Cancer Treatment Strategies

Editor
Dr Ahmed M Malki
Cancer Statistics

Cancer is a leading cause of disease worldwide and it is estimated that 12.7 million new cancer cases occurred worldwide in 2010. Lung (1.6 million, 12.7% of the total for men and women), female breast (1.4 million, 10.9% of the total for women), colorectal (1.2 million, 9.7% of the total for men and women) and stomach cancers (1 million, 7.8% of the total for men and women) were the most common, accounting for more than 40% of all cases diagnosed [1].

Currently, one in 3 women and one in 2 men in the United States will develop cancer in his or her lifetime. Increases in the number of individuals diagnosed with cancer each year, due in large part to aging and growth of the population, as well as improving survival rates, have led to an ever-increasing number of cancer survivors. The goal of treatment is to “cure” the cancer, or prolong survival in patients with advanced disease, while preserving the highest possible quality of life in both the long and short term [2]. Lung cancer has been the leading cause of malignancy in women since 1987, when it surpassed breast cancer. In 2010, it was estimated that over 116,000 men and 105,000 women would be diagnosed with lung cancer in the United States. Lung cancer is responsible for over 71,000 deaths per year in women. This number exceeds the mortality associated with both breast cancer (39,840 deaths) and colon cancer (24,790 deaths) combined, which are the second and third leading causes of cancer-related mortality in women, respectively [3]. Thus demands for efficient therapy are needed to control the growth and multiplication of cancer.

Cancers of the lung and bronchus, prostate, and colorectum in men and cancers of the lung and bronchus, breast, and colorectum in women continue to be the most common causes of cancer death. These 4 cancers account for almost half of the total cancer deaths among men and women (Figure 1). In 2012, lung cancer is expected to account for 26% of all female cancer deaths and 29% of all male cancer deaths. The data represented in Figure 1 revealed the most common cancer expected in men and women in 2012. In men, lung, prostate, bronchus and colorectum account 50% of all newly diagnosed cancer. In women, lung, breast, bronchus and colorectum also account the other 50% [4].

### Figure 1: Ten leading cancer types for estimated deaths by sex in 2012.

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Estimated Deaths</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>87,750</td>
<td>26%</td>
<td>72,590</td>
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<tr>
<td>Prostate</td>
<td>28,170</td>
<td>9%</td>
<td>39,510</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>26,470</td>
<td>9%</td>
<td>25,220</td>
</tr>
<tr>
<td>Pancreas</td>
<td>18,850</td>
<td>6%</td>
<td>18,540</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>13,980</td>
<td>5%</td>
<td>15,500</td>
</tr>
<tr>
<td>Leukemia</td>
<td>13,500</td>
<td>4%</td>
<td>10,040</td>
</tr>
<tr>
<td>Esophagus</td>
<td>12,040</td>
<td>4%</td>
<td>8,620</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>10,510</td>
<td>3%</td>
<td>8,010</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>10,320</td>
<td>3%</td>
<td>6,570</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,650</td>
<td>3%</td>
<td>5,980</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td><strong>301,820</strong></td>
<td><strong>100%</strong></td>
<td><strong>275,370</strong></td>
</tr>
</tbody>
</table>

Apoptosis

Most of cancer chemotherapeutics and chemopreventives exert their effects by triggering either apoptotic cell death or cell cycle transition, and accordingly, the induction of tumor cell apoptosis is used to predict tumor treatment response [5]. Apoptosis is a selective process of physiological cell deletion that plays an important role in the balance between cellular replication and death. Apoptotic signaling can proceed via two pathways, i.e., via death receptors expressed on the plasma membranes of cells or alternatively via mitochondria, which contain several proteins that regulate apoptosis. The death receptor pathway is initiated by the ligation of membrane bound Tumor Necrosis Factor (TNF) or Fas receptors, which result in a caspase-8-dependent cascade and subsequent cell death. During this cascade, caspase-8 cleaves Bid and induces cytochrome c release and/or directly activates caspase-3 [6-8]. On the other hand, the ‘intrinsic’ (or mitochondrial) pathway, which is triggered by diverse cellular stresses, such as cytokine deprivation, DNA damage or oncogene activation. The mitochondrial pathway involves cytochrome c release, which
leads to caspase-9 activation and a proteolytic caspase cascade. After reception of death signals, the outer mitochondrial membrane becomes permeable leading to a cascade of events. Cytochrome-c is released and subsequently activates caspase-9, which then activates caspase-3 that acts on specific apoptotic substrates these pathways are largely independent, each activating different initiator caspases, but they are connected through the cleavage of the BH3 only protein BID [9]. The two molecular mechanisms extrinsic and intrinsic phases of apoptosis are outlined in (Figure 2).

![Figure 2: Schematic representations of the main molecular pathways leading to apoptosis.](image)

There are two main pathways to initiate apoptosis. In the extrinsic pathway upon ligand binding to specific receptors the DISC complex is formed and caspase 8 activated. In the intrinsic pathway, the release of cytochrome c from the mitochondria results in the formation of the apoptosome and activation of Caspase 9. Caspase 8 and 9 then activate downstream caspases such as caspase 3 resulting in cell death [10].

**p53 and Mdm2**

The p53 tumor suppressor plays a pivotal role in regulating cellular processes including cell cycle arrest, apoptosis, cell metabolism and senescence. Mutation of the TP53 gene or inactivation of the p53 signaling pathway occurs at a high frequency in many human tumors, suggesting that p53 plays a critical role in preventing normal cells from becoming cancerous. p53 is a stress-inducible protein; it is inactive under normal physiological conditions and activated in response to various types of stresses such as DNA damage and ribosomal stress [11]. Activated p53 can either induce cell cycle arrest or inhibit cell growth or promotes cell apoptosis depending on different type of stress and the cellular context. Multiple mechanisms have been revealed to collectively accomplish the regulation of p53 activity which ultimately determines the selectivity of p53 for specific transcriptional targets, resulting in precise control of the p53 activity. p53 is the most frequently inactivated tumor suppressor gene in human cancer. Clinical studies have shown that p53 is mutated in approximately 50% of human cancers [12,13].

Mdm2 (Murine double minute 2) was discovered on double minute chromosomes in a derivative cell line of NIH-3T3 cells [14,15]. Mdm2 belongs to the family of E3 ubiquitin ligases that contain a RING domain16 and serves as the major E3 ubiquitin ligase for p53 degradation. Several studies have illustrated the importance of Mdm2 in the control of p53 activity. The mechanism by which Mdm2 suppresses p53 has classically been thought to occur by two distinct ways: by binding to the N-terminal domain of p53 and masking p53's access to transcriptional machinery, and by ubiquitinating p53 and targeting it for proteasomal degradation [16-20]. However, it was found that Mdm2-p53 binding alone in the absence of Mdm2 E3 ubiquitin ligase activity is insufficient to suppress p53 activity [21]. MdmX has been identified as a highly homologous gene that is closely related to Mdm2 [22,23]. Similarly to Mdm2, MdmX possesses a p53 binding domain at its N-terminus and a RING finger domain at its C-terminus through which it heterodimerizes with Mdm2. However, unlike Mdm2, MdmX does not have appreciable ubiquitin ligase activity. Because of its sequence similarity with Mdm2 and its ability to inhibit p53-induced transcription when overexpressed, MdmX has been hypothesized to act as a negative regulator of p53 through physical binding [24].

In response to stress, a decrease in Mdm2 protein levels and/or its activity and the interaction between Mdm2 and p53 lead to p53 stabilization. The mechanisms by which p53 escapes the detrimental effects of Mdm2 binding vary depending on the type of stress signals. The increase in p53 levels and in transcriptional activity of p53 leads in turn to increased production of Mdm2 (Figure 3). Elegant quantitative studies of p53 show that while an individual cell may have only one pulse of p53 activity, its neighbor might have several repeated pulses [25]. As the amount of radiation increases, the percentage of cells showing a high number of p53 pulses also increases. Interestingly, the mean height and width of a pulse is constant and independent of the damage level. Sister cells continue to oscillate in a correlated way even after cell division. These intriguing observations open new questions regarding the mechanism and function of p53 oscillatory dynamics, including the reason for the observed variation between cells [26]. Understanding why some cells respond poorly is of critical importance as these genetically unstable cells will continue to proliferate and eventually become the target of additional oncogenic mutations and the cell-of-origin of tumor development.
Prior to DNA damage, Mdm2 interacts with both p53 leading to their ubiquitination and targeting for proteasomal degradation. Mdm2 bound to p53 has been localized to regulatory regions of a number of p53 target genes, leading to repression of their expression. Upon DNA damage, both Mdm2 and p53 become post-translationally modified such that they no longer interact. p53 is now capable of influencing gene expression [27].

**p53 as Target for Cancer Therapy**

Cancer is usually associated with aberrant cell cycle progression and defective apoptosis induction due to the activation of proto-oncogenes and/or inactivation of tumor suppressor genes [28]. Given the central role of p53 in cancer prevention and suppression and in chemosensitization or radiosensitization, p53 has to be abrogated during carcinogenesis for most cancers to arise. Indeed, p53 is inactivated by point mutations in more than 50% of human cancers with a majority of mutations occurring in the DNA binding domain, which either change wt p53 conformation (conformation mutants, e.g., 175H, 249S, 281G) or abolish its DNA contact (contact mutants, e.g., 248W, 273H) [29]. Most malignant tumors that disrupt p53 signaling pathways remain addicted to p53 mutants. Various strategies have been successfully developed to reconstitute p53 functions in order to abrogate tumor progression [30,31]. Based on the action sites, these strategies can be briefly listed into three groups: replacement of wild-type p53 by gene therapy, augmenting of wild-type p53 by inhibition of MDM2-mediated degradation and reactivation of mutant p53 by alteration of protein conformation [32], the three major strategies to target the tumor suppressor p53 are outlined in (Figure 4).

A) Current strategies in reactivation of p53.

B) Targeting ceramide glycosylation to resuscitate p53 expression.
Ceramide modulates the expression of p53 to resuscitate wild-type p53 (phosphorylated, red fluorescence in the cell nucleus) and p53-dependent apoptosis, thus sensitizes mutant p53 tumors to therapies. Silencing of glucosylceramide synthase (GCS, Green Fluorescence in Golgi apparatus) with MBO-as GCS disrupts ceramide glycosylation to enhance endogenous ceramide. (+) - Increasing enzyme activity or synthesis; (−) - Inhibiting enzyme activity or synthesis; * - These genes are upregulated by mutant p53 in cancer cells.

A promising example of small molecules that restore the function of mutant p53 is PRIMA-1, p53 Reactivation and Induction of Massive Apoptosis (PRIMA)-1 is a small molecule that binds covalently with thiol groups in mutant p53 and restores DNA-binding activity to some mutant p53 proteins. PRIMA-1 preferentially suppresses the growth of tumor cell lines containing mutant p53, indicating that it functions by acting on mutant p53. PRIMA-1 is in a Phase I clinical trial [33]. The PRIMA-1 (p53 reactivation and induction of massive apoptosis) restores wild-type conformation to mutant p53 protein by covalent binding to and modifying the thiol groups of His175 and His273 in the core domain. The more potential PRIMA-1 analogue APR-246 that inhibits human tumor growth and is able to synergize with chemotherapeutic drugs is currently tested in a clinical trial [34]. The restoration of the impaired function of the p53 protein by disrupting the Mdm2–p53 or Mdmx–p53 interaction offers a fundamentally new avenue for the treatment of a broad spectrum of cancers [35,36]. The search for efficient p53–Mdm2 inhibitors has led to several small-molecule ligands. The most thoroughly studied among them is Nutlin-3 [37].

In conclusion, in cancer carrying a wt. p53, p53 is often nonfunctional as a result of either being degraded by overexpressed Mdm2 or being excluded from the nucleus where p53 acts as a transcriptional factor. There are various approaches targeting p53 1) to activate wt. p53, 2) to reactivates mutant p53 or selectively kill cancer cells with mutant p53, and 3) to temporarily inhibit wt. p53 for normal cell protection. Successful clinical development of these three classes of novel compounds would eventually revolutionize the current cancer therapies to benefit a majority of cancer patients. The approaches to activate p53 include the use of nongenotoxic small molecules to activate endogenous wt p53, chemoradiation to activate endogenous wt. p53, of gene therapy to introduce wt. p53 or modified adenovirus to kill cancer cells with mutant p53, and of synthetic peptides [38] (Figure 5).
As illustrated in the figure, three classes of p53 targeting compounds have been identified and characterized. The first classes are the compounds that activate or restore wild-type p53 function and can be used in human cancers harboring a wt. p53. The second class of compounds reactivates and rescues the mutant p53 with an application in human cancers carrying a p53 mutation. The third class is capable of inhibiting wt. p53 and can be used during chemoradiation to block p53 activation in normal cells, thus reducing cytotoxicity [38].

Mdm2 Targeted Drugs

These results have provided an encouraging direction for p53-target therapeutic strategy utilizing inhibition of MDM2. There are three main categories of MDM2 inhibitors: inhibitors of MDM2-p53 interaction by targeting to MDM2, inhibitor of MDM2-p53 interaction by targeting to p53, and inhibitors of MDM2 E3 ubiquitin ligase [39]. The binding sites and mechanism of action for these inhibitors are further illustrated in Figure 6.

Nutlin - cis-imidazoline; TDP - benzodiazepinedione; MI - spiro-oxindoles; PXN - isoquinolinone; HL198 - 5-deazaflavin; JNJ – 26854165 - tryptamine; RITA - thiophene; RING - Really Interesting New Gene (signature domain of E3 ligase). Binding of either HL198 or JNJ-26854165 to RING domain of MDM2 can block the interaction of ubiquitinated MDM2-p53 protein complex to the proteasome.

Nutlins, consisting of nutlin 1, 2 and 3, analogs of cis-imidazoline, fit in the binding pocket of p53 in MDM2 and inhibit the interaction between MDM2 and p53. Nutlin-3, an analog of the series, has the most potent binding capacity and lowest inhibition concentration, induced p53 levels, and activated p53 transcriptional activity. Nutlin-3 has been shown to exhibit a broad activity against various cancer models with wild-type p53 including cell lymphoma [40]. The major modes of inhibition of p53-mdm2 interaction by small molecules are outlined in Figure 7.

a) The p53 protein binds to Mdm2/x using a short helix with three hydrophobic residues Phe19 (orange), Trp23 (blue), and Leu26 (green) which fills the binding cleft.

b) Nutlin-2 is a close analogue of the most-studied Mdm2 inhibitor Nutlin-3.

c) Imidazole-indole compound WK23 in complex with Mdm2. WK23 possesses a 6-chloroindole group which is bound to Mdm2 in the same way as the Trp23 side chain of p53.

d) Benzodiazepinedione inhibitors utilize para-halogenated phenyl rings similar to those of the Nutlins. The Phe19 pocket is filled by the 7-iodobenzene ring.

e) A diastereomer of MI-63 positions the 6-chloroindole group in the Trp23 pocket. The Phe19 pocket interacts with the neopentyl group of the inhibitor and the 2-fluoro-3-chlorophenyl is situated in the Leu26 pocket.

f) Chromenotria-zolopyrimidines are also equipped with two halogenated phenyl rings that fill Trp23 and Leu26 pockets in a “Nutlin-like” fashion.
g) The imidazole-indole compound WW298 in complex with Mdmx. The 6-chloroindole group binds to Mdmx in the same way as the Trp23 side chain of p53 does. Note that in (c), (e), and (g) the 6-chloroindole group is used to bind in the Trp pocket, and that in (b), (d), and (f) a 4-halogenphenyl serves the same purpose [41].

![Diagram of molecular structures](image)

**Figure 7:** Low-molecular-weight inhibitors of p53–Mdm2/x binding (b-g, different categories for inhibition of interactions).

**Ceramide as Novel Messenger of Death**

Ceramides are a family of lipids that consist of sphingosine covalently linked to a fatty acid. Though ceramides were previously believed to be merely structural components of the cell membrane, discoveries over the last few decades reveal that virtually all stress stimuli (e.g., inflammatory mediators and oxidative stress) [42]. Three major pathways — de novo synthesis, sphingomyelin hydrolysis, and the salvage pathway — account for the production of ceramide within the cell [43] (Figure 8).

1. De novo ceramide synthesis depends on the availability of palmitate and serine
2. Sphingomyelin hydrolysis
3. Ceramide salvage both requires an initial supply of ceramide. Ss – Sphingosine; FFA - Free Fatty acid.
Recent studies revealed that another important ceramide binding protein, CERT, which specifically transports ceramide from the ER to the trans-Golgi for SM synthesis, plays a role in cancer drug resistance [44]. One of the well-described downstream targets of ceramide has been the protein phosphatases of the PP2A and PP1 family, also known as Ceramide-Activated Protein Phosphatases (CAPPs) [45]. PP2A is a tumor suppressor in cancer, and its activation regulates various downstream oncoproteins [46]. However, how ceramide mediates the activity of PP2A has been elusive. Most recently, ceramide was shown to directly bind to the oncoprotein SET/PP2A inhibitor 2 (I2PP2A) in A549 lung cancer cells. SET/I2PP2A is a nuclear protein and a known inhibitor of PP2A activity. Thus, this discovery gave insight into the possible mechanism by which ceramide regulates PP2A activity via binding to its biological inhibitor (SET/I2PP2A), which controls PP2A activity and its downstream targets, such as proto-oncogene c-Myc [47] thus describing a novel mechanism for regulating PP2A-dependent antiproliferative roles of ceramide (Figure 9).

In normal cells, ceramide and its binding protein, I2PP2A, which is the inhibitor for PP2A, are mostly in a 1:1 ratio. Therefore, it is believed to be the binding and inactivation of ceramide by I2PP2A that liberates the active form of PP2A, which, in turn, acts upon c-Myc, leading to the dephosphorylation and degradation. In cancer cells, elevated levels of I2PP2A were observed, which inhibits most of the available PP2A and results in stable (active) oncogenic c-Myc. The stable form of c-Myc can mediate tumor growth and cancer progression by upregulating expression of several oncogenes.I2PP2A: Protein phosphatase 2A inhibitor 2; PP2A: Protein phosphatase 2A [48].

Ceramide promotes apoptosis through the mitochondrial pathway, in part due to its effects on Bcl-2 family proteins [49]. Treatment of A549 lung adenocarcinoma cells with cell-permeable ceramide and/or agents that induce the synthesis of de novo ceramide downregulated Bcl-x (L) mRNA and protein levels and concomitantly increased Bcl-x(s) mRNA and protein [50]. This effect correlated with increased sensitivity of A549 cells to daunorubicin. Furthermore, A549 cells resistant to chemotherapeutic agents and cell-permeable ceramides demonstrated increased Bcl-x (L) levels. Others have reported that UV light-induced Bax activation and ensuing cytochrome C release and apoptosis, require the actions of A-SMase. Thus, in HeLa cells treated with siRNA against A-SMase or in A-SMase−/− cells from
NPD patients, UV light induction of Bax conformation change was drastically reduced. Further, restoration of A-SMase or addition of exogenous ceramide to A-SMase-deficient cells restored the UV pro-apoptotic response. These findings suggest that ceramide activates the intrinsic apoptotic pathway through its effects on Bcl-2 family proteins [51].

Apoptosis can also be activated through the extrinsic, or death receptor pathway (Figure 10). TNF receptor 1 and other members of the TNF family initiate this process when activated by ligand. Once activated, these receptors interact with an adaptor protein called FADD, leading to assembly of a protein complex that activates caspase-8, which in turn cleaves and activates the Bcl-2 family member, Bid. Bid then translocates to the mitochondrial outer membrane, initiating the intrinsic apoptotic pathway. Interestingly, Bid can also be cleaved by the lysosomal aspartate protease, cathepsin D. It was recently established that activation of cathepsin D by TNFα requires A-SMase activity. Further, ceramide was shown to bind directly to cathepsin D, causing autocatalytic proteolysis of the pre-pro-cathepsin D to form the enzymatically active isoforms of the enzyme, thereby implicating ceramide in regulation of Bid processing [52].

![Figure 10: Sphingolipid effects on intrinsic and extrinsic apoptotic pathways.](image)

The two pathways to apoptosis are shown. The extrinsic pathway begins by ligand binding to cell surface receptors such as FasR or TNFR, followed by Recruitment of Death Domain containing protein adaptors (e.g., TRADD) resulting in the formation of Death-Inducing Signaling Complex (DISC). This complex then activates the caspase cascade, culminating in cell death. The intrinsic or mitochondrial pathway begins when pro-apoptotic members of the Bcl-2 family cause mitochondrial release of cytochrome c, which binds to and activates Apaf-1 (apoptotic protease-activating factor), resulting in subsequent activation of the caspase cascade. Smac/DIABLO (Second mitochondria-derived activator of caspase/Direct Inhibitor Of Apoptosis-Protein with Low pI) is also released, inhibiting XIAP. XIAP (X-linked Inhibitor of Apoptosis Protein) functions as an inhibitor of caspases 3, 7 and 9. Shown are also the influence of ceramide, A-SMase and S1P on different components of the pathway.

**Antibody Drug Complexes as Target Therapy**

Although the last 50 years has seen remarkable progress in the prevention, detection and treatment of cancer, the most common methods (i.e., radiation, surgery and chemotherapy) often result in serious side effects. Additional deficits of current cancer therapies include non-specific systemic distribution, non-specific suppression of rapidly dividing cell types, inadequate drug concentrations at target tissues (i.e., tumors or cancerous cells), multi-drug resistance and a limited ability to monitor therapeutic responses [53].

An ideal anti-cancer therapeutic would be one that can be selectively concentrated in cancer cells while exerting minimal effects on normal tissues [54]. To achieve this, scientists are exploring biological molecules such as mAbs designed to target receptors on cancer cells or ligands relevant to cancer pathways that will facilitate delivery of cytotoxins, radioactive isotopes or chemotherapeutic drugs. The mAbs approved by the US Food and Drug Administration for cancer treatment is listed in (Table 1). The approach of conjugating bio-active anti-cancer molecules to mAbs has some limitations, e.g., low drug to mAb conjugation ratios. Increasingly, researchers are examining NMs to overcome some of the shortcomings of immunoconjugates [54].

<table>
<thead>
<tr>
<th>Year</th>
<th>International non-proprietary name/Trade name</th>
<th>Target</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Rituximab/Rituxan</td>
<td>CD20</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>1998</td>
<td>Trastuzumab/Herceptin</td>
<td>HER2</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>2001</td>
<td>Alemtuzumab/Campath</td>
<td>CD52</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>2002</td>
<td>Ibritumomab Tiuxetan/Zevalin</td>
<td>CD20</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>2003</td>
<td>Tositumomab/Bexxar</td>
<td>CD20</td>
<td>B-cell lymphoma</td>
</tr>
<tr>
<td>2004</td>
<td>Bevacizumab/Avastin</td>
<td>VEGF</td>
<td>Colon, lung, breast and renal cancer</td>
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<td>Cetuximab/Erbilux</td>
<td>EGFR</td>
<td>Colon; lung cancer</td>
</tr>
<tr>
<td>2006</td>
<td>Panitumumab/ Vectibix</td>
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<td>Colon cancer</td>
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<tr>
<td>2011</td>
<td>Ipilimumab/Yervoy</td>
<td>CTLA-4</td>
<td>Melanoma</td>
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**Table 1:** Monoclonal antibodies approved by the US food and drug administration for the treatment of cancer.
Gemtuzumab Ozogamicin

Gemtuzumab ozogamicin (GO, Mylotarg) (Figure 11) consists of a semisynthetic derivative of calicheamicin (N-acetyl-γ calicheamicin 1,2-dimethyl hydrazine dichloride), a potent enediyne DNA-binding cytotoxic antibiotic, linked to an engineered humanized monoclonal IgG4 antibody (hP67.6) directed against the CD33 antigen present on leukemic myeloblasts in most patients with AML (80%). IgG4 has interesting properties for a carrier. It has the longest circulating half-life of all isotypes, with limited ability for complement fixation and antibody-dependent cellular toxicity. The unconjugated antibody hP67.6 is not known to be cytotoxic.

The cytotoxic drug is attached to the antibody through a covalent linkage (condensation) of a bifunctional linker, 4-(4-acetylphenoxy) butanoic acid (AcBut linker), which allows stability in physiologic buffers (pH 7.4) and efficient calicheamicin release inside lysosomes (pH 4). The average loading of calicheamicin on the antibody is 2.5 mol/mol (drug-loading range of 2–3 mol of calicheamicin per mole of antibody). Calicheamicin binds to the minor groove in the DNA and causes double-strand DNA breaks, resulting in cell death. The custom-made, well-controlled, hydrolysable bond with the AcBut linker showed significantly more potent and selective calicheamicin conjugates of P67.6 against HL-60 cells in vitro. This effective intracellular hydrolytic release of the calicheamicin derivative was important for its intracellular trafficking and the subsequent access to its DNA target [55]. This indicates the importance of designing linkers that are specific for the individual target cell type [56].

Targeting HER2

Human Epidermal Growth Factor Receptor 2 (HER2), also known as ErbB2, c-erbB2 or HER2/neu, is a 185 kDa protein (p185) with an intracellular tyrosine kinase domain and an extracellular ligand binding domain. In humans, HER family includes four structurally related members, HER1 (ErbB1, also known as EGFR), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). Although HER2 is the only receptor which has no identified ligand, it is the preferred partner to form heterodimer with other HER members. HER2 involved heterodimerization is the most potent signal transduction pathway among all dimmers formed by the HER family [57]. HER2 plays important roles in cell growth, survival, and differentiation in a complex manner. The major signaling pathways mediated by HER2 involve mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3-kinase (PI3K) pathway. As a key gene for cell survival, HER2 gene amplification and protein overexpression lead to malignant transformation [58]. It directly associates with poor clinical outcomes in breast, ovarian, gastric, and prostate and other cancers.

Signaling occurs through both homo- and heterodimeric HER complexes (Figure 12) and can induce cell proliferation, motility, and invasion. Dysregulated expression and activity of HER-family members is prevalent in human neoplasia [59]. Strikingly, up to 30% of
breast carcinomas overexpress HER2, frequently as a consequence of genomic amplification of a region of the long arm of chromosome 17 (17q21) that includes the HER2 locus. HER2 overexpression may be more frequent in ER-negative than ER-positive cancers, drives aggressive disease, and thus represents an important therapeutic target. The humanized monoclonal antibody trastuzumab (Herceptin) was the first agent developed for HER2 targeting and has dramatically improved outcomes among women with HER2-positive (defined by HER2 overexpression and/or amplification) breast cancer [60].

A - Potential clinical preventive applications of lapatinib (along with known applications of trastuzumab, SERMs, and AIs). The major circle segments reflect HER2-positive cancer (violet; ~20% of total disease) and HER2-negative cancer (sky blue; ~80% of total disease). Divisions within segments reflect the rough proportions of HR (ER, PR)-positive and -negative disease, which, respectively, are 50%/50% in HER2-positive cancer and 75%/25% in HER2-negative cancer (the rough proportions in all cancers are 70% HR-positive and 30% HR-negative). Doubled blocking lines indicate settings of greater HER-targeting drug effect.

B - Signaling pathways involved in HER-targeting agent effects. MAPKs, mitogen-activated protein kinases; JNK, c-JUN N-terminal kinase; mTOR, mammalian target of rapamycin; S6K, p70 S6 ribosomal kinase; 4EBP1, 4E-binding protein 1; TF, tissue factor.

Conclusion

One of the major areas in cancer research is targeted delivery of drugs to cancerous cells, which can not only increase the therapeutic efficacy but also reduce the adverse side effects of the drugs [61,62]. Drugs or treatment strategies that can restore the apoptotic signaling pathways towards normality have the potential to eliminate cancer cells, which depend on these defects to stay alive. Many recent and important discoveries have opened new doors into potential new classes of anticancer drugs. The first report of p53 gene therapy in 1996 investigated the use of a wild-type p53 gene containing retroviral vector injected into tumor cells of non-small cell lung carcinoma derived from patients and showed that the use of p53-based gene therapy may be feasible [63]. As the use of the p53 gene alone was not enough to eliminate all tumor cells, later studies have investigated the use of p53 gene therapy concurrently with other anticancer strategies. For example, the introduction of wild-type p53 gene has been shown to sensitize tumor cells of head and neck, colorectal and prostate cancers and glioma to ionizing radiation [64]. Cancer treatment is entering new chapter in targeted therapies and personalized medicine due to the advance of molecular biology and medicinal chemistry. Most likely several compounds from this review will be approved for clinical application. Although the reports to date suggest that substantial potential exists for mAb combinations as future cancer therapies, there are numerous logistical hurdles that must be overcome. The mAbs therapies currently marketed are costly in part because of the large investment necessary for their development. Despite the challenges, innovative ways of combining biologic therapies...
are emerging. The ability to produce recombinant “polyclonal-like” antibodies is one mechanism for producing complex mixtures of antibodies to treat complex diseases. Research designed to deliver combinatorial targeted therapies appear to be moving rapidly toward multi-specific antibody-like therapeutics, for example, bispecific antibodies have been reported for some time [65,66]. The future design of potential drug combination therapies and the follow-up of their outcome will undoubtedly be facilitated by gene profilings. As the clinical trials of these inhibitors progress, more efforts should be directed to further unravel the complex biology and genetics, and the crosstalk signals of the cancer cells. Many questions remain to be answered:

(1) What are the long-term safety and toxicities of these inhibitors?
(2) How to use biomarkers to select patients who will benefit most from these inhibitors?
(3) How to combine these targeted therapies with cytotoxic agents or other treatment modality such as radiation?

References

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