

Emerging trends in HIV pathogenesis and treatment

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Introduction

This paper will discuss aspects of HIV pathogenesis and treatment approaches that have emerged in the recent period. This is not intended as a comprehensive review but rather attempts to provide a context for a broader understanding of these trends. While much of the data and clinical experience cited in this discussion derives from the highly-resourced world there are clear applications for planning and implementation in the developing world. This author draws from more than 25 years of experience as an HIV clinician, researcher and as one who has worked within the biotechnology industry in early drug development and novel approaches to the treatment of HIV.

Inflammation, Aging and HIV: Does HIV Lead to Premature Aging?

The chronic conditions and diseases that characterize the aging process are increasingly felt due to a state of chronic inflammation [1-3]. Markers of chronic inflammation have been associated with numerous conditions including cardiovascular disease, neurodegenerative diseases, osteoporosis and fracture, insulin resistance, diabetes and the metabolic syndrome as well as muscle wasting and frailty. Indeed elevated levels of one marker, IL-6, is a strong predictor of near-term all-cause mortality. The precise role – and the pathobiology - of the inflammatory cytokines as markers or initiators of the aging process have not been fully established. Nonetheless there is general agreement that chronic inflammation plays a notable role in aging. HIV, and likely other chronic infections, is associated with a generalized inflammatory process that persists throughout infection. While the successful treatment of HIV with antiretroviral agents reduces aspects of the inflammatory response, pro-inflammatory cytokine levels remain elevated above age matched HIV seronegative individuals. Many of the serious diseases of aging, along with alterations in tissue structure and function, have been noted in those with HIV infection. These diseases and conditions appear to occur earlier than chronological age would suggest, and many now hold the view that HIV itself promotes the aging process. The result is a second epidemic, premature aging, associated with HIV. The burdens of HIV infection must now be thought of to include not only those illnesses classically associated with the infection, but a host of additional chronic and debilitating conditions.

The inflammation hypothesis of aging posits that aging is characterized by the accumulation of cellular damage resulting from activation of acute and chronic inflammatory processes. These processes go beyond the well known characteristics of acute inflammation (heat, redness, pain and swelling) to include aspects of immune function, coagulation and numerous metabolic processes (notably those related to glucose and cholesterol metabolism). The putative explanation supporting the inflammation

hypothesis is that the short-term gain of a robust inflammatory response to infection or environmental stress is traded for longer-term tissue damage. As a number of workers in the field have noted, there is sound evolutionary advantage to such a strategy. Beginning early in life host responses permit the individual to survive infectious and environmental challenges, but at a cost of later tissue and organ damage brought about by the same local and systemic cellular responses that control the original insult. The inflammation hypothesis does not ignore the effects of diet/nutrition and genetics on morbidity and mortality, but rather views these as relative permissive or restrictive factors within the context of lifelong exposure to infectious diseases and other environmental challenges. Two ubiquitous herpes family viruses, cytomegalovirus (CMV) and Epstein-Barr virus (EBV), have attracted particular attention among researchers [4-6] and may play a central role in the aging process. Indeed markers of CMV and/or EBV are less often noted among those living to very old age, and evidence of CMV and EBV reactivation in the elderly is strikingly common. HIV is likely one such additional infectious challenge, and sets in motion an enduring cascade of cellular events that leads to a state of premature multi-system tissue dysfunction and disease. This may be demonstrated at a macro level in the developed world, where despite the widespread availability of antiretroviral therapy a 20 year old diagnosed with HIV has a life expectancy of 53 years, compared to 78 years for an age-matched HIV-negative person [7]. Clearly there is more to the “successful” treatment of HIV than the suppression of HIV itself. An understanding of the processes by which HIV accelerates human aging is a vital aspect of national and global planning for the epidemic.

HIV, Aging and Immune Function

While research in this area is relatively recent, HIV may hasten the aging process in several important ways. Immune deficiency – with the occurrence of malignancy and infection - is a clinical hallmark of the aging process. Several characteristics of the aged immune system share striking commonality with HIV immune deficiency. These changes include inversion of the normal CD4/CD8 ratio, diminished thymic output, CD4 lymphopenia, an increase in late differentiated CD4 and CD8 cells, and shortening of CD8 telomeres [8]. One particular aspect of age-related immune dysfunction is the limited ability of certain cells to respond to challenge. One such cell, the cytotoxic T-lymphocyte (CTL), is responsible for recognizing the presence of infection and responding by cellular (clonal) expansion, or proliferation. The potential for clonal expansion is understood to be limited by the number of cell divisions inherent in human somatic cells, termed the Hayflick limit. Each cell division is marked by the shortening of telomeres, a non-coding, terminal, region of DNA. Once telomeres reach a critical length, a DNA damage signal is triggered and results in a state of cell growth arrest, termed replicative senescence. Immune replicative senescence [9] is felt to account for the increased incidence and severity of a number of common infectious diseases of aging notably influenza, pneumococcal pneumonia, sepsis and herpes zoster, among others. An established marker of immune replicative senescence is a lymphocyte population that has lost expression of the cell surface marker CD28. As expected there is a normal loss of CD28+ cells with aging. An accelerated loss of CD28+ cells, however, is noted in those with HIV infection compared to a cohort of age-matched seronegative controls. Further, among HIV+ individuals those with the greatest loss of CD28 expressing lymphocytes demonstrate a more rapid progression of

their disease. Cells that have reached replicative senescence produce high levels of TNF α and IL-6, adding further to a physiological milieu rich in inflammatory cytokines. Telomere shortening, the signal for replicative senescence, has been noted to be greater in HIV-infected individuals when compared to HIV- controls [10]. This is likely reflective of a state of persistent cellular activation and proliferation, an important aspect of the pathobiology of HIV (see below). Thus it appears that HIV, in addition to directly depleting vital immune system elements, accelerates immune replicative senescence and the normal age-related process of immune decline. This would be expected to manifest by an increased frequency of infection, a hallmark of HIV disease, but also by an increased frequency of malignant diseases.

Malignancy and HIV Infection

HIV has been associated with a number of malignant conditions often termed AIDS-associated cancers. Among these are Kaposi's sarcoma, cervical cancer and non-Hodgkin lymphoma. Data now supports clinical observation that numerous non-AIDS cancers are more common in HIV-infected populations [11]. Among these are cancer of the lung, anus, testicle and certain hematologic malignancies. An increased frequency of these neoplasms is noted even after careful control for known risk factors, such as smoking and lung cancer. An independent role of immune deficiency states and subsequent malignancy has been demonstrated in a recent meta-analysis of standardized cancer incidence rates among HIV+ and solid organ transplant recipients [12]. Whether the state of immune deficiency is acquired in the setting of HIV, or iatrogenic through immunosuppressive therapy to prevent transplant rejection, an increased incidence of a number of malignant diseases is apparent. An important aspect of this study is the demonstration of enhanced cancer incidence despite dissimilar lifestyle and host risk factors. Most cancers found in these two populations were those induced by infectious causes, notably Epstein Barr virus (EBV), human herpes virus-8 (HHV-8), human papilloma virus (HPV), hepatitis B, hepatitis C and *Helicobacter pylori*. Other cancers such as those of the lip and non-melanoma skin cancers occur with greater frequency in both populations, while cancers of the colon, breast and prostate do not.

The reason for an increase in non-classic malignant diseases among those with HIV poses compelling research questions. The theory of immunologic surveillance of cancer was first proposed by Burnet in 1970 [13] and appears confirmed at least in part. Whether the leading non-infectious cancers will prove to be more common in those with HIV may require longer periods of observation than currently afforded by studies to date. The role of HIV in modifying extant cancer pathways [14] including regulation of apoptosis, cell growth, angiogenesis and cellular adhesion is a logical and potentially fruitful focus of study

Serious Non-Malignant Conditions in HIV

Following the introduction of highly active combination antiretroviral therapy a number of serious non-AIDS conditions were recognized to occur at rates above an age-matched seronegative population [15, 16]. In the main these conditions – atherosclerotic cardiovascular disease, renal, bone and hepatic disease – were felt due to traditional risk factors and/or adverse consequences of antiretroviral therapy. Indeed identifiable risk factors such as smoking have been noted to be more common among those with HIV infection than in the general population. And certain HIV therapies, both alone and when used in combination, are linked to clear evidence of metabolic, renal or hepatic perturbation. When clinical events and laboratory abnormalities bespeaking organ dysfunction occurred in those with HIV these occurrences were felt to confirm the adverse event profiles of the involved agent. Certain observational cohort studies detailed the incidence of these events, and the astounding clinical gains of antiretroviral therapy were now being viewed as a balance of benefit versus the risks and toxicities of therapy. Reflecting the belief that antiretroviral treatment contributed to the occurrence of metabolic, cardiovascular, renal and hepatic events, treatment initiation guidelines were altered and strategies designed to minimize drug exposure through treatment interruption were undertaken (see section on when to start antiretroviral therapy). The recalculated balance between benefit and risk proved incorrect as the pathobiology of HIV revealed a more complex interplay between host, drug and unanticipated viral factors.

Among the most important studies in the HIV field, the Strategies for Management of Antiretroviral Therapy (SMART) study [17, 18] demonstrated that untreated HIV, more than population risk factors, or antiretroviral agents, imparted an excess risk for all-cause mortality, cardiovascular, renal and hepatic events. Related to the degree of immune impairment, these excess risks remained even after adjustment for CD4. Designed to prospectively evaluate a strategy of intermittent CD4-guided use of antiretrovirals (so-called drug conservation group) versus continuous use (viral suppression group), the multi-year SMART study was prematurely discontinued after only 16 months following a data safety monitoring board review revealed an excess of non-AIDS deaths and serious clinical events in the drug conservation group. In understated fashion the authors summed up the impact of these findings:

“The excess of deaths from causes other than opportunistic disease in the drug conservation group was surprising... contrary to available data and to the assumptions underlying our study design, participants in the drug conservation group had a higher rate of major cardiovascular, renal, or hepatic disease than did those in the viral suppression group...We expected the risk of death...to decrease with the interruption of antiretroviral therapy, rather than to increase.”

Following termination of the study, the majority of subjects in the drug conservation group initiated or reinitiated antiretroviral therapy. While their risk of death and serious non-AIDS events fell, even after achieving and maintaining viral suppression this group demonstrated excess risk when compared to the continuous therapy - viral suppression group.

Plasma samples obtained during the study have provided SMART investigators the opportunity to prospectively evaluate various factors that may have affected clinical outcome. One such analysis [19] revealed that those in the drug conservation group had dramatically elevated levels of two markers of tissue inflammation, IL-6 and D-dimer (adjusted OR for IL-6 and D-dimer 11.8 and 26.5, respectively). IL-6 is synthesized in the liver and causes the release of Tissue Factor 1 and 2 from vascular endothelium. Tissue Factor release results in the cascade of events leading to micro- and macro-vascular coagulation. D-dimer is a marker fibrinolysis, the eventual breakdown of thrombi (clots). Both IL-6 and D-dimer levels were increased at study entry and rose during study observation in those with eventual death (all cause). There was a strong positive correlation between HIV RNA level and IL-6 and D-dimer. It should be noted that the IL-6 and D-dimer odds ratios noted in the SMART study are vastly higher than those seen in the decades of non-HIV studies for risk of cardiovascular deaths. These data provide strong support for the inflammation hypothesis and suggest that HIV replication is a potent driver of cellular activation, inflammation and the eventual non-AIDS clinical outcomes typically associated with aging.

HIV Immune Activation and Inflammation

The view that immune activation is a significant factor in the pathobiology of HIV is attributed to the immunologist Janice Giorgi. While understandably focusing on the profound immune deficiency of HIV, the first published report [20] noted the presence of an elevated lymphocyte subset now known to be CD38⁺ cells, lymphocytes showing evidence of activation. Working in a frightful, and self-renewing symmetry HIV replication stimulates the process of T cell activation which leads to increased transcription of integrated virus, and further viral replication. Additionally activation results in production and recruitment of more T cells, providing additional substrate for HIV and persistence of infection and an overall loss of T-regulatory cells. Immune activation has become understood as a crucial element of HIV pathogenesis, CD4 cell depletion and immune deficiency. The accepted dataset includes the key observations that immune activation is the most powerful independent predictor of disease progression and clinical AIDS [21], and that dampening of activation predicts the eventual rise of CD4 cell count more than the reduction of HIV RNA [22, 23]. The activation thesis also provides an explanation for the initially puzzling observation that among non-human primates infected with the simian immunodeficiency virus (SIV) a relentless course akin to HIV in humans was seen in some species, while in others the disease had a chronic and indolent course. Among SIV's so-termed "natural" hosts, the sooty mangabey and African green monkey, a high circulating levels of virus is noted, but without the expected loss of CD4 cells or evidence of immune activation. Among "non-natural" hosts such as the African rhesus macaque high levels of both viremia and immune activation are noted, and this animal has a rapid loss of CD4 cells and progression of disease. In humans infected with HIV-2 a milder and more slowly progressive disease is characteristic compared to those with HIV-1. In addition to lower levels of viremia and more robust immune responses HIV-2 infected individuals demonstrate lessened immune activation. These observations, from human and non-human primates alike support the importance of immune activation in CD4 cell depletion and clinical disease.

The question of how HIV induces immune system activation is not definitively established, but recent proposals combining work by Brenchley [24, 25] and Douek [26] has proven intriguing. These authors build upon several observations including: (1) that the earliest stage of HIV infection results in a massive depletion of CD4 cells located in gut associated lymphatic tissues (GALT) of the colon and distal small bowel and (2) damage to enterocyte mucosal barriers permits the gut to become a portal of entry for microorganisms. Termed bacterial translocation, cell wall and possibly other components of these microorganisms initiate a systemic immune response with lymphocyte activation and cellular expansion. Meant to contain infecting pathogens the response is mediated through Toll-like receptor-4 and other cellular defense systems. One product of bacterial translocation, lipopolysaccharide or LPS, is noted to be directly associated with plasma markers of immune activation, including several pro-inflammatory cytokines, and increased numbers of CD38+ cells. Central to this hypothesis is an explanation for the ongoing nature of immune activation throughout the course of HIV infection. For reasons that remain poorly understood, suppressive antiretroviral therapy leads to only partial immune restoration in GALT compared to other lymphatic compartments. While peripheral T cells often return to near normal levels, those in central lymphatic tissues may not. This is especially so in GALT¹. Lymph node collagen deposition and fibrosis are cited as one potential factor that might explain the attenuated localized T cell trafficking, renewal and homeostatic processes. In addition the integrity of gut mucosal cells may not return to normal following treatment with antiretrovirals. The impaired structural and immunologic aspects of the gut are held to permit the ongoing microbial translocation which results in a state of chronic immune activation.

Inflammation, Aging and HIV

While evidence supports substantial portions of the inflammation hypothesis of aging competing or complementary theories, including those attributed to mitochondrial damage and oxidative stress, have not been excluded. Inflammatory processes may prove to be the proximate mediators of aging and may at some point provide the basis for directed preventive and therapeutic intervention. Similarly the evidence linking immune activation to the pathobiology of HIV is robust but not yet complete. The 'leaky gut – bacterial translocation' theory provides an attractive mechanistic framework to conceptualize ongoing systemic immune activation. At a minimum the inflammation hypotheses of aging and HIV run in parallel, and provide a rich opportunity to explore questions of pathogenesis relevant to both the HIV infected and uninfected populations. At a symposium on HIV and aging held at the 2008 Conference on Retroviruses and Opportunistic Infections, one speaker noted that "the good news is that our HIV patients are aging. The bad news is that our HIV patients are aging." The truth in this statement should provide the basis for directed and cross-disciplinary basic and clinical research, but also the social and infrastructural planning in both the developed and developing world.

¹ One recent report noted that despite suppressive antiretroviral therapy ongoing viral replication could be demonstrated in GALT [27]

Implementation and evaluation of policies and programs of HIV therapeutics will need to extend beyond conventional measures to include the numerous indirect and collateral aspects of the disease.

When to Start Antiretroviral Therapy: Treatment Trends and Implications for Developing Nations

The most appropriate time for the initiation of antiretroviral therapy is not known. Three distinct treatment paradigms in the developed world have characterized the 13 years since the highly active combination therapies have been available. The pendulum has swung from treatment at the earliest stages of disease to delay of initiation until a point close to clinical AIDS. Newer data has caused a reevaluation of this later strategy and current guidelines recommend therapy at a point roughly midway between the two poles. In early 2009, well in to the era of highly active antiretroviral agents, the first large-scale, randomized clinical trial to evaluate the timing of treatment initiation will begin. Since the first US Department of Health and Human Services treatment guideline appeared in 1998 this panel has released 17 additional versions or revisions reflecting clinically important new information of pathogenesis or therapeutics. An examination of these documents [28] and those of the International AIDS Society [29] reveals the evolution of treatment imperatives based on clinical experience and the evolving clinical and basic sciences of HIV. The most recent International AIDS Society guideline [30] explicitly reaches beyond the developed nations:

"...the core principle... pathogenesis-directed therapy with regimens designed to achieve full virologic suppression with minimal toxicity and maximal simplicity, is applicable to the developing world...."

The discussion below will briefly review the evolution of the clinical imperatives underlying HIV treatment. The purpose is to place into historical context considerations that form the basis of current recommendations based on new insights into HIV pathogenesis. Such a discussion must also take into account that, at the time of this writing, there is neither an effective vaccine to prevent HIV, nor a microbicide to interdict the sexual acquisition of infection. This discussion will by necessity address treatment issues for adolescents and adults only. The important considerations for treatment of the pediatric populations are beyond the abilities of this author and the scope of this paper.

It must be acknowledged that a large portion of our initial understanding of antiretroviral treatment outcomes is based on experience reported from the developed world. Certainly clinical trials for investigational agents have included individuals outside of the Americas, Western Europe and Australia, but taken together these studies largely reflect the demographics of HIV infection in those geographies. Similarly the observational cohort studies that provided critical information on HIV progression and clinical outcomes reflect the experience in these same regions. Many aspects of the clinical biology and pharmacology of HIV appear to be the same regardless of race, sex and mode of acquisition, yet there are notable differences that may meaningfully impact populations of differing predominant demographics.

Treatment Imperatives of the HIV Epidemic: A View from the Developed World

Three periods have defined the therapeutic era of HIV. The first was driven by unbridled opportunistic infections and AIDS-defining malignancies, collectively termed AIDS defining events (ADEs). The second was conditioned by the frequent emergence of putative treatment complications, drug resistance and the demonstration of a reservoir of latently infected lymphocytes whose persistence precludes the possibility of viral eradication. The third period was established by the SMART study [17] which demonstrated that HIV replication was responsible for numerous serious non-AIDS conditions many of which had been erroneously viewed as treatment complications. Consistent with the prevailing imperatives, the therapeutic approach of the first period was characterized by early treatment initiation, the second by delay in treatment, and the third by a shift back to earlier intervention. CD4 cell count is conventionally used to define early versus late, though following the availability of direct quantitative measures of viral replication (HIV RNA) this was added as an additional consideration.

The First Period

1996 marked the beginning of the highly active antiretroviral therapies. Termed HAART, these therapies for the most part included a combination of nucleoside reverse transcriptase inhibitors² and a protease inhibitor. This combination therapy approach provided the first potent therapeutic regimens and resulted in the astonishing clinical gains of the period. HIV was now seen as a treatable condition and therapy was extended to virtually any individual regardless of CD4-gauged immune status or viral load.

² The nucleoside reverse transcriptase inhibitor (NRTI) is a misnamed class of antiretrovirals. NRTIs do not inhibit the viral enzyme reverse transcriptase, but rather inhibit the vital viral function of reverse transcription. NRTIs act by terminating viral chain elongation during reverse transcription. For the sake of clarity, and in keeping with convention, the traditional nomenclature of these agents, and the non-nucleoside reverse transcriptase inhibitors (NNRTIs), will be maintained.

The first U.S. Department of Health and Human Services treatment guideline suggested therapy for all individuals with less than 500 CD4 cells and an HIV RNA greater than 10,000 copies. Driving the widespread use of HAART were the disabling and lethal opportunistic infections and malignancies that characterize untreated advanced HIV disease. Despite numerous adverse drug events, inconvenient dosing and meal requirements that characterized the earliest regimens, the vast majority of patients receiving therapy gratefully soldiered on.

The Second Period

Several observations beginning in 1997 tempered the initial enthusiasm of HAART regimens, and formed the basis for the second period of antiretroviral therapy that was to follow several years later. Seminal work from the laboratory of Robert Siliciano [31] demonstrated the presence of a reservoir of HIV infected resting lymphocytes in patients on HAART. This reservoir – not affected by antiretroviral drugs - and the associated kinetics of integrated virus within these cells effectively ended any possibility of viral eradication or cure using conventional antiretroviral agents. While an understanding of the biology of the latent reservoirs remains incomplete, it appears that virus periodically discharged from these cells is responsible, at least in part, for the maintenance of infection in those effectively treated with current therapies. Three additional observations (1) frequent, and often severe adverse drug events; (2) the extraordinary levels of medication adherence required to effectively inhibit viral replication; and (3) the rapid development of drug resistance, and in many cases cross-class drug resistance, lead to a reconsideration of the timing of treatment initiation. Regrettably no randomized clinical trial data was available to guide the decision of when to commence antiretroviral therapy, and decisions were made on the basis of observational cohort data and expert opinion. The CD4 cell count used to commence therapy for the asymptomatic individual in this period was lowered to 200, and HIV RNA levels rose to 50,000 - 100,000 copies. The change in treatment guidelines were by-in-large reactive, and the limitations of the cohort datasets that provided the support for new CD4 and HIV RNA levels were infrequently acknowledged. Along with the known inherent biases of observational studies (principally unrecognized confounders and selection bias), the majority of the cohorts did not capture non-AIDS comorbid conditions or causes of death. Many while following a large number of individuals had a limited duration of follow-up.

Clinical trials in this period evaluated several strategies that attempted to limit drug exposure (and thereby adverse events and drug costs), and nearly all failed to demonstrate acceptable clinical outcomes. At the same time important advances in immune and viral pathogenesis, clinical virology (drug resistance mechanisms and the archiving of resistant viral variants) and pharmacology (intracellular and other compartment effects, and host genomic considerations) aided in a more complete appreciation of the complexity of HIV pathobiology. Certain viral and host factors were understood to have an effect on the occurrence and severity of treatment complications, and antiretroviral therapeutics improved considerably with a number of agents – and new classes of agents - that featured both enhanced potency and reduced toxicity. The pendulum began to swing back toward

an earlier, and now somewhat more individualized approach to treatment as reflected in the IAS Guidelines of 2004 and 2006 and the DHHS Guidelines of 2007.

The imperatives underlying the second era of HIV treatment were by no means trivial, though with the benefit of hindsight the deferral or interruption of therapy is now understood to be ill-advised. Apparent treatment-related adverse events, notably metabolic, hepatic and renal, often accompanied by marked body composition changes affected between 20% and 70% of patients. It is without doubt that the antiretroviral agents drove a portion of the events that conditioned the treatment decisions of the era. It is also equally apparent that HIV itself plays quite a notable role. This was made startlingly clear by early findings of the Strategic Management of Antiretroviral Therapy (SMART) trial first reported in early 2006.

The Third Period

The current period of antiretroviral therapy is supported by datasets reflecting a more robust understanding of HIV pathogenesis and an applied biologic basis for observational cohort studies that have expanded to include non-AIDS conditions. In addition, the interplay between HIV and comorbid conditions such as hepatitis B, hepatitis C, cardiovascular disease and nephropathy have provided the beginnings of a means to individualize antiretroviral treatment initiation. The essential observations supporting earlier and directed antiretroviral therapy can be summarized as follows:

- The SMART trial demonstrates the effect of uncontrolled viral replication and an increased risk of morbidity and mortality in all CD4 cell strata [17]
- Inflammatory biomarkers collected as part of the SMART trial are associated with HIV replication and predict the occurrence of death and serious non-AIDS events [19]
- Both randomized trials and cohort studies show that those who begin therapy with CD4 cell counts between 200 and 350 have lower rates of ADEs and death, are more likely to achieve maximal viral suppression, and achieve higher CD4 cell counts than those who begin therapy at lower CD4 cell levels [32-37]
- Observational cohort data from antiretroviral treatment naive individuals with CD4 cell counts greater than 350 demonstrates increased mortality compared with the general population [38] and an increased risk of non-AIDS cancers in those with CD4 cell count is less than 500 [39]
- Newer, less toxic antiretroviral agents and data that informs improved drug selection provides multiple durable and well tolerated regimens

The result is a movement towards earlier treatment initiation, reflected in several national and international guidelines, and the anticipation that this strategy could be adapted to the developing world. Compelling as they may be, these observations do not support an expectation that earlier treatment would provide the primary means of addressing the worldwide HIV epidemic. However, earlier treatment initiation may have meaningful impact and result in longer-term clinical gains for those most vital and productive elements of society, a reduction in mother to child transmission as suggested

by the 2007 Perinatal HIV Guidelines Working Group [40] , and a potential reduction of HIV transmission among adults [41].

The confirmatory evidence to inform such an approach is anticipated from the Strategic Timing of Antiretroviral Treatment (START) trial. This randomized international trial will evaluate the strategy of immediate (any CD4 cell count greater than 500) versus deferred therapy (when the CD4 cell count is less than 350). Planned in two phases, the first will enroll roughly 1000 subjects with an eventual total of 4000 in the second phase. The study is planned to follow subjects for 3 years; enrollment is anticipated to require 3 years. Several important sub-studies are planned including those addressing host genomics, cardiovascular changes and neurocognitive dysfunction. Results of the study will not be available until 2015 unless planned periodic data safety reviews reveal a meaningful difference in outcomes between the two study arms. If the START trial demonstrates the benefits of earlier treatment initiation how should this information inform policy and practice in the developing world? Given the inordinately high early mortality rates noted in resource-poor settings, the impact of earlier antiretroviral treatment might have even more benefit than in the developed world. Modeling and planning for a broadly inclusive approach, including earlier diagnosis of infection and the laboratory and clinical monitoring necessary for treatment decisions might permit actualization of any policy change in a time responsive fashion.

One observation from the developed world with great potential application to Sub-Saharan Africa and the Caribbean is the excess risk of kidney disease and progression to end-stage renal disease (ESRD) among HIV-infected blacks³. While the first observation of the adverse effect of race on progression to renal failure was noted early in the U.S. HIV epidemic, recent studies have quantified the risk in the era of antiretroviral therapy. HIV replication is known to promote renal dysfunction, even at low HIV RNA levels in populations at risk, and a clinical syndrome, HIV associated nephropathy (HIVAN) is well described. Untreated, HIVAN will rapidly progress to ESRD. Antiretroviral therapy is effective in slowing, even stopping, HIVAN. In one recent observational study [43] HIV-infected blacks were two times as likely to develop chronic kidney disease as whites, but once developed, nearly 18 times more likely to progress to ESRD. While both the DHHS and IAS treatment guidelines have suggested consideration of earlier initiation of antiretroviral therapy in those with HIVAN, or evidence of chronic kidney disease, there may be a role for earlier initiation of ART in high-risk patients, prior to the establishment of kidney disease [44].

While datasets remain limited, evidence to date suggests that the prevalence of HIV-related kidney disease in Sub-Saharan African populations is similar to that observed among American blacks. In this context efforts to develop simple and inexpensive laboratory methods to detect and manage early renal disease as well as the availability of accompanying renal-protective therapeutics (angiotensin converting enzyme inhibitors or angiotensin receptor blockers) should be pursued.

³ The gene (MYH9) conferring excess risk of renal disease among blacks has been recently identified [42]

Anticipating the Next Period in the Developing World

The present World Health Organization HIV treatment guidelines recommend initiation of antiretroviral therapy at CD4 counts of less than 200 cells. Earlier initiation is recommended for those with clinical stage 3 or 4 disease. The current era of HIV therapeutics based as it is on a more defined context of pathobiology, recognition of important comorbid conditions that impact the progression of disease, and the availability of increasingly effective and tolerable agents could arguably foster an environment where treatment is available more broadly, and at an earlier stage in the developing world. Earlier treatment initiation might be expected to have several immediate salutary benefits. The immune reconstitution inflammatory syndrome (IRIS) occurs frequently in those with more advanced disease and clinical or sub-clinical opportunistic infection [44], and likely contributes to the high early mortality rates currently reported. Earlier therapy would certainly reduce the overall burden of ADEs and the incidence of IRIS. Earlier treatment may also favorably impact mother-to child transmission and overall rates of transmission among adults. Current treatment guidelines in the highly-resourced nations suggest therapy commence when CD4 cell count falls to 350. This is the level used for the “deferred treatment” arm in the START trial and though not yet informed by a large randomized clinical dataset can thus be seen as a reasoned level. What are the implications of changing the CD4 cell count from less than 200 to less than 350 in the developing world? Clearly there are numerous substantial biomedical and sociopolitical issues that go beyond the availability of antiretroviral agents. It is certainly possible that the START trial will demonstrate that treatment initiation at levels greater than 500 CD4 cells leads to improved AIDS and non-AIDS clinical and surrogate marker outcomes. What then – actually what now - for the developing world?

Beyond When to Start – What to Start?

Three drug combination therapies have been the mainstay of antiretroviral treatment since 1996. Clinical trials to date have demonstrated that triple therapy must contain a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) plus two NRTIs. Combinations that feature three NRTIs alone are inferior in suppressing viral replication. These observations have formed the basis for guideline committee recommendations of treatment choices. Agents from novel classes, notably an integrase inhibitor and a CCR5 antagonist,⁴ have recently been tested in treatment naïve individuals with good success, and these agents will undergo evaluation by regulatory agencies and guideline committees for inclusion in the treatment naïve populations. These novel class agents and newer agents within the PI and NNRTI classes have renewed the question of whether three agents are required to affect a favorable clinical, immunologic and virologic response. There is interest in this question as a simplified therapeutic strategy, but also because the NRTIs as a class have lesser antiviral potency and greater toxicity. Clinical trials will address this question in the next several years in studies sponsored both by the pharmaceutical industry and the National Institutes of Health. A second question that will be reexamined is the strategy of induction – maintenance therapy. Earlier studies using the first

⁴ CCR5 is a co-receptor for HIV expressed on CD4 and other cells. CCR5 antagonists are the first antiretroviral agents to target a host receptor rather than viral enzymes or proteins.

generation of antiretroviral agents failed to demonstrate maintenance of viral suppression when treatment was reduced from three to two drugs. Using newer agents with increased potency could permit such an approach.

The treatment paradigm for HIV is poised for change based on a greater understanding of viral pathobiology and pharmaceutical advances. It appears that initiation of antiretroviral therapy earlier in disease leads to improved clinical outcomes, a question that will be definitively answered by the START trial. Importantly, earlier treatment may also be accompanied by reduced rates of transmission. Clinical trials are underway to answer this question as well. Newer agents both within traditional, and novel classes offer greater antiviral potency and less toxicity, and may permit a reconsideration of the standard triple agent combination approach. The next 5 to 10 years should see datasets to support the provision of a general as well as a more individualized therapeutic approach. It is hoped that the findings from these studies will have broad and meaningful application for nations of the developing world.

Considerations of Drug Development and Pharmacology

The current process of drug development follows an established course designed to fulfill the mandates of regulatory agencies while at the same time reflecting the dominant commercial imperatives of the pharmaceutical industry. In the HIV therapeutic arena this has resulted in the approval of 30 single or combination agents since 1987. The driving forces of the development process traditionally reflect predominant regional regulatory and market approaches more than a global view. Further, once an agent has been approved there is rarely a systematic reevaluation of fundamental pharmaceutical considerations such as dose optimization, drug formulation or process chemistry. The expiration of patent protection would be one logical time point for such an evaluation, but to date this has not occurred. This author does not maintain that fundamental considerations such as dose optimization or formulation are in any fashion faulty, but rather that recent biopharmaceutical advances might allow a more optimal understanding and solution to the invariable difficulties and limitations that characterize the initial process of a drug's development. The question posed is whether a reevaluation of dose-finding and optimization, and drug formulation methods might allow an improvement in an agent's efficacy and/or safety profile while realizing a reduction in overall cost. If true this might have special importance for nations attempting scale-up efforts for diseases such as HIV.

Dose Finding and Optimization

Dose finding and dose optimization are traditionally evaluated during phase II clinical trials. In fact the determinants of doses taken into phase II trials derive from earlier phase I safety and pharmacology

studies as well as animal toxicology studies. Animal toxicology studies attempt to define a dose and dose exposure leading to organ dysfunction or morphological change. Termed the no-observable-adverse-effect-level (NOAEL) this is the maximal level at which a cohort of animals exhibits no evidence of organ toxicity. The NOAEL provides an initial upper-bound for dosing in humans. Depending on the drug in development, and the disease under treatment, a 10-fold, or greater, difference between the NOAEL and the therapeutic dose in humans is sought. Not always does an animal NOAEL translate into an eventual human dose. Inter-species and disease-specific factors may alter such relationships and result in clinical doses above or below NOAELs. Indeed an exploration of such factors is part of the purpose of early phase human clinical trials. Phase I trials are designed to define the safety and pharmacokinetics of the drug and traditionally evaluate single and multiple drug administration at various dose levels. These studies are performed in healthy subjects initially and may be expanded to those with the disease under study.

Beyond the observed safety profile and pharmacokinetics of phase I studies a pharmacodynamic measure (drug activity) is incorporated in later phase trials. Since 1993 measures of antiretroviral drug activity have included reduction in HIV RNA as well as rise in CD4 count. Prior to the availability of viral load measures in the early 1990s, CD4 cell count was the singular parameter used in development trials. Dose selection in that period was based on a measure with substantial inter- and intra-patient variability, and one that did not reflect direct antiviral activity. In general a conservative approach has characterized dose selection for the antiretroviral agents. Preferring to err on the side of higher doses rather than risk inadequate response and the development of drug resistance, several important agents in use throughout the world might be administered at lower doses without compromising efficacy. This approach – if demonstrated - has the potential to realize cost savings through a reduction in overall cost of goods. Investigation of this approach is the subject of current interest by the Clinton and Gates Foundations. Agents considered for such an approach should be evaluated through adequately powered, carefully performed clinical trials after a thorough review of initial dose finding results. Adequate enrollment of women and those of diverse ancestry must be ensured. Among the agents that appear to fall into this category are the NRTIs stavudine and lamivudine, the NNRTIs nevirapine and efavirenz, the PIs lopinavir and darunavir and the recently approved integrase inhibitor raltegravir. Stavudine is widely used in the developing world, and like many of the early NRTIs has modest antiviral activity but is noted among the NRTI class for its high toxicity profile. There is suggestion from preclinical data and limited clinical experience that reduced doses may be associated with lessened toxicity without compromise of efficacy. The NNRTI agents, particularly efavirenz form the cornerstone of current first-line antiretroviral therapy. A reduction of dose without loss of efficacy would be a meaningful achievement. Several of the PIs and the integrase inhibitor currently approved for twice-daily dosing are being evaluated for once daily dosing in those without prior antiretroviral experience. This suggests that lower doses given twice daily may be possible.

At the opposite end of the dose finding spectrum an examination of dose dependency and the potential utility of using higher than recommended doses may prove beneficial in certain clinical settings. Like many agents, antiretroviral drug exposures are subject to substantial inter-patient variability (40% to 200%). In a setting of insufficient viral suppression there may be a rationale for raising doses of certain

agents rather than switching to another regimen. This point was demonstrated in a study that examined higher doses of the protease inhibitor, lopinavir/ritonavir (Kaletra) [46] in patients with detectable virus on conventional doses of the drug. While toxicity limited higher doses in some patients, others found the enhanced regimen tolerable, and noted control of their viremia without switching to a new regimen. This approach may not apply throughout the PI or other antiretroviral classes, but should be the subject of study. Indeed the completeness of dose finding and a full exploration of the dose-response relationship is often lacking during drug development. A recent article [47] points to the value of a more complete determination of dose-dependency and dose-response, and suggests that this approach may better inform drug selection and regimen optimization. While drug dosages are certainly limited by laboratory or clinical toxicity, a clear understanding of dose-response is necessary to maximize treatment approaches. With a limited number of regimens available in the developing world an approach that examines both lower and higher doses might prove clinically beneficial and cost effective.

Drug Formulation and Process Chemistry Considerations

Drug formulation and the process of drug manufacture evolve during the early phases of development. Prior to performance of phase III studies formulation is finalized and the process chemistry to permit fully scaled drug manufacture is set in place. Thus by the time of usual drug patent expiration the formulation and process chemistry work can be more than 20 years old. While certain chemistry considerations likely remain unchanged, advances in pharmaceutical sciences may provide for a more efficient solution to issue such as solubility, dissolution and stability. These in turn may affect manufacturing considerations and ultimately cost. Similarly central biopharmaceutical considerations including target organs and compartments of effect, factors affecting cellular permeability and drug transport as well as general pharmacokinetics can now be understood in far greater, and clinically relevant, detail. One application of newer technologies appears to enhance lipophilicity, the ability of drug to transit the lipid membrane of target cells. An approach such as this may permit use of a smaller drug quantity and realize improved efficacy while achieving cost savings. As part of efforts to provide antiretroviral agents to the developing world in the most cost effective fashion, incentives for research to reexamine certain drug attributes and properties should be established. One source of such support is global health agencies and foundations in collaboration with academic investigators and the private sector.

Genomic and Pharmacogenomic Considerations

More than other therapeutics areas, HIV has benefited from the early evaluation of genetic determinants of immune control, disease progression and treatment response. HLA B-57, HLA B-27, HLA C, CCR5 Δ 32, ZNRD1 and CCR2V64 have been identified and explain a clinically meaningful portion of commonly observed variation, notably among Europeans and Asians. The greater genetic diversity among those of African ancestry has made study in these populations more difficult. Among the genetic determinants of treatment response a frequently cited example of the successful application of

pharmacogenetics is the established correlation between the nucleoside reverse transcriptase inhibitor abacavir-induced hypersensitivity reaction and an ancestral haplotype, HLA-B*5701. Screening for the presence of HLA-B*5701 prior to use of abacavir has markedly reduced the incidence of a frequently serious hypersensitivity syndrome [48] and has been proven to be cost-effective [49]. As the experience with the abacavir hypersensitivity syndrome and the B*5701 haplotype have demonstrated, it is not a phenotypic characterization of the patient at risk, but rather the underlying genetic composition that provides the necessary understanding, and a possible means to individualize therapy. More individualized therapeutics could impact both drug response and safety. The antiretroviral agents appear no different from those of other therapeutic areas with large variability noted in treatment response rates and the occurrence of adverse events. Indeed, as noted above, inter-patient pharmacokinetic variability among antiretrovirals is high, regardless of the agent being studied. How much of this variability may prove to be due to underlying genetic factors remains the subject of ongoing investigation. However, the importance in HIV may be particularly acute where suboptimal drug exposure can rapidly lead to drug resistance and treatment failure.

The non-nucleoside reverse transcriptase inhibitor efavirenz (EFV) has proven to be a cornerstone of HIV therapeutics in the developed world, and provides a useful example of the complexities of variability in pharmacokinetics, genetics and clinical response [50]. All aspects of drug disposition: absorption, distribution, metabolism and elimination are subject to genetic (allelic) variability, some portion of which may be clinically meaningful. As EFV-based regimens have yielded consistently high response rates among patients initiating their first antiretroviral regimen, a full understanding of genetically based pharmacokinetic (PK) and clinical (pharmacodynamic) variability is important. Efavirenz as well as nevirapine (used extensively as a component of first-line treatment and MTCT regimens in the developing world) are principally metabolized by the CYP450 2B6 isoform. 2B6 is known to have substantial inter-subject variability in expression and catalytic activity. A polymorphism (516G>T, a marker for the 2B6*6 allele) yields an association with slower drug clearance and higher plasma concentrations. Some studies have found these PK parameters to be linked to EFV-associated adverse clinical symptoms, principally central nervous system events, while others have not. When using self-described race, altered EFV clearance and plasma concentrations appear higher among blacks and Hispanics when compared to whites. However when adjusting for the 516G>T genotype the differences between races was no longer apparent, and regardless of reported race, those with the 516T/T genotype have altered clearance and higher EFV and nevirapine plasma concentrations. In the U.S. the T/T variant 2B6 genotype is noted at a greater frequency in those of African ancestry than among those of European descent. The importance of these findings lies in the need to evaluate possible risk factors for elevated EFV (and nevirapine) concentrations, and adverse drug reactions in populations of differing allelic composition. Categorization by racial or ethnic phenotype (skin color or ancestry) is likely inadequate in determining potential differences between human populations. Genetically-based population study may define clinically relevant predictors of pharmacokinetic and pharmacodynamic response and permit a more tailored approach to treatment strategy. Prospectively planned population-based DNA sampling and biobanking may prove especially informative in targeted scale-up of ART in many parts of the developing world, and could provide the means for individualizing therapy with expected health outcome and financial benefits.

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