Is Cell Death a Critical End Point for Anticancer Therapies or Is Cytostasis Sufficient?

Olivier Rixe and Tito Fojo

Abstract
Since the discovery of conventional chemotherapy and the development of new target-based agents, the importance of cytostasis in anticancer activity has been debated. This review examines the relative importance of both cytostasis and cytotoxicity based on both preclinical data and clinical reports. Several limitations of our basic and clinical methods to evaluate cytostasis and cytotoxicity will be highlighted. Molecular mechanisms of cytostasis will be analyzed, including interference with the cell cycle as well as putative links with necrosis and autophagy. Finally, we will cite evidence that most older and newer compounds are both cytostatic and cytotoxic. The relative role of cytostasis and cytotoxicity on future drug screening and clinical development will be explored.

The understanding of mechanisms that drive malignant cell transformation, proliferation, and progression has identified targets against which new anticancer drugs are being developed. Agents targeting intracellular proteins as well as the surrounding tissues supporting the tumor or proteins involved in the initial steps of metastases are in preclinical and clinical development. Many of these compounds are said to be cytostatic.

By definition, cytostatic drugs do not kill cancer cells but instead stop cancer cells from proliferating. A true cytostatic agent should stop the growth of tumors and could prevent the development of metastases without affecting tumor shrinkage. Clinically, cytostasis would result in stable disease, presenting challenges in the evaluation of clinical activity (Table 1). Prolonged time to progression and improved overall survival have been recently reported using novel targeted therapies, and it has been argued that this effect is totally or partially mediated by cancer cell cytostasis without direct cytotoxic effects. We would argue that this latter conclusion is one that is at best difficult to draw (1, 2).

It should be noted that agents we now refer to as cytotoxic have for decades been observed to cause cytostasis (stable disease clinically) but were never described in those terms. Indeed, in most cases, cytostasis was viewed as ineffective and disregarded. Furthermore, whereas many agents currently entering clinical trials are said to be cytostatic, the data to be presented show that many if not all of these compounds possess cytotoxic activity that is at least as important if not more so than any cytostatic property. Examples include the multi-targeted kinase inhibitors sorafenib and sunitinib and the cyclin-dependent kinase (cdk) inhibitor flavopiridol.

For cytotoxic agents for which cures are considered valuable and evidence of activity and tumor shrinkage of some but lesser value, it is easier to ascertain the potential activity of a drug. But the value of tumor growth delay is more difficult to interpret, especially given the lack of a valid standard. The value in the clinical setting of a tumor growth delay of 7 days observed in a preclinical in vivo model is uncertain (3). Whereas this might be statistically significant in a xenograft model in which death occurs in less than a month, it nevertheless indicates that within a week the tumor managed to escape the drug effect. We would argue that this predicts that the ability of the drug to lead to cytostasis and stable disease clinically for a period much in excess of a few months would be unlikely. One might argue that the clinical data with sorafenib, with its very modest prolongation in progression-free survival, have underscored this concern and is an indication that a tumor growth delay of 1 week in a xenograft model that is ascribed to cytostasis is unlikely to translate into prolonged stable disease clinically.

This review examines the basic premises about cytostasis and its importance by stable disease clinically. We will argue that pure cytostatic agents may not exist but rather that when cytostasis occurs it will usually be followed by either cytotoxicity or cellular escape from the stasis. The relative importance of both cytostasis and cytotoxicity will be discussed and the limitations of our clinical tools for evaluating and describing cytostasis will be critiqued. We will cite evidence that indicates most if not all of our older and newer anticancer agents are both cytostatic and cytotoxic.

Cytostasis versus Cytotoxicity

Conventional chemotherapy agents
DNA damage and the subsequent induction of apoptosis is a primary cytotoxic mechanism of many anticancer agents,
Table 1. Conceptual differences between cytotoxics and cytostatics

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<th>Cytostatics</th>
<th>Cytotoxics</th>
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<tr>
<td>Agents</td>
<td>Molecular targeted therapies</td>
<td>Conventional chemotherapy</td>
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<td>Selectivity</td>
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<td>Indication</td>
<td>Low growth</td>
<td>Rapid proliferation</td>
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<td>Continuous administration</td>
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<td>Monotherapy</td>
<td>Monotherapy or combination therapy</td>
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<tr>
<td>Drug resistance</td>
<td>Combination therapy with cytotoxics?</td>
<td>Modifications of the target</td>
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<td></td>
<td>Unknown</td>
<td>Alteration of drug transport</td>
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including alkylating agents, platinum compounds, topoisomerase inhibitors, and the antimetabolites (4, 5). Because in many cases the DNA synthesis machinery is involved in this cytotoxicity, some evidence indicates that only S-phase cells are sensitive to these agents. Multiple effects occur during and after exposure to a drug as the treated cells flow through the cell cycle and encounter G1, S, and G2-M checkpoints. For example, the extent of cell cycle block and cell loss observed in vitro with the alkylating agent melphalan depends on the time course of drug administration and the drug concentrations (6). The response of the cell can consist of a G1 and/or a G2-M block, S-phase delay, recycling, or death, underscoring the occurrence of not only cytotoxicity but also cytostasis. Using a computational experimental approach and mathematical models, investigators have attempted to discern cytotoxic from cytostatic effects in cells that were in S phase at the time of treatment and cells that were in G1 and G2-M and identified cell cycle–dependent differences. With alkylating agents, at both high and low drug concentrations, cells are delayed in both S and G2-M, but lethality occurs only in S phase. By contrast, a cytostatic effect in G1, without lethality, occurs at intermediate drug concentrations. Similar observations have been reported with the topoisomerase I inhibitor topotecan in which a heterogeneous cellular response has been documented in ovarian cancer cell lines. Similarly, with the topoisomerase II inhibitor daunorubicin, in which differential effects have been reported depending on the dose—cytostasis and G2-M arrest at low drug concentrations and cytotoxicity and G1 and S arrest at high concentrations (7, 8). A final example consists of observations made when the cellular effects (cytotoxicity versus cytostasis) of antimetabolite anticancer agents have been evaluated. Although thymine-less death is an important response to antimetabolites and has been thought to lead to cytotoxicity through the induction of apoptosis, the outcome seems to depend on the status of the p53 tumor suppressor protein. In several models, the p53 status can determine whether deoxycytidine deprivation resulting from antimetabolites induces cytostasis or cytotoxicity as p53 protein may interfere with the G1 checkpoint (9, 10).

The potential to induce cytostasis, however, is not confined to agents that damage DNA. For example, the National Cancer Institute drug screen identifies both cytotoxic as well as cytostatic drug concentrations for our traditional anticancer drugs and thousands of other compounds, if one defines cytostasis as a concentration that prevents cell growth altogether, the total growth inhibition concentration (11). Furthermore, it could be argued that all microtubule-targeting agents from vincristine to paclitaxel and, more recently, the epothilones are inherently cytostatic (12, 13). None of these agents are intrinsically able to cause cytotoxicity. By interfering with microtubule dynamics, their mechanisms of action, these agents arrest cells in mitosis and induce cytostasis. Mitotic arrest is a condition that is poorly tolerated by any cell and must either be escaped or resolved by cellular death, hence the cytotoxic activity of these primarily cytostatic agents. Although we view these agents as cytotoxic, they are in fact cytostatic, and the arrest triggers cell death (14). Thus, in vitro cytostasis can occur with nearly all conventional anticancer agents. Although there is less in vivo data, there is nevertheless substantial evidence that drugs we view as cytotoxic can also be cytostatic and cause tumor growth delays in preclinical models (15, 16).

Before leaving conventional agents behind, we would note these studies have taught us that the demonstration of cytostasis or cytotoxicity often depends on the in vitro and in vivo assay conditions. Before concluding that a drug is not cytotoxic, a wide range of doses, times, and schedules of exposure and assessment intervals must be examined. We would also note that the relative importance of cytostasis in the anticancer activity of conventional agents has for the most part not been addressed in clinical trials. Nonetheless, the magnitude of the activity of conventional cytotoxic agents, many of which have led to prolonged progression-free survival and overall survival, is tightly correlated with tumor shrinkage and objective responses, underscoring the importance of cytotoxicity in their activity. In contrast to current thinking about targeted therapies, cytostasis after a conventional cytotoxic agent has often been viewed as an adaptive mechanism occurring in cancer cells to evade cytotoxicity, in effect, a mechanism of intrinsic or acquired drug resistance.

**Novel targeted agents: preclinical and clinical evidence of cytostatic and cytotoxic effects**

Approved in December 2005 by the Food and Drug Administration for the treatment of kidney cancer, sorafenib (Nexavar) is an orally active multikinase inhibitor with putative effects on tumor cell proliferation and tumor angiogenesis. In vitro, sorafenib inhibits Raf kinase, vascular endothelial growth factors receptors 1, 2, and 3, and platelet-derived growth factor receptor β (17). In vivo studies have shown a cytostatic effect on a large panel of tumors. The exact mechanism of cytostasis and its clinical effect has not been elucidated, however, perhaps because the essential target has yet to be identified.
In the clinical setting, although sorafenib was developed as a cytostatic agent, the accumulating evidence indicates clearly that it is also a cytotoxic agent. In four phase I studies that enrolled 163 patients, disease stabilization associated with clinical benefit encouraged investigators to continue drug development but objective antitumor activity was also noted in a small percentage of patients (18). A multicenter phase II randomized discontinuation study was then conducted at the 400 mg twice a day dose eventually approved by the Food and Drug Administration to distinguish cytostatic antitumor activity from spontaneous indolent tumor growth in metastatic clear cell carcinoma (19). Although the investigators concluded that "sorafenib has significant disease-stabilizing activity in metastatic renal cell carcinoma" consistent with its billing as a cytostatic agent, in fact, median progression was delayed only 18 weeks and 36% of patients had some degree of tumor shrinkage during the initial 12 weeks of treatment with a mean change of -18% for all patients, suggesting sorafenib had clinically measurable cytotoxicity. In a subsequent phase III study, treatment with sorafenib as initial therapy for metastatic disease prolonged progression-free survival (5.5 months for sorafenib versus 2.8 months for placebo, P < 0.01), a result interpreted by many as evidence of cytostasis. As in the randomized discontinuation study, however, objective responses were recorded, with partial responses reported as the best response in 10% of patients receiving sorafenib and in 2% of those receiving placebo (P < 0.001). Tumor shrinkage was observed in 76% of the patients in the sorafenib-treated cohort (Fig. 1). evidence that sorafenib has some cytotoxic properties (20). Finally, additional evidence of a cytotoxic effect can be found when higher doses are used. A recent abstract reported significant tumor shrinkage associated with a high response rate (52% objective response with 16% complete response) in metastatic clear cell carcinoma treated with 600 or 800 mg sorafenib twice a day (21). Thus, whereas some of the data suggest that sorafenib may have a cytostatic effect on some tumors, a conclusion that it has cytotoxic properties and that these might be responsible for at least some of its clinical activity is inescapable.

**Cytostasis and other targeted therapies: focus on protein kinase inhibitors**

Among the 518 kinases identified in the human genome are many exciting targets for cancer drug discovery (22). Molecular alterations in numerous kinases have been documented to drive malignant proliferation either via overexpression or activation, the latter secondary to an acquired mutation. Where dependence on a kinase is essential to the phenotype of a tumor, the term oncogene addiction has been coined. It is interesting that where such oncogene addiction is observed, the kinase inhibitors can have dramatic effects, whereas a lesser effect is observed on cells with mere overexpression of the target. An example of this is the activity of gefitinib or erlotinib in lung cancers with or without mutations in epidermal growth factor receptor (23). Can this be considered cytotoxicity on the one hand but cytostasis on the other? On examination of these agents, it becomes clear that the outcome, cytostasis or cytotoxicity, may depend less on the agent and more on the cellular context, especially the relative contributions of the mechanisms of cell death: apoptosis, necrosis, or autophagy as discussed elsewhere in this issue of *CCR Focus* (24).

**Cyclin-dependent kinases.** The cyclin-dependent kinases (cdks) are a family of enzymes encompassing the core components of the cell cycle machinery (25). Cyclin D-cdk4 and cyclin D-cdk6 as well as cyclin E-cdk2 complexes facilitate the G1-S transition, whereas cyclin A-cdk2 and cyclin B-cdk1 complexes are involved in S-phase progression and the G2-M transition, respectively. Several cyclin inhibitors have been identified, including the Cip/Kip proteins and the INK4 proteins (26). Flavopiridol was the first cdk inhibitor to reach the clinic (27). More recently, additional compounds that are 10 to 100 times more potent than flavopiridol as cdk inhibitors have been introduced into clinical trials, including CYC202 (28). Both flavopiridol and CYC202 act as ATP-competitive substrates but have a different spectrum of activity. Flavopiridol inhibits multiple cdks, including cdk1, cdk2, cdk4, cdk6, cdk7, and cdk9, whereas CYC202 primarily blocks cdk1, cdk2, cdk7, and cdk9. In *vitro*, both inhibitors cause arrest at both the G1 and G2 phases of the cell cycle, consistent with inhibition of cdk1, cdk2, cdk4, and cdk6. Cell death usually follows cell cycle arrest and is often delayed but may require concentrations higher than those needed to inhibit cdk activity. Based on experiments such as these, most investigators concluded that flavopiridol would cause cytostatic growth arrest. Consistent with these expectations, some of the patients enrolled in the early phases of flavopiridol development (phase I and II trials) experienced intervals of stable disease without significant tumor shrinkage (27). As clinical development progresses, however, it has become apparent that in the right setting flavopiridol is in fact a very cytotoxic agent. This cytotoxicity was most clearly shown when a change in the schedule of flavopiridol caused severe tumor lysis in patients with chronic lymphocytic leukemia, a disease where *in vitro* studies had shown flavopiridol induces apoptosis through the inhibition of positive transcription elongation factor b (cdk9/cyclin T; refs. 29, 30). These observations and other *in vivo* observations emphasize not only the inherent cytotoxic activity of flavopiridol but also the fact that with any agent the cellular context in which its activity is examined and the schedule of administration can be very important (29, 31). Indeed, flavopiridol, envisioned as a cytostatic agent, may well receive Food and Drug Administration approval because of its cytotoxic properties.

**Other targeted agents.** The type 1 receptor tyrosine kinase family, which includes epidermal growth factor receptor (HER1), HER2, HER3, and HER4, plays a crucial role in growth and differentiation of both normal and malignant epithelial cells (32). The mechanism by which the latter occurs is most likely mediated by the cyclins that function as the regulatory subunits for the kinases that control progression through the cell cycle. Previous studies have shown that specific cyclins, particularly cyclin D1, are targets for extracellular signaling and that HER2 expression is associated with increased levels of both G1-S-specific cyclins (cyclins D and E) and a G2-M-specific cyclin (cyclin A; ref. 33). Therapeutics targeting HER2-overexpressing breast cancers include trastuzumab, a humanized IgG1 monoclonal antibody that binds the HER2 ectodomain, and lapatinib, a selective, reversible inhibitor of both epidermal growth factor receptor and HER2 tyrosine kinases. By inhibiting
both epidermal growth factor receptor and HER2, lapatinib inhibits downstream activation of extracellular signal-regulated kinase 1/2 and AKT, leading to growth arrest and/or apoptosis in epidermal growth factor receptor and HER2-dependent tumor cell lines (34). Thus, even without an off-target effect, both cytotoxicity and cytostasis would be predicted in the clinical setting, with the balance dependent on dose and cellular factors. We would note that although a recent study using a mathematical model concluded that the predominant effect of lapatinib was cytostasis, the data analyzed were limited and the calculations were terminated as cytotoxicity began occurring (35). It seems that, despite claims that lapatinib preferentially affects cells growing in monolayer culture in G1-phase in a dose-specific manner by slowing the transition through G1 phase, there is a clear relationship between the strength of cytostasis and the drug concentration with cytotoxicity occurring after longer periods of drug exposure. Correlations for these preclinical observations can be found in the clinical setting (36). As with sorafenib, the clinical development of lapatinib has highlighted the cytostatic activity of the drug alongside limited cytotoxicity.

**HSP90 molecular chaperone.** HSP90 is an ATPase-driven molecular chaperone that has been found to be overexpressed in many malignant tumors. Chaperones are proteins required for the function, conformation, and regulation of many oncogenic proteins, including several kinases (e.g., HER2, cdk5, and MET), transcription factors (e.g., hypoxia-inducible factor 1α, mutated p53, and selected hormonal receptors), and catalytic subunits of telomerase. Inhibition of HSP90 exposes these oncogenic proteins to degradation through the ubiquitin-proteasome system (37). HSP90 inhibition leads to alterations in cancer cells, blocking essential processes of cell survival and proliferation. In fact, a drug that targets the HSP90 molecular chaperone uses a strategy that might be considered the ideal model of a molecular multitargeted therapy with a simultaneous attack on several steps in the process of oncogenesis by depleting multiple oncogenic client proteins. The natural molecule geldanamycin, its derivative 17-allylamino-17-demethoxygeldanamycin, and the orally bioavailable 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin are the lead HSP90 inhibitors in development (38). They induce cell cycle arrest in vitro and in human xenograft growth arrest in a spectrum of models. In addition to cytostasis, apoptosis (i.e., cytotoxicity) has also been reported in vitro (39). The latter is not surprising given the multiple downstream effects of HSP90 inhibitors compared with agents having more restricted effects (40). Clinically, cytostasis (stable disease) has been reported in the early phase of development of 17-allylamino-17-demethoxygeldanamycin; however, cytotoxicity in the right cellular context may yet be observed (41).
**Implication for Early Phases of Drug Development**

The premise that some agents would be preferentially cytostatic and would not lead to tumor shrinkage has led to arguments that the sequence and design of traditional phase I, II, and III trials used in developing traditional cytotoxic agents may not be appropriate with cytostatic agents. Lessons have been learned with positive studies (e.g., sorafenib) and negative experiences with drugs such as the matrix metalloproteinase inhibitors, proteolytic enzymes with different substrate specificities in the extracellular matrix shown to be important in its degradation (42). In the end, the value of a putative cytostatic agent needs to be evaluated in a randomized phase III study not unlike a cytotoxic agent (Table 2). For an agent that is exclusively or principally cytostatic, however, if such an agent exists, innovative approaches have yet to be designed to reduce the prolonged development of ineffective agents and to focus resources on more promising drugs.

Most often clinically defined stable disease is interpreted as a cytostatic effect, whereas cytotoxicity is inferred when objective tumor regression and necrosis are seen. This is a very simplistic analysis, however, that ignores the time course of cellular response and tumor heterogeneity. At the cellular level, cytostasis may be defined as the inhibition of cell growth and/or proliferation, and this initial event can be followed by cell death if the cytostasis is prolonged and profound. Alternatively, it could also be followed by cellular escape and regrowth. Clinically, stable disease could be the result of a prolonged cytostatic effect on a proliferating population, but it could also occur as a result of significant but transient cytotoxicity followed by tumor regrowth. These two effects, shrinkage and regrowth, could affect different cellular compartments in a heterogeneous tumor. Kinetic analyses of tumor measurements may help to discern among these possibilities. Surrogate end points including biomarkers (43) and dynamic imaging (44), considered important adjuncts in clinical trials evaluating putative cytostatic agents, may also help in distinguishing the two outcomes (Table 2).

**Table 2. Recommendations for clinical trials (cytostatics)**

<table>
<thead>
<tr>
<th>Method and design</th>
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<td><strong>Phase 1</strong></td>
<td>Dose-escalation based</td>
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<td><strong>Phase 2</strong></td>
<td>Target-bearing tumors</td>
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<td></td>
<td>Randomized discontinuation study</td>
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<td></td>
<td>Surrogate markers of activity (biomarkers, imaging, side effects) (RECIST criteria)</td>
</tr>
<tr>
<td></td>
<td>Randomized</td>
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Abbreviation: RECIST, Response Evaluation Criteria in Solid Tumors.

**Analysis and Perspective**

As drug development efforts have moved from conventional chemotherapies to targeted agents, we have faced the challenge of understanding the significance of cytostasis in anticancer drug activity. As the evidence cited clearly shows, there is still much to be learned about the relative effect of stasis and toxicity in the effect of a drug. It may be that experimental models can be established to help determine the predominant contributor to the regression of solid tumors, whether stasis or toxicity. In the end, however, the answer may lay in existing evidence that prolonged cytostasis can induce apoptosis and cell death. Originally reported with the Vinca alkaloids, similar observations have been reported recently with molecular targeted therapies. For example, the preventive agent monoterpene perillyl alcohol inhibits cellular proliferation in vivo and this is followed by the induction of apoptosis (cytotoxicity). This provides a clear illustration that inducing a block in the G2 phase of the cell cycle and slowing the G2-M transition by inhibiting cyclin D1–associated kinases induces indirect cellular damage leading to apoptosis (45).

Is a given compound definitively cytostatic or cytotoxic? As we have discussed, the existing preclinical and clinical data cannot support a well-delimited molecular-based classification. On the contrary, we could argue that whether a drug seems cytostatic or cytotoxic may be drug independent and instead depend on the dose used, the schedule of administration, the phase of the cell cycle in which the drug acts and in which the cell resides, and the cellular context. The complexity of the cell cycle can partially explain the pleiotropic effects of a given drug. As we noted, simple experiments using melphalan have shown a range of drug effects from arrest to lethality in all phases of cell cycle, the differences being strongly dose dependent (6). Finally, also important is the cellular context in which cytostasis occurs. It is well known that the propensity to apoptosis varies among cell types, with lymphoid cells most prone to undergo apoptosis. In cells more prone to apoptosis, one can often...
see cytostatic agents inducing apoptosis. In an apoptosis-prone cellular context, the cellular arrest, weak though it may be, is sufficient to trigger an apoptotic response. An illustration of this phenomenon at the molecular level has been the demonstration that the presence or absence of caspase inhibitors (such as Z-VAD-fmk) determines the ability of tumor necrosis factor to induce growth arrest or apoptosis in rhabdomyosarcoma cells (46). In the end, we may come to understand that prolonged cell cycle arrest in a phase other than G$_0$ is intolerable to a cell and must be resolved by either initiating a path to cell death or escaping the block, a decision that may depend in part on the cellular context in which the arrest occurs. Extensive data with microtubule-targeting agents that lead to G$_2$-M arrest and DNA-damaging agents that cause a p21-mediated G$_1$ arrest before apoptosis provide precedence for such a model (47). Were this true, clinically, we could see either cytotoxicity and tumor shrinkage when cells initiate a path to cell death or a period of slower growth followed by a documented recurrence when cells escape the drug-induced block, a prediction that fits the clinical experience with targeted agents in solid tumors.

Although the cytotoxic activity of a novel drug might synergize well with another cytotoxic agent, the occurrence of partial or complete cytostasis may result in a paradoxical cytotoxic effect so that combinations of agents with potential cytostatic properties with a cytotoxic agent should be evaluated carefully. An example is the first-generation cdk inhibitor flavopiridol. In preclinical studies, the combination of flavopiridol with docetaxel was shown to be antagonistic if flavopiridol was administered before or concomitantly with docetaxel. By contrast, synergistic cytotoxicity was observed when the cell cycle modulator was administered after docetaxel (48). This observation fits the paradigm of flavopiridol as a static agent and the well-established observation that cytotoxicity of a taxane is very vulnerable to anything that arrests cycling cells, if the arrest occurs before the taxane is added. For example, administering the cytostatic agent doxorubicin leads to a p21-mediated G$_1$ arrest and impaired taxane cytotoxicity (49). Allowing for differences in cellular context, similar results have been observed with the p21-mediated G$_1$ arrest induced by the histone deacetylase inhibitors (50, 51). In the case of flavopiridol, pretreatment prevented cells from entering mitosis by inhibiting cyclin B1/cdc2 kinase activity and, in this way, antagonized the docetaxel effect. As for the synergism observed when flavopiridol was administered after the taxane, the investigators noted that flavopiridol treatment of docetaxel-treated cells enhanced exit from mitosis but without cytokinesis, an exit path known to be lethal. Furthermore, independent of its cell cycle effect, flavopiridol, admittedly a promiscuous kinase inhibitor, has been shown to inhibit survivin phosphorylation on Thr 34, an effect that results in loss of survivin expression, as a consequence of accelerated survivin clearance (52). This ablation of survivin phosphorylation enhanced tumor cell apoptosis induced by several anticancer agents, including doxorubicin, independently of p53 and suppressed tumor growth in a breast cancer xenograft model in vivo. As noted by the investigators, “sequential ablation of p34cdc2 kinase activity after administration of spindle poisons, i.e., taxanes, may provide a rational approach to destabilize survivin levels in tumor cells and enhance the efficacy of common anticancer regimens in patients.” These examples underscore the complexity of novel targeted agents, many of which as kinase inhibitors may inhibit more than one of the >500 kinases in a cell, and also highlight the many ways that they may affect cell survival independent of cytostasis.

![Fig. 2. Interactions between the different processes leading to cancer cell death or survival. A and B, two opposite consequences of anticancer agents on cancer cells. Anticancer agents can induce cytostasis (A) or can lead to cytotoxicity (B) with necrosis, apoptosis, and autophagy involved in cell death. In vitro under certain circumstances prolonged cytostasis can induce necrosis and apoptosis leading subsequently to cell death. Dotted line, note that the relationship between cytostasis and autophagy is putative and remains to be shown. Autophagy can be paradoxically involved in cell protection and cell death.](image-url)
Finally, clarification of the relative importance of the multiple mechanisms involved in the activity of an anticancer agent can be difficult. As discussed elsewhere in this issue of CCR Focus, there are multiple pathways to cell death (24, 53–55). Tissue necrosis identified by the pathologist can be the end result of necrotic cell death, apoptosis, and even autophagy. Cytostasis can temporarily coexist with these events or can be the initial step for different mechanisms of cell death (Fig. 2). The putative link between cytostasis and autophagy remains to be studied, as autophagy may also protect cancer cells from the initiation of death pathways.

Conclusions

It is possible that in many ways our novel targeted agents are more similar to our conventional agents than we might have anticipated. An emphasis on cytostasis may have been driven initially by the recognition that the targeted pathways were often proliferative and the expectation that their inhibition would lead to reduced proliferation and, at an extreme, cytostasis. This, in turn, led to a clinical development strategy that sought a tolerable dose, not necessarily the most effective dose, thinking administration would be sustained over a long time with sorafenib as an example. Within the in vitro and preclinical data, however, consistent evidence of cytotoxic activity can be found, and this has been increasingly ratified in clinical trials. It may be difficult to discern whether the cytotoxic activity is mediated via a different target, an off target, or via the drug’s designed target when doses sufficiently high to maximally inhibit its activity are administered. As we have argued, however, it may be that a complete inhibition of growth resulting in cytostasis at various points in the cell cycle may be intolerable to a cell and a trigger for cell death if a way to escape cannot be found. If true, this would impart intrinsic cytotoxic activity to these cytostatic agents that would be a reflection of its ability to bring about effective cytostasis. According to this proposal, the higher activity seen with sunitinib over sorafenib at the Food and Drug Administration–approved doses might reflect the greater potency of sunitinib and, in turn, its ability to bring about more profound cytostasis from which cellular escape is less likely and a decision to follow the path to cell death more likely.

We have cited evidence that indicates that most if not all of our older and newer anticancer agents are both cytostatic and cytotoxic. The challenge going forward will be to dissect out these properties, if they can be dissected. Clarifying these distinctions is not trivial as they would clearly help us to design better drugs. An emphasis on cytostasis may not be ideal. Clinically, most such agents have performed not as cytostatic but at best as cytoslower or cytolytic agents, to borrow a term from Latin (the adjective lentus meaning sluggish). That the best that has been accomplished has been a slowing of tumor growth and not true cytostasis may be a reflection of the fact that cellular arrest at a phase in the cycle other than G0 was never part of nature’s design and, as we have noted, intolerable. It may then be that if we cannot design a drug that can affect a strong arrest that triggers cell death, the cancer cell will find a way to escape, resulting in, at best, a transient slowing of growth. Progress to be sure, but still a long way from a cure.

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Cytostasis in Cancer Therapy

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