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Fentanyl Contamination in Street Drugs

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Introduction and Background

By dose-equivalency, fentanyl is orders of magnitude less expensive to manufacture than heroin. The presence of fentanyl in street drugs has significantly contributed to the largest drug overdose death toll in United States (US) history. According to the Centers for Disease Control and Prevention (CDC); between April 2020 and March 2021, more than 100 000 Americans died of a drug overdose, with 66% of those deaths involving synthetic opioids such as fentanyl. The total number is increased by approximately 29% from the previous 12-month period from April 2019 to March 2020 and is greater than the number of deaths due to motor vehicle accidents or gun violence (1, 2). The biggest percentage increase in overdose deaths in 2021 occurred in Alaska, where deaths increased by 75.3% (1). While fentanyl and fentanyl analogs (fentanyls) are the main drivers behind the increase in drug overdose deaths, the CDC reports that many individuals that ingest the drug do so unknowingly because fentanyl-containing illicit drug products are often falsely represented as another drug (3). A large proportion of individuals who use fentanyl believe they are ingesting another drug and are unknowingly exposed.

Initially gaining prevalence as an additive in white powder heroin, fentanyl has since been detected frequently in other illicit drugs. Fentanyls are sold on the street as a powder, on blotter paper, mixed with or substituted for other street drugs, or as counterfeit prescription drugs (4). They can be smoked, insufflated, or injected and several different fentanyls have been found in cocaine, vape liquids, and methamphetamine (5). Recently, millions of counterfeit pills sold as OxyContin (oxycodone) or Xanax (alprazolam) have been found to contain fentanyl. In 2022, the US Drug Enforcement Administration (DEA) seized almost 10 million counterfeit pills, which was more than the previous 2 years combined (6). In mid-January 2023, 45 000 counterfeit oxycodone pills were seized in Oregon (7). The adulteration of street drugs with fentanyl is not limited to opioids and sedative-type compounds. In 2017, Florida law enforcement authorities revealed the widespread adulteration of cocaine with fentanyls, finding them present in 180 cocaine exhibits tested (8). Fentanyls have also been detected in cannabis, 3,4-methylenedioxymethamphetamine (MDMA), illicit antidepressants, and methamphetamine samples (9–12).

Illicit Fentanyl Dominates

While prescription fentanyl diversion does occur, most recent cases of fentanyl-related overdose are linked to illicitly manufactured fentanyl, which is distributed through illegal drug markets for its heroin-like effect (13). Prescription pharmaceutical preparations of fentanyl consist primarily of transdermal patches, transmucosal formulations, and intranasal sprays. Transdermal patches are not tamper-resistant and easily abusable (14). These preparations account for a small portion of seized products (15). Nonprescription formulations are readily available on internet websites. These formulations come in a wide variety of forms: powders, pills, nasal preparations, patches, capsules, lozenges, blotter paper, and liquids (14). Pills may easily resemble or mimic pharmaceuticals such as Oxycontin (oxycodone), Xanax (alprazolam), or Norco (hydrocodone) (15, 16). Thus, unsuspecting

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buyers may assume products are as advertised when obtained from their normal supplier. These products have no federal/FDA oversight, which means the product(s) may not be the desired drug but are more likely a mixture of drugs and/or drug classes, including other opiates or opioids, stimulates (cocaine and methamphetamine), and central nervous system (CNS) depressants (benzodiazepines). Depending on the physical state, these formulations lend themselves to be easily crushed, dissolved, diluted, or used native to be smoked, insufflated, injected, or ingested.

Effects of the COVID-19 Pandemic

Postpandemic US national drug summary data are currently largely unpublished, and the effects of coronavirus disease 2019 (COVID-19) on the illicit drug trade will likely be the subject of much research and analysis in the coming years. Anecdotally, evidence is available that indicates that the pandemic had profound effects on illicit drug availability and supply purity. Dr. Adina Badea is the Director of Toxicology for Lifespan Academic Medical Center in Providence, RI, and has been leading the substance analysis testing for National Institutes of Health and the Foundation for Opioid Response Efforts-funded research projects on drug use and supply variability patterns. Dr. Badea stated that pure drug compounds are rare and many of the mixtures are not what the purchaser believed them to be. Some contain large amounts of contaminants from poor synthesis techniques [often fentanyl precursors such as 4-ANPP (Siegfried method) or 4-ANBP (Janssen method)], and many also have small amounts of other drugs as a result of cross-contamination. The study participants describe the postpandemic drug supply as a game of "Russian Roulette," wherein they never know if the drugs they have purchased may have lethal concentrations of unexpected ingredients.

Fentanyl in Heroin

Substitution and Adulteration in Heroin Supply

Heroin is often used as a substitute for prescription opiates and many people who use drugs (PWUD) find that heroin is cheaper and more reliably available. Treatment community experts frequently find that once PWUD switch to heroin, they rarely return to prescription opiates. Given that much of the US heroin supply is now adulterated or frankly substituted with fentanyl, many of the current fentanyl users likely started out using prescription opioids and have transitioned to illicit fentanyl. Some authors have described the American opioid crisis as a triple wave epidemic with 3 distinct overdose death waves due to (a) the rise of prescription opioids, (b) the transition from opioids to heroin, and (c) overdose deaths that can be largely attributed to fentanyls. The number of deaths due to heroin overdoses eclipsed that of prescription opioid pills in 2015, followed by the still-increasing numbers of fentanyl deaths that exceeded that of heroin by 2016 (17–19). As the opioid epidemic continued to dominate US headlines in the period from 2010 to 2020, the increase in demand for potent heroin may have spurred the introduction of fentanyl and heroin mixtures in 2013. In 2014, fentanyl was only found in 0.5% of several hundred seizures analyzed by the DEA Heroin Signature Program (HSP). Fentanyl seizures increased to 0.6% in 2015, 14% in 2017, and the most recently published HSP data indicates that in 2018, 24% of heroin samples also contained fentanyl (19). Based on a large number of deaths due to synthetic opioids like fentanyl and its analogs in recent reports, along with data from small regions in the US, the average percentage of fentanyl-adulterated or substituted heroin is likely higher today. Pennsylvania is one of 4 US states that was in the top 5 for the most heroin reports and the most fentanyl reports in 2019 National Forensic Laboratory Information System (NFLIS) data that demonstrates the intertwined markets of both drugs (20). A partnership between the Center for Forensic Science Research and Education and the Philadelphia Department of Public Health has been established to accurately assess the drug supply in Philadelphia, PA. The third-quarter testing for 2022 indicated that 91% of tested drug samples the users thought to be heroin contained fentanyl and xylazine, a nonopioid veterinary tranquilizer often called tranq (21). While xylazine intoxication and its effects will not be explored in this article in depth, it is an emerging fentanyl combination that will undoubtedly be the subject of future discussions as xylazine use has its own significant and unwanted side effects.

Financial Incentive to Include Fentanyls in Heroin

Fentanyl is approximately 50 times more potent than heroin and is entirely synthetic. Heroin and other opium poppy-derived drugs like morphine and codeine are natural products whose price and availability will be affected by crop yields from plants with geographic growth constraints (22). Because fentanyl is entirely synthetic and extremely potent, it can be made at a fraction of the cost of heroin and by dose-equivalency, fentanyl is much less expensive to manufacture. One account estimates that a kilogram of fentanyl divided into 1 million 1.0 mg doses could sell for $10–30 million retail value, while a kilogram of heroin would sell for about $200 000. Using $20 million as a fentanyl retail estimate, a kilogram of fentanyl is 100 times more profitable than naturally derived heroin (19, 22). Additionally, there is evidence that a higher-potency heroin product will increase drug sales.

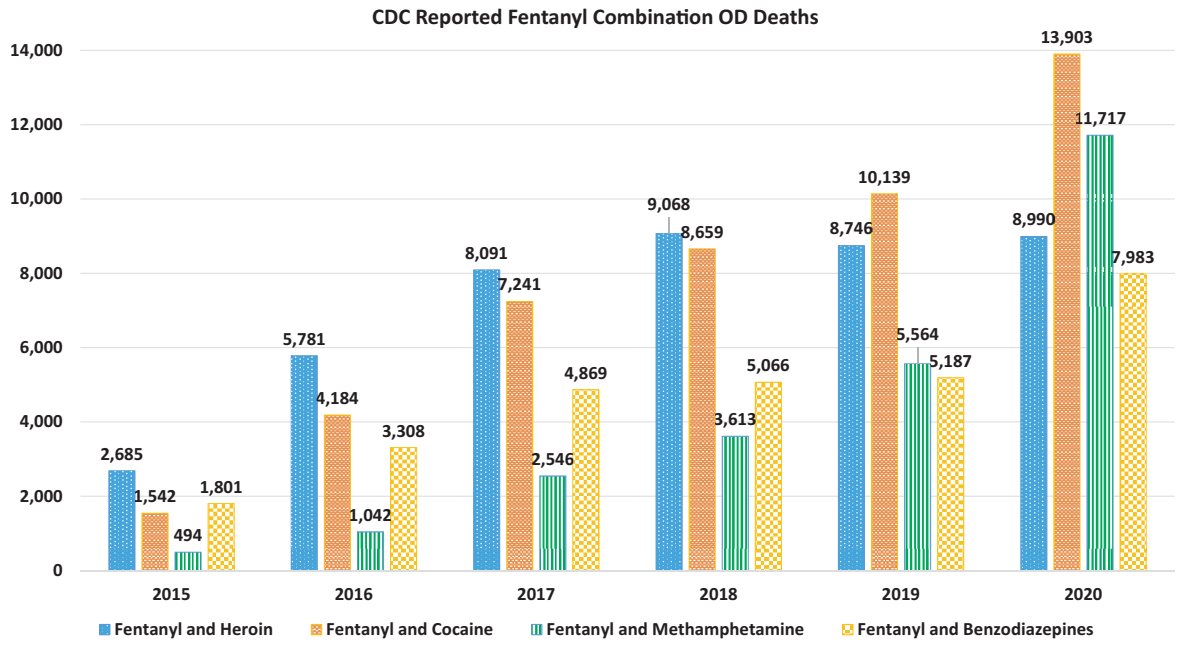

Fig. 1. Increasing combination drug overdose deaths over time. Drug overdose deaths are identified using underlying cause-of-death codes from the Tenth Revision of ICD (ICD–10). Drug overdose deaths involving selected drug categories are identified by specific multiple cause-of-death codes. Drug categories presented include: synthetic opioid analgesics other than methadone (primarily fentanyl) (T40.4), cocaine (T40.5), and psychostimulants with abuse potential (primarily methamphetamine) (T43.6). These numbers were extracted from a dataset provided at https://nida.nih.gov/research-topics/trends-statistics/overdose-death-rates and cite the National Center on Health Statistics, CDC WONDER as the source.

Previous findings have indicated that PWUD prefer to buy the strongest available heroin, even to the point of seeking heroin implicated in overdoses (22). The potent and synthetic nature of fentanyl has made it a popular substitute or adulterant for illicit drug distributors marketing a product as heroin. Additionally, as a synthetic compound, fentanyl has fewer vicissitudes in product availability that may be affected by environmental factors, disease, and national politics such as last year's Taliban-mandated destruction of the poppy fields in Afghanistan —the world's largest producer of opium (23, 24). Due to other types of political and agricultural constraints around opium poppy production, the period between 2001 and 2016 was marked by several sustained and widespread reductions in heroin supply and purity, which coupled with the increasingly opioid-addicted American population, likely resulted in the increase of fentanyl supply facing Americans today (13, 18, 19, 12).

Fentanyl in Cocaine

The presence of fentanyl in nonopioid drugs is particularly concerning as accidental poisoning is more likely among individuals who are not expecting to ingest opioids and could be opioid-naïve or have low opioid tolerance (12). The COVID-19 pandemic has adversely impacted multiple parts of the cocaine trafficking supply line, yet the pandemic has not significantly reduced the overall supply to the US (20). Drug overdose deaths involving cocaine rose steadily from 5419 in 2014 to 19 447 in 2020 (9; Fig. 1). This increase is driven largely by the combination of cocaine and synthetic opioids other than methadone, primarily fentanyl. These combinations account for approximately 75% of the cocaine-attributed deaths. According to the CDC, in 2020 overdose deaths due to fentanyl alone were the largest category of opioid deaths at 56 516; and the combination of cocaine and fentanyl was the most deadly at 13 903 attributed deaths (Fig. 1). This number exceeds that of fentanyl-laced heroin at 8990 deaths (Fig. 1). In the period from 2017 to 2020, the number of drug poisoning deaths involving cocaine and fentanyls was greater than the number of drug poisoning deaths involving cocaine *without* fentanyl. It is not exactly clear from this data if cocaine and fentanyl were co-ingested or consumed at different instances within a short time, but there is a growing body of evidence that cocaine supplies are adulterated with fentanyl and sold to retail consumers who may not be aware of the presence of the opioid. In February 2018, a DEA bulletin was released that described the widespread contamination of the cocaine supply with fentanyl in Florida (8). According to the report, fentanyls were found in 180 unique cocaine exhibits during the period of 2016–2017. The most prevalent

fentanyl analogue found in the Florida cocaine seizures was carfentanil, a substance that is approximately 10 000 times more potent than morphine. While fentanyl-laced cocaine may be more common in Florida than in some other areas, there is evidence that it has become a national issue. The National Forensic Laboratory Information System (NFLIS) reported fentanyls were detected in 1618 cocaine samples seized by law enforcement in 2019 (26). Recent national adulteration data has been difficult to obtain; however, isolated reports indicate adulteration of cocaine with fentanyl is still occurring. In December 2022, the DEA Rocky Mountain Division seized almost 45 pounds of a fentanyl and cocaine mixture and arrested 1 person with direct ties to the Sinaloa Cartel (29).

Canada is also experiencing a significant effect of cocaine adulteration with fentanyl. In 2020, 931 (2%) cocaine samples were positive for fentanyls. Of these, 116 (12%) were carfentanil. Health Canada's Drug Analysis Service data identified fentanyl or its analogues in nearly two thirds (62%) of opioid-containing and up to 3% of stimulant-containing samples nationwide. Fentanyl presence was particularly high in British Columbia, where fentanyl or fentanyl analogues were present in 91% of opioid-containing samples and up to 7% of cocaine-containing samples (n = 389/5,274). Nationally the proportion was 3% (12).

Adulteration of cocaine with fentanyl may be done to increase the drug's potency and profitability, or another possibility is cross-contamination from measuring equipment used to produce drug products. Given the high potency of the fentanyls, this theory is realistic due to the extremely small amounts that would be needed for pharmaceutical activity in a large batch of cocaine.

Fentanyl in Methamphetamine

Following closely behind cocaine and fentanyl mixtures in terms of overdose deaths, methamphetamines and fentanyl also significantly contribute. From less than 500 deaths attributed to this combination in 2015, there was an almost 2400% increase to 11 717 deaths in 2020 in the US. Illicit methamphetamine is increasingly available throughout the US, including the Northeast, which has not historically been a major market for the drug. In New York State in 2020, there were 184 methamphetamine-involved overdose deaths. The majority of these deaths (152, or 83%) also involved fentanyl (25). The crude rate of overdose deaths involving methamphetamine with fentanyl increased 1300%, from 0.1 per 100 000 population in 2016 to 1.4 in 2020. This increase was much larger than the increase in the crude rate for deaths involving methamphetamine without fentanyl, from 0.2 per 100 000 to 0.3, indicating that fentanyl is driving the increase (25).

The evidence for fentanyl adulteration before distribution to retail consumers is not as readily available for methamphetamine as it is for cocaine. The 2020 NFLIS annual report indicated that 1618 forensic drug samples were composed of methamphetamine and fentanyl in 2019, although this report did not explicitly state if the analyzed samples were counterfeit pills or methamphetamine powder (26). It is known that in the US pills are being sold as purported OxyContin (oxycodone), Xanax (alprazolam), or Adderall (mixed amphetamines); however, the pills frequently contain fentanyl and methamphetamine. In September 2021, the DEA issued a bulletin that there was a significant prevalence of these counterfeit prescription pills that contained both fentanyl and methamphetamine (6). According to the report, counterfeit pills have been seized by DEA in every US state in unprecedented quantities. These combinations are the attributed driver for the increase in methamphetamine-related deaths. Of the almost 24 000 US deaths in 2020 that involved methamphetamine, 11 717 (49%) were fentanyl and methamphetamine combinations (9). A Canadian study that reported methamphetamine samples collected between 2017 and 2018 in Vancouver, British Columbia reported that 5.9% (n = 15) of methamphetamine samples contained fentanyl (28). What is less defined is if typical methamphetamine users are aware that their powder or crystal product may also be adulterated with fentanyls.

Conclusions

The extensive prevalence of fentanyl in the US illicit drug market is the greatest threat to life facing PWUD today. While some people will actively seek out the potent opioid and prefer the fentanyl high, many are not aware that it is in the supply they have purchased. This unexpected presence is particularly dangerous for stimulant users who may be opioid naïve. Fentanyl is extremely potent and considerably less expensive to manufacture than heroin as it is entirely synthetic; thus, the adulteration of street drugs with it is practically ubiquitous. Starting with fentanyl's addition to the US heroin supply in 2013, it is now regularly detected in samples of cocaine and methamphetamine. Additionally, in 2022 almost 10 million counterfeit oxycodone, alprazolam, and Adderall pills were confiscated by the DEA and found to contain fentanyl. The most recent DEA Public Safety Alert indicated approximately 60% of these pills contain at least 2.0 mg of fentanyl, an amount that would be lethal to most people (2).

Total US overdose deaths almost doubled from 2015 to 2020; however, the fold-increase in death due to overdoses of fentanyl combined with other drugs such as heroin, cocaine, methamphetamine, and benzodiazepines range between 3.3 for heroin to 23.7 for methamphetamines (Fig. 1). Combined with the data

that the increase in any overdose death with fentanyl present during that time period is 5.9; it is very likely that fentanyl adulteration is the major factor behind the astonishing increase in these fatalities (9).

Harm reduction strategies such as the distribution of fentanyl drug testing strips to PWUD have been shown to be moderately successful. However, the most effective ways to reduce fentanyl-associated deaths is to reduce supply and importation to North America and educate the public about the extreme dangers of the presence of fentanyl in non-opioid street drugs (27, 30, 31). Supply limitation is difficult to achieve due to the influence of national politics and criminal organizations. Until an effective strategy is developed to reduce the prevalence of fentanyl in street drugs, clinical laboratories can contribute to fentanyl detection and surveillance by offering effective testing strategies and educational efforts. Several commercial fentanyl immunoassays are FDA cleared for use in hospital-based clinical laboratories and a recent publication indicates that at least two of them (ARK II and Immunalysis) are able to detect the majority of fentanyl analogs tested (32). Confirmatory assays are also available that can identify the specific fentanyl or fentanyl analogue that is present in the patient sample (33). Laboratory detection of fentanyl can be helpful to providers and to patients both of whom may be unaware of the presence of fentanyl in the substance that was ingested.

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Therapeutic Drug Monitoring for Kinase Inhibitors: Potential New Utility to Monitor Toxicity and Treatment Efficacy in Metastatic Melanoma

By Yifei K. Yang, PhD

Introduction

Targeted therapies have dramatically improved the treatment efficacy and reduced toxicity for cancer patients. Different from traditional chemotherapy drugs, targeted therapies utilize small molecule or monoclonal antibody drugs that inhibit proteins specifically involved in oncogenic pathways. Because they target specific molecular pathways in cancer cells, rather than all actively dividing cells, targeted therapies are generally perceived as less toxic compared with traditional chemotherapy. As a result, there is no widely adopted clinical practice to monitor their toxicity or treatment efficacy. During the treatment of metastatic melanoma with orally available small molecule kinase inhibitor drugs, certain severe toxicity side effects can lead to dose reduction in treated patients. The interplay between dose reduction and maximizing treatment efficacy suggests a potential clinical need to monitor the levels of these targeted therapy drugs and elucidate their association with treatment efficacy and toxicity.

Kinase Inhibitors and Targeted Therapy for Metastatic Melanoma

Based on their specific targets in cancer cells, targeted therapy drugs are considered less toxic compared with traditional chemotherapies. In metastatic melanoma, targeted therapy is applied when specific mutations in oncogenes (BRAF, NRAS, TRK gene fusions, and C-KIT) are detected in patients' tumor cells. In roughly 40%–60% of patients with metastatic melanoma, their tumor cells harbor mutations in the BRAF gene, and the most common BRAF mutations are V600E and V600K. BRAF is a protein kinase, and it can directly activate downstream MEK1/2 protein kinases. Through additional downstream MAPK signaling kinases, BRAF and MEK kinases are direct activating regulators of downstream transcriptional factors that can regulate genes involved in cell proliferation, differentiation, and cell survival. The BRAF V600E and V600K are both gain of function mutations that significantly potentiate BRAF kinase activity, resulting in a constitutively active state of its downstream kinases signaling pathway. Specific small molecule drugs, dabrafenib, encorafenib, and vemurafenib (Table 1), have been developed to target BRAF mutations BRAF V600E and V600K to inhibit their kinase activities. To further enhance the effects of kinase pathway inhibition, the treatments are also coupled with MEK1/2 inhibitors, trametinib, binimetinib, and cobimetinib (Table 1). Targeted inhibition of BRAF and MEK in metastatic melanoma has led to increased response rates and improved survival in patients harboring the BRAF V600 mutations (1, 2). The combination therapy of BRAF and MEK inhibitors can also be utilized in adjuvant therapy after surgical resection of melanoma.

Toxicities of Kinase Inhibitors

Although targeted therapies are less toxic compared with traditional chemotherapy, many patients develop and experience common side effects including pyrexia, skin rashes, increased liver

Table 1. Kinase Inhibitors for the Treatment of Metastatic Melanoma.

Drug generic name	Kinase targets	Standard dose	Molecular weight (Da)	Major metabolite/ metabolism pathways
Dabrafenib	BRAF V600E and K, D	150 mg orally taken twice daily	519.5	OH-dabrafenib, COOH-dabrafenib, desmethyl-dabrafenib
Encorafenib	BRAF V600E and K, D	450 mg once daily	540.0	N/A
Vemurafenib	BRAF V600E and K	960 mg orally taken twice daily	489.9	Hydroxyl-, phenolic hydroxyl-, glucuronide conjugation
Trametinib	MEK1/2	2 mg orally taken once daily, in combination with dabrafenib	615.4	Amide hydrolysis, aromatic hydroxylation, glucuronide conjugation
Binimetinib	MEK1/2	45 mg twice daily, in combination with encorafenib	441.2	Glucuronidation, N-dealkylation, amide hydrolysis
Cobimetinib	MEK1/2	60 mg once daily, in combination with vemurafenib	1178.7	Oxidation and glucuronidation

enzymes, and glucose levels in the blood, when treated with combination therapy for metastatic melanoma. Patients treated with combination therapy of BRAF and MEK inhibitors tend to exhibit lower occurrence of cutaneous adverse events, compared with patients treated with monotherapy of BRAF inhibitors. There are also rarer toxicities, such as cardiomyopathy (decreased ejection fraction), QTc prolongation, as well as pneumonitis, and ocular toxicities. Based on the severity of the side effects, the adverse events can be graded according to the National Cancer Institute Common Terminology Criteria for Adverse events. In patients experiencing more severe adverse events (generally when grade >2), the doses of the 2 drugs are both reduced. The doses of combination therapies involving BRAF and MEK inhibitors are designed based on maximal tolerated doses determined in phase I clinical trials of the drugs, required study component as part of the Food and Drug Administration approval. Nevertheless, it is common in clinical practice that many patients who require dose reductions or discontinuation due to toxicity still have clinically meaningful responses. Because of these severe adverse events, patients may experience treatment delays or dose reductions, potentially leading to decreased efficacy of the targeted combination therapies. In addition to toxicity, another reason that the targeted therapy may be discontinued is due to a lack of response as the result of developing drug resistance. As tumor cells evolve resistance mechanisms to targeted inhibitors, they can evolve molecular pathways that bypass the BRAF and MEK kinases in the MAPK signaling pathway to support tumor growth. The tumor cells can also bypass the MAPK signaling pathway to sustain cell proliferation and survival. As a result, some patients lose treatment response after several months of treatment, as BRAF V600E and V600K are no longer the dominant driver mutations in tumor cells. For patients who do not respond to kinase inhibitors, immunotherapy utilizing checkpoint inhibitors, such as pembrolizumab and nivolumab, is the first-line treatment.

Treatment Efficacy Related to Drug Exposure

For metastatic melanoma, the treatment efficacy is mostly evaluated by periodic radiographic imaging. Based on the modified Response Evaluation Criteria in Solid Tumors, the response criteria are mainly based on (a) changes in targeted lesions: complete response, partial response, progressive disease, and stable disease; and (b) the development of new lesions. In metastatic melanoma patients, the presence of intracranial brain metastases often indicates a more advanced disease and poor prognosis, and hence warrants more detailed radiological examination. Although cell-free DNA is used to identify eligible patients with targeted BRAF V600 mutations, cell-free DNA or circulating tumor DNA (ctDNA) levels in the blood are not yet established efficacy biomarkers for metastatic melanoma. A recent large study has examined the relationship between ctDNA and progression-free survival and overall survival (3). The study has identified that an elevated baseline BRAF V600 mutation-positive ctDNA concentration before dabrafenib and trametinib treatment was associated with worse overall survival outcome (hazard ratio [HR] 1.13 [95% confidence interval, CI 1.09, 1.18], $P < 0.0001$ by univariate analysis). The authors also showed that an undetectable ctDNA at week 4 of treatment was significantly associated with extended progression-free and overall survival, particularly in patients with elevated lactate dehydrogenase concentrations (HR 2.38 [1.24, 4.54], $P =$

0.0089 for overall survival). Pre-treatment and on-treatment ctDNA levels of BRAF V600 mutations can serve as a marker to monitor treatment efficacy and predict clinical outcomes.

Drug Monitoring of Kinase Inhibitors

The BRAF and MEK inhibitors have varied pharmacokinetic properties among individual patients due to varied metabolism enzymes and oral intake. Dabrafenib is primarily metabolized by CYP2C8 and CYP3A4. Strong inhibitors of CYP3A4 or CYP2C8 may increase concentrations of dabrafenib and strong inducers of CYP3A4 or CYP2C8 may decrease concentrations of dabrafenib. Vemurafenib is the substrate of CYP3A4, BRCP/ABCG2, and P-glycoprotein/ABCB1. Encorafenib is primarily metabolized by CYP3A4, CYP2D6, and CYP2C19, and is a substrate for P-glycoprotein/ABCB1. Trametinib is not a known substrate of CYP enzymes and is predominantly metabolized via deacetylation alone or with monooxygenation or in combination with glucuronidation biotransformation pathways in vitro. Cobimetinib is mainly metabolized by CYP3A4 and transported by P-glycoprotein/ABCB1, whereas binimetinib is the substrate of BCRP/ABCG2, CYP1A2, CYP2C19, P-glycoprotein/ABCB1, and UGT1A1. During clinical trials, the drug concentrations in patients were monitored to primarily understand the pharmacokinetic properties of the drugs, and they were not evaluated with toxicity and adverse events outcomes. To date, there are no established therapeutic and toxicity ranges for these kinase inhibitors. The recommended doses and subsequent dose reductions in clinical practice are designed empirically based on the toxicity experienced by individual patients. Recently, there have been several small patient group studies examining the use of drug exposure based on trough plasma concentrations and area under the curve (AUC), as biomarkers to understand their relationship with drug-related toxicity and treatment efficacy (4-7). In one study exploring the combination therapy of dabrafenib and trametinib, Goldwirt et al. explored the relationship between dabrafenib and trametinib exposure with progression-free survival, duration of response or all grades of treatment-related adverse events occurred in patients receiving standard care for metastatic melanoma (6). Following 50 patients over a 2-year period, they identified higher median trough dabrafenib concentrations in patients with higher progression-free survival and duration of response. Trametinib pharmacokinetic markers, tough concentrations, and AUC, were significantly higher in patients experiencing adverse events (mean ± SD, trough concentration: 13.1 ± 3.9 ng/mL; AUC: 384.1 ± 96.6 ng·h/mL) compared with patients without adverse events (mean ± SD, trough concentration: 11.7 ± 2.4 ng/mL; AUC: 339.1 ± 61.0 ng·h/mL). In a smaller group study (4), Rousset et al. evaluate the trough concentrations of dabrafenib and trametinib in 27 patients. Among these patients (13 males and 14 females) receiving the same standard dose (Table 1: 150 mg dabrafenib twice daily and 2 mg trametinib once daily), dabrafenib trough concentrations in plasma exhibited a high degree of interindividual variability, with the trough concentrations ranging from 15.4 to 279.6 ng/mL. Trough trametinib plasma concentrations ranged from 4.1 to 23.8 ng/mL. The authors identified a higher mean trough concentration of dabrafenib (118.6 ng/mL) in patients with significant adverse events related to combination therapy, requiring dose reduction (30%), compared with the patient subgroup without significant adverse events (33.5 ng/mL; $P < 0.0001$). Adverse events leading to dabrafenib dose reduction were all grade ≥2. The study did not identify differences in mean trametinib trough plasma concentrations between patient groups requiring dose reduction or not. The study further derived a dabrafenib trough plasma threshold of 48 ng/mL, as the predictor for the occurrence of significant adverse events requiring dose reduction. In another study based on 34 patients treated with dabrafenib and trametinib (7), the authors observed large inter- and intra-individual variations in the plasma concentrations of dabrafenib and trametinib among treated patients. Despite the high variability of dabrafenib and trametinib concentrations, the authors identified a relationship between plasma dabrafenib concentrations and patients' progression-free survival, when taking into account the follow-up time, which was close to statistical significance. The authors did not observe differences in dabrafenib and trametinib among patients with drug-related side effects and adverse events. The authors identified a minor decrease (0.9%) in the risk of disease progression, when there is a 1 ng/mL increase in plasma dabrafenib concentration. In a separate study based on 73 patients with BRAF V600 mutations (5), the authors also encountered great inter-individual variability in trough concentrations of dabrafenib and trametinib. They measured a major and active metabolite of dabrafenib, hydroxy-dabrafenib (OH-dabrafenib), and identified age and sex as determinants of dabrafenib and OH-dabrafenib clearance. They have also observed higher dabrafenib exposure (AUC: 9624 ng·h/mL) in patients experiencing severe adverse events requiring dose reduction than in the patient group without severe adverse events (7485 ng·h/mL, $P < 0.01$). In their analysis, the plasma ratio $AUC_{OHD}/AUC_{DAB} \geq 1$ was independently associated with shorter overall survival (HR: 10.61 [95% CI 2.34, 48.15], $P = 0.022$). They did not identify any association between trametinib exposure with treatment toxicity or efficacy. Results regarding treatment efficacy and adverse event occurrence varied widely

amongst existing studies, and their associations with either dabrafenib (BRAF inhibitor) or trametinib (MEK inhibitor) also differ. Blood collection time points were also variable in these studies and were not always collected at trough levels. Therefore, more data as well as a rapid test are needed to determine if therapeutic drug monitoring of BRAF and MEK inhibitors can guide therapy in metastatic melanoma. Although there are no uniform conclusions regarding treatment efficacy and the occurrence of severe adverse events drawn from the above studies, all the studies revealed large inter- and intra-individual variability in plasma trough concentrations and overall exposures in patients receiving combination therapy of dabrafenib and trametinib. While BRAF inhibitors and MEK inhibitors work synergistically to suppress the signaling pathway crucial for cancer cell survival and proliferation, their toxicity profiles appear to be independent of each other's plasma concentrations. Due to the large variation in drug and metabolite concentrations, prospective studies involving larger patient groups with more uniform evaluation time points for drug and metabolite trough concentrations are needed. The clinical utility of therapeutic monitoring of BRAF and MEK kinase inhibitors is still yet to be validated.

Analytical Methods for Kinase Inhibitors

Although the clinical utility of routinely monitoring kinase inhibitors in targeted therapies is yet to be validated, several analytical methods have been developed to quantify BRAK V600 and MEK inhibitors in human serum and plasma matrices (8-11). These analytical methods were developed largely based on liquid chromatography separation coupled with triple quadrupole mass spectrometry. From patient serum and plasma samples, the drugs and their major metabolites were extracted by protein precipitation or solid phase extraction, followed by separation using various gradient systems with increasing organic mobile phases on C18 columns. The eluted compounds were detected and quantified by triple quadruple mass spectrometry. The addition of isotope-labeled internal standards can further improve the accuracy and precision of the quantitative methods; however, they must be custom made and are not commercially available for all the inhibitors or their metabolites. Although BRAF and MEK inhibitors are used in combination when treating patients, their doses and elimination half-lives are significantly different (Table 1), hence the drugs in serum and plasma exhibited very different dynamic ranges. In addition to traditional venous-collected patient samples, additional studies were further carried out to evaluate specimen quality of dry whole blood samples collected via volumetric absorptive microsampling and capillary collection (12, 13). The current analytical methods can be further modified to accommodate the rapid turn-around time that may be required for the clinical application of therapeutic drug monitoring.

Conclusion

The treatment of metastatic melanoma has significantly improved patient survival outcomes and quality of life when combining 2 kinase inhibitors targeting the same cell signaling pathway. BRAF and MEK inhibitors are the first-line treatment for patients harboring BRAF V600 mutations. Although the drugs are highly effective in reducing tumor sizes and inhibiting disease progression, some patients may receive reduced doses due to the development of severe side effects. Therapeutic drug monitoring of kinase inhibitors in these patients may help maintain the delicate balance between toxicity and efficacy. However, larger studies with more uniform data points are needed to establish clinically meaningful therapeutic and toxicity ranges for these kinase inhibitors.

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Xylazine Prevalence in the Current Opioid Crisis

By Cate Omosule, PhD

Since its initial synthesis in 1962 as an antihypertensive drug by Bayer Corporation, xylazine, a nonopioid veterinary tranquilizer, has increasingly been associated with opioid drug overdose deaths across North America. Historically, intentional uses of xylazine were related to its sedative properties, with unintentional overdoses resulting when illicitly used for insomnia and for supplementation of alcoholic behavior (1). In recent times, xylazine is commonly used to potentiate the pharmacologic properties and duration of euphoria experienced with illegally manufactured synthetic drugs, and may confer a competitive market advantage for illicit opioids that contain it (2). In fact, 2020 data from a cross-national survey in the US revealed that xylazine was concomitantly detected in opioid overdose deaths containing illicitly manufactured fentanyl (98.4%), cocaine (45.4%), benzodiazepines (28.4%), or heroin (23.3%) (2).

Xylazine is an α2-andrenergic agonist (like clonidine). As such, it reduces the release of dopamine and norepinephrine in the central nervous system (CNS), which causes muscle relaxation, sedation, a reduction in the perception of painful stimuli, slowed breathing, and decreased heart rate and blood pressure. Using other CNS depressants concurrently, such as opioids, benzodiazepines, or alcohol, greatly enhances the risk of overdose and death. Repeated injection of drugs containing xylazine is also associated with skin ulcers and abscesses (2). In humans, exposure occurs via oral, intravenous, intramuscular, inhalation, and ocular routes. In multiple case reviews, autopsy findings reveal a distribution into multiple tissues: blood, urine, brain, kidney, liver, lung, and adipose tissues (1).

The opioid crisis has left no generational group untouched. According to several recent case reports, xylazine has been found in infants as young as 7 months old, adolescents, and adults, frequently as a result of accidental consumption, deliberate poisoning, or recreational usage (2-4). In 3 of these accounts, toddlers were being cared for by parents who had extensive histories of substance use problems and themselves had medical histories that were remarkable for prenatal substance exposure (3). Urine drug testing using GC-MS on expanded drug panels were positive for fentanyl, norfentanyl or acetyl-fentanyl, and xylazine (3).

Across the US, particularly in the Northeast, there has been a sharp rise in xylazine-present opioid overdoses (2). In 2020, xylazine was identified in 6.7% of overdose deaths, a sharp 20-fold rise from 0.36% in 2015 (2). Over a 6-month period in Canada from 2020 to 2021, xylazine was found in 7.2% and 12.5% of fentanyl- and methamphetamine-containing remnant samples taken from used drug paraphernalia (5). Xylazine is frequently coupled with opioids, so, although there is currently no pharmaceutical antidote and naloxone may not be as effective at fully reversing overdose symptoms when xylazine is present, experts recommend giving naloxone in suspected overdose (6). It is important to note, however, that patients with clonidine toxicity, for example, require much higher doses of naloxone to reverse solomnence as compared to fentanyl or heroin toxicity (7).

It can be challenging to diagnose xylazine intoxication, given that immunoassays for xylazine are not widely employed in standard urine drug screens, and specialized testing is not readily available in most clinical laboratories and hospitals. Therefore, clinicians should be aware of the increasing prevalence of xylazine in the illegal drug supply network and assess the presence of xylazine

exposure as well as exposure to synthetic opioids when suspected. The clinical laboratory must also keep up with reports of illegal synthetic drugs that may make the ongoing opioid crisis worse and implement testing that meets the needs of their communities by taking into account new and evolving drug use patterns in order to help combat the ongoing opioid epidemic.

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