated; the most common adverse events are stomatitis, skin rash, hyperglycemia, hyperlipidemia, anemia, thrombocytopenia, and interstitial pneumonitis.

mTOR inhibitors demonstrated anti-cancer activity in multiple tumor types, in particular in renal cell carcinoma (RCC), mantle cell lymphoma (MCL), and neuroendocrine tumors. Temsirolimus has been approved as first-line treatment in high-risk metastatic RCC and relapsed/refrac-

Drug interactions	Toxicity	Dose adjustment for toxicity
CYP3A4 inhibitors should be avoided (such as grapefruit) Otherwise a dose reduction to 12.5 mg/wk should be considered Also CYP3A4 inducers to be avoided	94% anemia; 89% hyperglycemia (16% G3); 87% hyperlipemia; 83% hypertriglyceridemia; 47% rash (5% G3); 41% mucositis (3% G3); 40% thrombocytopenia; 35% edema (3% G3); 21% hypokalemia; 20% infections; 20% back pain (3% G3); 14% nail disorders; 11% dry skin	Temsirolimus should be discontinued for ANC <1000/mm^3, PLT <75 000/mm^3, or NCI CTCAE ≥ G3 adverse reactions. Once toxicities have resolved to G ≤2, temsirolimus may be restarted with the dose reduced by 5 mg/wk to a dose of at least 15 mg/wk
Coadministration with strong CYP3A4 inhibitors (such as grapefruit) and CYP3A4 inducers to be avoided	77% hypertriglyceridemia; 76% hypercholesterolemia (3% G3-4); 50% hyperglycemia (12% G3-4); 40% stomatitis (3% G3); 37% fatigue (3% G3); 30% thrombocytopenia; 25% rash; 20% increase AST and ALT; 16% anorexia; 10% edema; 10% infection; 12% interstitial pneumonitis (3% G3)	Liver toxicity: dose to be reduced to half the normal Lung toxicity: everolimus should be discontinued, steroids may be indicated and everolimus can be restarted after resolution of symptoms at 5 mg/day
Coadministration with strong CYP3A4 inhibitors (such as grapefruit) and CYP3A4 inducers is not contraindicated but pts should be monitored for CAL101 adverse events	Pneumonia and colitis; stomatitis; renal dysfunction; rash; neutropenia, anemia, thrombocytopenia, febrile neutropenia; AST and ALT elevation	CAL101 should be discontinued for liver toxicity G3-4 and neutropenia and thrombocytopenia grade 4

tory MCL. Everolimus has been approved for the treatment of metastatic RCC as second line after TKI therapy.

Resistance to rapamycin is a common phenomenon related to the activation of the PI3K-AKT pathway due to inhibition of mTORC1. In fact, S6K1, activated by mTORC1, represses the PI3K-AKT pathway through inhibition of IRS1 (insulin receptor substrate). Inhibition of mTORC1 activates the IRS1 and the PI3K pathway upstream, diminishing the antitumor activity of rapalogs.

Second-generation mTOR inhibitors include: (a) mTORC1-mTORC2 inhibitors (AZD8055, OSI-027, MLN 0128); (b) dual PI3K-mTOR inhibitors. These drugs act by blocking the catalytic site. As expected, these molecules are more effective than rapalogs in preclinical models because they target concomitantly two nodal points of the pathway.

RAS-RAF-MEK-ERK Pathway

Ras proteins, localized to the inner surface of the cellular membrane, control the intracellular transduction of various signals of proliferation derived from growth factor receptors (EGFR, PDGFR), cytokines, and hormones (Figure 2). In mammalian cells, three Ras genes coexist, encoding for H-Ras, K-Ras, and N-Ras protein, respectively. Downstream from Ras, the Raf kinase pathway has a pivotal role in tumorigenesis through the activation of a series of pathways, including MAP kinase (including MEK/ERK) and PI3K pathways. Activating mutations in RAS genes, most often in KRAS, are found in about 30% of human cancers (pancreas 90%; colorectal 40%; lung 20%) and are generally acquired early in the tumorigenic process.

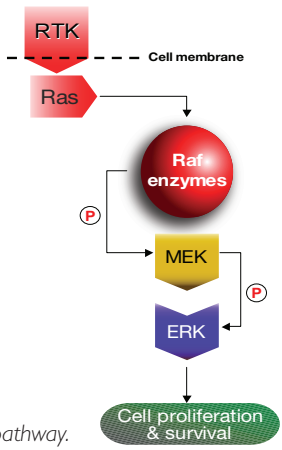

Figure 2 *RAS-RAF-MEK-ERK pathway.*

Table 2 *Pharmacological aspects of BRAF inhibitors*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism	Dosing when organ dysfunction
4. Vemurafenib	Potent selective inhibitor of oncogenic V600E BRAF	PO 960 mg 2/day	$T_{1/2}$ 30–80 hrs	Hepatic (mainly via CYP3A4)	Renal: no adjustment necessary Hepatic: if severe, dose to be reduced or held

Raf proteins exist in three isoforms: A-Raf (highly expressed in urogenital organs), B-Raf (expressed in neuronal tissues), and C-Raf (ubiquitously expressed). The activation of Raf leads to strong activation of the protein kinases MEK and ERK.

BRAF mutations are present with a high frequency in human cancer, particularly melanoma (50–60%), papillary thyroid cancer (35–70%), colorectal cancer (5%), and low-grade serous ovarian cancer (30%); mutations in *MEK* are not frequent (about 1% in lung, colon cancer, and melanoma). The substitution of a valine residue at position 600 for glutamic acid (*BRAF* V600E) accounts for about 90% of *BRAF* mutations. Vemurafenib, an orally available selective inhibitor of V600E BRAF, has shown an impressive overall response rate of about 60%, in addition to improved progression-free and overall survival, in V600E *BRAF* mutant melanoma patients (Table 2). The main side effects of the drug are proportional to the dose and exposure, the most frequent being rash, fatigue, arthralgia, and photosensitivity. The development of cutaneous squamous cell carcinoma (CSCC), with the histological appearance of keratoacanthoma, was described in up to 25% of patients. The molecular explanation of this event, which is due to ERK hyperactivation in the presence of BRAF inhibitors, is the transactivation of C-Raf and subsequently of the MAP kinase pathway in *RAS* mutant cells. Preclinical and clinical data on the combination of BRAF and MEK inhibitors confirmed the molecular hypothesis with no appearance of CSCC.

Several MEK inhibitors are in clinical development in combination with PI3K inhibitors or RAF inhibitors, because of the synergistic effect of the simultaneous inhibition of both pathways. In fact, the crosstalk between the PI3K and MAP kinase pathway can cause resistance to

Drug interactions	Toxicity	Dose adjustment for toxicity
Coadministration with drugs metabolized by CYP1A2 and CYP2D6 CYP3A4 should be monitored for adverse events Dose adjustments for medications predominantly metabolized via CYP1A2, CYP2D6, or CYP3A4 should be considered	57% arthralgia; 51% rash; 49% photosensitivity; 38% fatigue; 33% alopecia; 27% pruritus; 24% CSCC; 21% nausea	

MEK inhibitors; this resistance can be overcome in preclinical models by combining a MEK inhibitor with a PI3K inhibitor or with a RAF inhibitor (Figure 3).

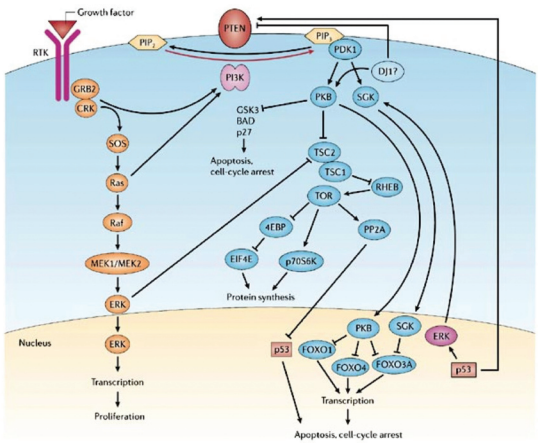

Figure 3 *Crosstalk between PI3K and RAS-RAF-MEK-ERK pathways. From Cully M, et al. Nature Reviews Cancer 2006;6:184–192 with permission.*

Further Reading

Dancey J. mTOR signaling and drug development. Nat Rev Clin Oncol 2010; 7:209–219.

Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009; 9:550–562.

Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 2010; 363:809–819.

Frémin C, Meloche S. From basic research to clinical development of MEK1/2 inhibitors for cancer therapy. J Hematol Oncol 2010; 3:8.

Heidorn SJ, Milagre C, Whittaker S, et al. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell 2010; 140:209–221.

Ribas A, Flaherty KT. BRAF targeted therapy changes the treatment paradigm in melanoma. Nat Rev Clin Oncol 2011; 8:426–433.

Shaw RJ, Cantley C. RAS, PI3K and mTOR signalling controls tumor cell growth. Nature 2006; 441:424–430.

Cancer-related Receptor Targeting: Bcr-Abl, KIT, MET

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Bcr-Abl (Table 1)

Bcr-Abl fusion gene derives from the t(9;22)(q34;q11) chromosomal translocation that juxtaposes Abl1 gene on chromosome 9 to Bcr gene on chromosome 22. This translocation generates a truncated chromosome 22, known as "Philadelphia chromosome", which is found in 95% of chronic myelogenous leukemia (CML) patients. The same translocation occurs with lower frequencies in other hematological malignancies such as acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML). Bcr protein is a Ser/Thr kinase and Abl1 is a proto-oncogene encoding a cytoplasmic and nuclear tyrosine kinase. The fusion protein resulting from the translocation is a constitutionally active tyrosine kinase that promotes cell proliferation, survival, and increased motility. Bcr-Abl, after its autophosphorylation on tyrosine 177, recruits a number of adaptor proteins which mediate the interaction and activation of different downstream factors.

Targeting Bcr-Abl

Since Bcr-Abl is the initial cause and the only driver of CML, it represents an ideal drug target. So far, three generations of synthetic Bcr-Abl inhibitors have been developed, the great majority being ATP-competitive inhibitors. Imatinib was the first drug to be marketed, followed by nilotinib and dasatinib for the treatment of imatinib-resistant CML. A third generation of inhibitors is currently being developed to target all identified mutated variants of Bcr-Abl.

Table 1 *Pharmacological aspects of Bcr-Abl inhibitors*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
1. Imatinib	Reversible TKI of Bcr-Abl, KIT, PDGFR	PO (with food) Single agent: Adults with Ph+ CML-CP: 400 mg/day Adults with Ph+ CML-AP or BC: 600 mg/day Adults with metastatic and/or unresectable GIST: 400 mg/day	T_{max} 2–4 hrs $T_{1/2}$ 18–40 hrs Excretion: feces (68%) and urine (13%) mainly as metabolites Food effect: bioavailability is 98% with food. CYP3A4 inhibitors increase imatinib levels; CYP3A4 inducers decrease imatinib levels	Mainly hepatic (via CYP3A4)
2. Nilotinib	Reversible TKI of Bcr-Abl, KIT, PDGFR	PO (empty stomach for at least 2 hrs before and 1 hr after dosing) Ph+ CML-CP: 300 mg 2/day Resistant or intolerant Ph+ CML-CP and CML-AP: 400 mg 2/day	T_{max} 3 hrs $T_{1/2}$ 16 hrs Excretion: mainly feces, unchanged and as inactive metabolites CYP3A4 inhibitors increase nilotinib levels; CYP3A4 inducers decrease nilotinib levels	Mainly hepatic (via CYP3A4)
3. Dasatinib	Reversible TKI of Bcr-Abl, KIT, PDGFR, SRC, src family	PO (with or without food) CML-CP: 100 mg/day CML-AP or BP and Ph+ALL: 140 mg/day	T_{max} 1 hr $T_{1/2}$ 5 hrs Excretion: mainly feces, unchanged and as metabolites CYP3A4 inhibitors increase dasatinib levels; CYP3A4 inducers decrease dasatinib levels	Mainly hepatic (via CYP3A4)

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Hepatic: pts with severe hepatic impairment should receive 75% of the recommended dose Renal: mild renal impairment (Cr Cl 40–59 ml/min): maximum dose 600 mg Moderate renal impairment (Cr Cl 20–39 ml/min): 50% of the recommended dose (maximum 400 mg) Severe renal impairment: maximum dose 100 mg/day	Caution when administering imatinib with CYP3A4 and CYP2D6 substrates that have a narrow therapeutic window	*60% fluid retention; 49% muscle cramps; 49% nausea/ vomiting; 45% musculoskeletal pain; 40% rash; hematological toxicity in the first months of treatment	†Hematological toxicity: If ANC <1000/mm^3 and/or PLT <50 000/mm^3: 1. Stop treatment until ANC >1500/mm^3 and PLT >75 000/mm^3 2. Resume treatment at the original starting dose of 400 mg 3. If recurrence of ANC <1000/mm^3 and/or PLT <50 000/mm^3, repeat step 1 and resume treatment at a reduced dose of 300 mg
Hepatic: moderate hepatic impairment: initial dosing of 300 mg 2/day followed by dose escalation to 400 mg 2/day. Severe hepatic impairment: initial dosing of 200 mg 2/day followed by dose escalation to 300 mg 2/day and then to 400 mg 2/day Cardiac: contraindicated with hypokalemia, hypomagnesemia, or long QT syndrome Pancreatic: contraindicated in pts with a history of pancreatitis	Avoid concomitant use of strong CYP3A4 inhibitors and inducers or antiarrhythmic drugs and drugs that may prolong QT interval	Myelosuppression, QT prolongation, and sudden death (2%) Hepatotoxicity (rare)	Withhold for G3/4 lipase elevations or QT prolongation
Cardiac: contraindicated with hypokalemia, hypomagnesemia, or long QT syndrome	Coadministration with simvastatin increases exposure to this drug. Avoid concomitant use of strong CYP3A4 inhibitors and inducers	Myelosuppression; 10% fluid retention and edema; QT prolongation	CML-CP with ANC <500/mm^3 or PLT <50 000/mm^3: 1. Stop until ANC >1000/mm^3 and PLT >50 000/mm^3 2. Resume treatment at the original dose if recovery occurs in <7 days 3) If PLT <25 000/mm^3 or recurrence of ANC <500/mm^3 for >7 days, repeat step 1 and resume at reduced dose of 80 mg once daily for second episode. For third episode reduce to 50 mg daily or discontinue
*Adverse reactions reported in newly diagnosed CML clinical trial †Dose adjustments for neutropenia and thrombocytopenia for chronic phase CML pts (starting dose 400 mg)			

1. Imatinib

Imatinib is an ATP-competitive inhibitor which reversibly binds to the ATP binding site of Abl1, preventing the phosphorylation of tyrosine residues on the Bcr-Abl downstream targets.

Imatinib was approved by the FDA in 2001 as first-line treatment of Philadelphia chromosome-positive (Ph+), chronic phase (CP) CML patients, following a trial that demonstrated its superiority compared to the standard treatment of interferon-alpha and cytarabine. The molecule shows a high specificity for other tyrosine kinases such as KIT and platelet-derived growth factor receptor (PDGFR). By 2011 imatinib had been approved for the treatment of the following malignancies: (Ph+) CML and (Ph+) ALL, KIT-positive gastrointestinal stromal tumor (GIST), and myelodysplastic/myeloproliferative diseases (MDS/MDP) associated with PDGFR gene rearrangements. The most common adverse reactions, reported in more than 30% of patients, are edema, nausea, vomiting, muscle cramps, diarrhea, fatigue, and myelosuppression. Concomitant CYP3A4 inducers or inhibitors can affect the biodistribution of the drug.

2. Nilotinib

Nilotinib (AMN107) is a rationally designed, reversible Bcr-Abl inhibitor. It shows high specificity, in the nanomolar range, for KIT and PDGFR-β as well. Nilotinib has been approved as the first-line treatment of Ph+, CP CML patients, and as second-line treatment of CML patients resistant or intolerant to imatinib.

Nilotinib is an oral drug and achieves peak concentration 3 hrs after administration. Nilotinib may cause QT prolongation and should be carefully administered to patients with liver impairment.

3. Dasatinib

Dasatinib (BMS-354825) is a potent Bcr-Abl reversible inhibitor with high efficacy also against KIT, PDGFR-β, ephrin receptor, SRC, and src-family kinases. Dasatinib has been approved as first-line treatment for CML; it received accelerated approval for the treatment of adults in all phases of CML with resistance or intolerance to imatinib therapy.

Dasatinib is orally administered and has linear pharmacokinetics between 50 and 110 mg. The T_{max} is 1 hr and the elimination half-life is 5 hrs. The most frequent adverse events are neutropenia and thrombocytopenia.

Third-generation inhibitors

Both dasatinib and nilotinib are effective against the majority of Bcr-Abl mutations. The most notable exception is T315I, which has a high recurrence in imatinib-resistant CML. The steric hindrance of Ile315 greatly reduces the affinity of imatinib/nilotinib/dasatinib for the ATP pocket, and patients carrying this mutation are resistant to both first- and second-generation inhibitors. Third-generation inhibitors are being developed to overcome this specific mutation; at present, none of them has reached clinical phase III.

KIT

The original KIT gene encoded for receptor tyrosine kinase (RTK) belonging to the type III family, the same family as PDGFR and CSF-I. KIT (mast cell growth factor receptor/stem cell factor receptor/CD117) is expressed on primitive mast cells and hematopoietic progenitor cells, and it regulates survival, self-renewal, and proliferation. Its expression is lost as these cells reach more differentiated stages. KIT activity is also required for the proliferation of germ cells, the migration of melanocytes from the neural crest to the dermis during embryonic development, and the function of interstitial cells of Cajal in the intestine.

Alterations of the KIT pathway lead to different types of tumor. Activating mutations of KIT determine cell proliferation and survival and usually occur in exon 11, which encodes the JM domain. Mutations arise rarely also in exon 9, encoding the extracellular domain, and in exon 13 and 17, encoding the kinase domains. These mutations are associated with larger and more aggressive tumors and tend to respond worse to therapy than those in exon 11. KIT mutations are found in GIST and with lower frequencies in AML, melanomas, germinomas, and mastocytosis.

Targeting KIT

The introduction of imatinib and other tyrosine kinase inhibitors (TKIs) in the treatment of GIST has dramatically improved the outcome of patients, especially those in the metastatic phase, for whom there was no effective treatment before TKIs. Imatinib and second-generation Bcr-Abl inhibitors display high specificity for KIT, because of the evolutionary conservation of the kinase domains of the two enzymes.

Imatinib was the first TKI to be approved for the treatment of GIST. Imatinib-treated patients tend to relapse because of a secondary KIT mutation, and second-generation Bcr-Abl inhibitors are being tested for their efficacy against KIT, mutant forms. Dasatinib is currently under investigation in patients with imatinib/sunitinib-refractory GIST. Nilotinib is in phase III as first-line therapy in inoperable/unresectable, metastatic disease.

MET (Table 2)

MET is the tyrosine kinase receptor for the hepatocyte growth factor (HGF). MET is structurally composed of an alpha (extracellular) and a beta (transmembrane) subunit linked by a disulfide bond. Upon ligand binding, transphosphorylation of tyrosine residues present in the catalytic subunit (C-terminal, intracellular part) occurs. This event in turn leads to the phosphorylation of two other sites present in the C-terminal part, which represents the signal for the recruitment of adaptor and transducer proteins.

MET is expressed primarily on epithelial cells, and drives different intracellular signaling pathways essential for the development and progression of many human cancers. The HGF/MET pathway regulates diverse biological activities, ranging from proliferation, motility, and invasion to survival and angiogenesis, many of which are hallmarks of cancer. Aberrant signaling of the MET pathway, a situation found in a variety of human malignancies, is associated with a poor prognosis, aggressive phenotype, increased metastasis, and shortened patient survival. Among the most frequent alterations of c-Met, gene amplification is found in gastric cancer, in non-small cell lung cancer (NSCLC), particularly in

those resistant to anti-EGFR therapies, and in esophageal cancer. Activating mutations in the gene encoding for MET can be found in some tumors, including renal carcinoma and hepatocellular carcinoma in children. In several tumors, however, constitutive activation of MET is found due to overexpression of the protein without gene amplification. This represents the major activation mechanism of MET.

The activation of MET leads to the activation of several important signaling pathways, including the MAP kinase cascade, the PI3K-AKT, the STAT, and the NFκB pathways.

Targeting MET

Several strategies have been developed to target the MET pathway. The majority of the compounds in development directly target the receptor, either through the use of monoclonal antibodies or with inhibitors, both ATP-competitive and non-competitive.

MET ATP-competitive inhibitors have shown high efficacy in preclinical systems, particularly in those "MET driven", i.e. in those cancer cells depending on MET for their growth. Non-competitive inhibitors are probably more specific than competitive inhibitors, although they are less potent as inhibitors of kinase activity.

Monoclonal antibodies have the advantage of being highly specific for the interaction between MET and its ligand.

4. Monoclonal antibodies (Onartuzumab, MetMab)

Onartuzumab is a monoclonal antibody which inhibits MET by preventing binding and activation of the ligand HGF. Onartuzumab has been evaluated in phase II in NSCLC in combination with erlotinib, where it was shown to prolong progression-free survival only in patients with high expression of MET. It has linear pharmacokinetics and the elimination half-life is 10–11 days with a clearance of 7–8 ml/kg/day. Adverse effects include grade 3 (dose-limiting) fever and grade 2 fatigue.

Table 2 *Pharmacological aspects of MET inhibitors*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
4. Onartuzumab	Inhibits the binding of HGF to MET receptor (monoclonal antibody)	15 mg/kg q 3 wks	$T_{1/2}$ 10 days	NA (opsonization via reticuloendothelial system?)
5. Tivantinib	Inhibits MET in a non-competitive way	PO: 360 mg 2/day	$T_{1/2}$ 4.5–6.5 hrs	Hepatic (mainly through CYP2C19)
6. Crizotinib	ATP-competitive inhibitor of MET and ALK	PO: 250 mg 2/day	$T_{1/2}$ 43–51 hrs	Hepatic (mainly through CYP3A)

5. MET non-ATP competitive inhibitors (tivantinib)

Tivantinib (ARQ 197) is an oral, small-molecule, non-competitive inhibitor of MET with a relatively high specificity for MET. It is being investigated in combination with erlotinib in advanced NSCLC. The pharmacokinetics is linear in the range of 100 to 300 mg, while for doses above 300 mg the linearity is lost. As adverse effects, tivantinib induces neutropenia, rash, fatigue, and diarrhea.

6. MET ATP competitive inhibitors (crizotinib, cabozantinib)

Crizotinib is an oral dual MET and ALK (anaplastic lymphoma kinase) inhibitor. It has been approved for the treatment of ALK-positive NSCLC, where a response rate of 57% (which reaches 84% including disease stabilization) has been reported. Crizotinib is a more potent inhibitor of MET than of ALK, and is now being tested as a MET inhibitor in several malignancies including NSCLC in combination with erlotinib.

Cabozantinib (XL184) is a multi-kinase inhibitor effective in inhibiting MET, RET, and VEGFR-2. It is being tested in medullary thyroid cancer (a phase III trial has ended) and in several other solid tumors.

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
NA	No known significant interactions	Fever; fatigue; abdominal pain; peripheral edema	NA
NA	No known significant interactions	15% G2 fatigue; 13% nausea; 12% vomiting	NA
NA	Moderate CYP3A4 inhibitor	54% nausea; 44% vomiting; 48% diarrhea; 41% visual disturbance; 16% peripheral edema All of G1-2	NA

Further Reading

Ganjoo KN, Patel S. Current and emerging pharmacological treatments for gastrointestinal stromal tumour. Drugs 2011; 71:321–330.

Kantarjian HM, Baccarani M, Jabbour E, et al. Second-generation tyrosine kinase inhibitors: the future of frontline CML therapy. Clin Cancer Res 2011; 17:1674–1683.

Lu XY, Cai Q, Ding K. Recent developments in the third generation inhibitors of Bcr-Abl for overriding T315I mutation. Curr Med Chem 2011; 18:2146–2157.

Peters S, Adjei AA. MET: a promising anticancer therapeutic target. Nat Rev Clin Oncol 2012; 9:314–326.

Sattler M, Reddy MM, Hasina R, et al. The role of the c-Met pathway in lung cancer and the potential for targeted therapy. Ther Adv Med Oncol 2011; 3:171–184.

Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol 2010; 11:834–848.

Weisberg E, Manley PW, Cowan-Jacob SW, et al. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. Nat Rev Cancer 2007; 7:345–356.

Epigenetic Therapy: DNA Methyltransferase Inhibitors and Histone Deacetylase Inhibitors

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Introduction

Epigenetics refers to inheritable changes in gene expression caused by mechanisms that do not involve a change in the nucleotide sequence. Examples include DNA methylation and post-translational histone modifications such as histone acetylation, histone methylation, and phosphorylation. Epigenetic mechanisms are essential in the normal regulation of gene expression and cellular differentiation and their disruption has been associated with the development and progression of cancer. In particular, aberrant DNA methylation and histone deacetylation have been associated with gene silencing and tumorigenesis in both hematological and solid tumors. Preclinical studies have demonstrated that it is possible to obtain gene reexpression, cell differentiation, and tumor growth inhibition by inhibiting the enzymes responsible for DNA methylation and histone deacetylation. These studies established that epigenetic changes, distinct from genetic changes, are reversible and have supported the development of agents with epigenetic effect as anti-cancer agents.

Four agents with epigenetic effect (two DNA methyltransferase inhibitors and two histone deacetylase inhibitors) have been approved by the

FDA for myelodysplastic syndromes and T-cell lymphomas and many others are currently in clinical development in different tumor types. Clinically, most of the epigenetic agents in development have shown a restricted activity in hematologic malignancies with limited or no single-agent activity in solid tumors.

DNA Methyltransferase Inhibitors (Table 1)

Generally, cancer cells present a decrease in DNA methylation. On the other hand, in addition to global hypomethylation, cancer cells present promoter hypermethylation, which results in silencing of the genes involved. Both global DNA hypomethylation and promoter hypermethylation can promote tumorigenesis.

Pharmacological inhibition of the enzymes responsible for DNA methylation (called DNA methyltransferases, DNMT) has been regarded as a method to develop anti-cancer agents. Preclinical studies have demonstrated that pharmacologically obtained DNA demethylation in cancer cells induces reexpression of silenced genes and results in tumor inhibition. These studies have supported the development of DNMT inhibitors (DNMTi) as anti-cancer agents.

5-Azacytidine (Vidaza) and 5-aza-2′-deoxycytidine (decitabine) have shown clinical antitumor activity and are now approved for the treatment of myelodysplastic syndrome (MDS). Both azacytidine and decitabine are nucleoside analogs. Their mechanism of action as demethylating agents is based on their incorporation after phosphorylation into DNA during replication.

5-Azacytidine was approved by the FDA in 2004 for the treatment of patients with all subtypes of MDS. The second DNMTi approved by the FDA in 2006 for MDS was decitabine.

Histone Deacetylase Inhibitors (Table 2)

Histones are small basic proteins that form a core around which DNA is wrapped into the nucleosome. The N-terminal tails of histones (which are rich in lysine and arginine residues) protrude from the nucleosome core

Table 1 *Pharmacological aspects of FDA-approved drugs with epigenetic targets: DNMT inhibitors*

Agent	Dosing	Pharmacokinetics	Metabolism	Dosing when organ dysfunction
1. Azacytidine	75 mg/m^2 SC, daily x 7, q 4 wks	$T_{1/2}$ 41 hrs	Hepatic	Caution in pts with liver disease (with albumin <3 g/L) or renal impairment
2. Decitabine	15 mg/m^2 continuously IV over 3 hrs q 8 hrs for 3 days q 6 wks OR 20 mg/m^2 continuously IV over 1 hr/daily x 5 q 4 wks	$T_{1/2}$ 0.62–0.78 hrs	Not known	Not known

Table 2 *Pharmacological aspects of FDA-approved drugs with epigenetic targets: HDAC inhibitors*

Agent	Dosing	Pharmacokinetics	Metabolism	Dosing when organ dysfunction
3. Vorinostat	400 mg/day continuously PO	$T_{1/2}$ 2 hrs	Hepatic	Not known
4. Romidepsin	14 mg/m^2 IV over 4 hrs days 1, 8 and 15 q 4 wks	$T_{1/2}$ 3 hrs	Hepatic	Not known

and are liable to post-translational modifications, consisting mainly of acetylation, methylation, and phosphorylation. These lead to changes in the secondary structure of the nucleosome and can modify gene expression by regulating access of transcription factors to promoter regions.

Acetylation of histones neutralizes the positive charge of histones tails and generates a more relaxed chromatin that allows transcription

Drug interactions	Toxicity	Approved use by FDA
Not known	71% hematological toxicity: 51% anemia (13% G3-4); 69% thrombocytopenia (58% G3-4); 65% neutropenia (61% G3-4); 61% GI events with nausea and vomiting (generally G1-2); 77% cutaneous reactions (generally G1-2)	MDS interm-2, high-risk IPSS CMML with 10–29% blasts AML with 20–30% blasts and multilineage dysplasia
Not known	87% G3-4 neutropenia; 85% thrombocytopenia; 23% febrile neutropenia; 22% leukopenia Less frequently: nausea; vomiting; diarrhea; constipation	De-novo and secondary MDS of FAB subtypes and intermediate-1, intermediate-2, and high-risk IPSS

Drug interactions	Toxicity	Approved use by FDA
Prolongation of QT in pts using coumarin derivative	49% diarrhea; 46% fatigue; 43% nausea; 26% anorexia; 24% dysgeusia; 20% thrombocytopenia; 66% hypercholesterolemia; 66% hypertriglyceridemia; 64% hyperglycemia; 45% increased creatinine	Cutaneous T-cell lymphomas
Prolongation of QT in pts using coumarin derivative Strong CYP3A4 inhibitors should be avoided if possible Caution with concomitant use of moderate CYP3A4 inhibitors Coadministration of potent CYP3A4 inducers may decrease concentrations of romidepsin and should be avoided	More than 15%: nausea; fatigue; vomiting; anorexia; anemia; thrombocytopenia	Cutaneous T-cell lymphomas

factors to bind to DNA, promoting expression of the corresponding genes (Figure 1). On the other hand, histone deacetylation results in a more tight binding of histones to the DNA with inhibition of transcription (Figure 1). The level of histone acetylation in normal cells depends on the activity of two groups of enzymes, histone acetyltransferase (HAT, responsible for acetylation) and histone deacetylases (HDACs, which cause deacetylation).

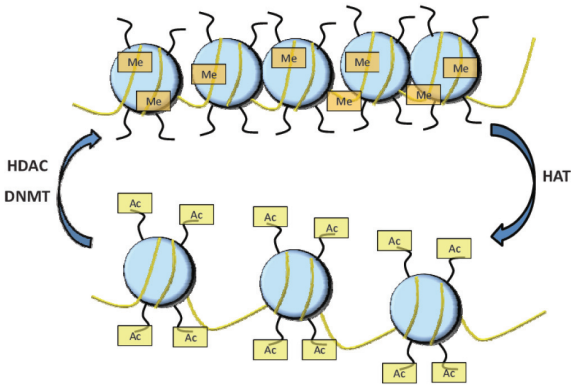

Figure 1 *Histone posttranslational modifications and DNA methylation. Upper part: deacetylation of histone tails by HDAC and methylation of cytosine in DNA by DNMT induce a tight chromatin configuration and transcriptional repression. Lower part: acetylation of histone tails by HAT and DNA demethylation relax chromatin and allow transcriptional activation.*

There is evidence that aberrant recruitment of HDACs and the resulting deacetylation of histones play a role in tumorigenesis. Cancer cells present high levels of HDACs and deacetylation of histones that result in silencing of tumor suppressor genes.

Preclinical studies have demonstrated that pharmacological inhibition of HDACs induces differentiation, cell-cycle arrest, and apoptosis and inhibit the growth of tumors in animal models. In addition to acetylation of histones, it is now clear that part of the antitumor activity of HDAC inhibitors (HDACi) is based also on acetylation of non-histone proteins (e.g. HSP90, p53, HIF-1α, α-tubulin).

Two HDACi have been approved by the FDA for the treatment of T-cell lymphomas. Vorinostat, an oral class I/II/IV HDACi, is approved for the treatment of cutaneous T-cell lymphoma (CTCL).

Romidepsin, a bicyclic depsipeptide, is an intravenous class I HDACi approved for the treatment of CTCL in patients who have received at least one prior systemic therapy.

Generally, it has been difficult to combine HDACi with chemotherapy due to the emergence of side effects that have resulted in significant dose reductions in comparison to single-agent HDACi; therefore the results of many of these studies have been inconclusive.

Further Reading

Esteller M. Epigenetics in cancer. N Engl J Med 2008; 358:1148–1159.

Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol 2009; 10:223–232.

Issa JJ, Kantarjian HM. Targeting DNA methylation. Clin Cancer Res 2009; 15:3938–3946.

Lane AA, Chabner BA. Histone deacetylase inhibitors in cancer therapy. J Clin Oncol 2009; 27:5459–5468.

Verbrugge I, Johnstone RW, Bots M. Promises and challenges of anticancer drugs that target the epigenome. Epigenomics 2011; 3:547–565.

Proteasome Inhibitors

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The Ubiquitin Proteasome Pathway

The ubiquitin proteasome pathway (UPS) is a key pathway for protein homeostasis in eukaryotic cells. Through this pathway the cell not only eliminates dysfunctional and misfolded proteins, but at the same time tightly controls the lifespan and therefore cellular levels of various key regulatory molecules for essential cellular functions.

In eukaryotes the UPS consists of a cascade of enzymatic steps by which ubiquitin, a small protein, is coupled via its lysine residue to the protein target scheduled for destruction. Proteins that are tagged by a chain of multiple ubiquitin molecules (i.e. polyubiquitinated proteins) are destroyed by a large multicatalytic protein complex, the proteasome.

The Proteasome

The proteasome is an abundant multienzyme complex of approximately 2 MDa size that resides in the cytosol and nucleus of eukaryotic cells. The 26S proteasome consists of a 20S core particle to which one or two 19S regulatory particles are attached. The 20S core contains the active proteolytic centers which hydrolyze the peptide bonds of proteins targeted for destruction, while the 19S particle is responsible for recognition, de-ubiquitination, and unfolding of protein substrates, which precede their translocation into the catalytic chamber of the 20S complex. The catalytic chamber consists of two outer alpha rings and two inner beta rings, of which three subunits contain proteolytically active centers (β1 caspase-like, β2 trypsin-like, β3 chymotrypsin-like).

The Proteasome as Target for Antineoplastic Therapy

Given the central role of the proteasome for the regulation of essential cellular functions, it is not surprising that proteasome inhibitors were shown to be cytotoxic against malignant cells in vitro.

Proteasome inhibitors showed a broad spectrum of antiproliferative and pro-apoptotic activities toward hematological and solid malignancies. Malignant cells were more sensitive toward proteasome inhibition than normal cells. The molecular mechanism of this finding is still poorly understood, but it is conceivable that it is based on the special conditions of metabolism and protein homeostasis of malignant cells. Most tumor cells have a high proliferation rate and therefore a high rate of protein biosynthesis. Key mechanisms of proteasome inhibition include the inhibition of NFκB (through decreased proteasomal destruction of the NFκB antagonist, IκB), and activation of the unfolded protein response.

Proteasome Inhibitors

Proteasome inhibitors are usually composed of an active electrophilic group which interacts with the active site threonines of the catalytic β-subunits, attached to a (usually peptide) backbone that fits into the corresponding substrate pockets surrounding the active subunits in the catalytic chamber of the 20S core particle. The most common active electrophiles used are boronates, epoxyketones, and B-lactones.

Bortezomib

Bortezomib is a peptide boronate reversible proteasome inhibitor. Bortezomib seemed to be particularly potent against myeloma, and a fast series of phase I–III studies in myeloma confirmed the clinical activity with approval of the FDA in 2003 for third-line therapy of myeloma. This approval was extended for first-line use and was followed by its approval for use in mantle cell lymphoma. The bortezomib dose and schedule established in phase I studies is 1.3 mg/m^2 D1, 4, 8, 11 q 21 days as intravenous bolus. Bortezomib distributes within minutes in the extracellular space and reaches concentrations of 20 nM at 30 mins, which leads to a 40–60% inhibition

of the rate-limiting B5 proteasomal activity. The drug is rapidly eliminated from the circulation independent of kidney function. However, the functional proteasome inhibition by a single dose of bortezomib is maintained up to 72 hrs due to the slow off-rate of proteasome-bound bortezomib.

Bortezomib monotherapy results only in a modest response rate of 27% in relapsed/refractory myeloma patients; however, the clinical value of the drug lies in its drug-sensitizing properties as a combination partner for drugs like dexamethasone, lenalidomide, melphalan, cyclophosphamide, doxorubicin, and others. As part of these combinations, bortezomib increases significantly the effect of the chemotherapy partner and is also able to overcome chemotherapy resistance. The addition of bortezomib to standard melphalan/prednisone in first-line myeloma therapy (VISTA trial) has resulted in a survival benefit and has replaced the old standard after almost five decades. The toxicities of bortezomib include thrombocytopenia (28% grade 3) and neuropathy (incidence approximately 30%, ca 10% grade 3). This mixed motor–sensory peripheral polyneuropathy is dose dependent and usually reversible. It is most likely not the result of a class effect of proteasome inhibitors, but of an off-target activity of bortezomib against non-proteasomal proteases. Neurotoxicity of bortezomib can be mitigated by subcutaneous application, which results in similar degrees of proteasome inhibition and activity, but less neurotoxicity. Bortezomib has a very narrow therapeutic window, and dose-limiting toxicity was observed at 1.6 mg/m^2 i.v.

In contrast to myeloma cells, characterized by a special biology of their protein biosynthesis machinery due to the high loads of paraprotein secreted, the clinical activity of bortezomib alone or in combination in solid tumors was disappointing.

Carfilzomib

Carfilzomib is the compound in the group of second-generation proteasome inhibitors at the most advanced stage of clinical development. It belongs

to the epoxyketone-type group and irreversibly inhibits the proteasome by forming a covalent bond with the active-site threonine. In preclinical models, carfilzomib showed a higher potency and longer activity on proteasome inhibition compared to bortezomib, which can mostly be attributed to the different type of electrophile used, so that bortezomib-resistant myeloma cells underwent cytotoxicity after carfilzomib. In phase I clinical trials, carfilzomib showed a very short plasma half-life time of <30 mins, but led to >75% target inhibition already after 1 hr. Only 4 days after carfilzomib treatment, the proteasome activity levels were back to baseline values.

The dose-limiting toxicity of carfilzomib was thrombocytopenia and leucopenia. Interestingly, phase I studies of carfilzomib have to date revealed no indications of neurotoxicity. In a phase II clinical trial in relapsed/refractory myeloma patients, single-agent carfilzomib demonstrated 24% of the activity with partial response or better.

Perspectives

Proteasome inhibitors have only one registered drug. The next generation of compounds will have higher potency (through better electrophiles and covalent inhibition), higher specificity, and more favorable toxicity (including lack of neurotoxicity), as well as higher bioavailability in the known indications.

It is still poorly understood to what extent solid tumors may be successfully treated with proteasome inhibitors in the future. Toward this goal, proper penetration of the inhibitor into the tumor tissue is crucial. Other, as yet unanswered, questions include the optimal pattern of inhibition of proteasome subunits for the different tumor entities, the biology of resistance against proteasome inhibitors, as well as whether proteasome inhibitors may be targeted more specifically toward the cell population of interest. In addition to their use as antineoplastic drugs, proteasome inhibitors may be of great value in the treatment of autoimmune diseases, graft-versus-host reaction, or chronic inflammatory conditions, for which there are very promising preclinical data.

Further Reading

Adams J. The development of proteasome inhibitors as anticancer drugs. Cancer Cell 2004; 5:417–421.

Molineaux SM. Molecular pathways: targeting proteasomal protein degradation in cancer. Clin Cancer Res 2012; 18:15–20.

Ruschak AM, Slassi M, Kay LE, et al. Novel proteasome inhibitors to overcome bortezomib resistance. J Natl Cancer Inst 2011; 103:1007–1017.

Monoclonal Antibodies for Targeted Treatment of Lymphoproliferative Neoplasias

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Monoclonal Antibodies for Cancer Therapy

Monoclonal antibodies (mAbs) against various cell types have been developed and antibody-based treatments now represent important therapeutic tools for an increasing number of hematopoietic malignancies. Unconjugated mAbs, including the anti-CD20 antibodies rituximab and ofatumumab and the anti-CD52 antibody alemtuzumab, which target surface antigens expressed in patients with non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL), have been approved for clinical use by the FDA and the EMA. Yttrium-90 (^{90}Y)-ibritumomab tiuxetan is a radiolabeled anti-CD20 monoclonal antibody FDA-approved for the treatment of relapsed or refractory, low-grade, or follicular B-cell NHL as well as of untreated follicular NHL in patients who achieve a partial or complete response to first-line chemotherapy. Recently, the antibody–drug conjugate brentuximab vedotin, containing an anti-CD30 antibody and monomethylauristatin E (MMAE), has been approved for use in refractory Hodgkin lymphoma (HL) and anaplastic large-cell lymphoma (ALCL).

Anti-CD20 Antibodies (Table I)

CD20 is a cell-surface glycoprotein that plays a key role in B-cell proliferation, activation, and differentiation. The antigen is expressed by normal pre-B cells and B cells until the immunoblast stage, as well as by the great majority (>90%) of B-cell NHL. Anti-CD20 antibodies induce B-cell lysis through different mechanisms, including complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and apoptosis. Additionally, anti-CD20 antibodies sensitize lymphoma cells to chemotherapy. Based on the mechanisms of B-cell lysis, anti-CD20 antibodies are divided into two types, i.e. type I (or rituximab-like) antibodies characterized by their ability to redistribute CD20 into lipid rafts and induce potent CDC, and type II (or tositumomab-like) antibodies which instead induce homotypic adhesion and programmed cell death.

1. Rituximab

Rituximab is a chimeric mouse anti-human CD20 monoclonal antibody that contains human IgG1 heavy chain and κ light chain constant regions (Fc) and murine heavy and light chain variable regions (Fab). The Fab domain of rituximab binds with high affinity to CD20 antigen expressed on B cells and the Fc domain forms crosslinks with Fcγ receptors on tumor cells and immune effector cells. This binding results in ADCC, whereas the binding of Fc domain to complement may result in lymphoma cell death via CDC. Single-agent rituximab therapy at the dose of 375 mg/m^2 weekly for 4 weeks has a limited efficacy in NHL (with overall response rate [ORR] of 30% and 50% in diffuse large B-cell lymphoma [DLBCL] and follicular lymphoma [FL], respectively). The greatest anti-lymphoma effects of rituximab are observed in combination regimens, likely due to rituximab-induced sensitization of tumor cells to the effects of chemotherapy. Serum levels of rituximab are dose proportional, correlate with patient response to therapy, and increase with each successive infusion. The half-life of rituximab is also proportional to the dose, increases with each subsequent infusion, and varies greatly from patient to patient. The wide variability in elimination half-lives may reflect differences in tumor burden and CD20+ B-cell populations

with repeated administrations. Though the mechanisms involved in the metabolism and elimination of rituximab are not fully understood, it is postulated that rituximab is degraded nonspecifically in the liver and excreted in the urine.

Rituximab is cytotoxic to both malignant and normal CD20-expressing B lymphocytes, but not hematopoietic stem cells. Rituximab induces a rapid and marked depletion of B lymphocytes in the peripheral blood lasting on average 6 months, with recovery of circulating B cells usually occurring 9–12 months after chemoimmunotherapy. More than 95% of rituximab-related adverse events are mild to moderate in severity, of brief duration, and observed during the first infusion. On average, 55% of patients have no adverse event on the first rituximab infusion. Serious adverse effects include infusion-related reactions, tumor lysis syndrome, mucocutaneous reactions, hypersensitivity reactions, cardiac arrhythmias, angina, and renal failure. Patients at increased risk of experiencing severe adverse events (grade 3 or 4) include those with high counts of circulating malignant lymphocytes (≥25 000/mm^3) as well as patients with a high tumor burden. The most common adverse reactions of rituximab (incidence ≥25%) observed in clinical trials of patients with NHL were infusion reactions, fever, lymphopenia, chills, infections, and asthenia. Hematological adverse events are limited with the exception of severe lymphopenia. In patients with NHL receiving rituximab monotherapy, grade 3 and 4 cytopenias were reported in 48% of patients. These included lymphopenia (40%), neutropenia (6%), leukopenia (4%), anemia (3%), and thrombocytopenia (2%). Cases of progressive multifocal leukoencephalopathy have been observed in patients treated with rituximab.

2. Ofatumumab

Ofatumumab is a type I human IgG1κ antibody that binds to a novel epitope of CD20 on both the small extracellular loop and the N-terminal region of the second large loop. Binding to the small loop of CD20 confers to ofatumumab a proximity to the cell membrane surface which may increase the efficiency of CDC activation. In vitro, ofatumumab induces ADCC at levels comparable to that observed for rituximab, whereas ofatumumab-triggered CDC occurs even at lower cell membrane levels

Table I *Pharmacological aspects of anti-CD20 monoclonal antibodies*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
1. Rituximab	Type I anti-CD20 antibody inducing B-cell lysis through ADCC and CDC	IV 375 mg/m^2 First inf: initiate inf at a rate of 50 mg/hr. in the absence of infusion toxicity, increase inf rate by 50 mg/hr increments q 30 mins, to a maximum of 400 mg/hr Subsequent infs: initiate inf at a rate of 100 mg/hr. in the absence of infusion toxicity, increase rate by 100 mg/hr increments at 30-min intervals, to a maximum of 400 mg/hr Premedication: paracetamol and antihistamine 30 min before administration	$T_{1/2}$ first inf: 76.3 hrs $T_{1/2}$ fourth inf 205.8 hrs	Not fully understood. Postulated that rituximab has a B-cell mediated metabolism, and is degraded non-specifically in the liver and excreted in the urine
2. Ofatumumab	Type I anti-CD20 antibody inducing B-cell lysis through ADCC and CDC	IV administration First inf: 300 mg Subsequent infs: 1000 mg or 2000 mg Slow inf (>6 hrs) Premedication: paracetamol and antihistamine 30 mins before administration	$T_{1/2}$ first inf: 1.3 days $T_{1/2}$ subsequent infs: 14.7 days	B-cell mediated
3. ^{90}Y-Ibritumomab tiuxetan	Anti-CD20 murine IgG1 antibody conjugated to tiuxetan, which chelates the high-energy pure β-emitter ^{90}Y	Single IV administration at 0.4 mCi/kg (maximum 32 mCi) over 10 mins Administration of rituximab 250 mg/m^2 1 wk and 4 hrs before treatment	^{90}Y-Ibritumomab tiuxetan $T_{1/2}$ 28 hrs ^{90}Y $T_{1/2}$ 64 hrs	^{90}Y tends to accumulate in the liver, is excreted through the biliary tract. Urinary excretion minimal
4. GA101	Type II anti-CD20 antibody inducing B-cell lysis through ADCC and caspase-independent mechanism	IV administration In phase II studies: for the first cycle, 1000 mg on days 1, 8, 21, then q 3 wks Premedication: paracetamol and antihistamine 30 mins before administration	$T_{1/2}$: Clearance dependent on number of infs and on tumor burden	B-cell mediated

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
No adjustment necessary	No known significant interactions	53% fever; 47% lymphopenia; 32% chills (4% G3–4); 31% infections (3% G3–4); 26% asthenia; 24% nausea; 15% rash; 15% neutropenia; 15% thrombocytopenia; 10% myalgia; 10% arthralgia; 10% hypotension	Interrupt the infusion or slow the infusion rate for infusion reactions. Continue the infusion at one half the previous rate on improvement of symptoms
No adjustment necessary	No known significant interactions	20% infections (12% G3–4); 18% neutropenia (15% G3–4); 12% fever; 10% dyspnea; 10% rash; 3% anemia	Interrupt the infusion or slow the infusion rate for infusion reactions. Continue the infusion at one half the previous rate on improvement of symptoms
Not known	No known significant interactions	57% neutropenia G3–4; 61% thrombocytopenia G3–4; 43% asthenia; 31% nausea; 24% chills	Pts with mild thrombocytopenia (PLT counts <150 000/mm^3) should receive a dose of 0.3 mCi/kg Pts with PLT counts <100 000/mm^3, ANC <1500/mm^3, or bone marrow involvement >25% should be excluded
No adjustment necessary	No known significant interactions	65% infusion-related reaction; 16% fatigue; 10% nausea; 15% neutropenia (10% G3-4); 10% thrombocytopenia; 7% headache	Interrupt the infusion or slow the infusion rate for infusion reactions. Continue the infusion at one half the previous rate on improvement of symptoms

of CD20 and does not seem to be dependent on cell-surface expression of complement regulatory molecules. Ofatumumab is eliminated through a target-independent route and a B-cell-mediated route. It also demonstrates a dose-dependent clearance with doses of 100 to 2000 mg. The most frequent adverse events are grade 1–2 fever, chills, dyspnea, pruritus, rash, and urticaria. More than half of adverse events are infusion related and are characterized by fevers, rigors, fatigue, rash, and sweating. Adverse events are usually recorded on the day of the first infusion and decrease in number and intensity at each subsequent infusion. Rare adverse events include tumor lysis syndrome and mucocutaneous reaction.

3. Yttrium-90 (^{90}Y) ibritumomab tiuxetan

^{90}Y-Ibritumomab tiuxetan consists of the anti-CD20 murine IgG1 monoclonal antibody (ibritumomab) conjugated to the second-generation chelator tiuxetan, which chelates the high-energy pure β emitter ^{90}Y for therapy or the γ emitter indium-111 (^{111}I) for imaging. The antibody binds to the CD20 antigen on the B-cell surface and the decay of accumulated ^{90}Y produces emission of β particles that release their energy to nearby cells ("crossfire effect"). The 5 mm average path length of the β particles, greater than the diameter of a cell, allows the radiation damage to reach cells remote from the site of antibody binding. In the past, the inability to perform imaging with ^{90}Y required the administration of the γ emitter ^{111}I chelated with ibritumomab before the infusion of the therapeutic dose of ^{90}Y-ibritumomab tiuxetan to evaluate biodistribution. Recently, the FDA has approved the removal of the bioscan and patients receive only administration of rituximab (250 mg/m^2) 1 wk and 4 hrs prior to injection of the radioimmunoconjugate to decrease circulating B-cell numbers and improve tumor targeting. ^{90}Y-Ibritumomab tiuxetan at the dose of 0.4 mCi/kg has been tested as a single agent in patients with refractory or relapsed follicular NHL, with an objective response rate of 73–83% and a complete response rate of 15–51%. Treatment with ^{90}Y-labeled antibodies results in emission of β radiation but there is no emission of penetrating γ radiation; therefore there is minimal risk of radiation exposure to health care workers or the patient's family. The most common adverse events of ^{90}Y-ibritumomab tiuxetan are hematological and include gradual cytopenia occurring 3 to 4 weeks after the

treatment, followed by a full hematological recovery by the third month post-radioimmunotherapy.

4. GA101 (Table 1)

GAG101 is a novel type II, humanized anti-CD20 monoclonal antibody with a glycoengineered Fc portion that confers to GAG101 a higher affinity and selectivity of the Fc gamma RIII (CD16).These structural characteristics are associated with a 10- to 100-fold increase in ADCC against CD20-positive NHL cell lines.

Anti-CD52 Antibodies

CD52 is a 21 kDa cell-surface glycoprotein expressed at high levels by both normal and malignant B and T lymphocytes, with lower levels found on monocytes, macrophages, and eosinophils and little expression on natural killer cells and neutrophils. CD52 is also expressed by epithelial cells in the epididymis and duct deferens. The exact biological function of CD52 remains unclear, but some evidence suggests that it may be involved in T-cell activation.

5. Alemtuzumab (Campath-1H) (Table 2)

Alemtuzumab is a humanized IgG1 monoclonal antibody with human variable framework and constant regions, and complementarity-determining regions from a murine (rat) monoclonal antibody (Campath-1G). When bound to the cell-surface glycoprotein CD52, alemtuzumab activates CDC and ADCC. In contrast, only limited pro-apoptotic effects of the antibody have been reported. The exact mechanism by which alemtuzumab mediates its biological effects in vivo is not clearly defined.

Alemtuzumab is currently approved for the treatment of patients with relapsed/refractory CLL who have failed prior fludarabine-based chemotherapy. Alemtuzumab has also been explored in a variety of hematopoietic malignancies. Because of the marked lymphopenia resulting from alemtuzumab therapy, the antibody has been used to prevent graft-versus-host disease following allogeneic bone marrow transplantation. Promising results have been reported in a small number of patients with refractory

Table 2 *Pharmacological aspects of alemtuzumab*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
5. Alemtuzumab	Anti-CD52 antibody inducing lysis of CD52-expressing cells through CDC and ADCC	IV or SC administration: thrice weekly for up to 12 wks, at an initial dose of 3 mg, escalated to 10 and 30 mg. SC route is preferred Premedication: corticosteroid, paracetamol, antihistamine, and ranitidine 30 min before administration	$T_{1/2}$ 11 hrs (range 2 to 32 hrs) after the first 30 mg dose $T_{1/2}$ 6 days (range 1 to 14 days) after last 30 mg dose	Not fully understood. Metabolism of alemtuzumab might be mediated by CD52+ cells Clearance decreases with repeated dosing due to loss of CD52 receptors

autoimmune thrombocytopenia. The currently approved schedule of alemtuzumab involves a dose of 3 mg delivered IV on day 1, escalating to 10 mg on day 2 and then to 30 mg three times weekly as tolerated. The duration of therapy is generally 12 weeks. Since subcutaneous administration of alemtuzumab at the same schedule has a comparable activity with fewer adverse effects, this route of delivery is largely preferred. The main complications of alemtuzumab treatment are caused by tumor necrosis factor (TNF)-α and interleukin (IL)-6 release and include pyrexia, chills, urticaria, rash, tachycardia, dyspnea, fever, rigor, nausea, vomiting, and hypotension responsive to steroids. These infusion-related side effects are common during the first IV administration and tend to decrease in frequency and severity during the subsequent weeks of treatment or using the subcutaneous route of administration. Opportunistic infections are the major adverse effects of alemtuzumab and are related to the decrease in CD4+ and CD8+ lymphocytes both during treatment and up to 9 months or more after completion of therapy. In addition to traditional bacterial infections, other more atypical infections are noted, including infections secondary to cytomegalovirus (CMV) or herpes simplex virus reactivation, *Pneumocystis (carinii) jeroveci* pneumonia

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
NA	No known significant interactions	Common adverse reactions: 70–80% infusion reactions (pyrexia, chills, hypotension, urticaria, nausea, rash, tachycardia, dyspnea); 15% neutropenia G3–4; 45% thrombocytopenia G3–4; 100% lymphopenia; 10% anemia; 5% infections (bacterial, fungal, and protozoan infections); 30-40% CMV reactivation; 5% GI symptoms Prophylaxis: trimethoprim/sulfamethoxazole 3/day three times per wk and valacyclovir 1000 mg daily CMV DNA load should be checked q 1–2 wks during treatment and for at least 3 months after therapy discontinuation	Withhold during serious infection or other serious adverse reactions until resolution. Discontinue for autoimmune anemia or autoimmune thrombocytopenia. There are no dose modifications recommended for lymphopenia

and aspergillosis. During treatment with alemtuzumab, prophylaxis with co-trimoxazole (trimethoprim/sulfamethoxazole) and valacyclovir is recommended. The pharmacokinetic profile of alemtuzumab was studied in NHL and CLL patients receiving the antibody once weekly for a maximum of 12 weeks. Following intravenous infusions over a range of doses, the maximum serum concentration showed relative dose proportionality. The mean $T_{1/2}$ over the dosing interval was about 12 days.

Anti-TNF Receptor Superfamily Antibodies

Tumor necrosis factor (TNF) receptor superfamily members are currently being explored as potential targets for lymphoma therapy. The TNF receptor superfamily includes a large variety of molecules whose engagement triggers pleiotropic biological effects. A strong preclinical rationale supports the development of antibodies targeting TNF-related apoptosis-inducing ligand (TRAIL) receptors as well as the CD30 antigen.

6. Brentuximab vedotin (Table 3)

CD30 is a TNF superfamily transmembrane receptor and is highly expressed in patients with HL and ALCL. Initial phase I and II clinical trials using first-generation naked anti-CD30 antibodies, such as SGN-30, were disappointing, especially in patients with relapsed HL. These poor results may be due to poor antigen-binding properties, ineffective activation of effector cells, and neutralization by soluble CD30. Since CD30 is internalized upon binding, it is a suitable target for ADCC strategies. Brentuximab vedotin (SGN-35) is an ADCC composed of the anti-CD30 antibody cAC10 (SGN-30) attached to the antimicrotubule agent, MMAE. After binding CD30, the antibody–drug conjugate is rapidly internalized and is transported to lysosomes, where MMAE is released

Table 3 *Pharmacological aspects of brentuximab vedotin*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
6. Brentuximab Vedotin (SGN-35)	cAC10 chimerized IgG1 monoclonal antibody SGN-30 linked to MMAE. Upon internalization, MMAE induces cell cycle arrest and cell apoptosis	IV inf MTD 1.8 mg/kg, administered q 3 wks	$T_{1/2}$ 4 days at 1.8 mg/kg MTD 1.8 mg/kg	MMAE is excreted in both feces (72%) and urine (28%)

Table 4 *Pharmacological aspects of mapatumumab and lexatumumab*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
7. Mapatumumab	Fully human IgG1 λ anti-TRAIL-R1 agonist monoclonal antibody; binding to TRAIL-R1 induces apoptosis mimicking the activity of the natural protein TRAIL	IV administration 10 mg/kg q 14 days	$T_{1/2}$ 18 days	–
8. Lexatumumab	Fully human IgG1 λ anti-TRAIL-R2 agonist monoclonal antibody; binding to TRAIL-R2 induces apoptosis mimicking the activity of the natural protein TRAIL	IV administration 10 mg/kg q 14 or 21 days	$T_{1/2}$ 15/16 days	

into the cell, binds tubulin, and prompts arrest of the cell cycle and cell apoptosis. Pharmacokinetic data revealed dose-proportional exposure to SGN-35 and MMAE with a mean $T_{1/2}$ of MMAE at 1.8 mg/kg of approximately 4 days. MMAE is excreted in both feces (72%) and urine (28%). Common treatment-related adverse events include peripheral neuropathy, hyperglycemia, diarrhea, pyrexia, and neutropenia.

Anti-TRAIL-R1 and TRAIL-R2

A strong preclinical rationale supports the development of antibodies targeting TNF-related apoptosis-inducing ligand (TRAIL) receptors, including mapatumumab and lexatumumab (Table 4).

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
Not known	Not known	Most common adverse events include: 36% fatigue, 33% fever, 22% diarrhea, 22% nausea, 22% neutropenia, and 22% peripheral neuropathy; followed by headache, vomiting, back pain, anemia, and alopecia. Most adverse events were G1 and 2	Dose reduction for peripheral sensory neuropathy. Dose delays for neutropenia, peripheral sensory neuropathy, and thrombocytopenia

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
–	–	Transaminase elevation (G1-2); fatigue; nausea; anorexia; diarrhea	–
–	–	Hyperamylasemia (G3); mild fatigue; nausea; anorexia	–

Further Reading

Brody J, Kohrt H, Marabelle A, et al. Active and passive immunotherapy for lymphoma: proving principles and improving results. J Clin Oncol 2011; 29:1864–1875.

Cheson BD. Ofatumumab, a novel anti-CD20 monoclonal antibody for the treatment of B-cell malignancies. J Clin Oncol 2010; 28:3525–3530.

Keating GM. Rituximab: a review of its use in chronic lymphocytic leukaemia, low-grade or follicular lymphoma and diffuse large B-cell lymphoma. Drugs 2010; 70:1445–1476.

Morris EC, Rebello P, Thomson KJ, et al. Pharmacokinetics of alemtuzumab used for in vivo and in vitro T-cell depletion in allogeneic transplantations: relevance for early adoptive immunotherapy and infectious complications. Blood 2003; 102:404–406.

Oldham RK, Dillman RO. Monoclonal antibodies in cancer therapy: 25 years of progress. J Clin Oncol 2008; 26:1774–1777.

Plummer R, Attard G, Pacey S, et al. Phase 1 and pharmacokinetic study of lexatumumab in patients with advanced cancers. Clin Cancer Res 2007; 13:6187–6194.

Tolcher AW, Mita M, Meropol NJ, et al. Phase I pharmacokinetic and biologic correlative study of mapatumumab, a fully human monoclonal antibody with agonist activity to tumor necrosis factor-related apoptosis-inducing ligand receptor-1. J Clin Oncol 2007; 25:1390–1395.

Witzig TE, Flinn IW, Gordon LI, et al. Treatment with ibritumomab tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. J Clin Oncol 2002; 20:3262–3269.

Younes A, Bartlett NL, Leonard JP, et al. Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. N Engl J Med 2010; 363:1812–1821.

Younes A. Beyond chemotherapy: new agents for targeted treatment of lymphoma. Nat Rev Clin Oncol 2011; 8:85–96.

Immunomodulatory Drugs and Cytokines

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1. Interleukin-2 (IL-2) (Table 1)

Recombinant IL-2 is a 133 amino acid protein of 15 000 Daltons. Interleukin-2 binds to the IL-2 receptor (IL-2R), consisting of three protein chains: the IL-2R alpha chain, the IL-2R beta chain, and the IL-2R gamma chain. The IL-2R beta chain and the IL-2R gamma chain belong to the cytokine receptor superfamily. The IL-2R gamma chain, also called common gamma chain, is also used by other interleukin receptors such as IL-4, IL-7, IL-9, IL-15, and IL-21. The IL-2R alpha and beta chains form the low affinity IL-2R and the IL-2R alpha, beta, and gamma chains form the high affinity IL-2R complex. Both receptors are able to signal upon IL-2 binding. The activation of naive T lymphocytes through T-cell receptor triggered by a major histocompatibility complex (MHC)–peptide complex results in both the upregulation of the IL-2 and IL-2R alpha genes (IL-2R beta and gamma chain genes are already expressed) and activated T cells secrete IL-2 for their own proliferative burst and for that of other cell types, such as B cells and NK cells. The three IL-2 receptor chains span the cell membrane and deliver signals to the nucleus. The alpha chain does not participate in signaling, but the beta and gamma chains do by interacting with tyrosine kinases Janus kinase 1 (JAK1) and JAK3. These enzymes are activated upon IL-2 binding to the IL-2R. As a consequence, three intracellular signaling pathways are initiated: the MAP kinase pathway, the PI3K pathway, and the JAK-STAT pathway. Together, these and other signaling events eventually promote cell-cycle progression and cell division.

IL-2 was first used for the treatment of melanoma and renal cell carcinoma (RCC). In 1992 and 1998 recombinant IL-2, given as high-dose bolus intravenous infusions, was approved by the FDA for the treatment of advanced stage RCC and melanoma, respectively. High-dose IL-2 results in about 15% objective response rates (World Health Organization response criteria) in both diseases, of which only 50% or less are complete.

The exact mechanism of action of high-dose IL-2 is still not fully understood. IL-2 induces (directly or indirectly) not only lymphocyte or NK-cell proliferation, but mediates many other effects on other cell types as well, which can result in severe toxicity that is accompanied by this treatment. IL-2 induces high-grade fever, chills, and severe capillary leak syndrome, leading to hypotension, oliguria and edema, rash, mental confusion, diarrhea, and dyspnea.

Intravenous (IV) infusion of IL-2 can be by bolus infusions (in 15 mins) every 8 hrs, or by continuous IV infusions. The latter appears to be more toxic than the first, which has become a standard of care in the USA. The $T_{1/2}$ of IV IL-2 is very short, 1.5 hrs versus more than 5 hrs after subcutaneous (SC) injection. The peak concentration of IV IL-2 is 20–30 times higher after IV than after SC injection.

IL-2 can affect multiple organ systems and, because of side effects such as nausea, vomiting, and diarrhea, the absorption of other drugs may be impaired. IL-2 may affect renal and liver function and, as a consequence, affect clearance or metabolism of other drugs. IL-2 may inhibit cytochrome P450 enzymes leading to altered pharmacokinetics of concomitant medications.

Some immunomodulatory drugs may influence IL-2 efficacy, such as corticosteroids that have immunomodulating effects. Nephrotoxic, hepatotoxic, or cardiotoxic drugs may increase IL-2 related toxicity.

In Europe, low-dose IL-2 has been used mainly for the treatment of metastatic RCC, but its use in advanced melanoma is controversial, since low-dose IL-2 does lead to toxicities such as fever, chills, rash, capillary leak syndrome, and fluid retention; however, these have no effect on patient outcome.

2. Interferon-alpha-2b/2a (Table I)

Interferon (IFN)-alpha exists as IFN-alpha-2a (Roferon) and as IFN-alpha-2b (Intron A), which differ only in one amino acid at position 23. Recombinant IFN-alpha is a 165 amino acid polypeptide with a MW of 19 300 Daltons. Slow-release IFN-alpha-2b (PEG Intron) can be given once weekly.

Interferon type I (to which IFN-alpha-2a and 2b belong) signals through specific heterodimeric IFN-alpha receptors, IFNAR1 and IFNAR2, present in most tissues. Upon receptor binding, several signaling cascades are triggered, leading to the activation of several hundreds of interferon response or stimulating genes (ISG). The first activated signaling cascade is the JAK-STAT pathway. Signaling through the IFNAR also leads to activation of MAPK, PI3K, and NFκB. Depending on the cell type, different signaling pathways prevail.

Type I IFNs directly or indirectly inhibit tumor cell growth and viral replication in virus-infected cells. IFN-alpha leads to upregulation of MHC class I molecules and thus antigen presentation. Type I IFNs, either through direct signaling effects on immune cells or through chemokine release and attraction of other immune cells, and through secondary activation and cytokine release, exert many effects on the immune system. IFN-alpha improves the killing potential of NK cells, stimulates the development of dendritic cells (DC) from monocytes, and improves migration of DC from the infected tissue to the draining lymph nodes by induction of CCR7 expression. IFN-alpha also influences behavior of CD4 and CD8 T cells. CD4 T cells are directed to a Th1-like differentiation, whereas IFN-alpha improves the function of CD8 T-cells (IFN-gamma secretion, survival, memory).

Activity of IFN-alpha-2b is universally measured in the "cytopathic effect assay". In this biological activity assay, the ability of IFN-alpha to protect cells from virus-induced cytopathology is being measured.

IFN-alpha is indicated for the treatment of RCC, hairy cell leukemia, chronic myelogenous leukemia, multiple myeloma, AIDS-associated Kaposi sarcoma, and polycythemia vera. The main adverse events

Table 1 *Pharmacological aspects of IL-2, IFN-alpha, and ipilimumab*

Agent	Mechanism of action	Dosing	Pharmacokinetics	Metabolism
1. IL-2	T-cell proliferation; NK-cell activation, growth	IV: 600 000 to 720 000 IU/kg q 8 hrs for a maximum of 15 followed, 10 days later, by another cycle SC: 18 $\times 10^6$ IU d × 5, followed by 2 days rest. For the following 3 wks: 18 $\times 10^6$ IU on days 1 and 2 followed by 9 × 10^6 IU on days 3–5. On days 6 and 7 no treatment. After 1 wk rest this 4-wk cycle should be repeated	IV: $T_{1/2}$ 1.5 hrs SC: $T_{1/2}$ 5 hrs	Kidney and receptor-mediated clearance (binding of IL-2 to IL-2R mainly on T cells and NK cells)
2. IFN-alpha	Inhibition of cancer cell growth and viral replication in virus-infected cells	IFN-alpha: 3 SC 3 ×/wk 3–9 MIU PEG-IFN-alpha-2b: 1.5–7.5 μg/kg/wk	IFN-alpha-2b: $T_{1/2}$ 4 hrs T_{max} 8 hrs PEG-IFN-alpha-2b: $T_{1/2}$ 40 hrs T_{max} 48 hrs	IFN-alpha is mainly cleared by the kidneys, partly by receptor-mediated clearance
3. Ipilimumab	Blockade of CTL A4	IV inf 3–10 mg/kg q 3 wks for 4 times	$T_{1/2}$ 16 days T_{max} 2.5 hrs	No metabolic studies performed. Independent from cytochrome P450 enzymes

reported in ≥50% of patients are associated with the flu-like syndrome commonly seen with interferon-alpha use, e.g. fever, fatigue, myalgia, arthralgia, and headache. Other adverse effects in >10% of patients are: anorexia, vomiting, back pain, dizziness, bone pain, depression, insomnia, viral infection, alopecia, pruritus, rash, and increased sweating.

Pegylated IFN-alpha-2b (PEG Intron) is a derivative of Intron consisting of a single straight-chain molecule of polyethylene glycol (PEG) with an average MW of 12 000 Daltons, attached by covalent linkage primarily to histidine 34 on IFN-alpha-2b. Pegylated IFN-alpha-2b has simi-

Dosing when organ dysfunction	Drug interactions	Toxicity	Dose adjustment for toxicity
In cases of cardiac disease, pulmonary disease, autoimmune disorders, renal insufficiency (Cr Cl <50 ml/min): no treatment	IL-2 may inhibit several cytochrome P450 enzymes: CYP3A4; CYP1A1. Drugs metabolized by these enzymes may require adjustments during IL-2 therapy	IL-2 may cause (≥10%): hypotension; dyspnea; cough; rash; anorexia; nausea; diarrhea; anxiety; confusion; fever; chills; headache; tachycardia; oliguria; ↑ creatinine; anemia; thrombocytopenia; fatigue; edema; weight gain; injection site pain (SC)	IV bolus: depending on the type of toxicity a bolus will be skipped in case of G3-4 toxicity. Previous bolus ≥24 hrs before: treatment stopped SC injections: pts who do not tolerate the recommended dose should undergo 25–50% dose reduction
Renal insufficiency (Cl below 50 ml/min) requires dose adjustment. IFN-alpha should not be administered in case of liver insufficiency, epilepsy, recent cardiovascular event, autoimmune disease. Pts known to suffer from depression should be monitored carefully for suicidal behavior	Induction of cytochrome P450 enzymes CYP2D6 and CYP2C8/9. Drugs metabolized by these enzymes may require dose adjustments during IFN-alpha therapy	Main toxicities: fatigue; chills; fever; myalgia; anorexia; nausea; vomiting; diarrhea; depression; sleeplessness; headache; dizziness; alopecia; rash; anemia; neutropenia	Dose reduction: neutropenia and thrombocytopenia G3 or more
Hepatic, renal, and cardiac dysfunction: no dose adjustment	No dose reduction needed because the drug is not metabolized via cytochrome P450 enzymes	MDX-010-20: any immune-related AE: 60% (G3-4 13%); 1% death 42% skin (1% G3-4); 28% GI (7% G3-4); 7% endocrine (4% G3-4); 3% hepatic (1% G3-4); 4% other AE (1.5% G3-4)	In case of G3-4 toxicity, treatment will be discontinued

lar pharmacodynamic and pharmacokinetic properties as IFN-alpha-2b delivered three times per wk. Pegylated IFN-alpha-2b delays renal clearance and allows drug intensification compared to IFN-alpha-2b.

From a pharmacokinetic perspective, 0.25 μg/kg pegylated IFN-alpha-2b is equivalent to 9 MIU IFN-alpha-2b per wk (3 MIU, 3/wk). Based on phase I clinical studies in cancer patients, the recommended dose of pegylated IFN-alpha-2b for phase II/III is 6 μg/kg/wk. This dose is much higher than that achieved by IFN-alpha-2b. However, the toxicity of pegylated IFN-alpha-2b is very similar to that of IFN-alpha-2b.

3. Ipilimumab (Table 1)

The cytotoxic T-lymphocyte antigen-4 (CTLA4) protein belongs to the so-called immune checkpoint molecules and plays an important role in T-cell-mediated immune responses. CTLA4 is expressed on both activated CD4 and CD8 T cells, more prominently on CD4 T cells. CTLA4 binds to the same ligands on antigen-presenting cells as CD28 does, being CD80/CD86. However, both the spatial and timely expression of CTLA4 is different from CD28. CD28 is expressed on naive T lymphocytes and triggering of CD28 by CD80/CD86 delivers the so-called second signal next to T-cell receptor engagement to fully activate the naive T cell. CTLA4, which is partly stored in intracellular vesicles, is expressed 24–36 hrs later and its expression on the cell surface forms a lattice-like grid bound to CD80/CD86 at the immunological synapse that is formed during T-cell activation, leaving little space for CD28 expression. In addition, the affinity of CTLA4 for its ligands CD80/CD86 on the antigen-presenting cell is about 10-fold higher than that of CD28; therefore CTLA4 outcompetes CD28 for binding to its ligands. Triggering of CTLA4 through binding to CD80/CD86 leads to an inhibitory signal, resulting in cell-cycle arrest in G1, and downregulation of IL-2 and IL-2R expression. Mice deficient in CTLA4 die within 3–4 weeks after birth due to a severe lymphoproliferative disease, indicating that the immune response against pathogens is uncontrolled.

In a B16 melanoma model, in which both vaccination with granulocyte–macrophage colony-stimulating factor-secreting inactivated tumor cells and anti-CTLA4 antibody treatment resulted in tumor control, autoimmune vitiligo developed. This indicated that the treatment of anti-CTLA4 could also break tolerance to self-antigens. Ipilimumab is the first fully human monoclonal IgG1κ (kappa) antibody, which presents high affinity for CTLA4 and efficiently blocks CTLA4 binding to its ligands CD80/CD86. In a dose escalating phase I study, ipilimumab was shown to be clinically active at doses of ≥3 mg/kg. Ipilimumab has a $T_{1/2}$ of about 15 days and its metabolism is independent from cytochrome P450 enzymes. Ipilimumab increases absolute lymphocyte counts (ALC) in a dose-dependent manner.

In several phase II studies, ipilimumab as single-agent treatment at 3–10 mg/kg has shown objective response rates in pretreated advanced stage melanoma in around 10% of patients, with long-term remission observed. Toxicity was most often immune related and consisted of colitis, dermatitis, uveitis, endocrinopathies (thyroiditis, hypophysitis), hepatitis, and, more rarely, Guillain–Barré syndrome, myasthenia gravis, meningitis, nephritis, sarcoidosis, and myocarditis. Toxicity has been reported in about 50–70% of patients treated with 3 mg/kg or 10 mg/kg (10% grade 3 or more). Toxic deaths (≤1% of patients) have been reported mostly in relation to severe colitis or intestinal perforation.

Activity of ipilimumab has been observed in melanoma, prostate cancer, RCC, and non-small cell lung cancer. In advanced stage melanoma, ipilimumab has been studied in two randomized controlled phase III studies. Study MDX-10-020 was a placebo-controlled, second-line study performed in HLA-A*0201-positive metastatic melanoma patients. In this study 676 patients were randomized in a 3:1:1 fashion to ipilimumab given at a dose of 3 mg/kg, for a total of four 3-weekly doses + gp100 peptide vaccine, ipilimumab alone, or gp100 peptide vaccine alone. Both ipilimumab treatment arms showed a statistically significant improvement in overall survival over the gp100 peptide vaccine arm (10.0 months and 10.1 months versus 6.4 months). Survival rates at 1 year were 44% and 45% versus 24% and at 2 years were 22% and 24% versus 15%. Based on the MDX-10-020 study, ipilimumab was approved by the FDA and EMA for the treatment of advanced stage (irresectable stage IIIc or stage IV) melanoma at a dose of 3 mg/kg for a total of 4 doses at 3-week intervals. In Europe, ipilimumab is approved for second-line treatment only.

Further Reading

Beck KE, Blansfield JA, Tran KQ, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol 2006; 24:2283–2289.

Brunet JF, Denizot F, Luciani MF, et al. A new member of the immunoglobulin superfamily - CTLA-4. Nature 1987; 328:267–270.

Bukowski RM, Tendler C, Cutler D, et al. Treating cancer with PEG Intron: pharmacokinetic profile and dosing guidelines for an improved interferon-alpha-2b formulation. Cancer 2002; 95:389–395.
Hervas-Stubbs S, Perez-Gracia JL, Rouzaut A, et al. Direct effects of type I interferons on cells of the immune system. Clin Canc Res 2011; 17:2619–2627.
Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711–723.
Isaacs A, Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci 1957; 147:258–267.
Maker AV, Phan GQ, Attia P, et al. Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. Ann Surg Oncol 2005; 12:1005–1016.
Morgan DA, Ruscetti FW, Gallo R. Selective in vitro growth of T lymphocytes from normal human bone marrows. Science 1976; 193:1007–1008.
Weber J. Review: anti-CTLA-4 antibody ipilimumab: case studies of clinical response and immune-related adverse events. Oncologist 2007; 12:864–872.
Weber JS, O'Day S, Urba W, et al. Phase I/II study of ipilimumab for patients with metastatic melanoma. J Clin Oncol 2008; 26:5950–5956.
Winkelhake JL, Gauny SS. Human recombinant interleukin-2 as experimental therapeutic. Pharmacol Rev 1990; 42:1–28.

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