REVIEW

Personalized medicine of non-gene-specific chemotherapies for non-small cell lung cancer

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Abstract  Non-small cell lung cancer is recognized as the deadliest cancer across the globe. In some areas, it is more common in women than even breast and cervical cancer. Its rise, vaulted by smoking habits and increasing air pollution, has garnered much attention and resource in the medical field. The first lung cancer treatments were developed more than half a century ago. Unfortunately, many of the earlier chemotherapies often did more harm than good, especially when they were used to treat genetically unsuitable patients. With the introduction of personalized medicine, physicians are increasingly aware of when, how, and in whom, to use certain anti-cancer agents. Drugs such as tyrosine kinase inhibitors, anaplastic lymphoma kinase inhibitors, and monoclonal antibodies possess limited utility because they target specific oncogenic mutations, but other drugs that target mechanisms universal to all cancers do not. In this review, we discuss many of these non-oncogene-targeting anti-cancer agents including DNA replication inhibitors (i.e., alkylating agents and topoisomerase inhibitors) and cytoskeletal function inhibitors to highlight their application in the setting of personalized medicine as well as their limitations and resistance factors.

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1. Introduction

Non-small cell lung cancer (NSCLC), because of its unrivaled prevalence and lethality, is by many measures the most urgent cancer-related challenge facing modern medicine. Not only is it already the most common type of cancer world-wide, increasing amounts of pollution and unhealthy life choices further drive its incidence rate upwards. It is now estimated that NSCLC accounts for over a quarter of all cancer-related deaths in the United States with an additional 1.8 million new known cases reported worldwide on an annual basis. Because of this, there has been a plethora of research into the deadly affliction with massive resources devoted to haling its advance. As these efforts began to mature and bear fruit, we saw the first chemotherapies developed for combating NSCLC come into use over half a century ago. Unfortunately, these early agents often elicited very low response rates, significant toxicity, and finally, great chance for relapse. Given these traditional “blind” treatments, patients generally did not survive past the first year after diagnosis. Without better options and out of desperation, patients and doctors came to accept that chemotherapy was a double-edged sword in the treatment of NSCLC.

The introduction of personalized medicine was a milestone for cancer treatment as incorporating knowledge of genetic factors into determining patient-specific regimens led to astounding improvements in treatment outcome. High failure rates of oncogene-targeting chemotherapies such as tyrosine kinase inhibitors and anaplastic lymphoma kinase inhibitors have been attributed to the treatments being mismatched to patients whose cancer lacked the drug’s genetic targets. However, the status of specific oncogenic mutations does not constrain the use of other drugs that target mechanisms common to all cancers, such as DNA replication inhibitors and anaplastic lymphoma kinase inhibitors. But their lack of specialized oncogenic targets, these chemotherapies are not entirely free from the effects of genetic factors and resistance mechanisms. Sensitivity to these drugs—and thus their effectiveness—depends on individual factors, including capability to metabolize specific substances and differences in genetic physiology resulting in varying expression levels of efflux pumps and stabilizer proteins. In this review, we discuss these factors and highlight the necessity of pharmacogenomic consideration in the decision-making process for physicians when prescribing non-oncogene-targeting chemotherapies for treatment of NSCLC patients.

2. Alkylating agents

DNA replication inhibitors are a diverse category of many smaller classes of drugs aimed at preventing ravenous tumor growth by interrupting the replication of DNA. Of these, alkylating agents, topoisomerase inhibitors, antimetabolites, are among the different approaches used by various chemotherapies aimed at preventing the critical DNA replication needed for (cancerous) cell division. From as early as the nineties, gemcitabine and cisplatin were used as anti-cancer compounds for the clinical treatment of NSCLC. Although gemcitabine is a topoisomerase I inhibitor instead of an alkylating agent, its discussion could arguably be made out of turn together with the alkylating agent cisplatin because the two are most often used in combination and as such, many studies apply to them both rather than individually. Because both gemcitabine and cisplatin are mainly metabolized independently of the cytochrome P450 system (cytidine deaminase for gemcitabine and cysteine S-conjugate β-lyase for cisplatin), they are excellent choices for combination therapy to avoid excessive stress on the typical hepatic enzymes that metabolize anti-cancer chemotherapies. Gemcitabine works by being incorporated into the DNA in place of its normal base pairs, preventing DNA polymerase from synthesizing the entire chain while cisplatin binds to DNA to cause DNA-lesions which trigger the DNA damage response leading to apoptosis. Loosely classified as a platinum-containing alkylating agent, cisplatin undergoes hydrolysis in the cytoplasm and binds to the N7 reactive center on purines in order to crosslink DNA. Due to its toxicity, derivatives of cisplatin exist, such as carboplatin which exhibit both reduced effect and toxicity compared to cisplatin. Gemcitabine, while also a DNA replication inhibitor, is instead an antimetabolite which disables topoisomerase I. A prodrug dependent on activation by deoxycytidine kinase, gemcitabine allows only one additional dNTP to be added after itself before replication stalls and DNA proofreading enzymes are unable to remove it from the replication complex.

Because DNA replication-inhibitors form DNA-level interactions as opposed to the protein-level interactions, resistance mechanisms are far different. Due to the lack of protein interaction, mutations causing conformational changes to the adenosine triphosphate (ATP) binding sites of target proteins were not identified as modifiers of drug effectiveness. With drugs that target DNA replication, the genetic components of resistance usually represent increased effectiveness or upregulation of the DNA-replication machinery, which more closely resemble intrinsic characteristics of individuals rather than definable mutations. DNA excision repair protein 1 (encoded by the ERCC1 gene) is a protein that forms a critical component of the DNA-repair/maintenance machinery whose natural activity level is implicated as an intrinsic factor affecting drug efficacy in both gemcitabine and cisplatin. Ribonucleoside-diphosphate reductase large subunit 1 (encoded by the RRM1 gene), which is identified as a tumor suppressor but is also critical in deoxyribonucleotide-synthesis prior to DNA replication, is another protein whose high expression is known to blunt the treatment effects of gemcitabine and cisplatin. Ceppi’s study showed that in a cohort of 156 NSCLC patients, not only did RRM1 and ERCC1 expression levels exhibit a substantial degree of correlation to each other (Pearson’s Rho = 0.624, P < 1 × 10^-4), their combined levels of expression were inversely correlated to patient survival time when treated with gemcitabine and/or cisplatin in that treated patients with low expression survived an average of 14.9 months vs. 10.0 months (P = 0.0345) for those with high expression. Between the two genes, ERCC1 seemed to hold more predictive power than RRM1 in that the difference in post treatment survival time between high expressers and low expressers was markedly greater when comparing ERCC1 (17.3 months in low expressers vs. 10.9 months in high expressers, P = 0.0032) than when comparing RRM1 (13.9 months vs. 10.9 months, P = 0.039). Furthermore, of the two drugs, cisplatin seemed to be more severely impacted by ERCC1 expression levels as the difference in post treatment survival between high expressers and low expressers was 23.0 months vs. 12.4 months (P = 1.0 × 10^-7). In addition to these, expression of several oncogenes as well as the tumor suppressor gene P53 and topoisomerase II alpha (TOP2A) may also affect cisplatin activity. These findings suggest that quantification of the mRNA of certain genes such as RRM1 and ERCC1 to determine their expression levels could yield valuable insight to...
developing a personalized treatment plan when considering DNA-replication targeting anti-tumor drugs such as gemcitabine and cisplatin. Other proteins that are involved with DNA replication that also play a role in treatment resistance include DNA polymerase kappa (encoded by the POLK gene) and ubiquitin-conjugating enzyme E2B (encoded by the UBE2B gene). While POLK is involved in creating a replication complex that can bypass the crosslinking disruption caused by cisplatin binding, UBE2B facilitates DNA synthesis across lesions triggered by DNA damage response27-29. Compromised mechanisms of uptake can also lead to individuals exhibiting resistance to treatment. Copper transport protein CTR1 (encoded by the SLC31A1 gene) is necessary for the uptake of cisplatin and its disruption or deficiency may lead to phenotypic resistance to cisplatin due to metabolic inability. Furthermore, transmembrane protein 205 (encoded by the TMEM205 gene) and associated vesicular trafficking RAS-related protein 8 (encoded by the RAB8 gene) were demonstrated by Shen et al.26 to confer cisplatin resistance during overexpression. Although the precise mechanism is unconfirmed, it is hypothesized that these proteins play a role in trafficking and localization of cisplatin. It was also found that certain cell survival/replication pathways such as nuclear factor kappa B (NF-kB) and AKT/P38 can defend tumor cells from entering apoptosis, causing resistance in some cases to gemitabine27,28.

Ifofsamide is another alkylating agent which was approved roughly three decades ago as a compound that is useful in treating NSCLC. As a produg that requires processing into its active form by cytochrome P450 enzymes, the mechanism of action is complex and not well-understood29. A study by Wang et al.30 found that in hepatoma-affected mice, ifosfamide treatment greatly caused a reduction in thioredoxin reductase, an enzyme that is often hyper-expressed in malignant tumor cells. Although the exact role(s) and mechanism(s) of the thioredoxin family are poorly-understood, the proteins are believed to be involved in defense of DNA against reactive oxygen species induced damage and are critically important in development as mouse knock-out models show embryonic lethality31. The study by Wang et al.30 found that ifosfamide reduced thioredoxin activity by 62% in solid tumors but most interestingly, those treated cells could no longer form new tumors when injected into other mice, suggesting a permanent or lasting alteration. Although the evidence shown would seem to suggest that ifosfamide achieves its anti-cancer effects as an antagonist to the critical thioredoxin pathway, the group never tested the effectiveness of ifosfamide when treating mouse models that are either externally-supplemented with thioredoxin reductase or with the TXNRD gene in hyper-expression to determine whether ifosfamide achieves its effects dependent on antagonizing thioredoxin reductase or if the down-regulation is an unrelated effect. From the evidence present, there are at least two genetic factors to account for when considering ifosfamide as treatment for NSCLC: patient levels of P450 enzymes as well as natural expression levels of thioredoxin reductase. Judging the effectiveness and determining the dosage of ifosfamide usage in patients from a personalized medicine point of view can be significantly complicated due to the relative lack of precise pharmacogenomic information applicable to this drug.

An interesting chemotherapy that is largely physiologically regulated is cyclophosphamide. This nitrogen mustard alkylating agent is administered as a produg which is metabolized into its active form, phosphoramide mustard, which then crosslinks DNA strands at the guanine N7 positions in order to disrupt DNA replication32. However, this process can be disrupted by an enzyme called aldehyde dehydrogenase (encoded by the ALDH gene), by converting cyclophosphamide into carboxycyclophosphamide, which loses its ability to convert into active phosphoramide mustard.33-35. Because this process only occurs in cells with low expression levels of ALDH, cyclophosphamide is both limited in use and low in overall toxicity as many of the traditional tissues that are vulnerable to the side-effects of chemotherapy exhibit high expression of ALDH. Therefore, patients presenting with naturally high expression of the ALDH gene will exhibit resistance to treatment with cyclophosphamide33,34. Similarly, it is known that due to the ability of glutathione S-transferase (GST) to facilitate the conjugation of glutathione with alkylating agents to inactivate them, it may facilitate resistance against all alkylating agents in individuals with hyperexpression of the enzyme and/or high levels of glutathione35. However, although dependent on biosynthesis pathways within the body, glutathione can also be influenced by dietary intake of its critical building blocks such as cysteine, glycine, alpha lipoic acid, vitamin D3, etc.36.

3. Topoisomerase inhibitors

In the non-oncogene-targeting chemotherapy field, botanically-derived compounds have played a significant role. One of the most impactful groups is the topoisomerase I inhibitors. Camptothecin is a cytotoxic alkaloid derived from the stem of the Camptotheca acuminata tree, which gave rise to the drug irinotecan.37. Taken as a produg, irinotecan is dependent on carboxylesterase for hydrolysis into its active metabolite form, SN-38. SN-38 attaches to the guanine base pairs surrounding the topoisomerase I cleavage complex and inactivates the topoisomerase I enzyme complex.37,38. Finally, SN-38 is glucuronidated by UDP-glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1) during inactivation37,38. It is commonly used in combination with cisplatin specifically for the treatment of NSCLC. Although little is known regarding the mechanisms of resistance to Irinotecan specifically and directly in regard to its treatment of NSCLC patients, several have been identified during the treatment of other types of cancer which may bear consequence to NSCLC patients as well. Metallothioneins (normally critical for metal storage and trafficking) and epidermal growth factor receptor (encoded by the EGFR gene) expression (both independently and through the Src signaling pathway) have been shown to interact with irinotecan in a self-inhibitory loop as their expression is upregulated by irinotecan and they seem to inhibit irinotecan activity in high concentrations39-40. The MAPK14/P38α pathway activates survival autophagy within the cell in response to Irinotecan to resist apoptosis41. Although the precise mechanism is unknown, it has been observed that overexpression of the heat shock protein 27 (encoded by the HSP27 gene) can cause resistance to irinotecan treatment, which can be reversed by antisense silencing of the HSP27 gene (in vitro)42. Clinically, patients with naturally higher expression of HSP27 tend to be non-responders to irinotecan treatment42. Finally, efflux pumps such as ATP-binding cassette subfamily (ABCC4 and ABCG2) have been shown to confer resistance to irinotecan in patients with high expression43-44.

An alternate semi-synthetic derivative of camptothecin is topotecan, which is commonly given with paclitaxel to treat advanced stage NSCLC. Topotecan binds the topoisomerase I enzyme complex to the lesion complex arresting it there and preventing the re-ligation of the DNA strand45-48. The
overabundance of arrested topoisomerase I complexes produces an apoptotic effect and eventually, their collision with the replication fork fragments the DNA. Known factors of genetic resistance to topotecan rely heavily on efflux pump expression. ATP-binding cassette subfamilies ABCB1, ABCC4, ABCG2 efflux pumps have all been implicated in topotecan resistance across multiple types of cancers treated but the resistance conferred by some can be countered by other drugs such as ritavatinib.

Etoposide is another chemotherapy approved for use in treating NSCLC but commonly presents with serious side-effects. This compound forms a bond with both the DNA and topoisomerase II enzyme complex, preventing re-ligation of the DNA and causing apoptosis-inducing breakage in a similar fashion to the mechanism of camptothecin-derived chemotherapies. Resistance generally comes in two forms: either by acquired activation of cell survival factors or by efflux pumps. In patients with NSCLC, it was observed that acquired resistance to etoposide can be caused by upregulation of DNA polymerase beta (encoded by the POLB gene) which is responsible for DNA excision-based repair and neuroendocrine transcription factor NK2 homeobox 2 (encoded by the NKX2-2 gene), both of which reduce apoptotic response in cancer cells. It was also seen in NSCLC cells that caveloae protein (encoded by the CAV1 gene) hyperexpression can promote the RAS–ERK cell cycle progression pathway, causing resistance to etoposide. Perhaps one of the most interesting finds regarding etoposide resistance is that the R428W mutation in the P53 gene produces an aberrant protein that transcriptionally activates the tyrosyl-DNA phosphodiesterase 2 (TDP2) DNA repair gene, causing resistance to etoposide. Efflux pump-induced resistance to etoposide is modulated by the ABCB1, major vault protein (MVP), and ABCG2 genes. Capicua transcriptional repressor 3 (CIC-3), a member of the CIC family, is an important component for drug sequestration as it regulates vesicular acidity; over-expression appears to correlate with etoposide resistance, possibly due to accelerated breakdown of the compound through upregulating P-glycoprotein expression. Hyperexpression of WNT5A and DKK1 in the WNT signaling pathway have also been correlated with etoposide resistance although curiously, WNT3A seems to correlate to sensitivity, which suggests that the regulation of cell cycle progression through the WNT pathway plays a role on the effects of etoposide. The scientific picture suggested by these studies shows a rather complex and diverse web of mechanisms that are likely to produce a cumulative effect on etoposide resistance.

Another chemotherapy that targets topoisomerase II is doxorubicin, which is an anthracycline that intercalates DNA, freezing the topoisomerase II complex to prevent re-ligation and reformation of the double helix. An additional effect that is unique to doxorubicin, however, is the occurrence of histone eviction from the duplicating chromatin, causing a myriad of issues for DNA repair genes that ultimately increase the final likelihood of apoptosis. Common efflux pumps ABCB1, ABCB8 and ABCG2 (with a likely mutational hotspot discovered at R482 in ABCG2 that increases anthracycline efflux at the expense of Topotecan efflux), contribute to doxorubicin resistance though they may be surmountable by other chemotherapies like bafetinib. Inactivating mutations in the PTEN tumor suppressor gene that drive AKT activation have been observed to cause resistance to doxorubicin although it may be reversed by treatment with berberine and/or rapamycin.

4. Cytoskeleton-targeting

Due to the complexity of the mitotic process that cancerous growth depends upon, there are multiple treatment targets to exploit, and with them, possibilities for future development. In case of failure of or developed resistance against DNA-targeting therapy, cytoskeletal-targeting drugs present another opportunity. Traditionally targeting tubulin, these drugs are isolated from various plants and work by preventing the cell from completing/entering mitosis through undermining the preparations of the crucial cytoskeleton. The pioneering drugs of the cytoskeleton-disrupting class are the vinca alkaloids derived from the Madagascar periwinkle (Catharanthus roseus). Vinblastine and vincristine are close chemical derivatives of each other differing in only one group (methyl for the former and aldehyde for the latter). Vinca alkaloids inhibit cellular proliferation by binding to tubulin and hindering the activities of the mitotic spindle in lower concentrations, though in higher concentrations, the entire microtubule can be destabilized. As is typically seen in anti-cancer chemotherapies, they are metabolized by CYP3A4 and CYP3A5 with the former being generally more critical for vinblastine and the latter for vincristine. The use of these vinca alkaloids for clinical treatment has been approved by the U.S. department of agriculture (USDA) as far back as 1963 due to their ability to prolong life in cancer patients however, they quickly evolved for use primarily in combination therapies (often with DNA-replication inhibitors) for NSCLC patients. Of the issues that have arisen from the use of vincristine and vinblastine, among the most prominent are, unsurprisingly, cases of drug resistance and excess toxicity in patients who are genetically lacking in their the CYP3A family enzymes. Reports of CYP3A5-deficient Japanese, Kenyan and Turkish patients suffering symptoms of excess drug toxicity from standard dosages of vincristine underline the importance of pharmacogenomic considerations even when using chemotherapies with universal targets and the rate of occurrence in various populations of patients highlights the need for population studies to improve the precision of physician guidelines. Interestingly, the over-expression of HSP27 is linked to vincristine resistance. HSP27 is known to inhibit caspase activation, which not only modulates apoptotic pathways but also tubulin clearance. In addition, the HSP27 protein has been shown to associate directly with tubulin and microtubules, strongly suggesting that the exact mechanism of HSP27-mediated resistance is likely to be universal to all cancer types. In many cases where the cause of resistance is not understood, the mechanisms to circumvent resistance commonly default on the use of chemicals to aid the mechanisms of the vinca alkaloids or to increase their cytotoxicity, in essence striking a blow to the patient’s resistance mechanisms rather than bypassing them. However, when the gene is identified, anti-strand oligonucleotides may hold value in their ability to down-regulate or shut off the gene. Additional causes of resistance to the microtubule-stabilizing vinca alkaloids can naturally be seen in genes that modulate microtubule formation. Microtubule-associated protein 4 (encoded by the MAP4 gene) is a microtubule stabilizing protein and variation in its expression levels have been shown to correlate inversely with sensitivity to general vinca alkaid treatment in different cell lines. On the other hand, mutations in the β-tubulin (TUBB) gene (such as L240H) have been demonstrated specific resistance to vincristine. Interestingly, WNT5A has shown that it can modulate vincristine sensitivity in much the same pathway as it did Etoposide although with the
inclusion of glycogen synthase (encoded by the GSK3β gene)107. And finally, vincristine and vinblastine are also affected by efflux pumps ABCC1 and ABCB1, respectively but new agents like erdafitinib can ameliorate the resistance conferred108,109.

Taxoids are another family of botanical tubulin-targeting compounds which consist of paclitaxel and docetaxel. Derived from the bark of the Pacific Yew tree (Taxus brevifolia) and approved by the USDA for chemotherapy in 1993, paclitaxel targets tubulin just like the vinca alkaloids but unlike vincristine and vinblastine which destabilize and deconstruct the microtubule, the taxoids bind to microtubules to induce a hyper-stable conformation preventing them from forming the mitotic spindle needed during metaphase105. An advantage of paclitaxel is that in addition to being metabolized by the CYP3A family of enzymes like the great majority of the compounds mentioned here, it can also be metabolized by the CYP2C8 enzyme, reducing metabolic stress when used in combination therapy with other drugs, (which often rely solely on the function of CYP3A family)106. Because paclitaxel tends to result in modest improvement and low response rate, it is usually used in combination with other treatments101,102. Resistance mechanisms for paclitaxel vary. It seems that several mutations in the TUBB gene are responsible for paclitaxel irresponsiveness most likely due to alterations of the binding site/binding pocket that diminish binding affinity, however, in addition to these mutations, it has been shown that just as in DNA-replication targeted therapies, high expression of β-tubulin also leads to low response rate103–105. Specific mutations in the TUBB gene such as A185T, A248V, R306C confer resistance to paclitaxel through natural low microtubule stability106. To add to this, mitotic centromere-associated kinesin (encoded by the MCAK gene) is a microtubule depolymerizing protein that is known to confer paclitaxel resistance when abundantly expressed107,108.

Tumor suppressor Parkin confers sensitivity to the microtubule hyper-stabilizing paclitaxel via TUBB and MAP4, previously shown to increase resistance to microtubule destabilizing vinca alkaloids109. In addition to the expected interactions with efflux pumps (in this case ABCB1, ABCB4, ABCCC2, and ABCC3), paclitaxel resistance was also seen in scenarios of increased expression of the Bcl-2 apoptotic regulator gene110–112. These studies suggest that sequencing for possible conformational mutations in tubulin and mRNA quantitative analysis of β-tubulin expression can be an effective way of choosing whether to use a vinca alkaloid or a taxoid like paclitaxel when treating cancer patients.

Docetaxel is a European Yew (Taxus baccata) isolated derivative of paclitaxel which enters the cell more readily and exhibits a greater retention time than Paclitaxel, allowing the dosage to be decreased113. Intracellularly, docetaxel binds reversibly to tubulin to facilitate hyper-stabilized microtubules that cannot disperse, resulting in apoptosis-inducing accumulation in the cytoplasm114,115. Because clinical use of docetaxel to treat NSCLC generally yields very low response rate, docetaxel is preferably utilized in combination therapy when used to treat NSCLC116. However, because metabolism depends on CYP3A4/5, careful consideration is required in drug pairing117. When combined with other chemotherapies such as cisplatin, the response rate would substantially (from ~30% to ~70%) improve in NSCLC patients as compared to docetaxel treatment alone118. Much of docetaxel’s functions also depend on resistance mechanisms such as expression levels of drug efflux pumps (ABCB1, ABCC10 which are surmountable by ibritinib), kinesins, and tumor suppressor gene P27107–111. While overexpression of the ABCB1 gene and kinesins are known to be detrimental to docetaxel efficacy, the inverse is true for PTEN (mechanism through P27)108–110. However, since P27 gene expression fluctuations in both directions have been reported to correlate with resistance to several chemotherapies without obvious connections to each other, the existence of a true causative relationship is questionable. As a direct challenge to the targeted molecular effects of taxoids, the forkhead box M1 protein (encoded by the FOXM1 gene) is capable of activating stathmin (encoded by the STMN1 gene), a protein whose role is destabilization of the microtubules, and as such, capable of conferring resistance to both docetaxel and paclitaxel112,113. As one would expect that due the universal mechanism of the taxoids, the resistance factors should be very similar as well, but surprisingly, the resistance mechanisms seen in docetaxel seem to be more widespread and scattered. Multiple outstanding factors have been identified in docetaxel resistance both in acquired and natural form. The NF-κB survival pathway as well as the interleukin 6 (encoded by the IL-6 gene) inflammatory regulators can be activated by docetaxel treatment and interfere with successful chemotherapy114. Hyper-expression of both the ribophorin II (encoded by the RPN2 gene) (role in peptide synthesis) and malate dehydrogenase 2 (encoded by the MDH2 gene) (role in citric acid cycle regulation) have shown to correlate to docetaxel resistance by unknown mechanisms115,116. Even symptoms as broad as aberrant methylation patterns which are commonly seen in cancer tissues may adversely affect docetaxel treatment success117. Extraordinarily, high levels of thioredoxins exhibited pretreatment can be correlated to docetaxel resistance, however, increases in response to chemotherapy do not yield the same118,119. This stresses the importance of establishing causation rather than accepting correlation when determining mechanisms of resistance as in this case, it is very likely that thioredoxin activity itself does not hinder the mechanism of docetaxel. Rather, it is some other factor possibly upstream that correlates to initially higher levels of thioredoxin but unlike thioredoxin, is not affected by docetaxel treatment.

5. Discussion

Although the application of personalized medicine to NSCLC has traditionally been focused on therapies designed to engage specific oncogenic mutations, pharmacogenomic considerations are not lost on other therapies that target the common themes of all cancers. However, unlike chemotherapies targeting specific driver mutations where most of the pharmacogenomic considerations go into whether a susceptible mutation is present for targeting, these non-oncogene-targeting chemotherapies are far more universal in their use. Therefore, most of the pharmacogenomic considerations that pertain to them will be avoidance of resistance factors that make them less effective or ineffective. Every patient is a unique individual to be especially resilient to the mechanisms of certain drugs. This unique personal genetic web that encompasses the tumor microenvironment is made of many components such as angiogenic factors, the various cells of the tumor stroma,
| Drug name        | Drug class               | Metabolic pathway                          | Mechanism of action                                                                 | Resistance                                      |
|------------------|--------------------------|--------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------|
| Cisplatin        | Alkylating agent         | Cysteine S-conjugate β-lyase               | Binds tN7 reactive center on purine to crosslink DNA, creating lesions that trigger apoptotic pathway | \textit{ERCC1, RRM1, POLK, UBE2B, RAB8, SLC31A1, TMEM205, TOP2A, RPN2} TXNRD family |
| Ifosfamide       | Alkylating agent         | P450 family                               | (?) Reduction of thioredoxin reductase, likely weakening defense against ROS damage DNA replication | \textit{ALDH, GST}                               |
| Cyclophosphamide | Alkylating agent         | CYP2B6, CYP2C9 and CYP3A4                  | Crosslinks DNA strands at the guanine N7 positions to disrupt DNA replication         | \textit{ERCC1, RRM1, NF-κB, EGFR, MAPK14/P38α, HSP27, ABCC4, ABCG2}                      |
| Irinotecan       | Topoisomerase I inhibitor| Carboxylesterase, UGT1A1, CYP3A4, CYP3A5   | Attaches to the guanine base pairs surrounding the topoisomerase I cleavage complex and inactivates topoisomerase I enzyme complex | Metallothioneins, \textit{EGFR, MAPK14/P38α, HSP27, ABCC4, ABCG2}                      |
| Topotecan        | Topoisomerase I inhibitor| P450 family                               | Binds topoisomerase I enzyme complex to the lesion complex preventing the re-ligation of the DNA strand; overabundance of arrested topoisomerase I complexes produces apoptotic effect and collision with the replication fork fragments the DNA | \textit{ABCB1, ABCC4, ABCG2}                   |
| Gemcitabine      | Topoisomerase I inhibitor| Cytidine deaminase                        | Incorporates into DNA in place of dNTP and stalls replication; DNA proofreading enzymes are unable to remove locked topoisomerase I from the replication complex | \textit{ERCC1, RRM1, NF-κB, AKT/P13K}              |
| Etoposide        | Topoisomerase II inhibitor| CYP3A4, CYP3A5, CYP1A2                    | Bonds DNA and topoisomerase II enzyme complex, preventing re-ligation of the DNA and causing apoptosis-inducing breakage | \textit{POLB, NKK2.2, CAV1, P53; p.R428W, TDP2, ABCB1, MVP, ABCG2, CIC-3, WNT5A, DKK1} |
| Doxorubicin      | Topoisomerase II inhibitor| CYP3A4, CYP2B6, CYP1B1                     | Intercalates DNA, freezing topoisomerase II complex to prevent re-ligation and reformation of double helix; causes histone eviction | \textit{ABCB1, ABCB8, ABCG2, P1EN, MVP, AKT}                                          |
| Vincristine      | Microtubule destabilizer | CYP3A4, CYP3A5                            | Binds tubulin to prevent dimerization into microtubules; caused microtubule detachment | \textit{HSP27, MAP4, TUBB;p.L240I, WNT5A, GSK3β, ABCC1, ABCB1, MVP MAP4, ABCC1, ABCB1} |
| Vinblastine      | Microtubule destabilizer | CYP3A4, CYP3A5                            | Binds tubulin to prevent dimerization into microtubules; caused microtubule detachment | \textit{ABCB1, ABCC3, ABCB4, ABCG2, BCL-2, MVP}                                        |
| Paclitaxel       | Microtubule hyperstabilizer | CYP2C8, CYP3A4                           | Binds tubulin, preventing disassembly; causes apoptosis by stopping mitosis and causing microtubule accumulation | \textit{TUBB;p.A185T, A248V, R306C, Kinesins family, ABCB1, ABCB3, ABCG4, ABCG2, BCL-2, MVP} |
| Docetaxel        | Microtubule hyperstabilizer | CYP3A4, CYP3A5                       | Binds tubulin, preventing disassembly; causes apoptosis by stopping mitosis and causing microtubule accumulation | \textit{ABCB1, ABCC10, kinesins, P27, FOXM1, STTM1, NF-κB, IL-6, RPN2, MDH2, MVP, P1EN;p.Y68fs} |

Summary of drug class, mechanism description, metabolic pathway, and genes/mutations to consider when determining usage and/or dose.
Figure 1  The types of genetic resistance factors within each patient and the available chemotherapies choices at the physician’s disposal that are appropriate for use.

Table 2  Mechanisms of resistance against non-oncogene-targeting chemotherapies.

| Gene      | Mechanism of resistance                      | Affected drug                                                                 |
|-----------|-----------------------------------------------|-------------------------------------------------------------------------------|
| ABCB1     | Efflux pump                                   | Topotecan, etoposide, vincristine, vinblastine, paclitaxel, docetaxel, doxorubicin |
| ABCB4     | Efflux pump                                   | Paclitaxel                                                                    |
| ABCB8     | Efflux pump                                   | Doxorubicin                                                                   |
| ABCCC1    | Efflux pump                                   | Vincristine, vinblastine                                                      |
| ABCCC2    | Efflux pump                                   | Paclitaxel                                                                    |
| ABCCC3    | Efflux pump                                   | Paclitaxel                                                                    |
| ABCCC4    | Efflux pump                                   | Irinotecan, topotecan                                                        |
| ABCCC10   | Efflux pump                                   | Docetaxel                                                                     |
| ABCG2     | Efflux pump                                   | Irinotecan, topotecan, etoposide, doxorubicin                                 |
| GSK3β     | Down-regulates efflux pump ABCB1              | Vincristine                                                                   |
| WNT5A     | Down-regulates efflux pump ABCB1              | Etoposide, vincristine                                                       |
| AKT       | Apoptosis disruption                          | Doxorubicin                                                                   |
| BCL-2     | Apoptosis disruption                          | Paclitaxel                                                                    |
| MAPK14/   | Apoptosis disruption                          | Irinotecan                                                                    |
| P38αa     | Apoptosis disruption                          | Gemcitibine                                                                   |
| AKT/PI3K  | Apoptosis disruption through AKT              | Doxorubicin, docetaxel                                                       |
| PTEN      | Apoptosis disruption                          | Gemcitibine, doxorubicin                                                     |
| NF-xB     | Promotes cell survival/growth/proliferation   | Etoposide, doxorubicin, vincristine, paclitaxel, docetaxel                    |
| MVP       | Indirectly promotes cell survival/growth/proliferation | Etoposide, doxorubicin, vincristine, paclitaxel, docetaxel                    |
| CAV1      | Indirect cell survival/growth/proliferation   | Etoposide                                                                     |
| RRM1      | DNA replication                               | Cisplatin, gemcitibine                                                       |
| ERCC1     | DNA replication/repair/maintenance            | Cisplatin, gemcitibine                                                       |
| POLB      | DNA replication/repair/maintenance            | Cisplatin                                                                     |
| POLK      | DNA replication/repair/maintenance            | Cisplatin                                                                     |
| RAD6B     | DNA replication/repair/maintenance            | Cisplatin                                                                     |
| TOP2      | DNA replication/repair/maintenance            | Cisplatin                                                                     |
| TOP2A     | DNA replication/repair/maintenance            | Cisplatin                                                                     |
| NKX2.2    | DNA replication/repair/maintenance            | Cisplatin                                                                     |
| FOXM1     | Microtubule destabilizer                      | Docetaxel                                                                     |
| STMN1     | Microtubule destabilizer                      | Paclitaxel, docetaxel                                                        |
| MAP4      | Microtubule stabilizer                        | Vincristine, vinblastine                                                      |
| TUBB      | Microtubule structure                         | Vincristine, paclitaxel                                                       |
| ALDH      | Drug metabolism disruption/inactivation       | Cyclophosphamide                                                              |
| CIC-3     | Drug metabolism disruption/inactivation       | Etoposide                                                                     |
| EGFR      | Drug metabolism disruption/inactivation       | Irinotecan                                                                    |
| GST       | Drug metabolism disruption/inactivation       | Cyclophosphamide                                                              |
| IL-6      | Drug metabolism disruption/inactivation       | Docetaxel                                                                     |
| SLC31A1   | Copper transport/drug uptake                  | Cisplatin                                                                     |

The main mechanisms of drug resistance to non-oncogene-targeting chemotherapies are efflux pump removal, disruption of apoptosis/promoting cellular survival/replication, repair/maintenance of DNA, microtubule stability adjustment, and disruption or inactivation of the drug.
immunological/inflammatory factors, etc... Forces in the tumor microenvironment that either feed tumor survival/replication or prevent the normal host tumor suppression mechanisms from their duties present quantitative challenges to all treatments. All of these factors come together to filter through the available chemotheraphy options that are appropriate for treating each patient (Fig. 1). Qualitatively, however, there remains much to be explored in terms of tailoring personalized medicine regimens to unique tumor microenvironments depending on individual genetic factors. Because these chemotherapies are not specific to oncogenic targets or their ATP-binding sites, they are naturally not susceptible to resistance from gatekeeper mutations and other changes that affect ATP binding site affinity. Therefore, the generic approach to resistance circumvention by conformationally altering the drug is usually not useful. Rather, most methods of resistance circumvention rely on employment of a second chemical, usually to block a host mechanism and/or allow the chemotherapy to increase its cytotoxicity. Since efflux pumps/cassettes are often the culprit, another possibility worthy of investigation is whether changing an efflux-pump binding site (assuming it is not a critical functional group) can help them circumvent this method of resistance. Great attention and further research are needed to gain a better understanding of the pharmacogenomic factors at play to aid physicians in making more accurate predictions on the effects of certain treatments to individual patients. Further studies on additional genes and pathways that moderate the multiple components of the DNA replication machinery and cytoskeletal function/production should enhance our ability to accurately predict the treatment values of DNA replication inhibitors and cytoskeletal function inhibitors. Higher resolution understanding of proteins is also critical to decision-making. Rather than clumping proteins as those related to microtubule assembly, categorizing them by role of microtubule stabilization or destabilization can help facilitate the decision to use vinca alkaloids or taxoids for patients with different expression profiles. Microtubule destabilizers like vinca alkaloids are expected to be less effective in patients with genetic factors that support greater microtubule stability (as they must first overcome the natural hyperstability before becoming effective) and more effective in patients with lower microtubule instability (as they add to the natural instability). The opposite would be expected to be true for toxoids; however, the genes to be analyzed and genetic variations to be interpreted to determine the collective level of microtubule stability would present a far more difficult challenge to our current understanding of genetics. Furthermore, caution must be exercised when assuming causation from correlation as there is a significant amount of literature making claims that the upregulation/downregulation of a certain gene confers resistance to some chemotherapy but through an unknown mechanism. Oftentimes, certain genes can be correlated with resistance to multiple (possibly unrelated) chemotherapies, but unless the mechanism is established, we should not assume a causative relationship as changes or differences in the gene’s expression can be due to upstream effects that are indirectly caused by the chemotherapy. A cumulative database of the genes known to cause resistance to chemotherapies, and their mechanisms of resistance would be a potential crown jewel to personalized treatments for NSCLC (and likely other cancers as well) (Table 2).

Furthermore, we must realize that although personalized medicine is an invaluable tool for enhancing precision health care, the ability to obtain detailed genetic information on a personal basis is a privilege that is often not available to many. In these circumstances, rather than defaulting to blind treatment, it would be greataly beneficial to have population data to guide physicians in making educated decisions on the compounds that are more likely to be effective based on the patient’s ethnicity/area of ancestry. To create such a library of common genetic profiles (for known treatment-modifying genes) based on ethnic background requires extensive data-collection and analysis on a large-scale effort that would undoubtedly be extremely costly in time and resources. Nonetheless, the benefits afforded to those around the world who don’t readily have access to genetic testing would be difficult to overstate.

Despite the successes in the implementation of personalized medicine on patients afflicted with NSCLC, our knowledge on the pharmacogenomics pertaining to drugs without specific oncogenic targets still needs improvement. However, due to the prominence of NSCLC as a global health issue, there is a great deal of effort dedicated to advancing our knowledge of its treatment, which corresponds to the ever-increasing quality and precision of therapies available. Thus, although there is no end in sight to the quest to understand how unique human physiologies best interact with different chemotherapy compounds, it is a struggle than modern medicine cannot afford to shy away from.

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Author contributions

Wenxiao Jiang drafted the manuscript. Guiqing Cai was genetics-related consultant. Peter Hu was cancer-related consultant. Yue Wang was responsible for final editing of this manuscript.

Conflicts of interest

The statement of conflicts of interest is attached.

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