Functional differentiation of cytotoxic cancer drugs and targeted cancer therapeutics

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A B S T R A C T

There is no nationally or internationally binding definition of the term “cytotoxic drug” although this
term is used in a variety of regulations for pharmaceutical development and manufacturing of drugs
as well as in regulations for protecting medical personnel from occupational exposure in pharmacy, hos-
pital, and other healthcare settings. The term “cytotoxic drug” is frequently used as a synonym for any
and all oncology or antineoplastic drugs. Pharmaceutical companies generate and receive requests for
assessments of the potential hazards of drugs regularly – including cytotoxicity. This publication is
intended to provide functional definitions that help to differentiate between generically-cytotoxic cancer
drugs of significant risk to normal human tissues, and targeted cancer therapeutics that pose much lesser
risks. Together with specific assessments, it provides comprehensible guidance on how to assess the rel-
vant properties of cancer drugs, and how targeted therapeutics discriminate between cancer and normal
cells. The position of several regulatory agencies in the long-term is clearly to regulate all drugs regard-
less of classification, according to scientific risk based data. Despite ongoing discussions on how to
replace the term “cytotoxic drugs” in current regulations, it is expected that its use will continue for
the near future.

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1. Introduction

There is no nationally or internationally binding definition of the term “cytotoxic drug”. Understanding of cytotoxicity as a prop-
erty of oncology drugs has evolved with increased appreciation of the molecular mechanism of generally toxic nitrogen mustard
beginning in the 1940s. Later development of therapies based on administration of mono- and bifunctional alkylating agents, natu-
ral Vinca alkaloids and anthracyclines took advantage of the prefer-
enial susceptibility of cancerous tissues to the toxic effects of agents that impaired cellular replication. Such selective suscepti-
bility was based on the high rate of cell division and poorly-
regulated growth in malignant tumors. Therapeutic use of these agents in oncology depends on careful selection of dose and regi-
men since selectivity is a relative concept considering the adverse
effect profiles of many chemotherapeutics. By the end of the
century, so-called targeted cancer therapy with reduced side
effects was made possible by advances such as specific monoclonal
antibodies that bound to unique epitopes on the surface of cancer-
ous cells and by small molecules such as selective tyrosine kinase
inhibitors that affected specific molecular pathways up-regulated
in certain cancers (Gottesman, 2002). More recently, targeted
monoclonal antibodies linked to a variety of microtubule-active
compounds (e.g. auristatins and maytansinoids) (ADC antibody
drug conjugates) have been developed conferring specificity for
cancer cells to non-selective anti-mitotic drugs (Chari, 2008).

Unfortunately, by common use, the term “cytotoxic drug” is
frequently used as a synonym for any and all oncology or antineo-
plastic drugs. It is formally a part of many regulations for pharma-
caceutical development and manufacturing of oncology drugs (ICH,
2000; ANVISA, 2010; WHO, 2010; EMA, 2012a,b). On the other
hand, the pharmaceutical manufacturers and regulatory agencies
are moving to clearly regulate all drugs based on scientific data
and risk assessment and not based on terms lacking a specific def-
inition. Respective guidances have been published (ISPE, 2010;
Bercu et al., 2013). In a draft of the European Medicine Agency
guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities (EMA, 2012a), reference is made to establishing safe exposure values based on scientific data. Oncology hospitals, pharmacies and caregiver organizations often have their own regulations (e.g. OSHA, 1999; NIOSH, 2004; ASHP, 2006; Green et al., 2009; HSE, 2003; HSE/MCA, 2003; Ziegler et al., 2013) for administering oncology drugs, designed to protect personnel from occupational exposure and safely dispose of contaminated waste. Adequate interpretation of monitoring results of exposed hospital personnel should take into account the actual hazard of oncology drugs dispensed. Finally, cytotoxic drugs are also a category of special concern mentioned in the recent publication for pharmaceutical quality and safety, especially as it concerns potentially genotoxic contaminants in medicinal products (ISPE, 2010; Bercu et al., 2013).

Pharmaceutical companies frequently receive requests from internal (good manufacturing practices [GMP] quality assurance; local production sites, medical safety departments and country organizations) and external (health authorities, contract manufacturers, hospitals) organizations for assessing the hazards of a variety of products – and a number of these concern whether a pharmaceutical agent could be defined as cytotoxic cancer drug for research, manufacturing, or other uses. The background is usually concern for determining and managing risk either for potential occupational exposure of employees in manufacturing, exposure of hospital and nursing staff or compliance with GMP guidance. Lack- ing a well-recognized and standard definition of “cytotoxic drug” makes very difficult the tasks of providing consistent advice and ensuring easily understood communications. The terms “non-specific” or “non-selective” cancer drugs have been used to describe generically-cytotoxic anticancer drugs in previous publications (Blagosklonny, 2004; Broxterman and Georgopapadakou, 2004). However, the authors also discussed the fact that “non-specific” or “non-selective” cytotoxic cancer drug drugs focus on targets such as DNA, microtubules or histone deacetylases, selectively. Specifically, these targets are not unique to cancer cells, but are also part of normally replicating cells. We have chosen to use the term “cytotoxic cancer drugs” in this manuscript for the sake of clarity. Other agents used in oncology form part of a broad group of “targeted cancer therapies”.

The purpose of this publication is to provide functional definitions that discriminate between cytotoxic cancer drugs and targeted cancer therapeutics for the purpose of guiding safe handling practice and some product quality decisions. The definition is used in Novartis and Patheon and in a similar format in other companies contacted.

Together with example assessments, the publication may be used to differentiate cytotoxic cancer drugs and targeted cancer therapeutic in a consistent way. The publication is intended to provide a comprehensible guidance for those involved in answering specific requests for relevant safety information from internal and external organizations.

2. Methods

Database searches were initiated in Embase, Medline and Biosis (OvidSP provided by Wolters Kluwer, Alphen aan den Rijn NL), by combinations of the keywords “cytotox*”, “oncology”, “regulation”, “definition” “properties”, “histopathology” and “mitosis” covering the span 1996–2013. Resulting hits were reviewed and integrated into this publication.

Besides the authors’ own experience with addressing requests concerning drug hazards, we contacted a number of pharmaceutical companies and contract manufacturers and conducted a survey on how similar requests regarding the cytotoxic properties of drugs are handled.

Additional data base searches in Embase, Medline and Biosis (OvidSP provided by Wolters Kluwer, Alphen aan den Rijn NL) were initiated combining the individual “drug name” with “pharmacology” and “mode of action” of select targeted cancer drugs. Resulting hits were reviewed and integrated into the individual assessments.

3. Results

3.1. Functional differentiation of cytotoxic cancer drugs

From database searches, a single scientific definition of the term “cytotoxicity” was retrieved: (OECD, 2010): “The adverse effects resulting from interference with structures and/or processes essential for cell survival, proliferation, and/or function. For most chemicals/substances, toxicity is a consequence of non-specific alterations in “basal cell functions” (i.e. via mitochondria, plasma membrane integrity, etc.), which may then lead to effects on organ-specific functions and/or death of the organism. These effects may involve the integrity of membranes and the cytoskeleton, cellular metabolism, the synthesis and degradation or release of cellular constituents or products, ion regulation, and cell division.” This OECD monograph is intended as guidance on using cytotoxicity tests to estimate starting doses of chemicals/substances in acute oral systemic toxicity tests in rodents. Although the term cytotoxic is mentioned in several other regulations, no definition is provided. A number of internet sites provide practical descriptions of cytotoxic cancer drugs.

Based on our collective experience and review of the literature, we have compiled an alternate definition of cytotoxic cancer drugs for the purposes of assessing safety risks: a therapeutic agent, whose primary activity is to indiscriminately and directly kill both healthy and cancerous cells in an effort to control the spread of cancer in the human body is considered to be cytotoxic if:

- the mechanism of action is to directly disrupt DNA structure or mitotic function (e.g., intercalation, clastogenicity, spindle destruction) causing cell death; and
- the above mechanism of action does not selectively target tumor cells or differentiate in susceptibility between tumor and non-tumor cells; and
- results of cell culture assays, genotoxicity and experimental animal studies or human clinical studies demonstrate that the drug’s toxicity is not specific to nor displays substantially different susceptiblity to tumor cells in comparison to non-tumor cells in living tissue.

To meet the definition, all three elements have to be present. Cytotoxic cancer drugs are usually of high acute toxicity. In preclinical studies, corroborative evidence can be provided by histopathology. Tubulin-binding cytotoxic cancer drugs such as maytansine, colchicine, DM1 and others are known to cause specific radiomimetic lesions indicative of cytotoxicity in numerous target organs. Lesions consist of mitotic arrest (aberrant mitoses) or apoptosis, which can be seen histologically (Melgoza et al., 2008; Barok et al., 2011; Poon et al., 2013).

Primary toxicity effects in rats between DM1 and maytansine are comparable. Aberrant mitotic figures in target organs e.g. liver have been described at intravenous doses of 1400–1600 µg DM1/m² in rats. This corresponds to similar DM1 plasma concentration achieved with therapeutic concentrations of 3.6 mg/kg trastuzumab-DM1 (corresponding to about 2300 µg DM1/m²) in clinical trials (Poon et al., 2013). Intravenous therapeutic maytansine
dose levels in clinical trials achieved dose-limiting toxic reactions at 750–1000 μg maytansine/m². Arrest of mitoses in metaphase stage (aberrant mitoses) 24 h after the injection was detected in bowel biopsy specimens (Franklin et al., 1980).

At therapeutic dose levels, similar radiomimetic lesions have also been observed in gallbladder epithelium biopsies after treatment with docetaxel in a human case study (Melgoza et al., 2008). In gastrointestinal biopsies of colchicine treated patients, abundant metaphase mitoses and apoptosis were present histologically (Iacobuzio-Donahue et al., 2001).

Thus, histopathological examination of target organ tissues in preclinical toxicity studies for the presence of specific radiomimetic lesions contributes relevant evidence for cytotoxicity. Comparison of exposure levels between preclinical animal species and human patients or, if exposure levels are not available, dose translation from animal to human studies, indicates a very narrow safety margin for dose-limiting toxic reactions (Reagan-Shaw et al., 2007) (see Fig. 1).

Examples of cytotoxic cancer drugs include DNA alkylating agents, topoisomerase inhibitors, antimetabolites, and microtubule-active agents, all of which affect cell survival, division or DNA synthesis in normal as well as tumor cells, their relative selectivity being dependent upon the rate of cell division rather than neoplastic state. In a number of publications this concept is discussed (Blagosklonny, 2004; Broxterman and Georgopapadakou, 2004). In addition, it is pointed out that cytotoxic cancer drugs may be very specific to certain cellular targets (e.g., DNA, tubulin) present in cancer as well as in normal cells.

From an active pharmaceutical ingredient (API) standpoint, in ICH Topic Q7 reflecting GMP for APIs (valid in three main regions (Europe, USA, and Japan)), which is also incorporated in the EU GMP guide as Part II (Section 4.4), it is stated that dedicated production areas should be considered when material of high pharmaceutical activity or toxicity is involved (e.g., certain cytotoxic cancer drugs) unless validated inactivation and/or cleaning procedures are established and maintained (ICH, 2000; Eudralex Volume 4 Part II, 2014). From a Finished Product stand point, in the current EU GMP Guide, Chapters 3 (Section 3.6) and 5 (Section 5.18), reference is made to the fact that “certain” cytotoxic cancer drugs should be manufactured in dedicated and self-contained facilities (Eudralex Volume 4 Part I Chapter 3, 2014; Eudralex Volume 4 Part I Chapter 5, 2014). Although in the draft revisions of these chapters, reference to such a product category is no longer visible, EU Member States may follow their own governance and still mention cytotoxics, for example Italy (Italian National Decree, 2007). Similar statements for cytotoxics can also be found in the GMP regulations for other countries, such as Canada, the US, and Brazil (Health Canada, 2013; Q7A, 2001; ANVISA, 2010). In addition, the World Health Organisation (WHO) GMP for pharmaceutical products containing hazardous substances from 2010, refers to certain cytotoxic anti-cancer agents in the Containment Section 4.41 (WHO, 2010). The overall industry trend observed to date is a risk-based approach combining GMP and toxicological parameters. For the sake of clarity, a cytotoxic cancer drug as proposed here can be defined as “a therapeutic agent, whose primary activity is to indiscriminately and directly kill both healthy and cancerous cells in an effort to control the spread of cancer in the human body”. This would include cancer drugs that kill cancerous cells via direct interaction with DNA or DNA-maintenance processes at doses which also kill healthy cells. In the spirit of the current GMP regulations in regards to “certain” cytotoxic cancer drugs, these drugs would not be acceptable for manufacturing in multi-product facilities. Targeted cancer therapeutics (i.e., non-cytotoxic drugs) do not meet this definition as these do not directly interact with DNA or DNA-maintenance processes, and act on targets that suggest a preferential or selective action on cancer cells as compared to healthy cells. In the spirit of the current GMP regulations, these drugs would be acceptable for manufacturing in multi-product facilities.

Each individual cancer agent is to be reviewed on a case-by-case basis with a high degree of professional judgment by individuals trained and experienced in risk assessment and knowledgeable about the biological and pharmaceutical properties of the substance considered.

It is important to distinguish that in the context of drug development certain in vitro cytotoxicity assays (for example focused on bone marrow progenitor cells) are used to screen drug candidates. In some considerations, the results of these in vitro cytotoxicity assays may be the focus questioning about how a drug should be identified as cytotoxic. For the purposes of this discussion, such results are considered relevant to determining whether a drug meets the basic definition of “cytotoxic” only if the mechanism of action suggests interference with DNA replication.

Finally, it should also be mentioned that a cell based impedance assay has been developed to differentiate cytostatic from cytotoxic effects in vitro by applying impedance-based real-time cell analysis and creating an algorithm to distinguish cytotoxic, cytostatic and non-toxic agents by the shape of the impedance curve (Kustermann et al., 2013). It appears to be a further development of the 3T3 NRU (neutral red uptake) assay with additional endpoints. The original assay was developed for assessment of in vitro cytotoxicity in the context of replacing rodent studies for acute toxicity (OECD, 2010) and for in vitro phototoxicity testing (Lasarow et al., 1992). Results from such assays could be considered in the assessment.

In the following section potentially cytotoxic cancer drugs will be compared individually against these criteria to determine whether these agents are properly defined as of concern as cytotoxic agents. In the following examples such considerations are discussed in context with relevant publications. Other examples of cytotoxic cancer drugs have been discussed previously by Blagosklonny (2004).
4. Examples of cytotoxic cancer drugs

4.1. Maytansinoids

Maytansine, like the Vinca alkaloids vincristine and vinblastine, possesses metaphase arrest antimitotic properties (Issell and Crooke, 1978). Flow microfluorimetry analysis of L1210 cells during exposure to maytansine indicated a shift in the distribution of DNA to a single peak, representing the DNA of cells in G2 and M phases (Wolpert-Defilippes et al., 1975), making mitotic and G2 cells most sensitive to maytansine cytotoxicity, while G1 phase cells are the most resistant, with S-phase cells being intermediate (Rao et al., 1979). Experiments with sea urchin eggs and clam eggs have suggested that it causes the disappearance of a mitotic apparatus or prevents one from forming if added at early stages. Maytansine does not affect formation of the mitotic organizing center but does inhibit in vitro polymerization of tubulin (Remillard et al., 1975).

In chronic studies in dogs and monkeys, maytansine produced target organ toxicity in the pancreas, esophagus, stomach, small and large intestine, adrenal cortex, kidney, bladder, liver and skin while the main dose limiting toxicities in human studies relate to effects on the gastrointestinal tract and nervous system (Issell and Crooke, 1978). An Ames assay with N2-deacetyl-N2-(3-mercapto-1-oxopropl) maytansine (DM1) showed negative results – consistent with targeted activity to the mechanism of mammalian DNA replication. This substance showed a positive result in the in vivo rat bone marrow micronucleus assay (FDA, 2013), which is in accordance with the mechanism of action which targets the mitotic apparatus and not the DNA.

Maytansine and its derivatives meet the functional definition of cytotoxic cancer drugs, because they target rapidly dividing cells in a specific mitosis phase and do not discriminate between cancer and healthy cells.

4.2. Topoisomerase Inhibitors

Topoisomerases are highly conserved enzymes essential for survival of all eukaryotic organisms and present in normal and cancer cells. Topoisomerase enzymes are categorized as topoisomerase I and II and both forms of the enzyme are validated targets for treatment of a variety of cancers. The mode of action of topoisomerases directly affects DNA replication, chromosomal condensation and chromosomal segregation (Hande, 2008).

The topoisomerase I enzyme acts to relax supercoiled DNA by inducing and then ligating single strand breaks (Binaschi et al., 1996). Inhibition of topoisomerase I by oncology agents such as irinotecan and camptothecin stabilizes DNA strands following initial scission required for replication, thereby fixing lethal single strand DNA breaks. Such breaks are detected as genotoxic and mutagenic events when prototypical topoisomerase I inhibitors are assessed in eukaryotic cell assays (Hashimoto et al., 1995). Clinical treatment with topoisomerase I inhibitors is associated with a constellation of adverse effects, including especially neutropenia and other hematological effects, indicative of effects on non-cancerous tissues. Several cancer drugs (and antibiotics), e.g., etoposide, ansamycin, act through topoisomerase II inhibition by inducing DNA breaks and apoptosis (Seiter, 2005). Topoisomerase II inhibitors are genotoxic in standard in vitro and in vivo studies. However, mutagenicity is usually restricted to eukaryotic cells (Binaschi et al., 1996; Boos and Stopper, 2000; Albanese and Watkins, 1985).

Toxicity of topoisomerase II inhibitors includes myelosuppression and gastro-intestinal disorders in the short-term. Cardiac toxicity and secondary leukemia have been seen in the long-term. Toxicity of topoisomerase II inhibitors indicates that topoisomerase II inhibitors do not discriminate between normal and cancer cells (Seiter, 2005).

Both topoisomerase I and II inhibitors meet our functional differentiation of cytotoxic cancer drugs since the mechanism of action does not selectively target tumor cells or differentiate in susceptibility between tumor and normal cells.

5. Examples of targeted cancer therapeutics

5.1. Selective tyrosine kinase inhibitors (TKIs)

TKIs do not meet our functional definition of cytotoxic cancer drugs due to their differential potency in normal vs. cancer cells. Nilotinib was not genotoxic in a standard battery of in vitro and in vivo studies (EMA, 2007). With imatinib, a positive effect was seen at the highest cytotoxic concentration and in the presence of rat liver S9 only in the in vitro chromosomal aberrations assay. Thorough in vitro and in vivo genotoxicity testing showed that imatinib is not genotoxic under the conditions of therapeutic use (EMA, 2004).

Imatinib and nilotinib potently inhibit the cytosolic ABL1 tyrosine kinase activity of Bcr-Abl (fusion oncogene), and to a lesser extent the tyrosine kinase activity associated with the platelet-derived growth factor receptor (PDGFR) and the stem cell factor receptor (KIT) (Broxterman and Georgopapadakou, 2004; Kantarjian et al., 2007). Bcr-Abl, PDGFR and KIT kinases are expressed in many normal human cell lines, but these enzymes are highly regulated and only active under certain stressed physiological conditions during which their receptors are stimulated by their respective ligands. Through such auto-regulatory mechanisms, as well as alternative signaling pathways, normal cells are not normally dependent upon the activity of a single kinase for survival. In contrast, a number of cancers require the continuous activity of a single oncogene for cell survival (‘oncogene addiction’), as is the case of ABL1 (via the chimeric BCR-ABL1 protein) in chronic myelogenous leukemia (CML) and for KIT (via mutations such as that leading to the K642E mutant) in the case of GIST (Garraway et al., 2005).

The comparative activities of imatinib and nilotinib in such cells are shown in Table 2 of Manley et al. (2010a). Although, imatinib and nilotinib will also inhibit these kinases to some extent under normal physiological conditions, when administered at therapeutic doses, this does not affect the viability of normal cells. Both imatinib and nilotinib have some, lesser effect on the PDGF and c-Kit kinases in normal cells, but their potency in this regard is much less than that against Bcr-Abl. The extended kinase profile of nilotinib has been analysed and published recently (Manley et al., 2010b). To confirm that the anti-proliferative effects were the direct result of kinase inhibition and not general cytotoxicity, imatinib and nilotinib were evaluated for their effects on parental Ba/F3 cells, cultured in the presence of interleukin-3; neither drug showed appreciable activity in this setting (Manley et al., 2010b).

Recently, a comprehensive review and comparison of the relative pharmacological properties of imatinib and nilotinib has been published (Manley et al., 2010a). Quantitative selectivity data for imatinib and 38 other kinase inhibitors have been presented in Karaman et al. (2008). In a mouse model of tumour growth with D27-expressing Ba/F3 cells, the authors examined the effect of twice daily, orally administered masitinib at 100 mg/kg, and found that tumour growth was blocked following 5 days of treatment. Masitinib significantly enhanced survival with no indication of general toxicity (Dubreuil et al., 2009).

In another study, a phosphatidylinositol 3-kinase inhibitor, BENC-511 displayed therapeutic efficacy in a PC3-derived prostate...
cancer model in nude mice. At an oral dose of 50 mg/kg, BENC-511 decreased tumor growth more than 50% in 27 days; however did not show overt toxicity (Shi et al., 2014).

TKIs meet our functional definition of targeted cancer therapeutics, due to their selective tyrosine kinase inhibition in cancers and due to auto-regulatory mechanisms, as well as alternative, compensatory signaling pathways available in normal cells.

5.2. Heat Shock Protein 90 inhibitors (HSP90)

Hsp90 inhibitors are non-genotoxic agents (Janz et al., 2007). HSP90 is an abundant protein, constituting approximately 1–2% of total protein in normal cells (Solit and Chiosis, 2008). When associated with its co-chaperones, HSP90, which has an ATP binding site at the N-terminal domain, exerts its folding activity via its ATPase activity (Kamal et al., 2003). Inhibition of ATPase activity leads to client protein misfolding, ubiquitination and proteasomal degradation. HSP90 is essential for eukaryotic cell survival. In many tumors, it is overexpressed and present in a complex “activated state” with its co-chaperones which has increased affinity to Hsp90 inhibitors (Kamal et al., 2003).

Small molecular weight HSP90 inhibitors competitively inhibit the ATPase activity of HSP90 resulting in degradation of client proteins. This translates into anti-tumor effects in non-clinical in vitro and in vivo studies. HSP90 inhibitors exhibit preferential cytotoxicity to cancer cells due to this enhanced susceptibility.

Although HSP90 is highly expressed in most cells, HSP90 inhibitors selectively kill cancer cells compared to normal cells. This has been attributed in part to selective accumulation of HSP90 inhibitors in cancer cells and to a 100-fold higher binding affinity of HSP90 inhibitors to cancer derived HSP90 as compared to HSP90 from non-transformed cells (Kamal et al., 2003; Solit and Chiosis, 2008).

HSP90 inhibitors meet our functional definition of targeted cancer therapeutics due to their discrimination between cancer and normal cells.

5.3. Inhibitors of Apoptosis Protein antagonists (IAPs)

Apoptosis is a physiological program for cell death, which is essential for maintenance of homeostasis. Cancer cells, but not normal cells, highly depend on aberrations in the apoptosis signaling pathway to remain viable. Drugs that can restore apoptosis in cancer cells might be effective in cancer treatment (Flygare et al., 2012).

Some IAP family members (e.g. XIAP, CIAP1, CIAP2) suppress apoptosis (and many other cellular processes), and thereby provide a mechanism for rescuing abnormal cells that would otherwise be destroyed. Many types of human cancer cells exhibit defects in apoptotic pathways and are dependent upon XIAP function for survival. Tumors of this type overexpress IAPs that enable growth and survival. Inactivation of IAPs does not appear detrimental for normal cells. Small molecule IAPs antagonists are potent cancer drugs in vitro and in vivo (Gyrd-Hansen and Meier, 2010). IAPs antagonists lead to decreased viability of breast cancer cells without affecting normal mammary epithelial cells (Flygare et al., 2012).

IAP antagonists thus discriminate between cancer and normal cells. By blocking IAPs, IAP antagonists selectively force cancer cells into apoptosis.

Since cancer cells as well as normal cells produce TNF-α (tumor necrosis factor alpha) when exposed to IAP antagonists, it is expected that the drug triggers both, efficacy and toxicity. In intravenous, repeated dose toxicity studies in rats and dogs with an IAP antagonist, dose related, acute systemic inflammation and hepatic injury was noted. Dogs were affected more severely by the findings attributed to the pharmacology (TNF-α-mediated toxicity) of the IAP antagonist. At equivalent doses, humans did not exhibit these findings (Erickson et al., 2013).

IAP antagonists do not meet our functional definition of cytotoxic cancer drugs. IAP antagonists meet our functional definition of targeted cancer therapeutics due their discrimination between cancer and normal cells.

5.4. Proteosome and histone deacetylase inhibitors

To reinforce the need for a cytotoxicity definition, some mechanisms of action and cancer drugs may be more complex than others to review. For example, proteasome and histone deacetylase inhibitors both inhibit ubiquitous cellular targets; however recent pre-clinical studies have demonstrated that malignant cells are more susceptible to the cytotoxic effects of proteasome inhibition or histone deacetylase inhibitors than normal cells (Crawford et al., 2011; Bolden et al., 2013).

The proteasome is a multicatalytic protein complex that causes turnover of cytosolic and nuclear proteins. Proteasome inhibitors do not directly disrupt DNA or mitotic function, and are reported to exhibit selective cytotoxicity to cancer cells over normal cells, by preferentially inducing apoptosis in proliferating or transformed cells, or by overcoming deficiencies in growth-inhibitory or proapoptotic molecules (Almond and Cohen, 2002). Carfilzomib is an example of a proteasome inhibitor that when administered at a dose that selectively inhibits chymotrypsin-like subunits in the proteosome, induced an antitumor response in multiple myeloma, non-Hodgkin lymphoma, and leukemia cells, with minimal effects in non-transformed cells (Parlati et al., 2009).

Histone deacetyleases are enzymes that catalyze the removal of the acetyl modification on lysine residues of proteins (including core nucleosomal histones). The deacetylation of histones in nucleosomes is an important factor regulating gene expression. Deacetylation of histones by histone deacetylase causes DNA to be tightly wrapped around the histone core resulting in the inhibition of gene expression. The inhibition of histone deacetylase promotes an increase in histone acetylation, causing the tightly wrapped DNA to relax. This leads to the expression of certain genes (tumour suppressor and/or cell cycle regulatory genes) which causes the inhibition of tumor growth (Richon and O’Brien, 2002). Vorinostat is an example of a histone deacetylase inhibitor that induced apoptosis in a tumor-cell-selective manner. Matched normal (BJ) and transformed (BJ LTSTERas) fibroblasts were treated with vorinostat, and following 24 h of treatment, there was a marginal increase in death of transformed BJ LTSTERas fibroblasts that increased substantially following extended drug exposure. This study found that BJ LTSTERas fibroblasts were significantly more sensitive to vorinostat than BJ cells. Pro-death gene expression changes may be important for the tumor-selective effects of histone deacetylase inhibitors (Bolden et al., 2013).

Proteasome and histone deacetylase inhibitors do not meet our functional definition of cytotoxic cancer drugs, but meet our functional definition of targeted cancer therapeutics due their discrimination between cancer and normal cells.

5.5. Functional differentiation of targeted therapeutics

For drugs that are early in development (prior to preclinical/clinical studies), the cytotoxicity evaluation will be based primarily on the mechanism of action. If new data become available that suggests the drug is not specific to cancer cells, a re-evaluation will be conducted and the cytotoxicity evaluation may change.

The mechanism for cancer selectivity may also be based on preferential exposure of cancer cells to the therapeutic agent, such as cell surface binding sites, transport mechanisms, tissue-specific activation or inactivation (metabolism) and subsequent degrada-
tion of cellular targets. A strategy to “target” cancer cells based solely on the rapid rate of cellular proliferation is not sufficient to obviate definition as cytotoxic. There are many normal tissues that contain cells that proliferate at a rate similar to, or exceeding that of some tumor cells, e.g. bone marrow and gastrointestinal epithelium. Hair loss as well as toxic effects on the reproductive organs, thymus, spleen and liver are known side effects associated with a lack of specificity for cancer cells. Cytotoxic cancer drugs cause adverse effects on cancer cells and healthy cells at similar doses. Although some targeted cancer therapeutics, such as kinase inhibitors, may cause these effects, studies have shown that the doses required to cause adverse effects on tumor cells would be lower than those on healthy cells (Dubreuil et al., 2009; Shi et al., 2014). The dose required to cause these effects should be taken into account, and a clear safety margin in dose between cytotoxic or growth inhibitory effects on cancer cells and normal, rapidly dividing cells must be observed in order to ascertain a conclusion of targeted cancer therapeutic. In the majority of preclinical studies in which animals do not have tumors, one may take into account the therapeutic dose and determine whether or not it is in the range of animal toxicity, using human equivalent dose conversions. In the event that the margin of safety is less than one, it will suggest that the toxicity occurs at therapeutic doses. The margin of safety would be greater for targeted cancer therapeutics. Nonetheless, although adverse effects on healthy cells may be observed in animal studies if dosed high enough, kinase inhibitors do not meet the first criteria of our functional definition of cytotoxic cancer drugs; namely, these do not directly disrupt DNA structure or mitotic function causing cell death.

Drugs which are potentially cytotoxic, specifically target cancer cells or are used in support of cancer therapies are compared individually against these criteria to determine handling requirements. In a number of examples below, such considerations are discussed in context with relevant publications. Other examples of targeted therapeutics have been discussed previously by Broxterman and Georgopapadakou (2004) and Blagosklonny (2004).

6. Discussion

The central purpose of this presentation is to bring increased focus and clarity in the use of the term “cytotoxic drug”. The definitions and descriptors of cytotoxic cancer drugs and targeted cancer therapeutics in this presentation provide guidance and facilitate a consistent classification of a variety of active agents used in cancer treatment. From the examples provided, it can be taken that there are abundant published references describing mechanism of action and the spectrum of therapeutic and adverse effects for already registered drugs. Drugs in development such as IAP antagonists are more difficult to assess due to a lack of safety related publications. For this purpose, (internal) pharmacology studies and publications as well as scientific judgment are required for assessing, how targeted therapeutics discriminate between cancer and normal cells. Usually, a first cytotoxicity assessment is requested for manufacturing and physicians, once manufacturing is scaled up and clinical trials are initiated. The authors have several years of experience in using the means of assessment presented here. Similar assessments have received positive feed-back and acceptance from internal and external organizations.

Faced with a large number of regulations in drug manufacturing and occupational health, pharmaceutical companies receive requests for cytotoxicity assessments regularly and for different purposes. GMP manufacturing of cytotoxic cancer drugs may require the use of segregated or self-contained facilities (ANVISA, 2010) or dedicated production areas (ICH, 2000 Q7). This substantially increases production costs for containment and cleaning validation. It appears unlikely that requests for statements which categorize drugs as possibly cytotoxicity will be reduced significantly in the near future.

In a draft of the European Medicine Agency guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities (EMA, 2012a), reference is made to establishing safe exposure values based on scientific data. The EU GMP Guide Chapter 3 and 5 ongoing draft revisions refer to Quality Risk Management principles, including a toxicological evaluation of the products being manufactured, and cross refer to the above toxicity guideline (EMA, 2012b). It is indicated that the outcome of the Quality Risk Management process should be the basis for determining whether equipment and facilities require dedication to a specific product or product family.

The position of EMA and other regulatory agencies in the long-term is clearly to regulate all drugs based on scientific data. Risk assessment for drugs with a thresholded mode of action or toxicity should be handled by calculating respective limits. Such scientific assessments would then be the basis for establishing safe limits for shared facilities. Despite ongoing discussions on replacing “cytotoxic drugs” by a risk based scientific approach and text in regulations and elsewhere, the global use of the label cytotoxic is not expected to disappear any time soon. There has been a long history of the regulatory use of the term “cytotoxic” without any clear definition, which has been a struggle for those impacted. In context with recently developed targeted monoclonal antibodies linked to a variety of microtubule-active antibody drug conjugates (ADC), such considerations have received renewed attention. GMP manufacturing of such combination drugs faces challenges regarding segregated, dedicated or self-contained facilities and related technical investments. In particular, the percentage of free microtubule-active compounds may be of concern, but cytotoxic properties of the ADC itself have to be assessed as well.

In the revised ICH S9 guideline (ICH, 2010) for nonclinical development of oncology drugs, the term “cytotoxic drugs” has been replaced by “genotoxic drugs targeting rapidly dividing cells”. In clinical development, however, the scheduled phases are different for cytotoxic cancer drugs (Kummar et al., 2006) and for molecularly targeted agents (Kummar et al., 2007; Li et al., 2012). In the same publication, the term “cytostatic agent” is defined as a drug, which “inhibits tumor growth without direct cytotoxicity” (Kummar et al., 2006).

In conclusion, the present paper provides definitions and examples for assessing properties of cytotoxic cancer drugs and targeted cancer therapeutics. In the short-term, the “cytotoxic drug” designation will still be used in a variety of regulations for pharmaceutical development and manufacturing of drugs. For this, a consistently applied functional definition is provided in this paper. It is consistent with the spirit of the regulations and should prove helpful for those involved in answering specific requests from internal and external organizations.

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References


