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Nicotinamide Adenine Dinucleotide (NAD+) and Enkephalinase Inhibition (IV1114589NAD) Infusions Significantly Attenuate Psychiatric Burden Sequalae in Substance Use Disorder (SUD) in Fifty Cases

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AUTHORS' CONTRIBUTIONS

KB developed the initial draft, which was reviewed, committed and edited by all authors. The statistical analysis was performed by Dr. David Han along with graphical representation.

CONFLICT OF INTEREST

It is to be noted that KB is the inventor of amino-acid enkephalinase therapy and, as such, holds a number of the USA and foreign patents issued and pending.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The use of intravenous administration of amino acids for the potential treatment of RDS was approved by the Path Foundation NY IRB along with approved consent forms. The IRB approval was in the form of exemption status that has been utilized in a number of studies. All subjects entered into the study meeting inclusion criteria, and all subjects signed an informed consent statement. The PATH Research Foundation approved the study (NIH registration # 00002334) for general research utilizing variants of the Pro-dopamine regulation in the infusion form. In addition, the IRB of Western University Health Sciences also approved a study related to GARS testing in RDS candidates with pharmaceutical treatment in 2021.

HUMAN AND ANIMAL RIGHTS

No animals were used for studies that are the basis of this research. All the humans used were in accordance with the approval of the Path Foundation NY IRB and with the Helsinki Declaration of 1975.

CONSENT FOR PUBLICATION

Subjects not only signed written consent to participate and allow publication but also agreed to volunteer for a feasibility study. For patient protection, the data will conform to standard HIPPA practices mandated by law. A distribution center provided NAD infusions to treatment facilities, involving the 50 subsets of patients derived from two programs located in the Orange County area, and their associated treatment facilities have now performed approximately 1,000 infusions on 900 patients without any serious side effects, pointing to the safety of this procedure.

STANDARDS OF REPORTING

STROBE guidelines were followed.

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Abstract

Background: There is a shortage of clinical studies examining the efficacy of Nicotinamide Adenine Dinucleotide and Enkephalinase infusions (IV1114589NAD) in treating Substance Use Disorder (SUD).

Objective: This study aims to provide evidence that IV1114589NAD infusions significantly attenuate substance craving behavior.

Methods: The study cohort consisted of addicted poly-drug, mixed gender, multi-ethnic individuals resistant to standard treatment. The investigation utilized Likert-Scales to assess behavioral outcomes.

Results: Using Wilcoxon signed-rank tests and sign tests, our team detected significant results by comparing baseline to post outcome scores after IV1114589NAD injections: craving scores (P=1.063E-9); anxiety (P=5.487E-7); and depression (P=1.763E-4). A significant reduction in cravings, anxiety, and depression followed a dose-dependent linear trend. Linear trend analyses showed a significant relationship between NAD infusions and decreasing scores for cravings (P=0.015), anxiety (P=0.003), and depression (P=8.74E-5). A urine analysis was conducted on a subset of 40 patients midway through the study to assess relapse; 100% of the urine samples analyzed failed to detect illicit substance use.

Discussion: The opioid crisis in America has claimed close to 800,000 lives since 2004; daily deaths are estimated to stand at 127, and in 2021, over 107,000 deaths were due to overdose. There is an urgency to find safe, side-effect-free solutions. Current interventions, such as Naltrexone implants, are invasive and may interfere with dopamine homeostasis leading to an anti-reward phenomenon. Larger randomized double-blinded placebo-controlled studies are needed to elucidate further the significance of the results presented in this study. The current pilot

study provides useful preliminary data regarding the effectiveness of IV1114589NAD infusions in SUD treatment.

Conclusion: This pilot study provides significant evidence that NAD infusions are beneficial in the treatment of SUD. This investigation serves as a rationale to extend these findings onto future research investigating the use of NAD/NADH as a stand-alone treatment, especially in patients showing high genetic risk as measured in the Genetic Addiction Risk Severity (GARS) test. Utilizing GARS will help provide a real personalized therapeutic approach to treat Reward Deficiency Syndrome (RDS).

Keywords

Nicotinamide adenine dinucleotide (ND+) infusions; cravings; anxiety depression; dopamine homeostasis; reward deficiency syndrome (RDS); Medication Assistant Treatment (MAT)

1. INTRODUCTION

1.1. Understanding Reward Deficiency and Required Dopaminergic-Homeostasis

This article of original research proposes a unique combination of coupling the compound Nicotinamide adenine dinucleotide (NAD+) with known Enkephalinase Inhibitors (EI), including DL-Phenylalanine, [1] to help detoxify and treat individuals diagnosed with reward deficiency as manifested by Substance Use Disorder (SUD). This article describes intravenous therapy of a specialized cocktail consisting of B vitamins, NAD and DLPA, and other amino acids (IV1114589NAD).

It is widely acknowledged that in both food and drug-addicted individuals, there is dopamine resistance because of an association with the DRD2 gene A1 allele [2–4]. Evidence is emerging for utilizing a natural, non-addicting, safe, putative D2 agonist as a means to recover from reward deficiency syndrome (RDS) for patients addicted to psychoactive chemicals [4]. Employing quantitative electroencephalography (qEEG) as an imaging tool, we show the impact of Synaptamine Complex Variant KB220™ containing DLPA as a putative activator of the mesolimbic system [3–5]. For the first time, it has been shown that the KB220z variant, given *via* intravenous administration, decreases or “normalizes” aberrant electrophysiological parameters of the reward circuitry site [6]. For that published pilot study [6], it has been found that the qEEG’s of a heroin abuser and an alcoholic demonstrate abnormalities (widespread alpha and theta activity respectively) during protracted abstinence; however, their qEEG’s were significantly normalized by the administration of 1 intravenous dose of Synaptamine Complex Variant KB220Z™. Specifically, both patients were genotyped for a variety of neurotransmitter reward genes to ascertain the extent of putative dopaminergic risk alleles they carry that may predispose them to heroin or alcohol dependence, respectively. The tested genes included the dopamine transporter (DAT1, locus symbol SLC6A3), DRD2 TaqIA (rs1800497), dopamine D4 receptor exon 3 VNTR (DRD4), monoamine oxidase A upstream VNTR (MAOA-uVNTR), serotonin transporterlinked polymorphic region (5HTTLPR, locus symbol SLC6A4), and COMT val158 met SNP (rs4680). It has been maintained that these are case studies, and it would be unlikely for all individuals to carry the relevant putative risk alleles. Based on

our previous research and qEEG studies, we cautiously suggest that long-term activation of dopaminergic receptors (*i.e*, DRD2 receptors) will result in their proliferation and lead to an increased "dopamine sensitivity" and a greater sense of happiness, particularly in carriers of the DRD2 A1 allele [7].

Moreover, this initial work is supported by a clinical trial on Synaptamine Complex Variant KB220™ utilizing intravenous administration in more than 600 alcoholic patients, resulting in significant reductions in RDS behaviors [8]. It is noteworthy that Substance Use Disorders (SUD) are quite heritable. Reward genes play a major role in the hypodopaminergic functioning observed in SUDs. We evaluated the natural dopaminergic agonist, KB220 intravenous (IV), and its oral variants, to enhance dopaminergic function in SUD. Our pilot experiment revealed a significant reduction of chronic symptoms, measured by the Chronic Abstinence Symptom Severity (CASS) Scale. The combined group (IV and oral) did significantly better than the oral-only group over the first week and a 30-day follow-up period. Following this, the combination was given to 129 subjects, and three factors were measured: Emotion, Somatic, and Impaired Cognition. Each had eigenvalues larger than one, and they were extracted for baseline CASS-Revised (CASS-R) variables. Paired sample t-tests for pre and post-treatment scales showed significant reductions ($p = .00001$) from pre-treatment to post-treatment. The values were $t = 19.1$ for Emotion, $t = 16.1$ for Somatic, and $t = 14.9$ for Impaired Cognition. A two-year follow-up of 23 subjects who experienced KB220IV therapy, which includes at least five IV treatments over seven days and oral treatments for 30+ days, revealed that 21 (91%) of the subjects were sober at six months and 19 (82%) had no relapse. 19 (82%) subjects were sober at one year, and 18 (78%) had no relapse. 21 (91%) subjects were sober two-years post-treatment, and 16 (70%) had no relapse. We await additional research and advise caution in interpreting these encouraging results. It is also confirmed by the growing number of oral studies, encompassing at least 38 on the Complex KB220Z™ [9].

Additionally, the powerful effects of KB220, as evidenced by more recent neuroimaging studies, have clearly shown the importance of pro-dopamine regulation along with the Brain Reward Cascade (BRC) (Fig. 1). There is evidence that modifications in synchronous neural activity between brain regions involved in reward and other cognitive functions may significantly contribute to substance-related disorders. Our previous work by Febo *et al*. [10] provided the first evidence demonstrating that the pro-dopaminergic nutraceutical KB220Z significantly increases (above placebo) the functional connectivity between reward and cognitive brain regions in rats. The following regions affected included: the nucleus accumbens, anterior cingulate gyrus, anterior thalamic nuclei, hippocampus, prelimbic and infralimbic loci. In addition, significant functional connectivity, increased brain connectivity volume recruitment (potentially neuroplasticity), and dopaminergic functionality were found across the brain reward circuitry. Most importantly, increases in functional connectivity were specific to these regions and were not broadly dispersed across the brain. Though these initial findings have been observed in drug naïve rodents, this robust yet selective response suggests clinical relevance for addicted individuals at risk for relapse, who show reductions in functional connectivity after protracted withdrawal.

Furthermore, our laboratory also showed the effect of KB220Z™ on reward circuitry for ten heroin addicts undergoing protracted abstinence (on average 16.9 months). In a placebo-controlled, randomized crossover study of KB220Z, five subjects participated in a triple-blinded experiment where the subject, the person evaluating the response to treatment, and the person administering the treatment were all blinded to the treatment that any individual subject was receiving. Additionally, nine subjects were genotyped utilizing the Genetic Addiction Risk Score (GARS) test. Previously, Blum's group [11] preliminarily reported that KB220Z induced an increase in BOLD activation in caudate-accumbens-dopaminergic pathways relative to placebo, following a one-hour acute administration. Moreover, KB220Z also decreased resting-state activity in the putamen of abstinent heroin addicts. For the second phase of this pilot study, we noted that three brain regions were significantly activated from the resting-state by KB220Z relative to placebo ($p < 0.05$) for all 10 abstinent heroin-dependent subjects. Enhanced functional connectivity was observed in a putative network, which includes the medial frontal gyrus, nucleus accumbens, posterior cingulate, occipital cortical areas, cerebellum, and dorsal anterior cingulate.

Future studies are necessary, utilizing both functional magnetic resonance imaging and positron emission tomography scanning, to ascertain the acute and chronic effects of oral and or intravenous KB220™ on numbers of D2 receptors and the direct interactions at the nucleus accumbens. Verification of these results in large, populationbased, case-controlled experiments is needed. These studies would offer important information that could eventually lead to significant improvement in recovery for individuals with RDS and dopamine deficiency, as a consequence of multiple neurotransmitter signal transduction breakdowns in the brain reward cascade (Fig. 1).

Fig. (1) illustrates the interaction of at least seven major neurotransmitter pathways involved in the Brain Reward Cascade (BRC). In the hypothalamus, environmental stimulation can cause the release of serotonin. After binding to the relevant receptors, for example, 5HT-2a receptors, this would activate (green equal sign) the release of opioid peptides from opioid peptide neurons, which are also in the hypothalamus. Subsequently, the opioid peptides can have two distinct effects, possibly *via* two different opioid receptors. One that inhibits (red hash sign) through the mu-opioid receptor (possibly *via* enkephalin) and projects to the substania nigra to GABAA neurons. Another stimulates (green equal sign) cannabinoid neurons (*e.g.*, 2-archydonoglcerol and anandamide) through beta–endorphin-linked delta receptors, which in turn, results in inhibition of GABAA neurons at the substania nigra. Activated cannabinoids, primarily 2-archydonoglcerol, can indirectly cause disinhibition (red hash sign) of GABAA neurons in the substania nigra by activating G1/0 coupled to CB1 receptors. Glutamate neurons within the dorsal raphe nuclei (DRN) can also indirectly disinhibit GABAA neurons in the substania nigra through activation of GLU M3 receptors (red hash sign). GABAA neurons, when stimulated, will powerfully (red hash signs) inhibit VTA glutaminergic drive *via* GABAB 3 neurons. It is also possible that stimulation of ACH neurons at the Nucleus Accumbens can stimulate both muscarinic (red hash) or nicotinic (green hash). Finally, glutamate neurons in the VTA will project to dopamine neurons through NMDA receptors (green equal sign) to preferentially release dopamine at the nucleus accumbens (NAc), shown as a bullseye, which indicates a euphoria or "wanting" response. The result is that when dopamine release is low, unhappiness or endorphin

Deficiency follows. At the same time, general (usual) happiness depends on the dopamine homeostatic tonic set point.

These results, along with other quantitative electroencephalogy (qEEG) study results, suggest a putative [12] anti-craving/anti-relapse role of KB220Z in psychostimulant addiction by direct or indirect dopaminergic interaction. Recently, Willuhn *et al.*, [13] reported that cocaine use, as well as non-substance-related addictive behaviors, increase as dopaminergic function is decreased. Chronic cocaine exposure has been associated with reductions in D2/D3 receptors and lower activation of cues in the occipital cortex and cerebellum, which was found in a PET study by Park *et al.* [14] Therefore, treatment strategies, such as dopamine agonist therapy that conserves dopamine function, could be an interesting approach to prevention of relapse in psychoactive drug and behavioral addictions.

1.2. Enkephalinase Inhibition (EI) and Addiction

There are a number of compounds that have been shown to inhibit the degradation of enkephalins [14]. Enkephalinase Inhibitors (EIs) appear to be promising as therapeutic agents due to their analgesic properties, which are accomplished by increasing enkephalins. Endogenous EIs include peptides like opiorphin and spinorphin. Endogenous and synthetic inhibitors of enkephalindegrading enzymes have been studied *in vivo* utilizing standard animal models. The potential EI targets seem to be APN (Aminopeptidase N), NEP (Neutral endopeptidase), and DPP-III (Dipeptidyl peptidase). EIs possess a distinct advantage in that they lack the side effects of opioids. Along these lines, it is important to understand the regulation, synthesis, and expression of receptors, particularly the catabolism (role of proteolytic enzymes) of brain endorphins and especially enkephalins [15, 16]. It is currently known that these enkephalinase inhibitors prevent the degradation of enkephalins, whereby these compounds produce naloxone reversible analgesia and potentiate the analgesia produced by enkephalins and acupuncture [17–20]. D-phenylalanine has proven to be beneficial in many human patients with chronic, intractable pain [21, 22]. Blum's group [2] proposed that enkephalinase inhibitors may be effective in a number of human "endorphin deficiency diseases" such as depression, schizophrenia, convulsive disorders, and arthritis. Cheng *et al.*, [22] revealed that the D-amino acids (DAA), D-phenylalanine, and D-leucine, produce naloxone reversible analgesia; electroacupuncture (EA) also produces analgesia, which is blocked by naloxone. Combining the two treatments produces an additive effect with larger analgesia than that produced by either treatment given alone. This combined effect is also blocked by naloxone. Moreover, only 62% of the mice show EA analgesia, and 53% show D-amino acid (DAA) analgesia; 80% of the animals show marked analgesia with both EA plus DAA treatment.

In addition, EIs may alleviate other conditions associated with decreased endorphin levels, such as alcohol/opiate withdrawal symptoms [23]. In the Blum *et al.* [24] review article, they point out that the consensus of the literature supports the concept that brain neurotransmitters and second messengers are involved in the net release of dopamine in the mesolimbic region, especially the nucleus accumbens (NAc). Furthermore, the release of neuronal dopamine is directly linked to motivation, anti-stress, incentive salience (wanting), and well-being. The role of dopamine in terms of alcohol withdrawal symptomology,

cocaine-craving behavior, dopamine-condensation products (TIQs), and, more recently, the genetic aspects of drug-seeking and pro-dopamine regulation, provide compelling evidence of the relevant molecular neurological correlates of dopaminergic/endorphinergic mechanisms in reward circuitry due to genetic polymorphisms and epigenetic insults.

Certainly, in the face of the American opioid epidemic, the clinical consensus is to treat opioid use disorder (OUD) with life-long opioid substitution therapy. However, it has been suggested that a paradigm shift involving novel modalities, such as targeting the endorphinergic system linked to dopamine release at the NAc, leading to the induction of "dopamine homeostasis [25]." Utilizing the known gene-environment interaction theorem P = G +E, Blum *et al.* [26] previously provided a clear rationale for the adoption of genetic risk testing coupled with endorphinergic/dopamine regulation to address dysfunction across the brain reward circuitry.

The goal of altering resting-state, functional connectivity may require a gentle "neurotransmitter fix" *via* enkephalinase inhibition (*e.g.* D-phenylalanine) to overcome self-induction of acute dopamine release *via* psychoactive substance misuse, resulting in chronic dopamine downregulation. As subsets of reward deficiency, we are poised to provide novel, genetically guided therapy for endorphinergic, opioidergic, and dopaminergic deficiencies and related syndromes, utilizing "Precision Addiction Management".

In terms of the therapeutic benefits of enkephalinase inhibition, there have been a series of articles that have focused on the role of endorphins in alcoholism. [27] One of these articles revealed that alcohol intake significantly reduces Leuenkephalin synthesis in brain circuits. [28] In a related article, it was also found that alcohol intake in genetically-bred ethanol-preferring or ethanol-averse mice was found to be an inverse function of the amount of brain methionine-enkephalin (METENK) present. [29] Simply, the lower amount of brain methionine-enkephalin, the higher the intake of alcohol in C57/Bl mice (low METENK), and the lower intake of alcohol in DBA mice (high METENK). Using these studies as a rationale, Blum *et al.*, [30] performed the first pharmacogenetic engineering experiment employing D-Phenylalanine, the enkephalinase inhibitor, to convert ethanol-preferring C57/Bl mice to behave like ethanol-averse DBA mice. These authors were able to significantly attenuate both volitional and forced ethanol intake respectively by acute and chronic treatment with D-phenylalanine. Since D-phenylalanine, through its enkephalinase inhibitory activity, raises brain enkephalin levels, it was shown that 18 days of treatment with D-phenylalanine significantly attenuated excessive alcohol intake in C57/BL to the same or even lower levels than its counter-part DBA mice. This suggests that alcohol intake can be regulated by alteration of endogenous brain opioid peptides.

1.3. NAD+ and Addiction

The significance of NAD+ in addictive disorders arises from the work of Paul O' Hollaren [31], who revealed to have successfully utilized IV NAD+ for the treatment and prevention of over 104 cases of addiction to alcohol, among other drugs of abuse, including opium extract, heroin, morphine, dihydromorphine, meperidine, codeine, cocaine, amphetamines, barbiturates, and tranquillizers. For his retrospective case series, IV NAD+ was given at a dose of 500–1000 mg, which was added to 300 cc normal saline daily for 4 days, twice per

week for a month. This was followed by a maintenance dose twice per month until addiction was ameliorated, with limited toxic effects. NAD+ is likely to represent an economical and holistic approach for the estimated millions of addicts worldwide; it may be an effective adjunct to psychotherapy, by ameliorating symptoms of physical addiction through a variety of mechanisms. While there have been animal studies showing the benefits of NAD with cocaine [32], there is a paucity of human studies on drugs of abuse. Nicotinamide phosphoribosyltransferase (NAMPT) is an important rate-limiting enzyme found throughout the body that converts the intracellular pool of nicotinamide adenine dinucleotide (NAD) into nicotinamide mononucleotide (NMN). A study published in Experimental Neurology by the Cen group demonstrated that NAMPT contributes to cocaine reward through sirtuin 1 (SIRT1) signaling in the ventral tegmental area. Thus, targeting the NAMPT/SIRT1 signaling pathway may provide a promising therapeutic strategy against cocaine addiction. [32] Additionally, Nicotinamide (NAM) is a small molecule that can oppose cellular adaptations observed following cocaine exposure in the rodent self-administration and reinstatement model of addiction. Furthermore, NAM has utility against symptoms of withdrawal and vulnerability to relapse to cocaine use; this has been suggested by case studies and anecdotal reports [33]. In fact, chronic NAM administered throughout extinction dose-dependently attenuated cue-primed reinstatement in male rats, but not female rats [33].

Nicotinamide adenine dinucleotide (NAD+) and its reduced form, NADH, both respond to cellular energy demands and redox states [34]. Other studies have shown that NAD+ is important for maintaining energy homeostasis in the brain, as well as calcium transport and mitochondrial respiration affecting dopamine neuronal release in the brain reward circuit [35].

While there is little evidence directly published on NAD/NADH, RDS, and associated drug and non-drug addictions, there is some rationale to utilize this substance based on metabolic effects. However, albeit emerging newer positive evidence regarding NAD and SUD, as an off-label modality. [36] The above brief historical information not only provides a rationale to incorporate NAD into clinical practice to treat SUD, but it begs for additional studies to support its potential benefits for this incredibly vulnerable population. The following is a detailed original executed pilot demonstration to assist in future larger required experiments in human and animal self-administration models.

1.4. Glycine

The work related to the possible role of the molecule glycine in alcoholism was initiated with the published works of Blum *et al.* [37], published in Science in 1972. They found both glycine and its precursor serine significantly enhanced the sleeping time (loss of the righting reflex) that was induced by ethanol in mice. The observed synergistic effect between ethanol and the amino acids is probably not related to an alteration of ethanol metabolism, but rather to an interaction of these compounds in the central nervous system. Moreover, Blum's group also reported on the antialcohol intoxication effects of these compounds. Specifically, both glycine and serine significantly reduced acute alcoholic intoxication in mice. [38] The enhancement of the soporific effects of ethanol in mice was confirmed in 1995 by Williams *et al.* [39] A Pubmed search using the words "Glycine and Drug Abuse" on November 26,

(th) 2020, resulted in 416 citations. Many of these articles involved glycine receptors and glycine transporters. In terms of the benefit of glycine as an antialcohol compound, there have been mixed results. The most recent study by Serrita *et al.*, [40] utilized glycine as an agonist of the glycine B coagonist site of the NMDA receptor on alcohol consumption and cravings. In this study, Glycine showed no benefit over placebo in the reduction of heavy drinking days or craving for alcohol over a 12-week treatment period.

However, there is important data related to the interaction of ethanol, glycine, and dopamine. Specifically, previous research has shown that strychnine-sensitive glycine receptors in the nucleus accumbens [NAc] are involved in regulating dopamine release and in mediating the reinforcing effects of alcohol. One noteworthy finding is that alcohol-induced dopamine release is blocked by local treatment with the glycine receptor antagonist strychnine (20 μM) or furosemide (100 μM or 1 mM [41]. There are also studies showing that Anandamide (AEA) and delta9-tetrahydrocannabinol (THC) are endogenous and exogenous ligands, respectively, for cannabinoid receptors in pharmacologically relevant concentrations. Utilization of these peptides per se administered into animals and humans was not followed by any side effects. No complaints from the subjects were noted. These results may suggest a safe and efficient use of Ala-Gln as a source of free glutamine in parenteral nutrition. Certainly, glutamine per se or its interaction at specific loci such as glutaminergic drive at the VTA, regulates dopamine release [42].

In contrast, the glycine transporter 1 inhibitor Org 25935 demonstrated no benefit over placebo in preventing alcohol relapse [43]. Despite this, other work showed that Org 25935 decreased alcohol intake and alcohol preference [44]. Other studies show that NMDA/glycine (glycine(B)) site antagonists attenuate wet dog shakes (withdrawal) and the development of addiction, induced by chronic morphine administration in rats [45].

The presence of GlyRs has been described in higher regions, such as the hippocampus and nucleus accumbens, with a prevalence of α2/α3 subunits. Burgos *et al.* [46], detailed the following aspects of alcohol effects on GlyRs: (1) direct interaction of alcohol with amino acids in the extracellular or transmembrane domains, and indirect mechanisms through the activation of signal transduction pathways; (2) analysis of α2 and α3 subunits having different sensitivities to alcohol, which allows the identification of structural requirements for alcohol modulation present in the intracellular domain and C-terminal region; (3) Genetically modified knock-in mice for α1 GlyRs that have an impaired interaction with G proteins and demonstrate reduced alcohol sensitivity without changes in glycinergic transmission; and (4) GlyRs as potential therapeutic targets. Of particular interest is the work of Michino *et al.*, [47], showing that a divergent glycine in extracellular loop 1 [EL1] is critical to the dopamine D3 receptor [D3R] over the dopamine D2 receptor [D2R] subtype selectivity. It should be noted that DRD3 genotypes are very relevant in African-Americans compared to Caucasians with regard to binge drinking [48].

Finally, the mesocorticolimbic dopamine system, originating in the ventral tegmental area (VTA), is regulated by GABA-mediated synaptic inhibition. Accumulating evidence indicates that long-term potentiation of GABAergic synapses (LTPGABA) in VTA dopamine neurons plays an important role in the actions of drugs of abuse, including

alcohol. It has been shown that a single infusion of glycine into the VTA of rats strongly reduces ethanol intake for 24 hours [49]. In follow-up studies, Gian & Ye [50] reported 10 μM glycine prevented trains of high-frequency stimulation (HFS) from producing LTPGABA, which was rescued by the glycine receptor (GlyR) antagonist strychnine. The authors suggested that the blockade of LTPGABA by glycine is probably resulting from suppressing glutamate release by activating the GlyRs on the glutamatergic terminals. This effect of glycine may contribute to the reduction in ethanol intake induced by intra-VTA glycine observed *in vivo*.

1.5. Alanylglutamine Dipeptide

A Pubmed search using the search term "Alanylglutamine-dipeptide and addiction or drug abuse" on November 26th, 2020 retrieved no results. However, its incorporation into the IV1114589NADASE infusion is predicated upon the potential release of glutamine *in vivo.* Using biotechnological and chemical methods, the stable and highly soluble peptide L-alanyl-L-glutamine (Ala-Gln) can be synthesized in substantial yields. Studies in experimental dogs and rats demonstrate the effective utilization of intravenously supplied Ala-Gln as well as the rapid provision of free glutamine for maintenance of the muscle-free, intracellular glutamine pool in catabolic conditions.

Subsequent studies in healthy volunteers provide strong evidence that the infused Ala-Gln is rapidly eliminated from plasma (t1/2:3.8 minutes), associated with a prompt equimolar increase in the concentrations of free glutamine and alanine. Continuous infusion and bolus injection of the peptide was not associated with any side effects, and no complaints by the subjects were noted. These results may indicate a safe and efficient use of Ala-Gln as a source of free glutamine in parental nutrition [51].

2. METHODS & MATERIALS

The primary objective of the present investigation is to provide some additional clinical evidence to show that IV1114589NADASE infusions significantly attenuate substance craving behavior and concomitant psychiatric burden sequalae in poly-drug abusers attending an out-patient chemical dependency program. IV1114589NAD [NAD] infusions are displayed in Table 1.

The study cohort (n=50) as a subgroup consisted of highly addicted, poly-drug, mixed-gender, and ethnicity individuals previously resistant to standard treatment with a failed range of treatment attempts. The incorporation of the NAD infusions involved detoxification, in-patient residence, and out-patients. Table 1 – provides the demographics of this cohort. Table 2. represents a summary status of the demographics.

Scrutiny of this data reveals that the various drugs of choice in these 50 patients include: opiates (heroin), methamphetamine, cocaine, ethanol, benzodiazepines, marijuana, and spray cans of air duster. The study consisted of twenty-one (n=21) females and twenty-nine (n=29) males. The age of the subjects ranged from 21 to 61 years, where the average for females was 34.7 ± 8.9 years and males were 34.3 ± 6.4. The study participants consisted of 84% Caucasians, 4% African-Americans, 2% Hispanic, 2% Arabic, and 8% Native Americans.

The years of use ranged from 5 to 33, with an average of 16.80 ± 6.83. The number of treatment facilities attended by these 50 patients ranged from the first time to as high as 10, with an average of 2.1 ± 2.1. In these 50 patients, the range of last use of any drug of abuse is self-reported from one day to 80 days, with an average of 21.76 ± 15.28. The longest sobriety of these patients ranged from 4 days to 7 years, with an average sobriety rate of 1.18 ± 1.41 years. In this cohort, the years of use varied from 5 to 33 years, with an average of 16.80 ± 6.83. Each patient included in this study received a minimum of 7 infusions for a duration of four weeks, with one to two infusions per week. The data on a number of variables included: craving scores, anxiety, depression, and sleep. While there are existing validated scales to measure the various behavioral-related levels, it has been decided to utilize the Likert scale [1–10] for self-reported responses, accomplished *via* a counselor-to-patient structured interview. It is to be noted that each of the two treatment centers used self-reported Likert scales to obtain the data on 50 patients. Throughout these 7 infusions of NAD, each of the subjects included in this study agreed, as per treatment policy, to be urine screened for the presence or absence of non-prescribed psychoactive drugs. The first and last urine samples on these subjects were analyzed and presented herein.

2.1. Statistical Analysis

In terms of statistical analysis, we utilized the Fisher Exact test to evaluate the extent of utilizing NAD infusions (10 per patient over 5 weeks), comparing self-reported pre-baseline Likert scores and post-infusion scores. We also employed the Mantel–Haensel test for linear trends showing a potential consistent relationship between the effect of these NAD infusions on craving anxiety, depression, and sleep. P-value is the level of marginal significance within a statistical hypothesis test, representing the probability of the occurrence of a given event. In terms of P values indicating E means exponential to the power of 10.

3. RESULTS

Using Wilcoxon signed-rank tests and sign tests, it has been found the following significance comparing the baseline scores to post outcome scores after IV1114589NAD infusions; craving scores (P=1.063E-9); anxiety (P=5.487E-7); depression (P=1.763E-4), sleep outcome was found to be non-significant. The significant reductions in cravings, anxiety, and depression followed a linear trend whereby with cravings (pre 5.90, post 0.22); anxiety (pre 4.66, post 1.54); and depression (pre 2.58, post 0.88). Linear trend analyses showed a significant association of NAD infusions with decreasing scores of cravings (P=0.015), anxiety (P=0.003), and depression (P=8.74E-5) (Figs. 2–5).

The significant reductions in cravings, anxiety, and depression followed a linear regression (Fig. 6) with a) cravings (pre 5.90, post 0.22); b) anxiety (pre 4.66, post 1.54); and c) depression (pre 2.58, post 0.88).

The results of urine drug screens. A subset of 40 out of 50 patients during the infusion period was provided by each treatment center. The urine analysis was performed by Southwest Medical Laboratory, which is a CLIO-certified toxicological reference site. The panel included: 6-Acetylmorphine, Amphetamine, Barbiturate, Benzodiazepine, Buprenorphine, Cocaine- Fentanyl- Methadone, Methamphetamine- Opiate, Oxycodone,

THC, and Tramadol. 100% of those patients tested midway into the infusion period tested negative. Therefore, 80 percent (40/50) of all subjects of the total of fifty cases were tested (Table 3).

4. DISCUSSION

The present study demonstrates a method to treat and detoxify patients with Substance Use Disorder (SUD), utilizing a series of Nicotinamide adenine dinucleotide (NAD+) and Enkephalinase (IV1114589NADASE) infusions in subjects attending chemical dependency programs. A PUBMED search using the term “NAD and addiction” resulted in fifty-one articles and using the term “NAD and Substance Abuse” resulted in three hundred and seventy-one. However, there is a paucity of clinical studies on humans with SUD and the incorporation of NAD infusions as developed in this current cohort. The earliest known published clinical study on NAD for addiction was authored by Paul O’ Hollaren in 1961 [30]. Since then, many treatment programs have adopted its use [30–36, 52–76].

The primary objective of the present investigation is to provide some additional clinical evidence to show that NAD+ and other amino acids, including D-phenylalanine [77], glycine, ananylglutamine dipeptide, and Myers cocktail (B complex) infusions, significantly attenuate substance-craving behavior and concomitant psychiatric burden sequalae in poly-drug abusers attending both in-patient and out-patient level of care in a number of chemical dependency programs in Orange County.

Importantly, urine analysis of a standard panel of illicit drugs of abuse [78, 79] was utilized during NAD infusions. A subset of 40 patients was tested midway during the infusions resulting in 100% of these patients testing negative. The remaining ten subjects were not urine screened.

To reiterate, after obtaining the baseline measurements, each subject was followed throughout the experiment of five weeks and ten infusions. Using the statistical analyses based on Wilcoxon signed-rank tests and sign tests P, we found the following significance comparing the baseline scores to the post outcome scores after NAD infusions: craving scores (P=1.063E-9); anxiety (P=5.487E-7); depression (P=1.763E-4), with sleep levels showing a trend to a higher number of hours slept post infusions [80], though this trend was found to be non-significant (Figs. 2–5).

4.1. Limitations

In the face of the current opioid crisis in the US, killing close to 800,000 people since 2004 and accruing an estimated death rate daily of 127, there is a pressing need to find a safe solution free of side effects [81]. We are cognizant that, for example, Naltrexone pellets have heuristic value but may interfere with dopamine homeostasis and induce anti-reward benefits [82]. This pilot demonstration study with a small N requires future larger, randomized, double-blinded, placebo-controlled studies enabling a clearer interpretation. However, it does contribute to the emerging literature concerning NAD efficacy in SUD. One other limitation relates to the fact that many studies show that NAD alters dopaminergic signaling.

[83–85], there is a paucity of neuroimaging reports related to addictive behaviors and reward processing [86].

CONCLUSION

These robust pilot results demonstrate the significant positive effects of NAD, amino-acid, and enkephalinase inhibition infusions in treatmentresistant probands showing reward deficiency. This provides the rationale to extend these seemingly clinically relevant findings in extended future investigations utilizing NAD/NADH alone coupled with the GARS test as a DNA guided precision-matched pro-dopamine regulator to help induce required “dopamine homeostasis”.

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AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available within the article.

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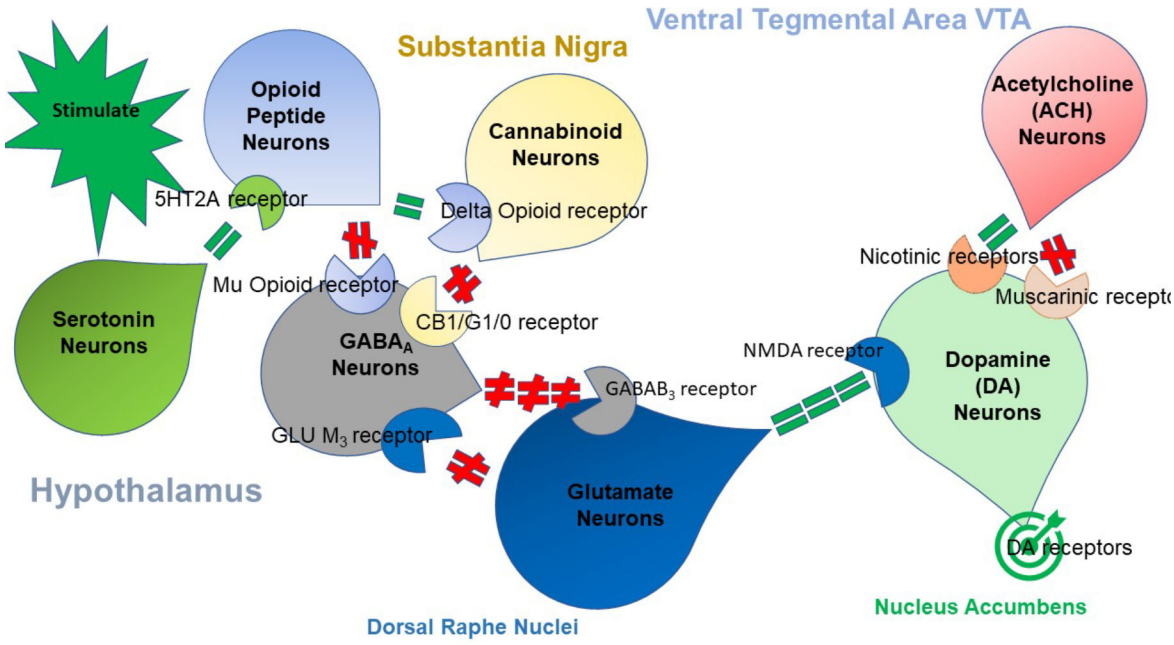

Fig. (1).

The brain reward cascade. “Reprinted from Publication Author(s), Gold MS, Baron D, Bowirrat A, Blum K. Neurological correlates of brain reward circuitry linked to opioid use disorder (OUD): Do homo sapiens acquire or have a reward deficiency syndrome? J Neurol Sci. 2020 Nov 15;418:117137. doi: 10.1016/j.jns.2020.117137. Epub 2020 Sep 15. PMID: 32957037; PMCID: PMC7490287.with permission.

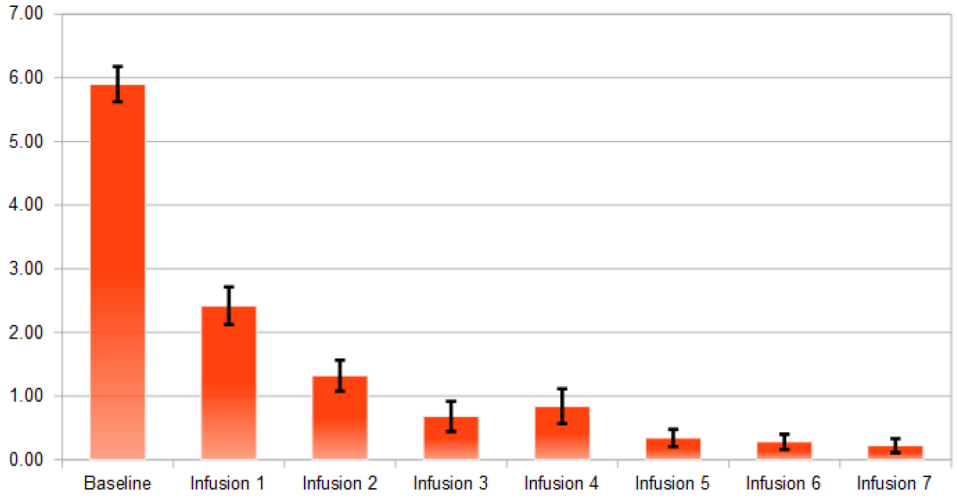

Fig. (2).
Average of cravings scores after each NAD infusion (n=50).

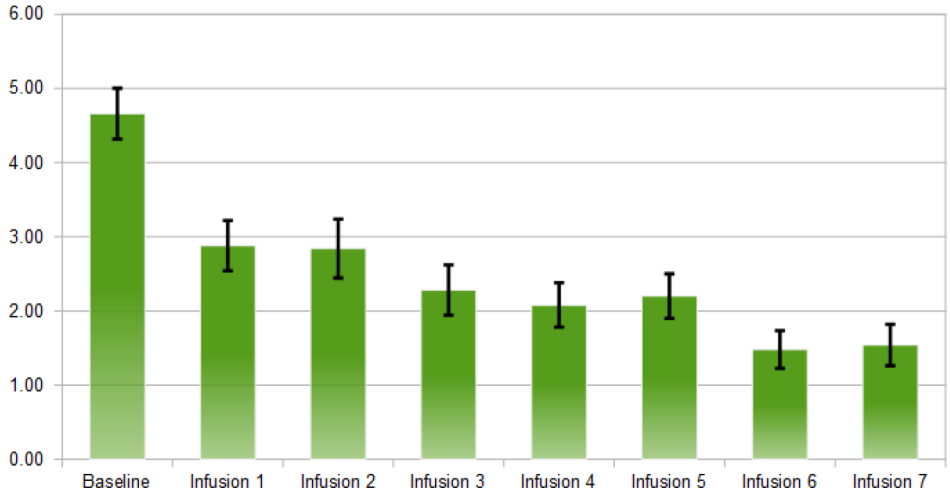

Fig. (3).
Average of anxiety scores after each NAD infusion (n=50).

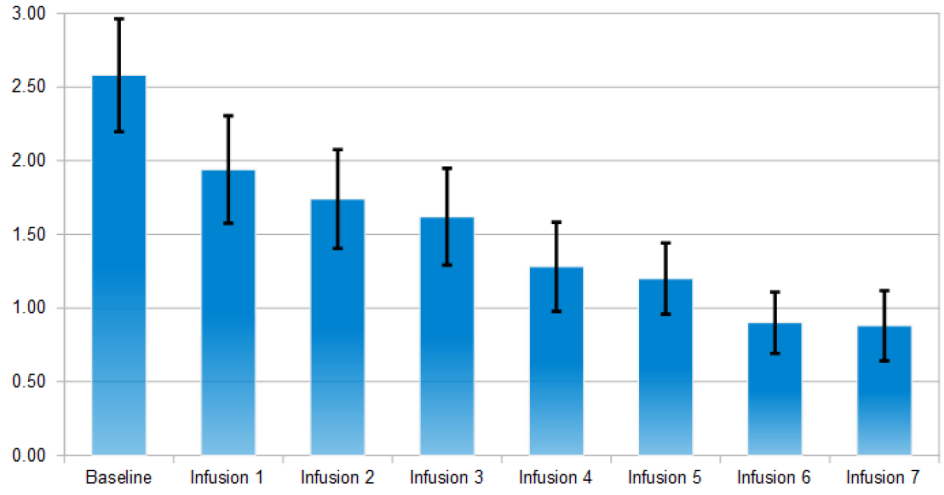

Fig. (4).
Average of depression scores after each NAD infusion (n=50).

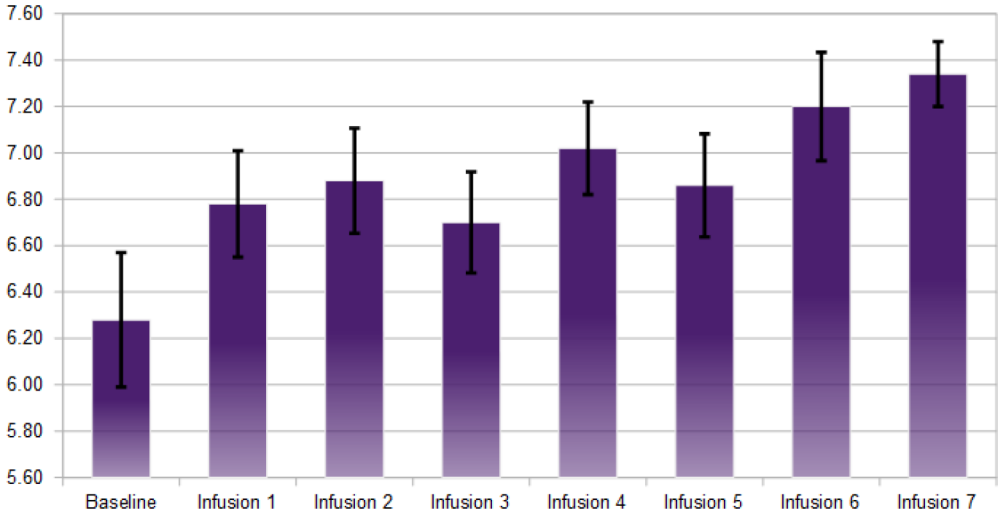

Fig. (5).
Average hours of uninterrupted sleep after each NAD infusion (n=50).

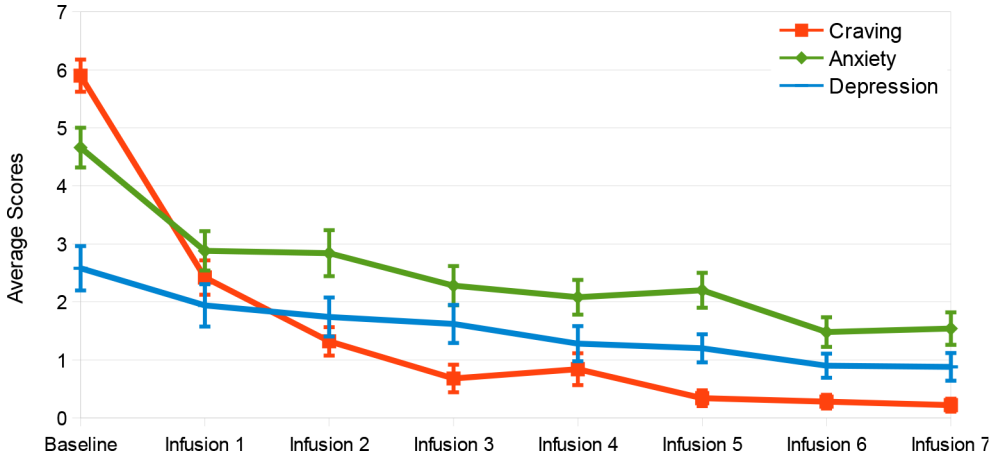

Fig. (6).

Averages of outcome measures after each NAD infusion (n=50).

Table 1.

Detail of Infusions per session.

-	**Infusion #1**	**Infusion #2**	**Infusion #3**	**Infusion #4**	**Infusion #5**	**Infusion #6**	**Infusion #7**
Hydration Multi-Vitamin Immune Support with Amino Acids	X	X	X	X	-	-	-
Hydration Multi-Vitamin Immune Support	-	-	-	-	X	X	X
Alpha Lipoic Acid	X	X	-	-	-	-	-
Glutathione	-	-	X	X	X	X	X
NAD +	X	X	X	X	-	-	-
NAD + with Amino Acids	-	-	-	-	X	X	X

Table 2.

Demographics of the Fifty patients in this cohort.

Gender	**29 Male (58%), 21 Female (42%)**
Age (years)	34.48 ± 7.46 (range 21 to 61 years)
Drug of Choice (DOC)	3 Air Duster (6%), 8 Benzodiazepines (16%), 1 Cocaine (2%), 1 Crack (2%), 16 Ethanol (32%), 16 Heroin (32%), 4 Marijuana (8%), 38 Methamphetamine (76%), 5 Opiates (10%), and 1 Suboxone (2%); one substance: 22 (44%), 2 substances: 17 (34%), 3 substances: 8 (16%), and 4 substances: 3 (6%)
Days since Last Use	21.76 ± 15.28 (range 1 to 80 days)
Level of Care	11 Detox (22%), 4 IOP (8%), 12 PHP (24%), and 23 Residential (46%)
No. Treatment Facilities	one facility: 32 (64%), 2 facilities: 6 (12%), 3 facilities: 5 (10%), 4 facilities: 2 (4%), 5 facilities: 1 (2%), 7 facilities: 1 (2%), 8 facilities: 2 (4%), and 10 facilities: 1 (2%)
Longest Sobriety (years)	1.18 ± 1.41 (range 4 days to 7 years)
Years of Use	16.80 ± 6.83 (range 5 to 33 years)
Random UA Screening	40 negative UASO (80%)
Ethnicity	2 African American (4%), 1 Arabic (2%), 42 Caucasian (84%), 1 Hispanic (2%), 4 Native American (8%)

Table 3.

Urine analysis results.

-	Gender	Age	DOC	Last Use	Level of Care	# of Treatment Facilities	Longest Sobriety	Years of Use	Random UA Screening	Ethnicity
-	Gender	Age	DOC	Last Use	Level of Care	# of Treatment Facilities	Longest Sobriety	Years of Use	Random UA Screening	Ethnicity
Patient 1	Female	42	Meth, Benzos	22 Days	PHP	1 (st) Time	1 Year	20 Years	Negative	Native American
Patient 2	Female	35	Meth, ETOH, Duster	15 Days	Residential	3	9 Months	19 Years	Negative	Caucasian
Patient 3	Female	27	Meth	19 Days	PHP	1 (st) Time	7 Months	11 Years	Negative	Caucasian
Patient 4	Female	41	ETOH	7 Days	Detox	1 (st) Time	6 Months	20 Years	Negative	Caucasian
Patient 5	Female	45	Meth, Marijuana	24 Days	PHP	1 (st) Time	24 Days	23 Years	Negative	Caucasian
Patient 6	Female	45	Meth, Duster, ETOH, Marijuana	27 Days	PHP	4	1.5 Years	33 Years	Negative	Caucasian
Patient 7	Female	29	Meth, Opiates, Benzos	4 Days	Detox	1 (st) Time	5 Years	9 Years	----------	Caucasian
Patient 8	Female	28	Suboxone	12 Days	Residential	1 (st) Time	32 Days	5 Years	Negative	Caucasian
Patient 9	Female	38	Meth	4 Days	Detox	1 (st) Time	4 Days	5 Years	Negative	Caucasian
Patient 10	Female	32	Meth, Opiates, Benzos	22 Days	Residential	1 (st) Time	9 Months	19 Years	Negative	Caucasian
Patient 11	Female	34	Meth, Heroin, ETOH	23 Days	Residential	1 (st) Time	4 Years	11 Years	Negative	Caucasian
Patient 12	Female	32	Meth, Heroin, ETOH	30 Days	Residential	1 (st) Time	30 Days	27 Years	Negative	Native American
Patient 13	Female	21	Meth, Benzos	10 Days	PHP	1 (st) Time	28 Days	5 Years	Negative	Hispanic
Patient 14	Female	61	Opiates, Meth	22 Days	Residential	3	90 Days	20 Years	Negative	Caucasian
Patient 15	Female	29	Meth	1 Day	Detox	1	1 Year	11 Years	Negative	Caucasian
Patient 16	Female	28	Meth, ETOH	46 Days	PHP	7	46 Days	13 Years	Negative	Caucasian
Patient 17	Female	36	Cocaine, Meth	30 Days	PHP	1 (st) Time	30 Days	16 Years	Negative	Caucasian
Patient 18	Female	30	Heroin	21 Days	Residential	2	2 Years	11 Years	Negative	Caucasian
Patient 19	Female	29	Meth, Benzos, ETOH, Marijuana	17 Days	Residential	1 (st) Time	17 Months	16 Years	Negative	Native American
Patient 20	Female	41	Heroin	80 Days	IOP	2	80 Days	30 Years	Negative	Caucasian
Patient 21	Female	26	Meth, Heroin	17 Days	Residential	1 (st) Time	17 Days	9 Years	Negative	Caucasian
Patient 22	Male	42	ETOH, Benzos	22 Days	Residential	1 (st) Time	1 Year	26 years	Negative	Caucasian
Patient 23	Male	33	Meth, ETOH	26 Days	Residential	2	2 Years	21 Years	Negative	Caucasian

-	Gender	Age	DOC	Last Use	Level of Care	# of Treatment Facilities	Longest Sobriety	Years of Use	Random UA Screening	Ethnicity
Patient 24	Male	42	ETOH	28 Days	Residential	10	2 Years	26 Years	Negative	Caucasian
Patient 25	Male	34	Meth	19 Days	Residential	3	2 ½ Years	17 Years	Negative	Caucasian
Patient 26	Male	35	Heroin, Opiates	23 Days	Residential	2	2 Years	13 Years	Negative	Caucasian
Patient 27	Male	43	Meth, Heroin	23 Days	Residential	1 (st) Time	7 Years	20 Years	Negative	Caucasian
Patient 28	Male	40	Meth	19 Days	Residential	1 (st) Time	17 Months	20 Years	Negative	Caucasian
Patient 29	Male	36	Meth, ETOH	28 Days	Residential	3	5 Months	23 Years	Negative	Caucasian
Patient 30	Male	32	Meth, ETOH	22 Days	Residential	1 (st) Time	1 Year	16 Years	Negative	Caucasian
Patient 31	Male	28	Meth, Heroin	13 Days	Residential	2	100 Days	12 Years	Negative	Caucasian
Patient 32	Male	23	Heroin, Meth, Crack	50 Days	IOP	5	1 Year	7 Years	Negative	Caucasian
Patient 33	Male	31	Meth	1 Day	Detox	1 (st) Time	30 Days	15 Years	Negative	Caucasian
Patient 34	Male	30	ETOH, Marijuana	30 Days	Residential	1 (st) Time	30 Days	15 Years	----------	Caucasian
Patient 35	Male	37	Meth	20 Days	PHP	3	3 Years	25 Years	Negative	African American
Patient 36	Male	30	Heroin, Meth, Duster, ETOH	30 Days	PHP	4	2 Years	15 Years	Negative	Caucasian
Patient 37	Male	31	Meth, Heroin, Benzos	23 Days	Residential	1 (st) Time	9 Months	16 Years	Negative	Caucasian
Patient 38	Male	32	Meth	20 Days	PHP	1 (st) Time	1 Year	16 Years	Negative	Caucasian
Patient 39	Male	41	Meth	29 Days	PHP	1 (st) Time	4 Years	21 Years	Negative	Caucasian
Patient 40	Male	26	Meth, Heroin	1 Day	Detox	1 (st) Time	6 Months	10 Years	----------	African American
Patient 41	Male	42	Meth	1 Day	Detox	2	8 Months	24 Years	----------	Caucasian
Patient 42	Male	29	Meth	1 Day	Detox	1 (st) Time	5 Months	15 Years	----------	Caucasian
Patient 43	Male	40	Meth	57 Days	IOP	1 (st) Time	57 Days	26 Years	----------	Native American
Patient 44	Male	24	Heroin, Meth	41 Days	IOP	1 (st) Time	41 Days	10 Years	Negative	Caucasian
Patient 45	Male	30	Heroin, Opiates, Benzos	29 Days	Residential	1 (st) Time	29 Days	18 Years	Negative	Arabic
Patient 46	Male	32	Heroin	22 Days	Residential	1 (st) Time	22 Days	20 Years	Negative	Caucasian
Patient 47	Male	46	Meth	5 Days	Detox	1 (st) Time	2 Years	28 Years	----------	Caucasian
Patient 48	Male	45	ETOH	5 Days	Detox	8	1 ½ Years	8 Years	----------	Caucasian
Patient 49	Male	28	Meth, ETOH	40 Days	PHP	1 (st) Time	9 Months	14 Years	----------	Caucasian
Patient 50	Male	33	Heroin	7 Days	Detox	8	1 ½ Years	10 Years	----------	Caucasian