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Death and All Its Friends

The Role of Programmed Death-1 in T-cell Exhaustion During Chronic Viral Infection

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Introduction

Programmed death-1 (PD-1) is a CD28-family protein receptor, composed of 288 amino acids, that is expressed on the surface of B and T-cells (Riley 2009). Its main purpose is the regulation of the immune system, primarily through the process of peripheral tolerance. Through this mechanism, self-reactive T-cells that stochastically pass through self/non-self selection in the thymus and progress to peripheral circulation get deactivated into a state called T-cell anergy. In this state, cell proliferation and cytokine secretion is arrested. PD-1 is one such means of regulation, ensuring the prevention of self-reactive immunity and maintenance of T-cell homeostasis (Patsoukis, et al. 2012). Extensive research conducted on PD-1 and other receptors such as CTLA-4 has found that the PD-1 upregulation mechanism causes T-cell exhaustion and anergy, or even apoptosis during chronic viral infection. The PD-1 receptor is made of an N-terminal immunoglobulin-V type domain, a 20 amino acid stalk, a trans-membrane domain, and a cytoplasmic tail with tyrosine signaling motifs (Riley 2009). PD-1 is activated by its ligands, PD-L1 and -L2, which are on antigen-presenting cells. As seen in Figure 1, when they bind to PD-1, the protein inhibits the PI3K/Akt signaling pathway and affects T-cell activity.

During chronic viral infections such as HIV or hepatitis C, the ability of cells to secrete cytokines and replicate is often impaired, a condition called T-cell exhaustion (Petrovas, et al. 2006). Particularly in the case of HIV, where the virus infects T-helper (T\(_H\)) cells that are critical for promoting the immune response, cytotoxic T (T\(_C\)) cells are weakened due to the lack of T\(_H\) support despite high viral load in the bloodstream. Research has shown that during chronic viral infections, PD-1 becomes highly upregulated.
on T-cells and contributes to T-cell suppression through the same mechanism as peripheral
tolerance (Muthumani, et al. 2011). This correlation has become so well established that higher PD-
1 expression is now considered a marker of decreased homeostatic and antigen-induced

One of the central pillars of human immunodeficiency virus (HIV) research has been
centered on the phenomenon of ‘HIV controllers’. These individuals have a genetic ability to resist
HIV infection and, despite exposure to the virus, have anti-HIV antibodies or viral load lower than
detectable levels even months afterwards. In studies of their immune response, the controllers
were found to have much lower levels of PD-1 and CTLA-4 than HIV sufferers. Studies have
suggested that these lower levels allow the controller’s immune system to remain active and

Due to its protective effect in HIV controllers and potential in pharmaceutical anti-viral
therapies, the path through which PD-1 affects T-cell exhaustion has become the center of
research. The use of anti-PD-L1 antibody blockades in mice has been shown to restore T-cell
function while reducing viral load (Day, et al. 2006). More specifically, studies found that the PD-1
pathway blockade created an increase in T_c cells that were able to overcome exhaustion and
secrete cytokines (Walker and Kaufmann 2009).

This paper will discuss three research articles exploring “Death and All Its Friends” or rather,
the role of Programmed death-1 and similar receptors in arresting T-cell activity during chronic viral
infection and attempt to shed light on the little known mechanism behind these effects. Following
my analysis and synthesis of the three papers, I will suggest future experiments to explore and
develop the use of receptor blockades in anti-viral therapy to complement drugs that suppress the virus with treatment that maintains the strength of the immune system.


In this paper, Petrovas et al. explore the roles of PD-1 and CD57 (a cell adhesion molecule) in apoptosis. They found that PD-1 is linked to increased spontaneous and C95/Fas-induced apoptosis in HIV-infected patients, decreasing the efficacy of the immune system. This correlation was reduced in cells with high expression of CD57. However, they found that there was no correlation between PD-1 expression and secretion of cytokines, contrary to conventional evidence linking PD-1 to T-cell exhaustion.

With blood collected from HIV+ individuals, the authors analyzed cell sensitivity to apoptosis using polychromatic flow cytometry with fluorescent-conjugated monoclonal antibodies for various markers to differentiate between cells in the sample. In this process, a sample of cells is inserted into the FACS (fluorescence activated cell sorter) machine. Since cells have already been fluorescently tagged for receptors of interest, the FACS analyzes the sample and sorts each cell based on high/dim/low expression of the selected receptors, graphed on a logarithmic scale. This allows researchers to analyze an entire population of cells highlighting specific characteristics. In this case, cells are selected for CD27 and CD45RO to identify memory T cell subsets. CD27 is a tumor necrosis factor (TNF) receptor that is necessary for T-cell immunity and is involved in apoptosis when bound by apoptosis-regulatory protein, SIVA (National Center for Biotechnology Information 2012). CD45 is a receptor common to all leukocytes and was used as the primary way to identify immune cells. Apoptosis was measured using cell culture in presence or absence of annexin V and
an anti-caspase 3 antibody. Annexin V is often used as a probe for phosphatidylserine, which is expressed on the surface of cells during apoptosis. Similarly, caspase-3 is another protein associated with apoptosis. During their studies, Petrovas et al. found that the presence of annexin V was consistently associated with the presence of caspase-3. This shows that the apoptosis was caspase-3 mediated.

The authors first used naïve T-cells (CD27^hiCD45RO^lo) to set a gating scheme for expression of PD-1, as can be seen in Figure 2A across the top. As CD8+ cells matured (shown from left to right in Figure 2B), sensitivity to both spontaneous and CD95/Fas-mediated apoptosis increased, as shown by increased presence of both annexin V and active caspase-3. In the bar graph of Figure 2B, the various cell phenotypes are organized according to apoptosis, spontaneously or in the presence of CD95/Fas. This is one shortcoming in the methodology. In measuring cell death, they assume that all the apoptosis that occurs in the presence of C95/Fas is protein-mediated. However, they overlook the possibility that some of the apoptosis under those conditions could still be spontaneous. This makes the number for C95/Fas-induced apoptosis artificially inflated since it potentially includes both forms of apoptosis that took place in the presence of C95/Fas. In Figure 2C, this data is combined with the expression rates for PD-1. PD-1^hiCD27^hiCD45RO^hi/D^lo cells had the greatest sensitivity to apoptosis, especially C95/Fas-induced. In agreement with commonly accepted mechanisms, there was a higher percentage of PD-1^hiCD8^+ cells in patients with higher HIV+ viral load. Furthermore, authors found that there were fewer PD-1^hiCD27^hiCD45RO^hiCD8^+ T-cells in patients undergoing Highly Active Anti-Retroviral Therapy (HAART) compared to untreated HIV patients. They also found that PD-1^hiCD27^hiCD45RO^hi cells from the same donors, undergoing the treatment, were more susceptible to C95/Fas-mediated apoptosis, an interesting correlation that
the authors weren’t able to explain. It would be fascinating to see why the anti-retroviral drugs cause this increased susceptibility in T-cells.

2: PD-1 is a primary indicator of ex vivo apoptosis of CD8+ T-cells in HIV Infection. A: Flow cytometry gating of CD8 T-cells expressing L, D, or H levels of PD-1. B: Flow cytometry plots with annexin V binding and caspase 3 levels in CD8 T-cells from HIV-positive donor. C: Percentage of cells undergoing apoptosis in relation to PD-1 expression. Apoptosis was measured based on binding of annexin V or expression of active caspase 3 (Petrovas, Chaon, et al. 2009).
However, here is another shortcoming of this paper. The data for HAART-recipients and untreated patients are pooled for the charts and data analysis. While the authors include their observations from the data of the subgroups, they overlook those differences in their data publication, a significant oversight. In the chart, they should have shown the two groups separately in every category since the effects of HAART on the PD-1 pathway have not been established yet. By pooling them, they assume that there is no difference between both samples, yet they prove their own assumption wrong when they observe the differences in susceptibility to C95/Fas apoptosis between treatment groups.

The next step that the Petrovas et al. took was studying the association of PD-1 and CD57 with the different forms of apoptosis using flow cytometry. Figure 3A shows the amount of CD57 as a function of time or maturation of T-cells. As can be seen, CD57 is markedly upregulated over time, progressing from 0.43% in younger cells to 60.1% in memory cells. In the lower panel of 3A, the authors make the observation that the two receptors peak at opposite ends of the maturation spectrum, with CD3 being highest in young cells and CD57 highest in memory cells. They conclude their analysis with this; however, they should have included more analysis of this data to draw conclusions about why PD-1 peaks earlier and then decreases, as well as why the presence of PD-1<sup>H</sup> lingers after the fading of the PD-1<sup>L</sup> phenotype. In Figure 3B, the sensitivity to apoptosis is examined in the same combinations of PD-1 and CD57 cells. As can be seen, PD-1<sup>H</sup>CD57<sup>L</sup> cells showed the highest sensitivity to both spontaneous and CD95/Fas-mediated apoptosis except in CD27<sup>H</sup>CD45RO<sup>L</sup> cells. In that case, the PD-1<sup>H</sup>CD57<sup>H</sup> cell count is significantly higher. Furthermore, apoptosis rates for the PD-1<sup>L</sup>CD57<sup>H</sup> subgroup are fairly constant throughout T-cell types. The researchers do not explore this further, providing no explanation for these phenomena. Regardless of this question, the data clearly shows that PD-1 and CD57 have a differential association with
apoptosis, because CD57<sup>H</sup> is consistently associated with low amounts of annexin V or active caspase-3.

Petrovas et al. then establish how many HIV-specific CD8<sup>+</sup> T-cells express the PD-1<sup>H</sup>CD57<sup>L</sup>

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3: Differential association of PD-1 and CD-57 with spontaneous and CD95/Fas-mediated apoptosis. A: In the upper panel, flow cytometry showing PD-1 vs. CD57 expression in the entire CD8 T-cell population as well as CD57 expression in the memory CD8 T-cell population. Lower panels show relative expression of PD-1 and CD57 in relation to maturation. B: Upper panel shows expression of PD-1 in relation to CD57 at stages of maturation. Lower panel shows percentage of apoptotic memory CD8 T-cells with respect to PD-1 and CD57. (Petrovas, Chaon, et al. 2009)
subgroup that was associated with sensitivity to apoptosis in Figure 3, as opposed to the previous consideration of all CD8$^+$ cells before. In Figure 4A, the authors analyze the HIV-specific CD8 T-cells and use a gating strategy to measure cytokine expression of IFN-γ, TNF-α, and IL-2, which were used to select virus-specific cells after stimulation. In the lower panel of Figure 4A, the relative percentages of cells expressing PD-1 and CD57 are shown based on the cytokines used to select group-antigen (gag) specific cells for either HIV or cytomegalovirus (CMV). The writers found that PD-1$^{1}\text{CD57}^{1}$ was the most common HIV-specific T-cell found. PD-1$^{1}\text{CD57}^{1}$ was consistently the lowest percentage among T-cells. This data was in line with existing research showing that in chronic viral infections, T-cells expressing high amounts of PD-1 become the dominant phenotype. However, in the right panel for CMV, there were slightly different results. PD-1$^{1}\text{CD57}^{1}$ and PD-1$^{1}\text{CD57}^{1}$ were the highest expressed phenotype for specifically the IFN-γ and IFN-γ/TNF-α groups. On the other hand, cells expressing both IFN-γ and IL-2 expressed PD-1$^{1}\text{CD57}^{1}$ or PD-1$^{1}\text{CD57}^{1}$. This leads us to believe that CMV-specific T-cells overwhelmingly express a PD-1$^{1}$ phenotype, in direct disagreement with existing research. The significance of this data is questionable considering the far smaller sample pool of CMV-specific cells than with the HIV-specific cells. Furthermore, the testing of CMV is strange since the article is about HIV. Its inclusion only serves to confuse the author’s conclusion that HIV and CMV cells both exhibit no association between PD-1 expression and cytokine production since the same trend of receptor and cytokine expression cannot be seen between HIV and CMV-specific T-cells. This difference could be due to the small sample size, making the result misleading.

Using the same data, authors sought to compare the cytokine response between PD-1 positive and negative cells. Figure 4B shows the pie chart illustrating the proportion of cells expressing each cytokine. Contrary to the extensive documentation suggesting the role of PD-1 in T-
cell exhaustion, including depressed cytokine secretion, the authors found that there was not a link between PD-1 expression and cytokine secretion for virus-specific cells. When comparing the pie

4: HIV-specific CD8 T-cells express predominantly a PD-1^+CD57^+ that is not associated with the ability to produce cytokines. A: Flow cytometry plots showing expression of PD-1 and CD57 in Gag-specific T-cells identified by IFN-γ, IFN-γ/TNF-α, or IFN-γ/IL-2 production. Lower panel shows phenotypes of HIV and CMV specific T-cells with respect to PD-1 and CD57 expression. B: Composition of HIV and CMV specific T-cell response in relation to PD-1 expression. Pie denotes total fraction corresponding to a given number of cytokine functions. Right panel shows MFI values of PD-1 for all combinations of cytokine response. Boxes represent interquartile range. (Petrovas, Chaon, et al. 2009)
charts for both PD-1^+ and PD-1^- cells, one can see that there is no observable difference between cytokine expression, regardless if they are HIV or CMV-specific. In the right panel of Figure 4B, the authors show the MFI (mean fluorescence intensity) of PD-1 with a variety of cytokine combinations. They observe that no significant difference can be seen between cells specific for one virus, regardless of the cytokine combination tested. While their observation for HIV-specific cells is accurate, the same for CMV-specific cells is questionable because there is deviation for some of the cytokines combinations. Nevertheless, I think that it is less a result of actual difference and more due to the small sample size that the authors used. As a result, they should have disregarded their data for CMV and only concentrated on HIV-specific cells.

Overall, Petrovas et al. found that PD-1 and CD57 were differentially associated with spontaneous and C95/Fas mediated apoptosis in HIV cells. This is significant because high expression of PD-1 is found to be the dominant phenotype in HIV-specific cells during chronic infection as the cells mature. These findings are in line with various existing papers. However, the data diverges from convention in terms of cytokine products. The authors find that there is no link between PD-1 expression and cytokine production in either HIV or CMV-specific cells. This is in direct contrast to papers explaining that PD-1 expression has been directly linked to T-cell exhaustion during chronic viral infection. One of the points to be considered in the methodology is the donor population size. The researchers collected blood from only 32 HIV^+ donors and pooled the samples. However, as discussed earlier, these HIV^+ donors consisted of a mix of those undergoing HAART as well as those that were untreated. Ten of the donors were still asymptomatic. Therefore, they cannot even really be considered sufferers of chronic HIV infection. The researchers’ choice to pool blood from patients in different stages of HIV infection for
experimentation was unfortunate. The authors should have ensured that their sample pool was either separated or was limited to either treated or untreated patients.

*Synergistic Reversal of Intrahepatic HCV-Specific CD8 T-cell Exhaustion by Combined PD-1/CTLA-4 Blockade* (Nakamoto, et al. 2009)

Nakamoto et al, study the role of PD-1 and CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) in T-cell exhaustion during chronic hepatitis C (HCV) infection. Hepatitis C is a highly pathogenic virus that infects the liver and leads to chronic liver disease. As with other similar viral infections, PD-1 expression is upregulated on virus-specific T-cells. The authors cite previous papers that show that an in vitro PD-1 blockade results in restoration of effector function. However, in acute HCV infection, exhausted CD8 T-cells do not react to PD-1 blockade alone, suggesting the involvement of other mechanisms in the regulation of T-cells.

To look for other mechanisms, they studied the CTLA-4 protein. Existing research showed that, in HCV-specific T-cells, CTLA-4 is upregulated in addition to PD-1. Due to this, Nakamoto et al. explored the use of a combined PD-1/CTLA-4 blockade to reduce T-cell exhaustion as a therapy for viral infection. For the study, blood from 47 HCV patients was collected, including 33 that were cirrhotic and undergoing a liver transplant and 14 that had chronic but stable HCV. The control groups consisted of ten seronegative patients (no detectable antibodies), four seropositive (detectable antibodies) but RNA-negative, and 6 with acute HCV. In the paper, the authors were really clear about their sample size and distinguished their various control groups which I appreciated in terms of research method; I will address this in more detail later in my paper.

In their first experiment, shown in Figure 5, the authors used flow cytometry to study CTLA-4 expression specifically in hepatic HCV-specific T-cells. In Figure 5A, they show the percentage of
CD8 cells positive for CTLA-4 within C (chronic patients), R (recovered patients), N (seronegative patients), and A (acute patients). As can be seen, R and N controls show very low expression of CTLA-4. Interestingly, in acute patients, peripheral blood lymphocytes (PBLs) with CTLA-4 are quite high, especially in comparison to PBLs in chronic patients. However, as the HCV infection progresses, one can see that levels of CTLA-4+ cells drop in the blood stream. Alternatively, there are very high levels of CTLA-4+ liver infiltrating lymphocytes (LILs), an extremely significant observation (p-value <0.0001).

In Figure 5B, the researchers study the levels of CTLA-4 between HCV and other viral infections including influenza, Epstein-Barr virus (EBV), and CMV (cytomegalovirus). They measure CD8 cells that are tetramer+, a refolded peptide:MHC complex that is specific to a particular epitope. This technique allows scientists to quantify the number of T-cells in a sample that target a particular antigen. The data shows that, in the blood, CTLA-4 levels remain about the same, but, in the
liver, a higher percentage of HCV-specific cells express the protein. This demonstrates that this observation is unique to HCV. In the lower panel of Figure 5B, the authors show the data arranged by the patient group. As expected, there is no significant difference between blood and liver CTLA-4 levels in recovered patients; both are very low. But, once again, there is a very large difference between levels of CTLA-4\(^+\) T-cells in the liver versus blood of chronic HCV patients. Nakamoto et al. note that this contrasts with known levels of PD-1 expression in viral infection, where PD-1 is expressed highly in circulating lymphocytes as well.

In Figure 5C, authors show flow cytometry dot plots of their gating strategy to visually demonstrate the difference between the T-cell populations, especially comparing the levels of CTLA-4 and PD-1 in row two. In the first row, one can see the small proportion of the virus-specific CD8 cells in the blood and the significantly higher percentage for HCV in the liver. When levels of CTLA-4 and PD-1 are compared, the researchers found that CTLA-4 is a barely expressed in the absence or low-expression of PD-1 (Figure 5C/D). Consequently, CTLA-4 is only significantly found on the chronic HCV-specific LILs and the acute HCV-specific PBLs; the cases in which PD-1 was expressed in higher proportions as well. This correlation led credence to the author’s hypothesis that CTLA-4 plays a role in the cell exhaustion mechanism along with PD-1. In Figure 5E, data shows that there is a strong correlation between PD-1 and CTLA-4 expression among all cells during acute infection (red circles indicate patients with acute infection). This confirms the notion that this upregulation of both receptors continues in the bloodstream until onset of chronic infection, where CTLA-4 and PD-1 dual-positive cells become sequestered in the liver. In terms of data, a similar chart, showing the correlation between PD-1 and CTLA-4 in LILs of chronic HCV patients, would have been beneficial. Having established the link between CTLA-4 and PD-1 expression in liver-based CD8 T-cells of chronic HCV patients, the authors sought to test the effects of a blockade.
Figure 6 shows the results following CTLA-4/PD-1 receptor antibody blockade of HCV-specific LIL and PBLs in culture medium. Following blockade with αPD-L1 and αCTLA-4, the cells were stimulated with viral peptides spanning the entire HCV pNS3 or flu matrix and then underwent intracellular cytokine staining to identify antigen specific IFN-γ or TNF-α expression. As can be seen in Figure 6A, there was little, if at all, production of cytokines with the isotype, PD-L1, or CTLA-4 blockade. However, with antibodies to blockade both receptors in the presence of viral peptides, cytokine production for the pNS3 peptide jumped. This showed that the dual blockade was the

6: Intrahepatic HCV-specific T-cell dysfunction can be reversed synergistically by combined PD-1/CTLA-4 blockade.
A: Effect of receptor blockade on HCV-specific cytokine production by T-cells from liver and blood. B: Flow cytometry plots showing 7 days of culture with NS3 or flu peptides in the presence of isotype or blocking antibodies. On far left, plots show PD-1 and CTLA-4 expression in liver CD8 T-cells in vivo. (Nakamoto, et al. 2009)
most effective. Interestingly, the dual blockade had little difference to single blockade in PBLs, which responded in small degrees to both individual and dual blockades alike. In CD4 cells, this pattern was not seen. The PD-L1 blockade actually produced the highest cytokine levels, even higher than the dual blockade. The blockade of just CTLA-4 was no higher than the isotype only group. This could indicate that the role of CTLA-4 on T-cell exhaustion is limited to CD8 cells. In Figure 3B, the flow cytometry plots for HCV as well as flu blockades are shown. Results for HCV are, of course, the same: dual blockade is the most effective at increasing cytokine production. Interestingly enough, with the flu, there was no increase in cytokines when a dual blockade was implemented. If anything, the production seems to have decreased by over half from 1.52% to 0.59% of cells. This confirms the previous conclusion that PD-1 and CTLA-4 both have roles in T-cell exhaustion only in chronic hepatitis C infection.

Earlier in the paper (figure not shown), Nakamoto et al. established that the PD-1HCTLA-4H cells also expressed high levels of the co-stimulatory molecule CD28, even more than in cells with either PD-1H or CTLA-4H. It is already known that, like PD-1, CD28 plays a role in T-cell anergy. However, unlike PD-1, it is a positive stimulatory molecule involved in T-cell activity. T-cell activation requires two signaling events: binding with antigen presented on dendritic cells as well as CD28:B7 interaction, to prevent the T cell from falling into an anergic state.

Due to this observation, the authors sought to clarify the role of CD28+ cells and see whether CD28 was required for the CTLA-4/PD-1 blockade to be effective. Figure 7 shows the results of this experimentation. Figure 7A shows the HCV NS3-specific T-cells once again, except this time those exclusive to IFN-γ. The populations of CD4-deprived lymphocytes were separated into one group with and one without CD28 subset depletion. As can be seen in this figure, even without
CD4 T-cells, the cytokine response was significantly higher with the dual CTLA-4/PD-L1 blockade. However, when CD28 was depleted, the effect of the blockade was much reduced in all three patients regardless of whether they were chronic or acute sufferers of HCV. The authors conclude that CD28 is therefore required for the blockade to be effective.

Nakamoto et al. then decided to study a specific epitope of the HCV virus, NSC-1073. Figure 7B sets the background for the assay in ex-vivo conditions by showing the respective proportions of cells. As can be seen, 28% of the CD8 T-cells expressed CD28. 97.3% of the cells express PD-1, alone or with CTLA-4, and 20.5% of cells have CTLA-4 (all of these express PD-1 too). In the right-most graph, the numbers of cells expressing each receptor are compared to CD28 expression. The green line represents the number of PD-1^+CTLA-4^- cells (19.5% express CD28). The blue line shows PD-1^-CTLA-4^- (12.2% express CD28) and the red line shows PD-1^+CTLA-4^- cells (50% express CD28), clearly indicating a majority of cells positive for both receptors. These individual cell populations are
analyzed separately in Figure 7C. Carboxyfluorescein succinimidyl ester (CFSE) dilution is used to track proliferation of T-cells in the presence of receptor blockades, with and without CD28. CFSE is a dye that stains lymphocytes. When lymphocytes divide, the concentration of CFSE in their daughter cells is cut in half. This allows researchers to use this dye to track proliferation of cells for several divisions. Due to this, cells in the upper quadrant of the dot plots are newer cells (with lower concentration of CFSE, an indicator of cell proliferation) that are still specific to the HCV 1073 epitope. As can be seen, when the dual blockade is carried out, the proportion of new cells is 3.65%. This is compared to the 0.58% of CTLA-4 blockade alone and close to zero for the isotype or PD-L1 blockade alone. However, when CD28 cells are depleted, the impact of the dual blockade results in only a 0.13% new HCV1073-specific cell population. This clearly shows that a dual CTLA-4/PD-L1 blockade not only inhibits apoptosis, but also revives both cytokine secretion and proliferation of virus-specific T-cells in the HCV infection specifically. The authors also show how this process must be mediated by CD28 CD8 T-cells to have an effect. They further discuss how PD-1 inhibits the activation of T-cells through the CD-28 mediated phosphatidylinositol 3-kinase (PI3K) pathway and that CTLA-4 “activates the type II serine/threonine phosphatase PP2A, both leading to the inhibition of Akt phosphorylation” (Nakamoto, et al. 2009). As such, CTLA-4 directly competes with CD28 and reduces its mediation of PI3K-activation. In the presence of PD-1, CTLA-4 further inhibits the pathway.

Looking at the big picture, the authors confirm previous data that PD-1 impacts T-cell exhaustion; however, they prove that CTLA-4 has a crucial role in depressing the immune response to hepatitis C, but not to other viruses that they tested. Their finding regarding PD-1 directly contradicts Petrovas at al, by showing that PD-1, with or without CTLA-4, does inhibit antigen-specific cytokine (TNF-α and IFN-γ) secretion in T-cells.
In further comparison to the first paper by Petrovas et al, this paper was far clearer in its analysis and description of figures. Figure 5 is an example of how Nakamoto et al. were very thorough in keeping extensive track of their control populations and establishing a baseline for expression. Rather than pool the data together, they separated groups that had chronic, acute, or resolved expression, in addition to being seronegative or positive. This attention to detail makes the data in the second paper more valuable when they draw conclusions based on a specific population profile. For some of their later experiments, they also took specific patients and analyzed their lymphocyte populations independently, again keeping careful track of their state of infection. This methodology was very thorough and minimized potential critique of their conclusions. One of the few insufficiencies was that some of the later figures (6 and 7) rely on only a few patients to establish their conclusions. While this analysis on a patient-specific basis was also a strong aspect of the paper, the authors should have carried out this separate analysis for more individuals. I recognize that conducting the data collection for every patient is difficult, but a sample size of only three (in Figure 7) is not enough to draw conclusive results. This is a significant shortcoming, especially considering that, despite having data from only one acute patient, they assume that the exhibited effects of CD28 are universal among other acute sufferers.

Despite this flaw, this paper is an excellent example of effective and clear research methods. Nakamoto et al. provide data that sheds important light on the therapeutic potential of a receptor blockade of CTLA-4 and PD-1 to reduce the impact of exhausted cells in chronic sufferers of viral infection. This blockade would supplement the use of anti-retroviral drugs, which slow viral replication, by reducing the exhaustion of the CD8 T-cells that combat the virus. With increased proliferation and cytokine secretion, the patient would be able to more effectively fight the disease. They clearly discuss how the two receptors redundantly cause T-cell exhaustion, but that “immune
exhaustion at the site of antigen expression may be reversed by combined inhibitory receptor blockade” (Nakamoto, et al. 2009). This observation, shared by with Petrovas et al, illuminates a potential path for therapy research and development.

**HIV-Mediated Phosphatidylinositol 3-Kinase/Serine-Thereonine Kinase Activation in APCs Leads to Programmed Death-1 Ligand Upregulation and Suppression of HIV-Specific CD8 T-cells**

(Muthumani, et al. 2011)

Muthumani et al. explore the role of HIV in depressing T-cell function during chronic HIV infection. However, in contrast to the previous two papers, this lab takes a very different approach to the problem. Rather than look at the role of HIV infection on upregulation of PD-1 in T-cells, they look at the role of HIV infection on antigen presenting cells’ (APC) expression of PD-L1 and PD-L2.

They start their project on the basis that the administration of antibodies that interfere with the binding of PD-1 to its ligands results in more functional PD-1\(^{\text{H}}\) T-cells, a conclusion reached in the previous paper that I discussed. The authors note that existing studies have found an increase in PD-L1 and PD-L2 expression in APCs infected by HIV, even though the mechanism remains unknown. On this basis, the researchers hypothesize that HIV is able to “disrupt the generation and/or activation of HIV-specific T-cells by attenuating/disrupting Ag presentation via upregulation of PD-L1/2 expression on APCs and hence signaling through PD-1 on T-cells” (Muthumani, et al. 2011). To study this hypothesis, the authors disrupted the interaction between PD-1 and PD-L1 using PD-L1 small interfering RNA (siRNA), molecules that can prevent the receptor-ligand interaction using complementary base pairing. They also sought to show that APCs in HIV infected humans express higher levels of PD-1 than uninfected donors.
By using flow cytometry, Figure 8A identifies that levels of PD-1 ligands increase in HIV infected patients. Their gating strategy for separating HIV-specific cells was on the basis of whether they were HIV-1 p24 Gag⁺ or HIV-1 p24 Gag⁻. In the second gating, cells were separated on the basis of positivity for CD4 and CD14; a double positive indicated an antigen presenting cell, specifically a monocyte. In the upper dot plots, among the cells that were specific for HIV, there are cells with a much higher level of PD-L1 and PD-L2 expression compared to the monocytes from uninfected cells (lower panel of 8A).

Similarly, Figure 8B shows the difference in PD-L1 mean fluorescence intensity (MFI), between monocytes in HIV-positive and HIV-negative individuals. As can be seen, with a p value of 0.0001, the PD-1 levels in HIV-positive monocytes are far higher than in HIV-negative individuals. The authors wanted to determine if the effect was a result of higher gene expression, so they carried out reverse-transcription polymerase
chain reaction (RT-PCR). Figure 8C shows the relative gene amounts in the samples from infected patients and the uninfected (u.i.) control. The labeled fold change refers to the amount of PD-L1 or PD-L2 DNA present in relation to the uninfected control. Since the u.i. was taken as baseline 1 fold, all the patient samples (1-8) are expressed in folds of the uninfected sample. As can be seen, both PD-L1 and PD-L2 expression was increased in most patients compared to the u.i. control. From both Figures 8A and B, it is clear that HIV significantly upregulates PD-L1 and PD-L2 expression on monocytes.

To further study the effect of HIV on APCs, the authors separately examined two subsets of dendritic cells, myeloid (mDC) and plasmacytoid (pDC). Figures 8 D-G show the results of this experiment. Muthumani et al. stained the samples to identify CD80 and CD86 (costimulatory markers) in the monocytes. Figure 8D shows how almost all CD14+ monocyte cells expressed the two receptors. The authors found that there was no significant difference in marker expression between infected and non-infected individuals (Figure 8E). This established that the HIV virus was not changing the expression of co-stimulatory molecule and weakening the APC activation of T-cells in that way. The researchers studied the effects of HIV on PD-L1 expression in both subsets of dendritic cells and found that it was largely responsible for the suppression of the T-cell response. Figure 8G shows the results of this specific experiment: the mean PD-1 expression for both mDCs and pDCs in infected vs. health patients. It becomes clear that the infection HIV results in a significant rise in PD-L1 expression in both dendritic cell subsets, but especially in pDCs.

At this point, the lab had successfully demonstrated a rise in PD-L1. However, the question remained whether this upregulation had an effect on T-cells. To determine this, the authors correlated their data of PD-1 with PD-L1 expression on cells within the same sample using flow
cytometry. As can be seen in Figure 9A, the expressions of both receptors are directly correlated with a positive slope. In Figure 9B, they show that, as PD-L1 levels increased, counts of CD4+ cells, including the critical T-helper cells, decreased with a negative slope. Finally, the researchers found an even steeper decline (slope more negative than Fig. 9B) of IFN-γ secretion. This directly shows the link between upregulation of PD-L1 and increased T-cell exhaustion, which decreases the ability of the patient’s immune system to fight the virus.

Muthumani et al. then sought to collect more information about this effect: whether it could be observed in multiple cell populations and suppressed. Using known gene sequences for PD-L1 and 2, they researchers created target siRNA for both proteins. Using pools of three to five PD-L1 and PD-L2 specific siRNAs and a non-specific siRNA control, the authors could see whether they could stop the upregulation of PD-1 ligands. Furthermore, they could also see if the prevention of the PD-L1 and 2 upregulation could decrease the inhibition of T-cells. Figure 10A shows the Western blots of protein samples along with the β-actin control gel. The effects of the PD-L1 siRNA clone sequences 1 and 2 are shown. It seems that clone 1 has no difference in band darkness to the negative control or siRNA control, indicating no effect on PD-L1 expression. The relative increase in darkness of the control siRNA band is correlated to the corresponding increased darkness in the β-acting control for the same lane. This means that perhaps the amount of sample loaded was
accidentally more and is not suggestive of an actual increase in PD-L1 expression for the control.

The important aspect of this figure is the drop in PD-L1 expression when the siRNA clone 2 is used. Presumably, the siRNA clone 2 bound to mRNA for PD-L1 expression, blocking its translation into the receptor protein.

In Figure 10B, the results are confirmed through a different medium: flow cytometry. As can be seen, PD-L1 expression in monocytes remains fairly constant throughout the various controls and siRNA clone 1, but for siRNA clone 2, the expression drops to 2.0%! This confirms the conclusion that the use of siRNA clone 2 is able to stop the upregulation of PD-L1. The authors then had to study whether or not this limiting of PD-L1 expression has an effect on cytokine expression by T-cells. Figures 10C and D show the levels of IFN-γ specific to HIV group-specific antigen (or gag-antigen within the core protein of the virus) or envelope (env-antigen on the external protein coat) measured by ELISPOT after stimulation in culture.

ELISPOT, an adaptation of the ELISA (enzyme-linked immune sorbent

10: PD-L1 mediated functional suppression of HIV-specific CD8 T-cells. A: Western Blot for PD-L1 content of whole cell lysate with β-actin controls. B: FACS plot with PD-L1 expression on CD14+ cells 5 days after siRNA treatment. C and D: ELISPOT analysis of IFN-γ production by Gag-specific (C) or Env-specific (D) monocytes when cultured with PD-L1-blocking siRNA or antibodies. E: Blockade of PD-L1 can restore immune cytokine production after PD-L1 silencing. Env-stimulated samples were tested for cytokine responses and are shown. (Muthumani, et al. 2011)
assay), is a procedure using monoclonal antibodies, specific for the cytokine of interest, that are conjugated to a fluorescent tag. The cytokines are captured on a special membrane and following addition of color sensitive reagents, the color change and spots are used to measure the number of colonies producing the given cytokine and quantify the amount being produced.

Figures 10 C and D both show a very similar trend of cytokine production. The culture of siRNA-silenced monocytes and monocytes with blocking antibodies for PD-L1 produced the highest level of IFN-γ for either HIV antigen. This indicates that both are effective in reducing T-cell exhaustion. siRNA stops the upregulation of PD-L1 in the first place, compared to the anti-PD-L1 antibodies that only prevent the PD-1/PD-L1 interaction. Finally, Figure 10E shows the impact of PD-L1 siRNA broken down specifically by cell type: T<sub>C</sub> cells (CD3/CD8), T<sub>H</sub> cells (CD3/CD4), and dendritic cells (CD14). For every single cell type, the introduction of siRNA resulted in an almost uniform 3-fold increase in secretion of pro-inflammatory cytokines (IL-2, IFN-γ, and IL-12). Furthermore, secretion of the anti-inflammatory cytokine, IL-10, decreased by the same degree, indicating the revival of lymphocyte and dendritic cell function, as well as the increase of general inflammatory effects.

The lab similarly shows that the use of siRNA increases the proliferation of T-cells. Figure 11A illustrates how, through the use of decreasing CFSE concentration as a marker for increased T-cell division, 11: Increased T-cell proliferation after PD-L1 silencing. A and B: Mixed cells incubated in medium only, siRNA control only, or PD-L1 siRNA. Flow cytometry carried out displaying CFSE as a function of CD8 on CD3 lymphocytes. C: Proliferative responses for Gag (top) and Env (bottom) with value for siRNA control subtracted. (Muthumani, et al. 2011)
the PD-L1 siRNA results in a three to four fold increase for the gag-specific T-cells (A) and up to a 4.4 fold increase for the env-specific T-cells (B). The data is alternately displayed according to the percentage of cells containing CFSE (greater percentage implies that T-cells have divided, conferring daughter cells with CFSE at lesser concentrations). As can be seen, both gag-specific (top) and env-specific (bottom) CD8 and CD4 cells showed an increase in cell proliferation when PD-L1 expression was blocked by siRNA.

Having established conclusively that HIV viral infection does upregulate the expression of PD-L1 and PD-L2 and that siRNA or antibody interference with PD-1/PD-L1 expression and/or binding is effective in reviving T-cell activity, the authors wanted to explore the mechanism through which this occurs. They hypothesized that the PI3K/Akt pathway played a strong role in the upregulation of PD-L1 by HIV. As can be seen in Figure 1, when PI3K is activated by its ligands and co-receptors, it phosphorylates Akt. To study the role of this mechanism in HIV’s effects (1), the researchers used a PI3K specific signaling inhibitor, LY294002, and an Akt inhibitor, Akt inhibitor-IV. Muthumani et al. harvested proteins from cultured U937 (a model monocyte cell line) cells periodically following infection with HIV. Using ELISAs, these samples were then analyzed for Akt and pAkt. They found that from 12-hours post-infection onwards, there was a large jump in
amount of phosphorylated Akt (pAkt), while the level of regular Akt rises only slightly during the time period (Figure 12A). This indicates that the levels of non-phosphorylated Akt are not changing, confirmation that the increase in pAkt is not due to increased expression but rather increased phosphorylation of existing Akt. This conclusion is verified in the Western blot results of Figure 12B. Levels of Akt are not significantly changed, however, at 12 hours from infection, the amount of pAkt is much higher than at 6 hours. This indicates a role of the pAkt process in HIV infection. The researchers are able to confirm that this pAkt process is involved in specifically the PD-L1 process when inhibition of PI3K and Akt both lead to decrease in number of cells expressing high levels of PD-L1. The HIV+DMSO control shows two cell population groups: one with lower PD-L1 (uninfected cells, thin lines) and one with higher PD-L1 (infected cells, bold line). In the presence of either pathway inhibitor, the infected cell population is shifted left, representing moderate levels of PD-L1 expression (Figure 12C). This data is directly indicative that the PI3K/Akt pathway is the mechanism through which PD-L1 is upregulated by HIV in antigen presenting cells.

In Figure 12B, the researchers should have included the control bands of β-actin to provide the context for analyze the darkness of each lane. Also, the data in C does not necessarily prove that the PI3K/Akt pathway is the only way in which PD-L1 is upregulated by HIV. There is a high likelihood that other mechanisms are involved as well. This is indicated by the fact that in the presence of signaling inhibitors, levels of PD-L1 don’t return to uninfected levels. Granted, this could be impossible due to the inability to block the effects of HIV in every single cell. An ideal next step to confirm the scope of the PI3K/Akt signaling pathway would be to flood the culture with the signaling inhibitors and see whether PD-L1 amounts are comparable to that of uninfected cells. The authors did indeed carry out this step (data not shown). They conducted an assay in which they slowly increased the concentration of both inhibitors. The levels of PD-L1 activity slowly decrease
until they become zero. This indicates that the PI3K/Akt pathway is critical for HIV’s upregulation of PD-L1 in antigen presenting cells.

In conclusion, Muthumani et al. make significant progress in explaining the inability of HIV+ immune systems to mount complete effector responses. In part, this can be explained by the unusual role of HIV in not only exploiting PD-1 expression but also upregulating PD-L1 expression. The paper cites that little is known about the function and distribution of PD-L1 and PD-L2, limiting how we understand the receptor/ligand interaction. It is understood that the loss of T\textsubscript{H} cells is one of the main debilitating aspects of HIV infection, a primary reason that the body’s own immune system is crippled. However, this emerging research shows how HIV uses alternate pathways as well, such as expression of PD-L1 on antigen-presenting cells, to weaken the immune system in different ways. There is scope for therapeutic development in this regard, which will be covered more in the synthesis section.

**Discussion and Synthesis**

The combination of the three papers provides a broad overview of the role of HIV and other viruses in the weakening of the immune system, more specifically on T-cell exhaustion during chronic infection. PD-1 is a necessary part of the immune system and is involved in regulation of the immune response by balancing the need to prevent auto-immunity with maintaining basic immune efficacy. However, viral infection seems to utilize this pathway to weaken the immune system. All three papers center around the basic premise that programmed death-1 plays a significant role in limiting the T-cell response. Each of the papers takes a similar experimental response to the problem: first to identify the correlation (if any) between viral infection and expression of PD-1, and then to study co-surface molecules and the extent and ways in which the PD-1 expression affects T-cell exhaustion (measured by cytokine expression, proliferation, or apoptosis). While the papers’ collective results can be aggregated as a model, there are significant areas of difference.

The first paper, by Petrovas et al, studies the role of PD-1 and CD57, both surface molecules, in T-cell survival during chronic viral infection. During infection, PD-1 is significantly upregulated on CD8 T-cells. The authors find that PD-1 expression is linked to a higher prevalence of both spontaneous and C95/Fas mediated apoptosis. The paper shows that PD-1 and CD57 are oppositely linked to T-cell survival. In fact, expression of CD57 was an indication of resistance to apoptosis. However, strangely enough, the researchers find that there is no connection between PD-1 expression in T-cells and secretion of cytokines, in particular IL-2, IFN-γ, and TNF-α. This was directly contradicted in both the following papers where the same cytokines were tested, but resulted in opposite conclusions.
The second paper studied the role of PD-1 and CTLA-4 in mediating the immune system and regulating T-cell functionality in hepatitis C infection. The researchers study the expression of PD-1 and CTLA-4 and found that, for HCV, PD-1 and CTLA-4 are significantly upregulated in hepatic lymphocytes but not in blood lymphocytes. In patients with acute infection, these expression levels are comparable in the blood as well. However, this similarity ended when the disease progressed to chronic stage as the upregulated cells were almost exclusively present in the liver. While this was surprising at early stages, it later seemed normal that the virus was suppressing T-cells the most in its primary area of infection. For HCV, the liver was the site of T-cell exhaustion. However, for HIV, the bloodstream was the area of action and therefore was the site of PD-1 upregulation.

The second and third papers explored the potential therapeutic implications of this upregulation. Nakamoto et al, studied the effects of an individual and combined PD-1 and CTLA-4 blockade on T-cell proliferation and cytokine expression. They found that a dual antibody blockade, not just anti-PD-1 alone, was required to both reverse T cell dysfunction and renew cell proliferation. This paper established a biochemical interaction mechanism between PD-1/CTLA-4 and CD28, a co-receptor. They mention that the PI3K/Akt is involved in this process but they don’t elaborate further. This segues well into the study conducted in the third paper two years later.

Muthumani et al carry out a well-designed study to identify the exact mechanism through which HIV acts to induce T-cell exhaustion. However, they take a very unique approach to the problem. Rather than study the upregulation of PD-1 on T-cells as the previous papers did, the authors study the role of HIV in upregulating PD-L1’s ligands (PD-L1 and PD-L2) on antigen-presenting cells. They hypothesize that HIV infection of dendritic cells results in a rise in PD-L1 and PD-L2 levels and that this increase is responsible for the T cell exhaustion that is exhibited during
chronic infection. As described earlier in this paper, they are able to prove this upregulation and then link it to decreasing cytokine levels and T cell counts.

This agreement between the Nakamoto et al and Muthumani et al contradicts the assertions made in the first paper that the PD-1\(^H\) phenotype doesn’t influence cytokine secretion. This finding, by Petrovas et al, is weakened by evidence from multiple research papers showing the role PD-1 has on T-cell exhaustion, including cytokine suppression. The sheer number of papers that contradict the findings of Petrovas et al, only lends further credence to this conclusion.

To move from the effects of PD-1 and PD-L1 to practical application, Muthumani et al, take the novel approach of using siRNA to stop the translation of the PD-L1 and L2 RNA into protein. This silencing does significantly increase the levels of cytokine secretion and T cell proliferation. However, the most effective treatment seemed to be the use of blocking antibodies to prevent the binding of PD-1 to its ligands. The authors then prove the conclusions of Nakamoto et al by linking the PI3K/Akt pathway to the expression of PD-L1 in antigen presenting cells. They use inhibitors of PI3K and Akt and find that HIV is unable to upregulate PD-L2 in APCs without the activation of this pathway.

The model that the three papers together provide has a significant implication on treatment options for HIV. It establishes the way through which HIV causes CD8 T-cell exhaustion: not only due to lack of sufficient CD4 T-cell support, but also through upregulation of the PD-L1/2 and PD-1 pathway due to the increase in expression of the both the receptor and its ligands. The research shows that HIV, as well as other viruses, has been able to exploit the body’s natural regulatory mechanisms in order to weaken the immune system and further replicate.
The papers’ focus on chronic viruses shows that, despite their common use of PD-1, many of the viruses act through slightly different activation mechanisms. Some involve CTLA-4 as a co-receptor in the activation pathway while others require molecules such as T-cell immunoglobulin mucin domain-3 (Tim-3), a T-cell surface receptor. Regardless, once these mechanisms of action are better understood, they could be a target for therapeutic intervention. The second and third papers show how use of anti-receptor antibodies (such as PD-1) or siRNA specific to receptors or ligands can stop the activation of the pathway. Elite controllers, those individuals that are genetically able to control viral replication without medication, have been reported to be able to maintain their immune response by preventing the upregulation of PD-1 (Baker, et al. 2009). This research sheds light on the ability of the individuals self-sufficiently fight the virus and resist its effects on T-cells. Possible therapies for HIV could mimic this advantage that elite controllers have and minimize damage of the virus.

During these infections, HIV or others (such as CMV, HCV, or LCMV), the effect of the virus on individual cells is devastating. To control the spread of viral infection, it is crucial that the body is able to mount a strong immune response. However, the opposite often happens. In an HIV infection, as CD4 T-cells die, the Tc cells would ideally step in and fight the infection. But, at the same time, CD8 T-cells start to weaken and become anergic due to the interaction between upregulated PD-1 and PD-L1 and L2. This makes HIV immunologically crippling. Treatment with siRNA or anti-PD-1/PD-L1 blockades, in addition to HAART, would allow the patient to fight the disease on two fronts. HAART would slow down viral replication while, concurrently, the siRNA or blockade would reverse the inhibition of CD8 T-cells, allowing the body to fight the disease naturally.
Future Experiments

While the three papers fit in well together in terms of new knowledge of PD-1 activity, there is some key experimentation to be done that would take this knowledge forward.

A potential first step would be to explore the role of CTLA-4 and CD28 in other viral infections, not just HCV, to identify whether these effects are unique or uniform for other major viruses including HIV, SIV, and HCMV. As can be seen from Figure 1, CTLA-4 is present in the same signaling pathway. As a result, it probably has a role in mediating the T-cell exhaustion. I would use the same methods used by Nakamoto et al when they test for a correlation between CTLA-4 and PD-1 upregulation for HCV and apply them to HIV and other viruses as well. The other two papers establish that PD-1 is upregulated; however none of them study whether CTLA-4 expression increases along with PD-1 for non-HCV cases.

To further study the upregulation of CTLA-4, I would take chronic patients and, using flow cytometry, test their blood for the percentage of CD8 cells that express CTLA-4 to see whether this receptor is upregulated too. The paper by Muthumani et al showed that use of PD-L1 antibody blockades was the most effective way to revive the T-cell population. If CTLA-4 does have a role in this regulation in HIV too, then the inclusion of a dual PD-L1/CTLA-4 blockade would be so much more effective, especially if CD28 promoted the T-revival. If we can identify a constant role for CTLA-4 expression in various viruses, we could use that information to create better therapies to combat this inhibition of T-cells.

I also want to explore the role of PI3K/Akt in the PD-1 upregulation processes. From these three papers, we can conclude that this signaling pathway is responsible for the upregulation of both PD-1 in T-cells and PD-L1 and PD-L2 in antigen-presenting cells. In order to better understand
this mechanism, we would need to identify whether that is the only path through which these receptors and ligands are upregulated. This could be done through blocking of various common immunological signaling mechanisms, with siRNA or antibody blockades, and, through RT-PCR, ELISPOT, or Western Blot, observing whether upregulation still takes place in cell culture.

Another interesting step would be to establish a model for developing medication for these diseases. SIV (simian immunodeficiency virus), a cousin of HIV, might be a potential target in this case. SIV can be characterized in the same way researchers study HIV. If they can establish that, like HIV, SIV upregulates PD-1 in culture conditions, researchers should attempt to identify key co-receptors involved in the signaling process in a human-like system. This information could further help in developing therapies to block the role of these molecules in a simian model.

Using this information, we could develop siRNA and antibody receptor-blockades specific to SIV, using similar procedures used by Nakamoto et al and Mutumani et al. This would allow us to move from in-vitro to in-vivo experimentation. Since a simian model is very complex, it could provide a good basis from which to establish effectiveness and safety of therapy. One such concern of using siRNA or antibody blockades to prevent PD-1 activation is that that T-cells might become too uninhibited, leading to increased autoimmunity. It is important to remember that PD-1 and CTLA-4 are crucial regulatory molecules. Nakamoto et al cite that knock-out mice for these genes suffer from lupus-like autoimmune conditions or eventual fatal lymphoproliferation (Nakamoto, et al. 2009). As such, the PD-1 pathway should not be completely silenced.

A similar problem is seen with the role of the PI3K/Akt signaling pathway. Muthumani et al showed that the suppression of the signaling molecules completely prevented the upregulation of PD-L1. While this seems like an easy therapeutic intervention, it is important to note that this
pathway has major roles in other processes in the body. Inhibiting its function could have disastrous effects on other body systems. Therapies that strike this balance between preventing over or under expression could be achieved within an animal model to ensure that it was safe and effective before starting clinical trials in humans.

In conclusion, like most other HIV and other chronic viral infection research that is emerging, these conclusions require extensive further exploration and development to have greater potential as therapeutic paths. The role of the ‘HIV controller’ needs to be studied further, by identifying if the prevention of PD-1 upregulation is a key factor leading to their resistance. If so, the prevention of PD-1 upregulation during chronic viral infection could be a central means through which patients could be treated. In addition to standard therapy, such as the use of HAART, this treatment could prevent the weakening of a patient’s immune system, allowing the body to naturally help fight HIV itself. This research is thus a crucial link in the fight against HIV/AIDS and, pending further study, has tremendous therapeutic potential.

References


