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Effect Of Antiretroviral Drug Dolutegravir On Mitochondrial Function

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Abstract:

Background: Though antiretroviral therapy (ART) regimens have become progressively less toxic, ART-associated toxicity is still pervasive. Dolutegravir (DTG) is an HIV integrase strand transfer inhibitor (INSTI), a class of antiretrovirals that are generally well-tolerated by patients. However, in the clinic there are reports of unexpectedly high rates of toxicity in cohorts of pregnant women such as neural tube defects, and in older patients' adverse effects such as neuropsychiatric disorders. We hypothesized that these adverse effects could be due to DTG-induced mitochondrial toxicity in the central nervous system.

Methods: Human neuroblastoma cells were treated with ART regimens that are currently considered standard of care for HIV management. The cells were passaged every 4 days with standard growth media. Neuroblastoma cells were exposed to 1X or 4X-Cmax of tenofovir, emtricitabine, and DTG in single or in combination for 24 hours. We harvested the cells after 24 hours of treatment to determine the effect of treatment on cell growth, mitochondrial protein presence, mitochondrial expression levels, and NMDA receptor protein levels.

Results: Our preliminary results indicate that DTG has a potentially appreciable effect on both mitochondrial function and cholesterol biosynthesis. ATP synthase expression levels appears to be relatively unaffected by treatments while expression levels for the other mitochondrial complexes appear to be decreased from DTG treatment compared to other single treatments.

Conclusion: Though DTG in clinical trials has been demonstrated to be a potent treatment for HIV viral load, have a high resistance barrier, and low interaction potential, there is still much to be understood in terms of toxicity mechanisms. Our study indicates that the clinical safety profile of DTG and DTG-combination therapies need to be further evaluated.

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Abbreviations

AIDS – Acquired Immunodeficiency Syndrome

HIV – Human Immunodeficiency Virus

ARTs – Antiretroviral Therapies

NRTIs – Nucleoside Reverse Transcriptase Inhibitors

NNRTIs – Non-Nucleoside Reverse Transcriptase Inhibitors

PIs – Protease Inhibitors

INSTIs – Integrase Strand Transfer Inhibitors

HAART – Highly Active Antiretroviral Therapies

Introduction:

Brief introduction to HIV/AIDS

Acquired immunodeficiency syndrome (AIDS) is a condition caused by infection by the human immunodeficiency virus (HIV)[1]. The infection leads to a decrease of CD4+ T cells in humans which results in a compromised immune system and increased susceptibility to common infections. There are many theories about the origin of HIV, but the most supported theory is that the virus originated from monkeys in West Africa[2]. AIDS was officially described in 1981 as a new disease when unusual clusters of Pneumocystis pneumonia, Kaposi's sarcoma, and other opportunistic diseases began to spring up in generally healthy men in cities across the United States[3,4]. French scientist Françoise Barré-Sinoussi was the first to isolate the causative agent of AIDS in 1983, followed by further research in 1984 by Robert Gallo and Jay Levy who made the link between HIV and AIDS[5].

As of 2018, 37.9 million people globally are living with HIV[6]. The global burden of HIV can be measured by both the number of HIV-related deaths (770,000 in 2018) and the number of people newly infected with HIV worldwide (1.7 million in 2018). The World Health Organization (WHO) has stated that they are aiming for worldwide HIV-related deaths to drop to below 400,000 and number of people newly infected with HIV to below 200,000. To reach this goal, one of the most important tools to combat the mortality and new infection rates are antiretroviral therapy (ART) drugs. ART drugs are life-saving treatment regimens that help people who are infected survive significantly longer periods of time. About 62% of people living with HIV are on some form of ART[7]. The functional benefit of ART is two-fold; the treatment

helps increase life expectancy by slowing the loss of CD4+ T cells and also suppresses viral load in individuals which makes it much less likely to transmit the virus to another person[8].

Though the overall global rate of new cases of HIV infection is slowing, there are some areas that are still observing disproportionately high levels of HIV incidence and deaths from AIDS due to lack of access to ART drugs. People in the countries of sub-Saharan Africa alone account for 17% of all people in the world living with HIV[8]. Access to frontline ART drugs is difficult for countries that do not have the requisite healthcare and economic infrastructure to sustain a robust testing, education, and treatment support system.

HIV life cycle

The HIV life cycle consists of seven stages: 1) binding, 2) fusion, 3) reverse transcription, 4) integration, 5) replication, 6) assembly, and 7) budding[9]. At the beginning of infection, the virus binds to the CD4 receptors and CXCR4 co-receptors, utilizing the resulting cross-linkage to gain access to the CD4+ T cell via fusion. During fusion, the HIV envelope and CD4 cell membrane fuse and allow for the entry of the virus' functional parts: the HIV RNA, reverse transcriptase, integrase, and other necessary viral proteins. Once the virus is inside the cell, it utilizes its own reverse transcriptase to form viral DNA via reverse transcription. The resulting viral DNA is then integrated into the host DNA by way of the viral integrase. At this point, the host cell begins to produce more of the viral RNA and proteins, allowing for the assembly of non-infectious HIV at the surface of the cell. Once the new virus is leaves the host cell, HIV enzyme protease cleaves long chains of protein to complete the mature infectious virus. The HIV life cycle has been an intense area of focus in research as the various classes of ART drugs target different parts of the life cycle.

Evolution of anti-HIV medications

At the beginning of the AIDS epidemic in the U.S., massive drug screening programs were conducted to find possible candidates to combat HIV/AIDS. The first candidate to be approved was azidothymidine (AZT). Originally developed as a cancer therapy drug, AZT was deemed ineffective against cancer. However, in clinical trials for treating people with AIDS, the drug decreased deaths and opportunistic infections at the cost of serious adverse effects[10]. Lower doses of the drug proved successful in delaying the onset of AIDS after infection by HIV in asymptomatic people, albeit with serious side effects. Based on the mechanism of action, AZT falls under a class of drugs known as nucleoside reverse transcriptase inhibitors (NRTIs). After the approval of AZT as an anti-HIV medication, additional NRTIs were subsequently discovered and approved.

Though AZT generally worked as intended, HIV accumulated small mutations at a high rate due to a high replication rate. The resulting variants developed resistance to AZT sometimes in a matter of days[11]. To combat the resistance developed from single-drug treatments, combination therapy of two drugs was first used in 1995. For the most part, people who had never previously used ART and were placed on a two-drug regimen had better outcomes in terms of the development of resistance when compared to those that only used AZT. The advent of dual drug (two NRTI) combination therapy eventually led to the development of highly active antiretroviral therapy (HAART). HAART is a treatment regimen that uses three or more ART drugs from different classes that target distinct parts of the HIV replication cycle[12]. A new ART drug class called protease inhibitors (PIs) was discovered to complement the NRTIs in the triple-drug therapy. The first protease inhibitor to be introduced

was saquinavir. In comparison to dual drug combination therapy with the two standard of care NRTIs at the time, zalcitabine (ddC) and AZT, the triple-drug combination was more effective in health outcomes as well as staving off the development of resistance.

The next drug class to be discovered was the non-nucleoside reverse transcriptase inhibitors (NNRTIs). Based on cost of production and efficacy of treatment, NNRTIs were favored over PIs, which has led to an increased prevalence of NNRTI resistance in resource-limited settings[13]. Later in the 1990s, it was discovered that in order for HIV to gain access to CD4+ T cells, they had to bind to both the main CD4 receptor and a co-receptor, CCR5. Drugs such as maraviroc, a CCR5 antagonist, act as a binding inhibitor, preventing HIV from binding to the necessary CCR5 co-receptor and thus preventing entry[14]. The most recent class addition to the ART drug repertoire are the integrase strand transfer inhibitors (INSTIs)[15]. In 2007, first-generation integrase inhibitors raltegravir and elvitegravir became important components of HAART regimens. Though they are effective drugs, there are several pathways for the virus to develop resistance. The newest frontline drug is second generation integrase inhibitor dolutegravir (DTG). Touted by WHO as the preferred HIV treatment in all populations[16], DTG has a relatively low production cost, adherence friendly once-daily dosing, and a high barrier to the development of resistance.

Anti-HIV targets

The different classes of ART drugs target various points of the HIV life cycle to try and mitigate the damage the virus will cause. Based on Figure 1, the process of HIV infection to replication to dispersal is shown with the different classes of ART labeled according to the mechanism of action they undergo[17]. NRTIs interfere with the reverse transcriptase from the

virus, rendering it unable to be transcribed into DNA. By functioning as chain terminators, the viral DNA does not have a chance to be formed, which subsequently prevents them integrating. There are two known pathways of drug resistance that have been developed against NRTIs. The discrimination pathway is when the reverse transcriptase enzyme has a change in primary structure that decreases the binding affinity for the foreign NRTI-triphosphate compared to the natural nucleoside[18]. The other pathway is the excision pathway, which is when the reverse transcriptase has the ability to remove chain terminators from the 3' end of the DNA chain after it has been incorporated. This prevents early termination and the viral DNA is still able to form. As mentioned previously, protease is an important enzyme in the final step in the formation of the HIV mature infectious form. PIs prevent the cleavage of HIV Gag and Pol polyproteins, preventing the conversion into functional infectious virions[19]. Mutations that change the confirmation of the substrate binding site for proteases can lead to cross-resistance to PIs as they will not be able to bind the active site[20]. The mechanism of action for NNRTIs are different from NRTIs though the target remains the reverse transcriptase enzyme. NNRTIs bind to an allosteric non-nucleoside binding component, causing a confirmation change in the active site of the transcriptase.

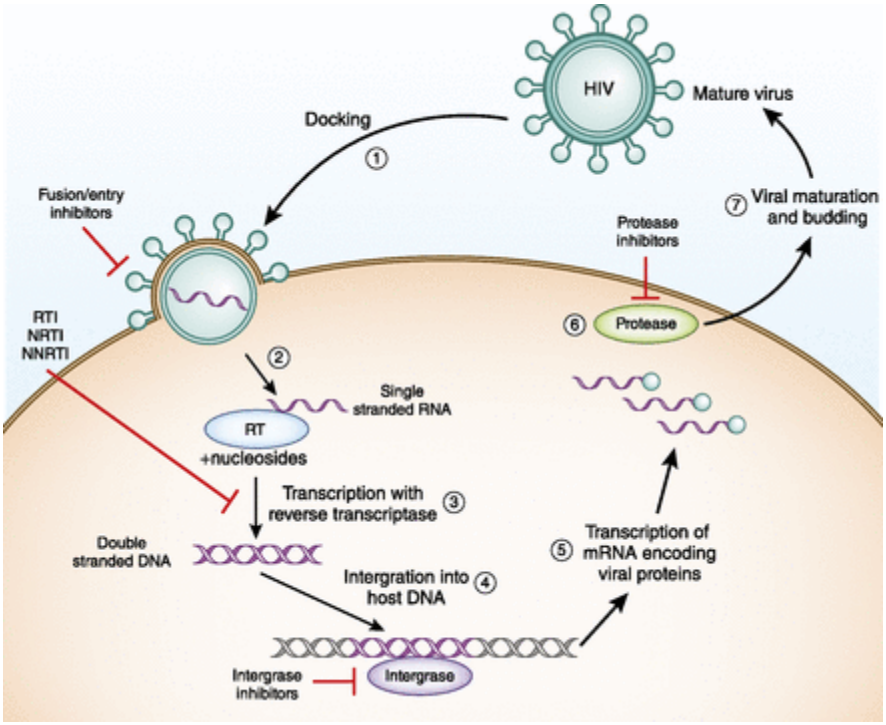

Figure 1 Different Mechanisms of Action for Various ART Classes

INSTIs are the newest group of ART; they target the HIV enzyme integrase, the enzyme responsible for integrating the reverse transcribed viral DNA into the host DNA for further replication[21]. The focus of this paper will be on DTG, the latest INSTI to be approved for the treatment of all populations. Though there are many documented benefits associated with the drug, including a high barrier against development and resistance, there have been reports of side effects that include neuropsychiatric symptoms, including depression and/or suicidal thoughts or actions[22]. In a small Dutch cohort of 387 patients on DTG, the researchers observed a 14.2% discontinuation rate due to psychiatric side effects[23]. Other cohorts had found discontinuation rates between 10 to 14%[23]. Out of the three main INSTIs, DTG reports the highest number of cases of neuropsychiatric side effects. Combined with the recent cluster of cases of neural tube defects in infants exposed to DTG during peri-conception in Botswana,

there is evidence to suggest that DTG safety for pregnant women and infants may need to be examined further[24].

A possible mechanism that could cause the neuropsychiatric side effects observed in DTG patients is NMDA receptor activation that leads to glutamate induced reactive oxygen species (ROS) production. Studies have shown that the mitochondria may play an important role in the overproduction of ROS with an association with glutamate excitotoxicity[25].

Hypothesis:

Our hypothesis is that the observed neuropsychiatric and neural tube defects in patients on regimens that contained DTG may be due to the effect of DTG-induced mitochondrial toxicity. The mitochondrial complexes are governed by mitochondrial genes with the exception of Complex II, which is coded by nuclear DNA. What we expect to see is that Complex II remains relatively unaffected by DTG treatment because it is reliant upon nuclear DNA as opposed to mitochondrial DNA. The central nervous system effect of DTG could be mediating mitochondrial and other sources of ROS production such as NMDR glutamate excitotoxicity.

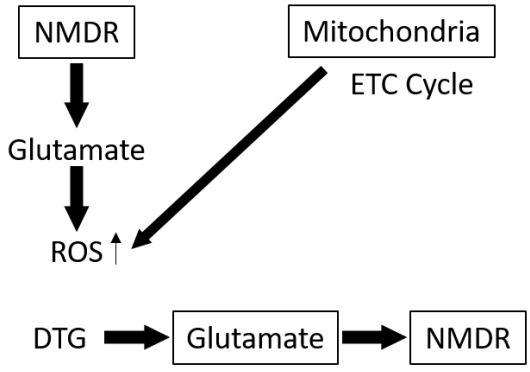

Figure 2 Possible mechanism of the relationship between DTG, NMDA receptors and ROS production

Methods:

We cultured SH-SY5Y undifferentiated human neuroblastoma cells, a line of neuroblastoma cells that were derived from a 4-year-old Caucasian female suffering with neuroblastoma. In previous studies, the cells have been used as a target cell in cell-mediated cytoxicity tests[26]. We treated them using three treatment regimens. We used three standard of care drugs for HIV treatment: Emtricitabine (FTC), Tenofovir (TDF), and Dolutegravir (DTG). They were also used in combinations of TDF/DTG and TDF/FTC/DTG. We used DMSO as our control. Each drug or drug combination was administered at the Cmax 1X and 4X concentrations. We counted the cell numbers before and after treatment, then examined each of the treatment groups by Western Blot and qPCR, looking specifically for the five mitochondrial complexes and NMDA receptor levels.

Results:

We counted under a microscope the number of cells that remained alive after treatment. Our control was DMSO with 60 cells remaining alive, as indicated by the black line in Figure 3.

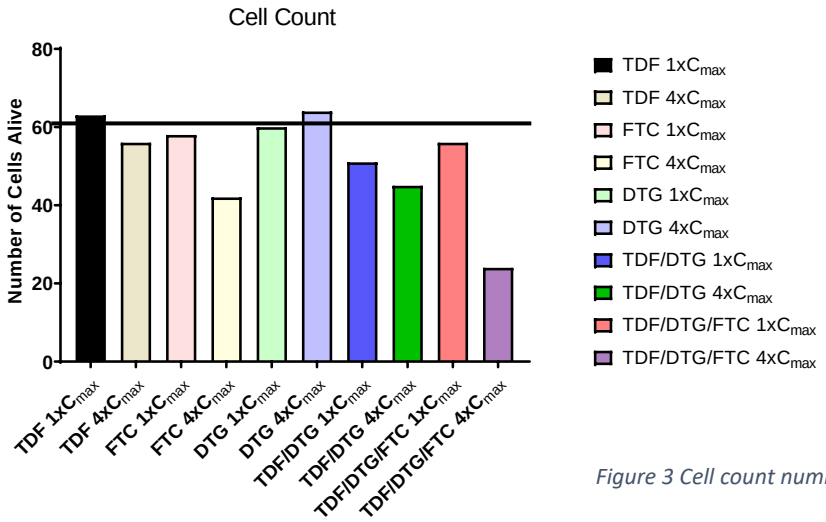

Figure 3 Cell count numbers for cells that survived treatment

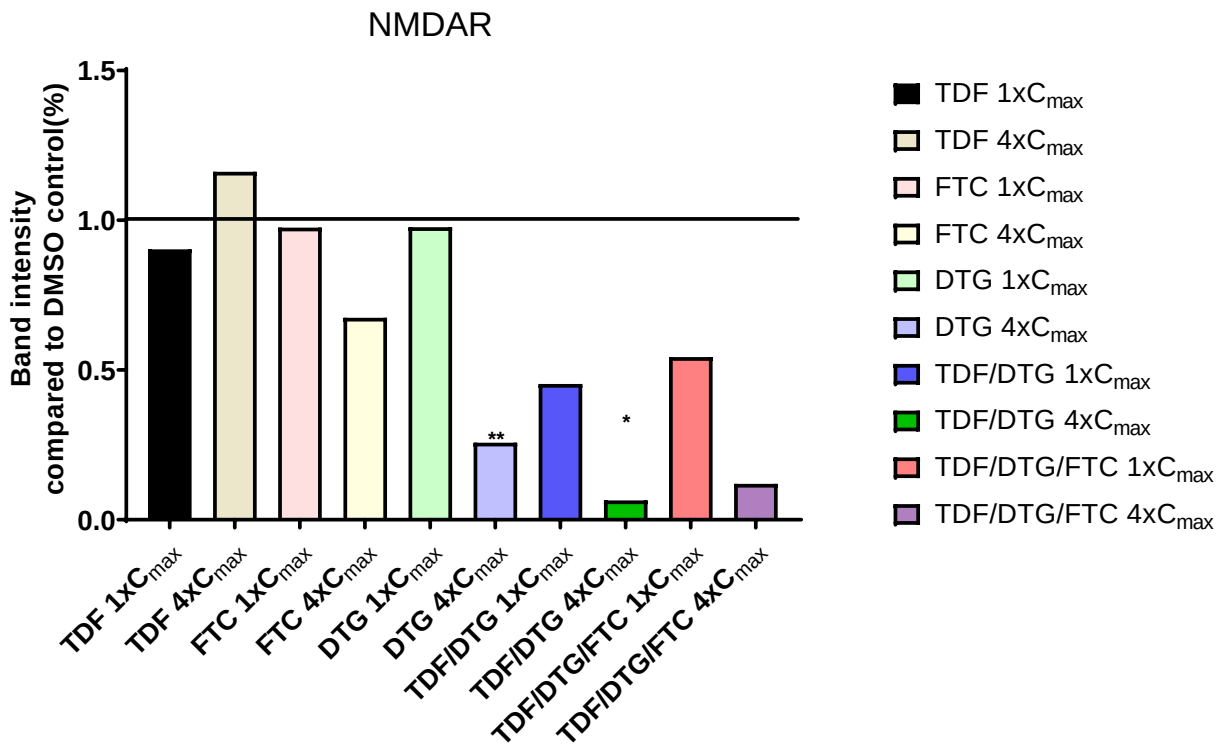

Figure 4 Western blot results for NMDAR protein levels in undifferentiated neuroblastoma cells

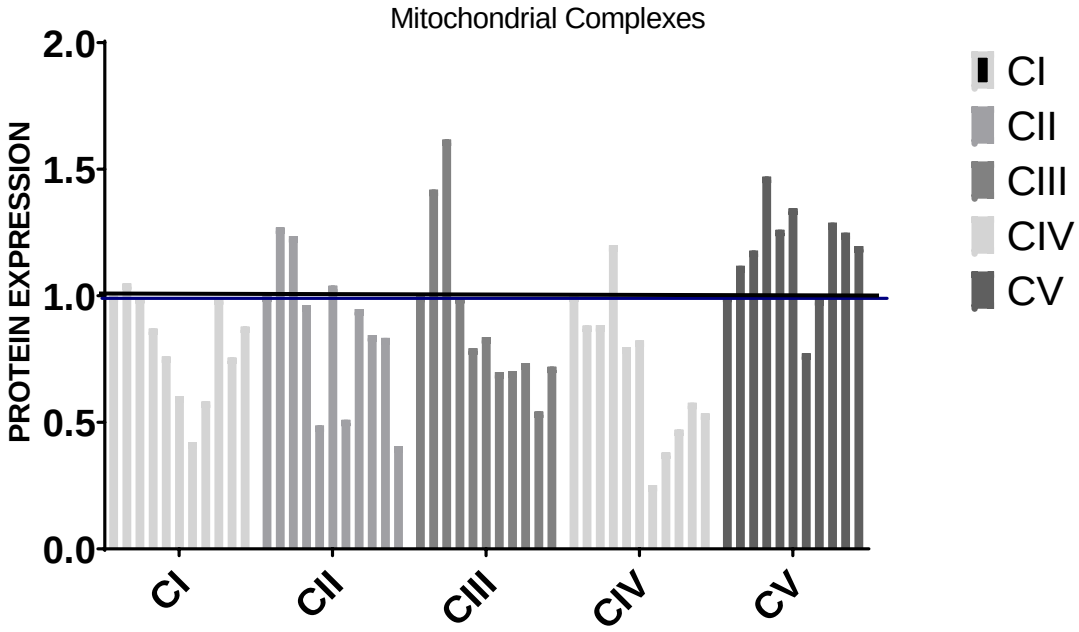

Figure 5 Western blot results for mitochondrial complexes protein levels in undifferentiated neuroblastoma cells. The order of the treatments by complex are: DMSO, FTC 1X, FTC 4X, TDF 1X, TDF 4X, DTG 1X, DTG 4X, T/D 1X, T/D 4X, T/D/F 1X, T/D/F 4X.

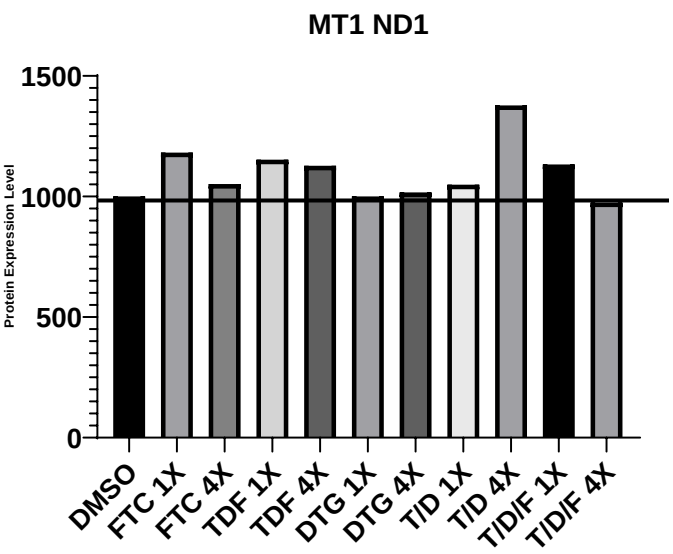

Figure 6 qPCR results for mitochondrial complexes gene expression levels for mitochondrial complex I NDI

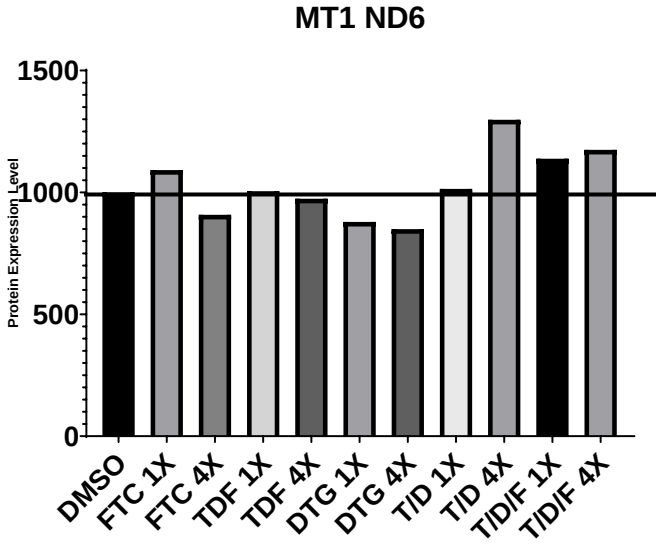

Figure 7 qPCR results for mitochondrial complexes gene expression levels for mitochondrial complex I ND6

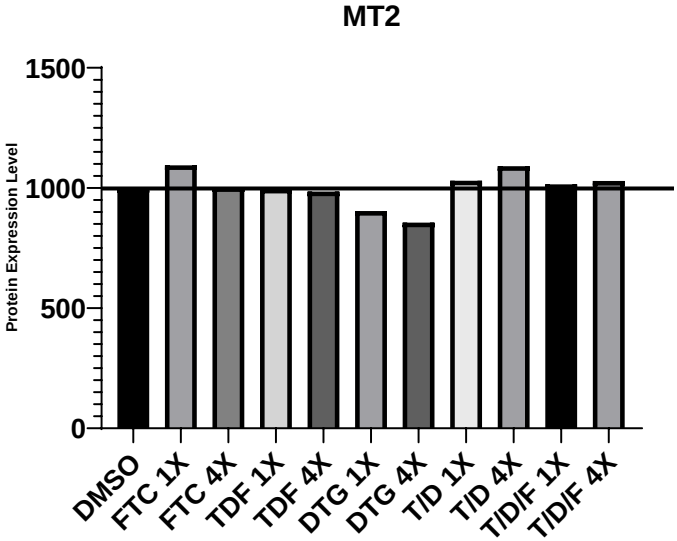

Figure 8 qPCR results for mitochondrial complexes gene expression levels for mitochondrial complex II

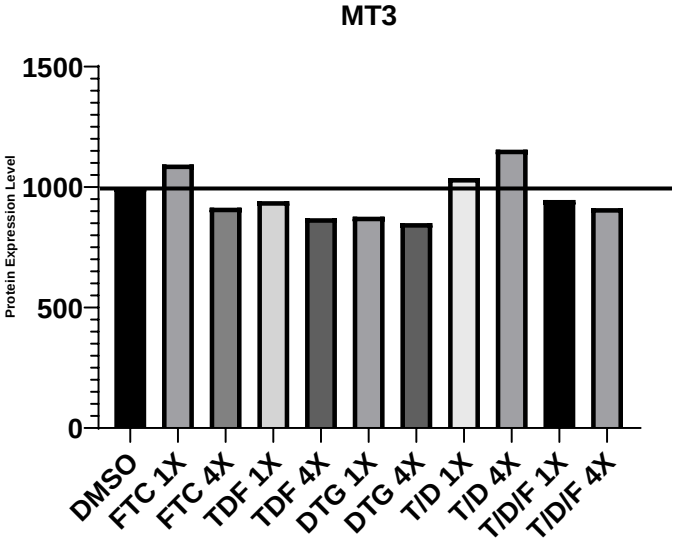

Figure 9 qPCR results for mitochondrial complexes gene expression levels for mitochondrial complex III

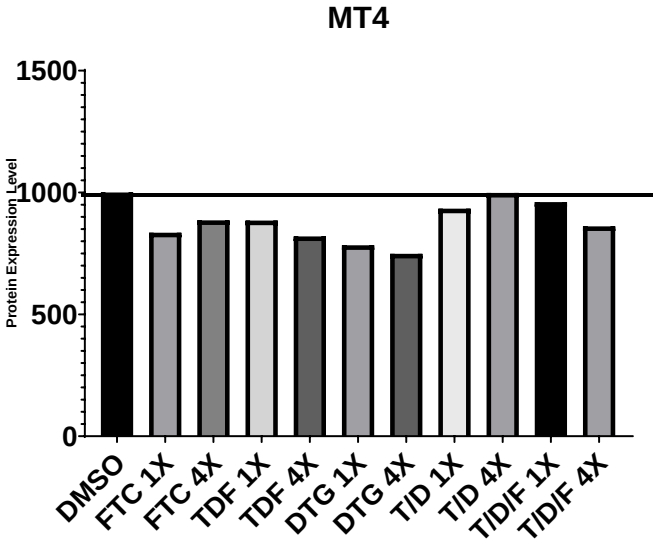

Figure 10 qPCR results for mitochondrial complexes gene expression levels for mitochondrial complex IV

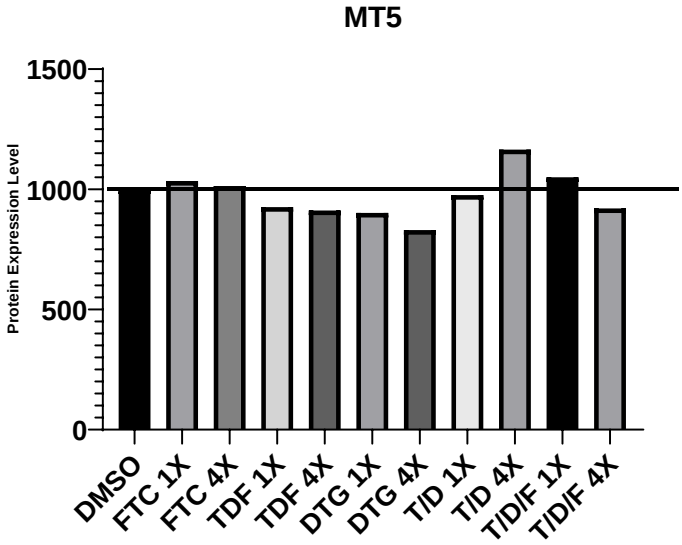

Figure 11 qPCR results for mitochondrial complexes gene expression levels for mitochondrial complex V

The three drug combination at 4X Cmax had the lowest number of remaining cells. In Figure 4, the DMSO control is represented by the black line at one. As a relative measure, all treatments that involved high concentrations of DTG had much lower NMDA receptor concentration. Complex V is relatively unaffected by the drug treatments in Figure 5 while the other complexes decrease significantly with a sharp drop in those treatments that included DTG. Figures 6 through 11 show the results of qPCR for the mitochondrial complexes. The expression levels of all complexes appear to be quite similar. Whether DTG has any adverse effects in terms of gene expression remains to be seen.

Discussion:

Though there is evidence of neuropsychiatric effects resulting from the use of DTG, the mechanisms that drives these effects are still in question. Glutamate driven excitotoxicity is an important player in signaling within neuronal cells[27]. Though we saw an appreciable effect of DTG treatments on NMDA receptor levels, it is hard to draw conclusions as whether DTG directly affects their production. Because we used neuroblastoma cells, signaling pathways are not representative of in vivo conditions for neuronal cells. The lack of difference between the gene expression levels of the mitochondrial complexes suggest that DTG may not have a direct impact on mitochondrial function. Due to the coronavirus pandemic, the potential for this project was cut short, with many trial yet to be completed. Both social distancing and stay at home orders cut into the time that could have been spent running more trials. With more data and more treatments, we would be able to better determine if there is in fact a trend.

Conclusion:

ART drugs have evolved considerably over time and treatment regimens have taken advantage of combination therapies using drugs from different classes to target various points in the HIV life cycle and to avoid building resistance. However, to move forward and try to attain the WHO goals of 90% of all people living with HIV knowing their HIV status, 90% of all people with diagnosed HIV infection receiving sustained antiretroviral therapy, and 90% of all people receiving antiretroviral therapy having viral suppression by 2020[28] , we will need to gain a better understanding of how frontline treatments for HIV affect those from vulnerable populations. DTG has shown great promise in terms of its high barrier to developing resistance and efficacy in suppressing viral load. However, the unusually high number of discontinuations due to neuropsychiatric adverse effects should not be ignored. The link may not be direct, but the neural tube defects seen in Botswana[29] with an association to mothers exposed to DTG peri-conception is more than likely related to the neuropsychiatric effects. Future studies should continue to focus mitochondrial dysfunction as a link to neural tube defects[30] and how integrase inhibitors such as DTG be higher risk for those with a history of neuropsychiatric disorders and developing children.

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