Newer Cytotoxic Agents: Attacking Cancer Broadly

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Abstract

The plasticity and instability of the cancer genome is impressive and is characterized by gene amplifications and deletions, rearrangements, and many silent and active mutations. Although targeted therapeutics have had effect in some diseases, there remains a large role for new cytotoxic agents that have the potential to be broadly active across multiple cancers. Platinum-based regimens are the basis for treatment of several common tumors. Sratraplatin and picoplatin are newer platinum complexes that form bulkier lesions in DNA than their forerunners. Microtubules are a key target for anticancer agents. Vinca alkaloid and similar compounds fragment these critical structures, whereas taxanes stabilize them. Vinflunine is a new fluorinated Vinca alkaloid derivative with vascular disrupting effects, as well as antitumor effects. Epothilones are a new class of microtubule stabilizers. Mitosis has been targeted directly and indirectly by many anticancer agents. The aurora kinases are new targets in this class. Inhibitors of aurora kinases are likely to be cytotoxic. Finally, protein regulation is essential for cellular integrity. With the approval of bortezomib (Velcade, PS-341), the proteosome, a master protein regulator, has been validated as an anticancer target. The five articles in this issue of CCR Focus present the current status of these next generation cytotoxic agents.

Since medicinal chemists discovered that it is possible to selectively target differences in the ATP-binding pockets of kinase enzymes with small molecule inhibitors, much of cancer research has been racing forward with the notion that targeting one critical normal, mutant, and/or aberrantly expressed enzyme could have a major therapeutic effect in many malignant diseases (1–5). In a few cases, inhibition of a single receptor tyrosine kinase has proved to have a major therapeutic benefit; however, in many situations, the clinical benefit of these compounds as single agents has been modest (6).

In parallel with drug discovery efforts, translational investigators have been probing deeper and with more detail in the genomes of malignant diseases. The malignant genome is now described in gene amplifications and deletions, rearrangements, and in many silent and active mutations (7–12). The plasticity and instability of the cancer genome is impressive. These findings clearly show that most tumors are unlikely to yield to single enzyme–targeted agents. Although in the near future therapeutic regimens may be selected based upon the gene expression pattern or molecular signature of an individual tumor, there will continue to be a long-term need for broadly active anticancer agents.

The current armamentarium of medical oncology includes many active anticancer agents that are applied across tumor types. None of the broadly active anticancer agents are ideal medications. They are curative as sole therapy in some diseases and as adjuvant therapy in others. In the rush to develop molecular targeted therapies, the successes of established therapeutic strategies should not be discounted. It is likely that combination regimens, including cytotoxic anticancer agents and molecularly targeted agents, will comprise the next generation of successful cancer treatments. This CCR Focus describes several next-generation cytotoxic anticancer agents. Some of these molecules are directed toward highly validated cancer targets, and some are directed toward newer molecular targets. The commonality is that all can be described as cytotoxic agents and have the potential to be broadly active across multiple malignant diseases.

Targeting DNA

Over the past 30 years, platinum-based therapeutic regimens have been the basis for treatment of many common solid tumors (13, 14). Cisplatin is one of the most widely used and effective anticancer agents. Its efficacy, however, is limited by acquired or intrinsic resistance, the mechanisms of which are still under investigation. Cisplatin is a simple inorganic platinum salt (Fig. 1). Inside cells, the chlorines on cisplatin are exchanged with water molecules to form a short-lived diaquo-platinum complex. The water molecules then exchange with nitrogens and sulfurs but, most importantly, with the N-7 nitrogen of adjacent guanines forming intrastrand coordination bond lesions in DNA, as well as several other less abundant bifunctional lesions (Fig. 2; refs. 15–17). All of these lesions contribute to inhibition of enzymes involved in RNA transcription, DNA replication, and chain elongation of DNA polymerization. The cellular repair of cisplatin DNA lesions occurs by two major mechanisms: nucleotide excision repair and recombinational repair. In addition, correlations have been established between loss of mismatch repair and cisplatin resistance (18–22). Intrastrand platinum DNA adducts are primarily repaired by nucleotide excision repair mechanisms.
and double-strand breaks are repaired through recombinational repair. Expression of ERCC1, one of the components of nucleotide excision repair, correlates with resistance to platinum-based therapy (23, 24). Transfection with small interfering RNA for ERCC1 to eliminate ERCC1 protein from cells can sensitize cells to cisplatin (25). Interstrand platinum DNA adducts are handled by homologous recombination. The mismatch repair machinery is critical for ensuring replication fidelity and requires the tandem action of multiple protein complexes (26). Mismatch repair corrects mismatched insertion and deletion loops introduced by DNA polymerases. Alt et al. (27) analyzed four crystal complexes, along with biochemical information, and provided a detailed description of the mechanism by which DNA polymerase η is able to replicate through cisplatin-induced 1,2-d(GpG) adducts (Pt-GGs) formed during anticancer therapy with cisplatin (28, 29). Resynthesis and ligation are done by DNA polymerases and ligases with the assistance of single-strand binding proteins. Loss of mismatch repair has been associated with drug resistance by impairing the ability of tumor cells to detect mismatch-induced DNA adducts and trigger proapoptotic signaling (18, 21). High-mobility group proteins (HMGa, HMGB1) which are often highly expressed in cancers can potentiate the effects of cisplatin by modulating repair of damaged DNA producing double-strand breaks (29, 30). The most common marker for selective action of cisplatin is the specific deficiency of some tumors in one or many elements of the complex DNA repair machinery required for the repair of the various adducted DNA bases induced by cisplatin. Despite the significant potency of platinum-based drugs in these tumors, acquired resistance mediated by DNA repair enzymes is the major cause of relapse after chemotherapy.

Successive anticancer platinum complex carboplatin produces an identical lesion in DNA, as cisplatin and oxaliplatin produces a similar DNA lesion, having a flexible diamino-cyclohexane ring (Fig. 1). Carboplatin and oxaliplatin have different pharmacokinetics, tissue distribution, and toxicity profiles than cisplatin; therefore, carboplatin and oxaliplatin have found important clinical application in a different spectrum of malignant diseases from cisplatin. Satraplatin (JM118) and picoplatin, now in clinical trial, produce bulkier lesions in DNA that may be more difficult for cellular machinery to repair. Each of these new platinum complexes has a different spectrum of normal tissues effects, pharmacokinetics, and biodistribution (13, 31). Each, therefore, is a
different drug. Choy and colleagues (13) provide a concise history of the complex development history of satraplatin. The availability of an oral platinum agent is critical for reducing the problems related to i.v. administration and for decreasing the side effects related to peak blood concentrations resulting from i.v. dosing of platinum-based agents. Proving equivalent efficacy, however, will be an important factor needed to enable clinical success for an oral platinum complex. The development of satraplatin in prostate cancer is at a critical juncture.

Targeting Tubulin

The cellular requirement for the dynamic instability of microtubules is one of the key targets of anticancer therapies. Although principally recognized as important in mitotic function for their role in separating the duplicate set of chromosomes during cell division, microtubules are an essential component of the cytoskeleton and are critical in many interphase functions, including maintenance of cell motility, cell shape and scaffolding, intracellular transport, secretion, neurotransmission, and relay of signaling between cell surface receptors and the nucleus (32, 33). Most antimicrotubule agents are structurally complex, naturally occurring alkaloids or semisynthetic compounds.

Microtubules are composed of molecules of tubulin that are composed of two tightly linked globular protein subunits called α-tubulin and β-tubulin. Microtubule assembly and disassembly are in dynamic equilibrium; the direction of which is determined by several factors, including the concentrations of free tubulin, GTP, calcium, and magnesium. Cells modify the rate of dynamic instability of microtubules to perform specific functions. During mitosis, the rates of both microtubule assembly and disassembly are increased so that chromosomes can readily capture growing microtubules forming mitotic spindles. Microtubule-associated proteins suppress dynamic instability during differentiation by binding to the microtubules and preventing depolymerization, thus allowing cellular cytoplasm to organize.

The Vinca alkaloid anticancer agents, vincristine and vinblastine, are cytotoxic by interacting with tubulin and disrupting microtubules (Fig. 3A and C; ref. 34). The cytotoxicity of these
compounds is primarily due to disruption of microtubules comprising the mitotic spindle apparatus, thereby inducing metaphase arrest in dividing cells. The **Vinca** alkaloids also affect microtubules involved in chemotaxis and direction migration; intracellular transport and movement of organelles, such as mitochondria and secretory granules, especially in neural cells; secretory processes; membrane trafficking and transmission of receptor signals; and structural integrity in some cells. Platelets, in particular, are rich in tubulin and depend upon microtubules for structure. The relationships between the inhibitory effects of **Vinca** alkaloid on cell proliferation, mitotic arrest, mitotic spindle disruption, and depolymerization of microtubules have been described in detail (35, 36).

Endothelial cells in tumors have proved to be another important target for microtubule-binding agents (37). These molecules have been described as vascular-disrupting agents. Vascular-disrupting agents target established tumor vasculature and produce an acute, pronounced shutdown of tumor blood flow leading to tumor necrosis. In endothelial cells, depolymerization of the microtubules and disorganization of tubulin in the cytoskeleton produce conformational changes in the cells and decreased blood flow. Vinflunine, a difluorosemisynthetic **Vinca** alkaloid derivative currently in phases II and III clinical trials, destabilizes microtubule dynamics by binding to tubulin, leading to cellular mitotic arrest (38). Vinflunine not only targets the highly proliferative tumor cells but also endothelial cells of the tumor vasculature. Compared with other microtubule-disrupting agents, vinflunine has an improved safety profile. Bennouna and colleagues in this issue of CCR Focus argue that several properties of vinflunine make it likely to be a better clinical agent than vinorelbine or vinblastine. These properties include a weaker affinity for tubulin, more rapid reversibility of centrosome separation and mitotic block, and a broader concentration curve for the antivascular effects (38). If confirmed, these differences could portend some clinical differences, although finding that niche may be difficult. This strategy may be more rewarding, however, than attempting to prove vinflunine superiority in diseases wherein **Vinca** alkaloids are already widely used.

Taxanes, specifically paclitaxel, was discovered during a National Cancer Institute screen of plant extracts for anticancer activity (Fig. 3B). It was later found that paclitaxel is produced by Taxomyces andreanannae, a fungal endophyte endemic to the inner bark of the Pacific yew (39). The binding site of paclitaxel binding on microtubules is different from the binding sites for **Vinca** alkaloids. Paclitaxel binds to the NH₂ terminal 31 amino acids of the β-tubulin subunit in tubulin oligomers or polymers rather than to tubulin dimers. Thus, taxanes shift the dynamic equilibrium between tubulin dimers and microtubules toward microtubule assembly and stabilize microtubules preventing depolymerization (Fig. 3C). The taxane-bound microtubules are very stable, and the dynamic reorganization of the microtubule network is inhibited (40). Taxanes induce microtubule bundling in cells and formation of numerous abnormal mitotic asters. At low concentrations, taxanes kill cells by producing a sustained mitotic block at the metaphase-anaphase boundary and triggering apoptosis.

The epothilones are nontaxane microtubule-stabilizing agents (Fig. 3B; refs. 41, 42). Like taxanes, epothilones induce microtubule bundling, formation of multipolar spindles, and mitotic arrest. Epothilones and taxanes, however, do not bind to tubulin polymers in an identical manner (43). Five epothilone analogues are in phases I and II clinical trials. Epothilones have shown clinical activity in patients whose
tumors are refractory to anthacyclines and taxanes; however, normal tissue toxicities are complex (41, 42). The epithilone ixabepilone was approved by the Food and Drug Administration in 2007 for treatment of breast cancer. This is likely to be approval of a first in class agent with several other examples to follow, given that differences in chemical structure and pharmacology for other classes of anti cancer agents have led to different spectrums of clinical activity. The epithilones are discussed in this issue of CCR Focus by Lee and Swain (41). Competition for ixabepilone not only arises from other epithilones but also from several improved formulations of taxanes, such as nab-paclitxael (ABI-007, Abraxane), a nanoparticle albumin-bound formulation of paclitaxel (44–46). Abraxane is approved for treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. It is not clear, however, that taxanes and epithilones will be equivalent clinically. Despite the identical target and mechanism of action, the marked differences in chemical structure between taxanes and epithilones along with the differences observed in activity in preclinical models indicate that it is likely that these agents will have different spectrums of clinical activity.

Targeting Mitosis

Over the past few decades, investigators have identified several classes of anticancer agents that block key pathways of cell cycle and apoptosis control. A key goal of current cancer research is to build upon molecular knowledge to develop a next generation of mechanism-based, rationally designed anticancer agents. New drugs specifically target key intermediates of cell fate decision pathways. The aurora kinases A (STK15, BTAK), B, and C are recent additions to cancer cell cycle targets (47). The three aurora kinases localize to the centrosome during mitosis (Fig. 4). Aurora kinase A is associated with centrosome maturation and separation and regulates spindle assembly and stability. Aurora kinase B is a chromosome passenger protein involved in chromosome segregation and cytokinesis. The role of aurora kinase C has not been fully elucidated. Aurora kinase A localizes next to the...
centrosome late in G1 and early in S phase. The expression of aurora kinase A increases as the kinase associates with the mitotic poles and adjacent spindle microtubules through telophase (47). As mitosis completes, aurora kinase A localizes to the midzone of the microtubule spindles (48). Without aurora kinase A, the centrosome does not accumulate γ-tubulin properly before entering anaphase and the centrosome has fewer aster microtubules than normal (49). Aurora kinase A is necessary for the proper separation of the centrosome after mitotic spindle formation and for proper organization and alignment of the chromosomes during prometaphase. In the absence of aurora kinase A, the mitotic spindle do not separate or collapse. Aurora kinase B cooperates with aurora kinase A in the kenetocore, the part of the chromosome at which the mitotic spindle’s extended microtubules attach and pull (48).

Lastly, aurora kinase A participates in the completion of mitosis by contributing to the cytokinesis process that is the division of the cytoplasm of the mother cells into the two daughter cells.

Aurora kinase A dysregulation and overexpression is frequent in cancer (50–52). Most solid tumors are highly aneuploid. The chromosome gain or loss can frequently be attributed to chromosome segregation errors during mitosis (53, 54). Mitotic aberrations associated with numeral and functional abnormalities of centrosomes can trigger spindle checkpoints, leading to mitotic catastrophe and cell death. Downstream, aurora kinase A phosphorylates p53 at S315, thus priming p53 for MDM2-mediated degradation that may contribute to the generation of amplified centrosomes (55). Furthermore, both inactive CDK1–cyclin B and CDC25B are targeted to centrosomes by aurora kinase A-mediated phosphorylation (53). Aurora kinases, polo-like kinases, and cyclin-dependent kinases, like the mitotubulin targets discussed above, are critically involved in cell cycle and thus in proliferation. Overexpression of aurora kinase A disrupts the spindle checkpoint activated by paclitaxel exposure resulting in resistance to paclitaxel (56). Small molecule inhibitors of aurora kinases may provide clinical benefit in combination with drugs that target microtubules. As discussed elsewhere in this issue of CCR Focus, aurora kinases may be considered modern targets because the goal is to target them with highly conserved 76–amino acid protein that is covalently ligated to target proteins by a multienzyme process consisting of ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligating (E3) enzymes acting sequentially (60). Polyubiquitin chains of more than four ubiquitins must be assembled through K48 linkage to target the substrates for proteasome degradation (61). The S19 regulatory particle recognizes polyubiquitinated proteins, guides entry into the core particle, and unfolds the substrate. The 20S cylinder-like complex contains the proteolytic chamber. There are four ring structures in the cylinder. The upper and lower rings are called the α rings, and the center rings are the β rings. Each ring has seven protein subunits. Three subunits in each β ring have proteolytic activity constituted by caspase-like, trypsin-like, and chymotrypsin-like catalytic activities. The products of proteasome degradation are peptides of 3 to 22 amino acids in length and intact ubiquitin protein (Fig. 5; ref. 61).

The abnormal proliferation rate of many tumors makes malignant cells more dependent upon the proteasome to remove misfolded or damaged proteins. The accumulation of such proteins will likely induce apoptosis. Thus, tumor cells are more sensitive to proteasome inhibition than normal cells. The ubiquitin-proteasome pathway is vital in the degradation of proteins involved in cell cycle progression, proliferation, and apoptosis and a vast majority of abnormal proteins that results from oxidative damage and mutations. With the approval of bortezomib, the enzymes comprising the ubiquitin-proteasome system has become a validated anticancer target (Fig. 4A; ref. 62). Exposure of cells to bortezomib markedly induces the proapoptotic protein NOXA in a tumor cell–restricted manner (63). The induction of NOXA is dependent upon the c-MYC oncogene in several cell types. C-MYC binds to the NOXA promoter, so down-regulation of c-MYC decreases NOXA induction in tumor cells and expression of c-MYC in normal cells increases levels of NOXA in cells. This pathway reveals an unexpected role for c-MYC in bortezomib antitumor activity. As discussed by Orlowski and Kuhn (62) in this issue of CCR Focus, the field has evolved from the original thought that bortezomib would act primarily through promotion of nuclear factor-κB degradation. Numerous proteins, such as c-MYC, have been found to be altered as well. It remains to be definitively shown whether there is one

Targeting Protein Regulation

The regulation of cellular metabolic activities by the proactive synthesis and degradation of specific proteins is vital to cellular integrity and proliferation (57–59). Along with transcription and translation, degradation/recycling of proteins and amino acids is essential for life. While lysosomes, cytoplasmic membrane-bound vesicles, degrade extracellular and transmembrane proteins, the proteasome is responsible for degradation of intracellular proteins. Because the ubiquitin-proteasome pathway is the main mechanism for proteolysis in eukaryotic cells, the function of this pathway is critical for controlling the intracellular levels of varied proteins and maintaining normal cellular function (60). The proteasome is a very large, abundant multiprotein catalytic complex located in the nucleus and cytoplasm of eukaryotic cells. The functionally active form 26S proteasome is composed of a 20S core catalytic complex capped on both ends with 19S regulatory subunits (Fig. 5). The distribution of proteasomes between the nucleus and cytoplasm of cells varies with cell type, and proteasomes redistribute in a cell cycle–specific manner (57, 58). Active proteasome complexes can enter the nucleus through pores or during reassembly of the nuclear envelope after mitosis. Many proteasome substrates, such as cyclins, are important in cell cycle progression.

For proteasome-mediated degradation, proteins are commonly conjugated to several ubiquitins. Ubiquitin is a highly conserved 76–amino acid protein that is covalently ligated to target proteins by a multi-enzyme process consisting of ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligating (E3) enzymes acting sequentially (60). Polyubiquitin chains of more than four ubiquitins must be assembled through K48 linkage to target the substrates for proteasome degradation (61). The S19 regulatory particle recognizes polyubiquitinated proteins, guides entry into the core particle, and unfolds the substrate. The 20S cylinder-like complex contains the proteolytic chamber. There are four ring structures in the cylinder. The upper and lower rings are called the α rings, and the center rings are the β rings. Each ring has seven protein subunits. Three subunits in each β ring have proteolytic activity constituted by caspase-like, trypsin-like, and chymotrypsin-like catalytic activities. The products of proteasome degradation are peptides of 3 to 22 amino acids in length and intact ubiquitin protein (Fig. 5; ref. 61).

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main target triggering apoptosis after proteasome inhibition or whether the effect on several proteins simultaneously triggers cell death.

**Conclusion**

Recent studies of the cancer genome have reinforced the knowledge that cancer is a heterogeneous accumulation of mutations and other genetic abnormalities in cells that through unlucky chance has resulted in a malignancy with a proliferative and invasive thrust. Although a subpopulation of the malignant diseases may thrive through addiction to a specific abnormally functioning growth factor pathway, many malignancies are multifactorial in origin. Thus, there is a continuing need for improved broadly effective anticancer agents. Several of those at the near horizon are described in this CCR Focus.

**References**

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