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TAG is glad to resume publication of *TAGline* after a year-long hiatus. We have restructured *TAGline* and hope to publish it on a quarterly basis. Longer versions of some articles and related research and policy updates will also be available on the website at www.treatmentactiongroup.org. TAG thanks Mike Barr for his many years editing *TAGline*, welcomes interim editor Jen Curry, and expresses gratitude to layout genius Lei Chou and our webmaster, Joel Beard, for their expert work.

SMART Stops Stopping

By Richard Jefferys

The Strategies for the Management of Antiretroviral Therapy (SMART) trial was designed to investigate whether antiretroviral therapy could be used on an as-needed basis rather than continuously. The protocol involved interrupting therapy when the CD4 count was confirmed to have crested 350 cells/mL and restarting therapy when it fell below 250 cells/mL. The impetus for the trial came from the well-documented association between CD4 count and risk of clinical disease, and from a number of studies suggesting that people who had begun therapy too early could safely interrupt treatment for prolonged periods without obvious harmful consequences.

SMART was sponsored by the Community Programs for Clinical Research on AIDS (CPCRA) and led by investigators Wafaa El Sadr and Fred Gordin. The late, desperately missed AIDS activist Carlton Hogan—who taught many fellow activists the occult art of statistics—was also part of the protocol team. In a remarkable testament to the engagement and commitment of those involved, SMART began in 2002 and enrolled 5,472 participants in 33 countries, making it the largest treatment strategy trial ever undertaken.

On January 10th of this year, the disappointing news emerged that SMART's Data **Cont. Page 2**

HCV at CROI: Growing Chasm Between Bench and Bedside

By Tracy Swan

Hepatitis C virus (HCV) and HIV/HCV coinfection were prominently featured at this year's Retrovirus conference. Coverage ranged from scientific progress in the laboratory to treatment access in the clinic. During the opening plenary, Takaji Wakita detailed the development of an important new tool for hepatitis C research: a cell culture system in which hepatitis C virus can replicate. The ability to "grow" hepatitis C virus represents a significant breakthrough with the potential to accelerate drug and vaccine development. Previously, the study of HCV replication was limited to synthetic replicon systems incapable of producing infectious particles. The only available animal model for hepatitis C research is the endangered, prohibitively expensive chimpanzee. The novel cell culture system enables the study of each step involved in viral replication. Researchers are currently using the system during preclinical drug development to identify new anti-viral targets and assess activity of anti-HCV drugs.

The closing CROI Symposium, *Advances in the Understanding of HCV Biology and Treatment*, included a detail of the HCV protease and polymerase inhibitor pipeline. Ann Kwong from Vertex Pharmaceuticals reviewed clinical development of protease inhibitors, describing **Cont. Page 5**

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and Safety Monitoring Board (DSMB) had recommended that the trial stop due to roughly twice the number of progression events in the intermittent therapy arm, including deaths and serious complications associated with drug toxicity. The lead investigators concurred with the DSMB and promptly advised that all study participants restart antiretroviral therapy. Though initial confusion abounded as to precisely why the study was stopped, it later became clear that the DSMB had met and requested additional data in November and made the recommendation to terminate the study upon review of the new information in January.

In order to grasp the rationale behind the DSMB decision, it's necessary to understand the intent of the SMART protocol design. The hypothesis was that people receiving intermittent treatment in what was called the drug conservation (DC) arm would fare slightly better over the long term than those receiving continuous therapy in the virological suppression arm due to a reduction in serious ARV-related complications. SMART was statistically equipped with 80% power to detect 20% superiority of the drug conservation arm, as determined by the trial's primary clinical endpoints: death, progression, and serious complications. The study protocol makes it very clear that the design team expected the incidence of toxicity-related events to be lower in the drug conservation arm, and that, if both progression and toxicity events favored one arm over the other, this would be cause for study re-evaluation and, potentially, cessation. As the preliminary data at CROI revealed in February, this is exactly what happened.

The SMART Data

At the time the study was stopped, 2,720 participants were enrolled in the DC arm and 2,752 in the VS arm. The average age was 46; about a quarter of the total enrollees were women and roughly a third were black. Participants had been receiving antiretroviral therapy for an average of six years prior to joining the study. Baseline characteristics were similar between the arms, as seen below.

Baseline Characteristic	Drug Conservation (DC)	Virological Suppression (VS)	Total
Median baseline CD4 count	596	599	598
Median CD4 nadir	250	252	251
% with viral load < 400copies/mL	71	70.8	70.9
Prior clinical AIDS	24.7	23.4	24.1
Antiretroviral naïve	4.5	4.8	4.7
Years of prior antiretroviral therapy	6	6	6

Median follow up was 10.1 months in the DC arm and 9.4 in the VS arm. Participants in the conservation arm spent just 33% of their time on antiretroviral therapy compared to 93% for people in the VS arm.

The DSMB decision to stop the study was driven by divergent outcomes between the two arms in terms of disease progression, death, and serious complications. There were 117 disease progression events in the DC arm (3.7%), versus 47 (1.5%) in the VS arm. Crucially, there were also 68 serious complications in the DC arm versus 46 in the VS arm (2.1 vs. 1.4%). Serious complications included cirrhosis of the liver, coronary artery disease requiring surgery, heart attack, stroke, and kidney failure. Although the difference between the arms in terms of these complications was of borderline statistical significance, the fact that this difference also favored the virological suppression arm suggested to the DSMB that it was highly unlikely that the excess progression events seen in the drug conservation arm would ultimately be compensated by a reduction in serious complications (as the SMART protocol had projected).

Endpoint	DC	VS
Progression of disease or death	117	47
Death	55	29
Serious disease progression	18	3
Serious complications	68	46

While the majority of progression events were relatively clinically innocuous (e.g. oral thrush), a significant number of more serious opportunistic infections also occurred, including bacterial pneumonias and lymphoma.

In terms of mortality, the most significant difference between the arms was a disproportionate number of deaths for “Other” and “Unknown” reasons in the DC arm. More details surrounding these deaths will hopefully become available once the study results are published (expected soon in *The Lancet*). However, the fact that the precise cause of death is unclear for these two categories does not necessarily undermine the study results. Since the association is statistically significant, it is extremely unlikely that the increased risk of death seen in the DC arm resulted from chance. This point regarding “all cause mortality” was made with typical acuity by Carlton Hogan in an article he wrote for the GMHC Treatment Issues newsletter (“Death as an Endpoint,” September 2001, <http://www.gmhc.org/health/treatment/ti/ti1509.html#4>).

As Wafaa El Sadr highlighted in her CROI presentation, the VS arm performed superior to the DC arm for just about every other possible endpoint. The only findings favoring the DC arm—beyond the reduction in antiretroviral usage and associated costs—were self-reported improvements in body shape changes and reduced use of lipid-lowering drugs. SMART also had a number of sub-studies including a fully enrolled investigation into the quality of life of the participants, for which data have yet to be presented. Quality of life is a critical parameter that informs the real world decisions that people make about starting and stopping therapy, so these data will be extremely valuable.

Understanding the Results

The investigators were obviously dismayed by these results, although it is worth stressing that the vast majority of SMART participants (~95%) remained healthy regardless of the arm to which they were assigned. Efforts are now underway to try to understand the multiple factors that contributed to the

study outcome. At CROI, investigators offered a glimpse at some of the work they have already completed with respect to this question.

The first and perhaps most obvious variable to consider is the lowest-ever CD4 count (CD4 nadir), but analyses did not reveal an association between this parameter and the events that occurred. Factors that did turn out to be associated with disease progression in the DC arm included the CD4 count immediately prior to the progression event (the proximal CD4 count), age (every 10 year increment in age was associated with an increased risk of progression) and a prior AIDS diagnosis. The same held true for the VS arm, which included the additional factor of viral load. Importantly, the investigators noted that these associations could not entirely explain the differential risk of disease progression between the two arms; even individuals with higher CD4 counts in the DC arm appeared more at risk (in the strata of 350-499 CD4s, for example, there were 28 events in the DC arm and 8 in the VS arm), suggesting that further analyses will be required to understand the SMART study outcome.

One strange but potentially critical finding which emerged from this work was that controlled viral load at study entry in the DC arm predicted an increased risk of disease progression and complications. Participants who entered the conservation arm with a viral load greater than 400 copies/mL did not experience more progression or complication events than those in the VS arm. The increased risk was confined to those who enrolled in the DC arm with viral loads <400 copies/mL and faced a nearly fourfold greater chance of an event than participants in the VS arm.

This finding left many people scratching their heads. The most widespread hypothesis—albeit speculative—is that the data may indicate that the inflammation that occurs as a result of viral load rebounds is not benign, as many people had surmised, but is in fact clinically very significant. In this scenario, the acute inflammatory response to interrupting antiretrovirals is more harmful than the low-level inflammation that accompanies persistently

detectable viral load. It is also possible that the individuals with detectable viral loads had stronger HIV-specific immune responses, as Steve Deeks recently reported in his cohort of people who only partially control viral load on antiretroviral therapy (see Deeks et al., *J Virol.* 79;22:14169-78). Better HIV-specific immune responses could potentially serve to blunt the inflammation that accompanies a treatment interruption. Further, ongoing analyses of immune responses and known inflammatory markers may shed more light on these questions, but as it stands, it appears that the desperately unfashionable idea of using immune-based therapies (e.g. “immunosuppressant” drugs like cyclosporine and/or therapeutic HIV vaccines) in the context of treatment interruptions may be due for reevaluation.

The Future

Trials like SMART, which investigate treatment strategies using available drugs, tend to be the least sexy studies imaginable for the majority of HIV researchers (several of whom are known to have referred to it as “The Dumb Study”). Calls for a similar size study to investigate the question of when it is best to start highly active antiretroviral therapy have gone unheeded since 1996. In this context, it is important to stress that SMART was in no way a failure; it addressed a crucial “real world” question about the management of HIV disease and obtained an answer swiftly. Furthermore, the study represents a trove of data that will allow additional questions to be addressed. TAG and other community organizations have co-signed a statement from the Center for AIDS in Texas recommending that NIAID continue to support SMART as a large cohort study of treatment experienced individuals receiving antiretroviral therapy.

One prudent, immediate consequence of SMART’s termination is that researchers are carefully evaluating ongoing studies of interrupted therapy. An international trial known as DART, which included

an arm using fixed three-month treatment interruptions, recently announced that its DSMB recommended discontinuing this arm due to a higher incidence of clinical events compared to those seen during continuous therapy. A French study in Abidjan, Cote D’Ivoire, stopped its CD4-guided therapy arm a few months before SMART ended, also due to a higher rate of progression events. Conversely, follow up continues in the multi-national STACCATO trial, which includes a CD4-guided arm wherein therapy is reinitiated when the count dips below 350 cells/mL (as opposed to the 250 cells/mL cutoff used in the SMART and Abidjan trials). In an update on STACCATO at CROI, Jintanat Ananworanich noted that, based on the incidence rates seen in SMART, 17 events should occur in the CD4 guided arm of STACCATO. However, there have not been any AIDS-defining illnesses, and only a single death has occurred. In addition to the CD4 criteria, Ananworanich highlighted the fact that the average time spent on antiretrovirals differed between the studies, with STACCATO participants averaging just 15 months on therapy prior to enrollment.

The results of SMART and other relevant studies like STACCATO will need to be considered carefully when designing future clinical trials of intermittent therapy. Available data imply that higher, conservative CD4 count thresholds should be considered. The extensive treatment experience of the SMART study participants strongly suggests that any future exploration of therapy interruptions in a similar population should be undertaken with great caution. Conversely, it also suggests that it would be premature to generalize the SMART results to individuals who are less treatment experienced (a point supported by the STACCATO data). SMART clearly buttresses the rationale for studying whether immunological interventions can improve the safety and efficacy of intermittent antiretroviral therapy, so paradoxically, one valuable outcome of the study may be to reinvigorate this marginalized area of HIV research.

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the active site of the HCV protease as a lousy target: “landing an inhibitor on the top of that enzyme is like landing on a piece of pizza – it’s just greasy and [the inhibitor] flies right off.” Previously, Boehringer Ingelheim had established proof-of-concept with BILN-2061, but development was halted in 2004 due to the drug’s cardiac toxicity. Two candidates have recently completed early Phase I studies: Vertex’s 950 and Schering’s 503034. Data appears below in Table 1.

Table 1. Antiviral Activity of Two HCV Protease Inhibitors (With or Without Peg-IFN) vs. PEG-IFN Monotherapy

Agent (s)	Population	Duration	Dosing	HCV RNA Response
SCH 503034	N=12 HCV genotype 1; non-responders to peg-ifn + rbv	14 days	400 mg/tid	Mean maximum reduction: 2.06 log ₁₀ (range: 1.1-2.07)
SCH 503034 + Peg-IFN alfa 2b	N=12 HCV genotype 1; non-responders to peg-ifn + rbv	14 days	400 mg/tid; + Peg-IFN 1.5 µg/kg once weekly	Mean maximum reduction: 2.9 log ₁₀ (range: 2.3-4.1)
Peg-IFN alfa 2b	HCV genotype 1; non-responders to peg-ifn + rbv	14 days	1.5 µg/kg once weekly	Mean maximum reduction: 1.1 log ₁₀
Vertex 950	N=8 HCV genotype 1; treatment-naïve	14 days	750 mg/q 8 hours	Median reduction: 4.0 log ₁₀
Vertex 950 + Peg-IFN	N=8 HCV genotype 1; treatment-naïve	14 days	750 mg/q 8 hours; + Peg-IFN alfa-2a 180 µg/week	Median reduction: 5.5 log ₁₀
Peg-IFN	N=4 HCV genotype 1; treatment-naïve	14 days	Peg-IFN alfa-2a 180 µg/week	Median reduction: 1.0 log ₁₀

Sources: Abstracts 94 and 201, 56th Annual Meeting of the American Association for the Study of Liver Diseases; November 11-15th, 2005; San Francisco, CA; and Vertex Pharmaceuticals, press release. January 9th, 2006. Available at: <http://www.vrtx.com/Pressreleases2006/pr010906.html>

Promising results from these early trials have inspired considerable excitement and speculation. As indicated in Table 1, both PIs were active against HCV genotype 1, and in treatment naïve persons (Vertex) and non-responders (Schering). Genotype 1 requires 48 weeks of treatment and is less responsive to current therapy than genotypes 2, 3, and 4.

The spoiler is resistance. A single mutation at position A/156/T creates cross-resistance to HCV protease inhibitors. Additional mutations conferring low and high-level resistance to Vertex 950 have been identified. Vertex’s early research suggests that the replicative capacity of resistant virus may be poorer than that of wild-type virus. The crucial question is whether an HCV protease inhibitor-based regimen can eliminate the virus before resistance develops.

Phase I data have been used to estimate the impact of HCV protease inhibitors on hepatitis C treatment duration. These models ambitiously predict a significant treatment abbreviation from 48 to 12 weeks, but more data are needed. As Kwong rightly noted, “we’ll just have to see how it turns out.”

Daria Hazuda from Merck closed the symposium with an update on preclinical development of HCV polymerase inhibitors. HCV’s polymerase enzyme is a mother lode for anti-viral drug development; five classes

of polymerase inhibitors have already been identified. Preclinical data indicate that these drugs may be combined with one another, and some may offer additive or synergistic effects. The drawback is resistance. In preclinical testing, a single amino acid change caused high-level resistance, although cross-resistance may be less likely with polymerase inhibitors than protease inhibitors.

These exciting developments bode well for the future of HCV therapy but contrast sharply with today's clinical reality for HIV/HCV coinfecting patients. Several posters at CROI documented high rates of HCV treatment ineligibility, low response to HCV therapy, and significant liver-related morbidity and mortality among coinfecting persons. In particular, two HIV clinics based in Baltimore and Seattle reported that less than one third of their coinfecting patients had been evaluated for HCV treatment; of the third who underwent evaluation, less than 20% began treatment. Not surprisingly, at the end of the day, the number of patients who achieved SVR during treatment represented an abysmal .7 and 1.6 percent of the entire Baltimore and Seattle-based cohorts, respectively.

Table 2. Hepatitis C Evaluation, Treatment and Response Rates At Two HIV Clinics

Cohort Description	Referred to HCV Care*	Evaluated for HCV TX	Treated	Achieved SVR
Baltimore 1998-2003 Coinfecting HIV clinic patients; N=845	33% (277/845)	66% (185/277); 22%(185/845) of total	16% (29/185) of evaluated patients; 3% (29/845) of cohort	21% (6/29) of treated patients; 0.7% (6/845) of cohort
Seattle 1997-2004 Coinfecting HIV clinic patients; N=369	Data not provided	28% (103/369)	18% (19/103) of evaluated patients; 5% (19/369) of cohort	32% (6/19) of treated patients; 1.6% (6/369) of cohort

Sources:

Mehta S, Lucas G, Torbenson M, et al. Barriers to referral for hepatitis C care among HIV/HCV coinfecting patients in an urban HIV clinic. 13th Conference on Retroviruses and Opportunistic Infections; February 5-8th; Denver Colorado. 2006. Abstract 884;
Scott J, Wald A, Kitahata M, Drolette A, Corey L, Wang, C. HCV is evaluated and treated infrequently in an HIV/HCV coinfecting population. 13th Conference on Retroviruses and Opportunistic Infections; February 5-8th; Denver Colorado. 2006. Abstract 882.

Addressing Barriers

Current barriers to HCV treatment are substantial and must be surmounted prior to the advent of new therapies. Despite accumulating evidence that HCV can be successfully treated in multiply-diagnosed, mono- and coinfecting persons, some clinicians remain reluctant to treat people with histories of drug use and/or psychiatric co-morbidities. Coinfecting individuals, who often reside in at least one of these two categories, have additional treatment challenges, including worse experience of side effects and toxicities, and decreased likelihood of viral clearance.

More effective, less toxic therapies will undoubtedly increase treatment uptake among coinfecting people. Still, new drugs alone won't dispel existing barriers, particularly since the clinically demanding, and difficult-to-tolerate, pegylated interferon is likely to remain the therapeutic backbone of HCV treatment for the next few years. Successful HCV treatment programs offer integrated psychiatric care, drug treatment, and include strong peer education and support components. Such programs are few and far between. The infrastructure necessary for delivering HCV treatment to coinfecting people must be developed now, in anticipation of improvements in therapy.

Overlapping Epidemics: TB, HIV AND VIRAL HEPATITIS

By Tracy Swan

Disturbing reports of overlapping TB, HIV, and viral hepatitis epidemics emerged at the 2005 International AIDS Society Conference. In the newly independent states of Eastern Europe and Central Asia, HIV incidence continues to rise, and viral hepatitis is highly prevalent among prisoners, injection drug users, the homeless, and people in tuberculosis treatment programs.

HIV and Viral Hepatitis Among Prisoners, IDU, the Homeless, and Persons in TB Treatment Programs

Population	%TB	% HIV	% HBV and % HCV
People in TB treatment programs, Republic of Georgia N=272	100%	1.1%	HBV: not reported HCV: 22.4%
Injection drug users, Republic of Georgia N=926	Not reported	0.5%	55% HBV 58.2% HCV
Prisoners, Samara, Russia N=1334	100%	12.2%	HBV and/or HCV: 24.1%
Homeless adults and adolescents, St Petersburg, Russia N=312	Not reported	27.2%	4.8% HBV 43.6% HCV

Hepatitis B and C are more easily transmitted through injection drug use than HIV. Disproportionate HBV/HCV and HIV prevalence rates among IDU in the Georgian Republic [55/58% v .5%; and 22.4% v 1.1%] reflect this reality. In regions where injection drug use is the primary mode of HIV transmission, an increase in new hepatitis B and/or C infections among high risk populations commonly precedes a spike in HIV incidence. If drug users in these regions are denied access to the information and apparatus necessary to prevent HIV—sterile injection equipment, opiate substitution therapies, including methadone and buprenorphine, and available drug detoxification/treatment services—they will continue to be needlessly infected.

Globally, tuberculosis is the leading cause of death among HIV-positive people. Poverty, incarceration, homelessness, and poor nutrition increase the risk for TB in populations where HIV and viral hepatitis are already endemic. Although TB is curable, co-infection with HIV complicates the diagnosis of TB and the treatment of both TB and HIV. Interactions between TB medications and antiretroviral agents restrict HIV treatment options. Additionally, certain drugs used to treat HIV and TB can cause hepatotoxicity. Coinfection with viral hepatitis increases the risk for antiretroviral-induced hepatotoxicity, and may, in turn, increase the risk for hepatotoxicity from TB therapy.

Tuberculosis can be cured. HIV and viral hepatitis can be prevented. When prevention is not possible, thorough screening, healthcare, and treatment services must be made available. Strategies to reduce the incidence and mortality of HIV, TB, and viral hepatitis in these regions will fail unless they consider the complex prevention, care and treatment needs of drug users and other groups vulnerable to this triple threat.

References available online at: www.treatmentactiongroup.org

TB Diagnostics: A Crisis for People Living with HIV/AIDS Worldwide

by Javid Syed

Globally, TB is the most common infection and a leading cause of death among people living with HIV. Despite being curable, TB accounts for two million deaths each year. Thirteen percent of deaths among HIV+ people are TB-related, and some autopsy studies have seen rates as high as 50%. TB disproportionately affects poor people; 95% of the disease is concentrated in the developing world. Of the estimated 14 million people coinfectd with TB/HIV, 10 million reside in Africa. Insufficient resources, neglect, and challenges posed by TB/HIV coinfection have collectively contributed to a diagnostic emergency in tuberculosis, uniquely jeopardizing people with HIV.

HIV increases the risk of developing TB disease by 50% and is a primary driver in the global increase in TB prevalence, along with multi-drug resistant TB (bacterial strains resistant to isoniazid and rifampicin, the two most potent anti-TB drugs). The most frequently utilized TB diagnostics lack the sensitivity to consistently detect the bacteria in an HIV coinfectd population. These tests are also incapable of identifying drug resistant strains of TB. Consequently, TB diagnosis is often delayed in coinfectd people and people with MDR TB, resulting in needless illness and death. Excess mortality in HIV+ people can range up to 33% in the first two months of TB treatment. Some individuals die of late-diagnosed tuberculosis, while others die from HIV-related complications. Effective TB control requires, among other advances, access to novel and improved diagnostic tools.

The increase in HIV-associated TB requires rethinking the global strategy for TB control: directly observed, short course therapy (DOTS). Introduced by WHO in 1993, DOTS is now used in 191 countries worldwide. This schematic for TB surveillance, treatment, and monitoring represents a dramatic improvement over the dismal days prior to 1993, when no global standard existed for TB. Since 1994, more than 17

million people have been treated under DOTS, of whom 80% have completed treatment. Despite its success, DOTS is limited by reliance on passive case finding, where individuals presenting with an unexplained cough lasting longer than three weeks receive a clinical exam and sputum smear microscopy. A central limitation is that these tools focus on TB in the lung, and hunt for bacilli in the sputum. Since HIV-positive people often have less bacilli in their sputum and are more likely to have extra-pulmonary TB, the most commonly performed tests cannot consistently detect TB disease in HIV+ people.

The availability of diagnostic tests is closely linked with access to various resources required to administer them, including trained personnel, clean water, electricity, budget for testing equipment and reagents, and equipment maintenance costs. Tests will be utilized less when fees are associated with their use. Furthermore, existing diagnostic recommendations (codified in decision-making flow-charts and algorithms), used to guide health care providers in the diagnosis of TB, are chiefly oriented toward smear microscopy and chest X-rays, and do not address the particular challenges posed by coinfection with HIV.

Common diagnostic tests can be categorized into the following three sets based on their respective mechanisms for identifying TB infection and disease: clinical examination based on symptoms; a measure of immune response to TB; and tests that detect the TB bacteria itself.

Clinical Examination and Symptom Assessment:

Clinical examination remains an important initial screening step in TB control. A simple symptom questionnaire assessing the presence of chest pains, night sweats, weight loss, fever and other common symptoms should be administered routinely in settings where TB is endemic. Despite its simplicity, the combination of exam plus symptom questionnaire is surprisingly effective at raising the clinical index of suspicion. Many individuals with an unexplained cough lasting longer than three weeks will turn out to have pulmonary TB.

Immune Response Based Diagnostic Tests:

The tuberculin skin test (TST), also called the Mantoux test, is one of the more common immune based diagnostic tests. TST involves injecting a substance called purified protein derivative (PPD), which is isolated from the mycobacterium tuberculosis (MTB) cell wall, under a person's skin. If the person is infected with MTB, immune cells such as macrophages and lymphocytes migrate to the injection site, causing a marked swelling (induration) in the skin, which appears within two to three days. The presence or absence of TB infection is judged based on size of the swelling; very large indurations are often associated with active TB.

Unfortunately, TST may be harder to interpret among individuals who were immunized with *Bacillus Calmette-Guérin* (BCG) vaccine, a live attenuated mycobacterial strain often administered to children under age five in order to prevent TB. The TST may also be non-reactive among individuals unreactive to skin tests. People successfully treated for tuberculosis will test positive using TST. Often, tests looking for signs of active TB disease (such as chest X-ray) are required to distinguish between latent and active TB infection following a positive TST result. Thus, while useful, TST requires follow up, and results are occasionally misleading, particularly among people with HIV and low CD4 counts.

Chest X-rays look for cavities and abnormalities in the lung, both of which are components of the immune response to pulmonary TB. Chest X-ray is a diagnostic tool frequently used to confirm active, pulmonary TB following positive immune based tests. Unfortunately, it is equally spotty in its ability to catch TB disease in HIV+ people. TB infection and the accompanying immunologic response typically lead to holes in the upper portion of the lungs. In people with HIV, TB related cavities are either deficient or clustered instead near the middle chest region, often evading detection by lab workers

trained to scrutinize the upper lung portions of chest film. Though these differences in TB-related lung disease between HIV-positive and negative people are well documented, diagnostic technicians in endemic areas are not routinely and consistently trained to hunt for them. The consequences can be fatal.

However, in the remaining half of cases evaluated using smear microscopy, TB is present in the lungs despite negative results on sputum samples.

The QuantiFERON-TB test (QFT) is a more recent method used to detect TB infection. QFT measures the amount of gamma interferon (a cell-mediated immunity associated cytokine) released in whole blood following stimulation with purified protein derivative (PPD). Approved by the FDA in 2001, the QFT test is not yet widely used in rich or poor countries, due to its costliness, technically demanding nature, and lack of guidelines about its specific usefulness relative to existing diagnostic tests.

Due to their collective reliance upon host ability to mount a cell-mediated immune response, immune-based tests for TB infection are of less utility in HIV+ people, particularly among the more immunologically compromised, including persons with AIDS. Thus, this highly common method for diagnosing TB may be least useful among the people who are at greatest risk for TB related progression and death.

Diagnostic Tests that Detect TB Bacteria:

Sputum smear microscopy is the most standard diagnostic recommended by DOTS. The method involves collecting three sputum samples over at least two days. Samples are then stained with a dye and washed with acid. This dye adheres to mycobacteria such as TB and other 'acid-fast bacilli' (AFB) and remains visible under the microscope even after an acid wash. In order to detect the elusive acid-fast TB bacterium, laboratory workers must examine each slide using 100 different microscopic fields over a ten minute period. An individual is recorded as a 'smear positive case of

pulmonary tuberculosis' when technicians detect AFB in two of three samples collected. This time-consuming, tedious process dates to the discovery of *Mycobacterium tuberculosis* (MTB), announced by Robert Koch on March 24, 1882 – the original world TB day.

Cumbersome methodology aside, smear microscopy frequently yields false negatives and is further limited by its singular focus on TB in the lung – the most infectious form of disease. Evidence suggests that only 40-60% of pulmonary TB cases are AFB positive by sputum smear microscopy. Smear positive cases exemplify typical pulmonary TB, which causes cavitory lesions visible in chest X-rays. However, in the remaining half of cases evaluated using smear microscopy, TB is present in the lungs despite negative results on sputum samples. Additionally, TB can manifest as extra pulmonary disease, involving the lymph nodes, bones, joints, central nervous system tissue, or pleural space. Studies from Malawi and southeast Asia indicate that up to two thirds of HIV-associated TB cases are either sputum smear negative or extrapulmonary. Thus, even when available, sputum smear microscopy is inadequate for capturing the majority of HIV/AIDS-related TB cases. In addition, the method cannot detect TB in children, who constitute one-sixth of the global burden of disease and are generally under-diagnosed and under-treated.

Some tests diagnose tuberculosis by detecting the presence of TB causing bacteria, bacterial proteins (antigens), or bacterial nucleic acids (DNA or RNA). Two examples include the sputum smear microscopy test for acid fast bacilli (AFB) and the nucleic acid amplification tests (NAAT). At times, HIV infection can complicate interpretation of these diagnostics as well. As discussed above, HIV-positive people are less likely to have abundant bacilli in their sputum (possibly leading to false negative on smear microscopy); and often have extra-pulmonary TB disease.

Culture tests, which sample infected tissue or fluid from an individual and then culture the TB organism in a test tube, diagnose TB with far great accuracy, even among HIV-infected people. The disadvantage is that cultures are time-consuming, requiring four to eight weeks – an unacceptable long window of time for coinfecting TB/HIV patients, who may die waiting. Faster tests that use liquid media can grow

the culture in as short a period as 12 days. Paradoxically, these tests have far greater efficiency and sensitivity but are expensive, technically demanding, and less widely available in resource-poor settings. Yet, despite both time and expense required, a clear advantage to culturing TB

A clear advantage to culturing TB is that results also provide drug susceptibility profile of the bacilli, enabling the detection of drug resistant TB

is that results also provide drug susceptibility profile of the bacilli, enabling the detection of drug resistant TB. For these reasons, it is imperative that liquid culture tests become available as a component of treatment for patients with MDR TB. While such culture-based diagnostics are a vast improvement over smear microscopy in terms of accuracy, their utility is currently limited due to the length of time they take and the amount of resources they require.

Policy and Advocacy Issues

The solution to the current diagnostic crises is two-pronged. At a bare minimum, currently available technologies must become more accessible in resource poor countries. This includes broader use of CXR, PPD, rapid culture, and nucleic acid amplification technology. Meanwhile, there is an urgent need for heightened investment in the development of cheaper, easier-to-use diagnostic tools capable of detecting (with greater sensitivity) sputum smear negative, extrapulmonary, pediatric, and/or multi drug resistant TB. Such tests must be designed for use at the point of care in resource-constrained settings of the developing world. Additionally, diagnostic algorithms must be modified to hasten the accurate diagnosis of TB in people living with HIV/AIDS, children, and others.

Continued success of TB control programs depends on thorough and meaningful community education and empowerment. Community involvement can significantly contribute to earlier detection of TB and higher treatment completion rates, thereby reducing transmission, morbidity, and mortality. Advocacy on the part of people living with HIV and TB is vital to ensure that:

- basic, applied, and operational TB research is adequately funded;
- education and support is available for people in treatment or at risk for TB infection;
- algorithms are adapted;
- diagnostics are improved; and
- access to these tests is expanded.

There is an urgent need for heightened investment in the development of easier-to-use diagnostic tools capable of detecting sputum smear negative, extrapulmonary, pediatric, and/or multidrug resistant TB.

Finally, the discovery and development of TB diagnostics requires consistent, adequate funding to support TB research, including basic science research to enhance our understanding of the bacilli, develop new methods for distinguishing between latent and active TB, and identify characteristics that predict conversion from latent to active TB.

Despite recent, significant increases in resources for TB research and control (including from the Gates Foundation and the Global Fund to Fight AIDS, TB, and Malaria), the field of TB diagnostics continues to be drastically under funded. In 2004, the NIAID had just eight grants focused on TB diagnostics. In its January 2006 *Global Plan to Stop TB*, the *Stop TB Partnership*—a global network of 400 public and private institutions working to develop a global TB control strategy—identified a very modest resource

gap of 163 million for TB diagnostics over the next ten years (2006-2015). Such projections, which influence funding allocations, must increase to reflect the true cost of scaling up current diagnostics, as well as costs associated with developing new diagnostics capable of being used in the settings where they are most needed. Additionally, there is a need to develop consistent regulatory standards to ensure

high quality TB diagnostics.

Nascent coordination efforts have begun through the public-private partnerships such as the Foundation for Innovative New Diagnostics (FIND). However, we need to address this matter with far greater urgency, and people living with TB and HIV must

play a central role in pushing the agenda forward.

Conclusion

TB is a preventable and curable disease that still kills one person every 15 seconds, yet it has failed to receive due political and financial attention. The inadequacy of current diagnostics contributes to great illness and death, especially among people living with HIV and AIDS. Active engagement is needed on the part of all people of conscience in order to change this situation. Networks of people living with HIV must assume a leadership role, given that TB is a leading cause of death among us. Sadly, TB has not been consistently prioritized on the HIV advocacy agendas to date. It is imperative that civil society and activists, especially people living with HIV, engage this issue in order to ensure that TB—a disease which has been with us since before the time of the ancient Egyptians—finally becomes history.

Co-Receptor Conundrum

By Richard Jefferys

Since the late 1980s researchers have recognized that HIV isolates (viruses sampled from infected individuals) can be divided into one of two categories based on their ability to replicate in particular cells in the laboratory. Originally, a variety of terms were used to describe this phenomenon. One of the most common was the designation of viruses as either syncytium-inducing (SI – these viruses caused cells in the lab dish to clump together and form clusters of dying cells called syncytia) or non-syncytium-inducing (NSI). The classification of SI and NSI viruses was subsequently aided by the discovery that SI viruses could replicate in specific laboratory cell line—known as MT-2 cells—whereas NSI viruses could not. Confusingly, an alternative classification dubbed these viruses T cell tropic (T-tropic) or macrophage tropic (M-tropic), respectively, even though both types could replicate in T cells.

In early 1996, the underlying biological basis for this distinction became clear when researchers discovered that, in addition to latching onto the CD4 molecule in order to gain entry into T cells, HIV isolates utilized one of two different co-receptors: either CCR5 or CXCR4. It quickly became apparent that CCR5 use correlated with the NSI/M-tropic classification while viruses that used CXCR4 were SI/T-tropic. As it turns out, the MT-2 cell line only expresses CXCR4. The older nomenclature has been replaced by the simpler designations of R5-tropic or X4-tropic. Rare “dual tropic” viruses that can use either co-receptor have been reported, but this designation is more commonly (and perhaps misleadingly) applied to mixed populations of R5-and X4-tropic HIV.

One salutary result of this progress is the development of pharmaceutical compounds designed to inhibit the interaction between HIV and its various coreceptors. However, the larger questions of why HIV bifurcates into two variants with differing tropisms and how these variants relate to disease

pathogenesis remain unanswered, leaving a cloud of uncertainty looming over the clinical development of both R5 and X4 inhibitors. Similarly, now that large human studies of coreceptor inhibitors are underway, outstanding questions regarding the biological functions of CCR5 and CXCR4 receptors in humans have inspired research and regulatory interest in the possibility of unpredictable toxicities. The recent termination of the development of GSK's CCR5 inhibitor, aplaviroc, due to several cases of severe liver toxicity further underscores the need for vigilance in this regard.

Co-Receptor Biology

Both CCR5 and CXCR4 belong to a family of molecules known as 7-transmembrane receptors. These receptors are snake-like structures with portions both inside and outside of the cell (think of a picture of the mythical Loch Ness monster with a trail of humps visible above the water line – 7-transmembrane receptors have seven loops protruding from the cellular membrane). The primary function of CCR5 and CXCR4 is to interact with specific chemical messengers called chemokines; binding of the chemokine to the receptor causes the chemokine/receptor complex to submerge into the cell and initiate a cascade of signals that affect the cell's behavior. A ligand is a substance capable of binding to a receptor. For example, binding of the chemokine CCL5 (which stands for chemokine ligand 5, also known as RANTES) to CCR5 can trigger the cell's migration to specific locations within the body. The chemokine SDF-1 binds to CXCR4, and these interactions are important in many settings, including embryonic development (mice genetically lacking CXCR4 die in utero).

Notably, chemokine receptors can be rather promiscuous in their ability to bind different chemokines. CCR5 is known to interact with CCL3, CCL4, and CCL8 in addition to CCL5. The reverse is also true; certain chemokines can interact with more than one receptor. CCL5, for example, can bind CCR1 and CCR3 in addition to CCR5. The functions of all the known chemokine receptors

and chemokines are not fully characterized, but broadly speaking, they seem to be involved in cell migration and/or inflammation.

In terms of which cells possess the two HIV co-receptors, CCR5 is primarily found on activated T cells but CCR5 expression has also been reported (primarily from mouse studies) on multiple other cell types including macrophages, dendritic cells, neutrophils and hepatic stellate cells. CXCR4 is broadly expressed in cells of both the immune and the central nervous systems. A critical question facing developers of co-receptor inhibitors is whether CCR5 and CXCR4 inhibitor compounds interfere with chemokine/receptor interactions, and if so, whether such interference has harmful consequences.

Immunotoxicities

On December 14th 2005, the Forum for Collaborative HIV Research held a roundtable discussion concerning the potential immunotoxicities of entry inhibitors (copies of the presentations are available at <http://www.hivforum.org/projects/CCR5.htm>). Mark Swain reviewed two recent studies that investigated the role of CCR5 in a mouse model of hepatitis. The model involves administering the drug Concanavalin A (Con A) to mice, which triggers an immune system attack on the liver leading to hepatitis. Swain's research group compared the severity of hepatitis in normal mice compared to mice genetically bred to lack CCR5 receptors (called CCR5^{-/-} or CCR5 knockout mice). The study found that knockout mice who received Con A developed profound hepatitis that progressed rapidly to fulminant liver failure. Three of six knockout mice died within eight hours compared to no deaths among the normal mice. Researchers searched for an explanation and found that a specialized group of T cells called natural killer T cells (NKT cells for short) appeared more resistant to death (apoptosis) in the knockout mice. The NKT cells from knockout mice also produced more of the cytokine interleukin 4 (IL-4) compared to their CCR5 possessing counterparts. Blocking IL-4

or depleting NKT cells using antibodies reduced the extent of the liver damage in knockout mice. This study was published in June 2005 in the *Journal of Immunology* (J. Immunology, 174: 8027-8037, 2005), <http://www.jimmunol.org/cgi/content/abstract/174/12/8027>

The second study discussed by Swain was conducted by a group of Belgian researchers led by Christophe Moreno and involved the same mouse model of Con A-induced hepatitis. The researchers reported similar findings to Swain's group, namely, increased mortality in CCR5 knockout mice. They also reported that, in normal mice, serum levels of the CCR5 ligands CCL3, CCL4, and CCL5 were significantly increased following Con A injection and that CCR5-expressing liver mononuclear cells (comprising T cells, macrophages, natural killer cells and NKT cells) were recruited to the liver. The CCR5 knockout mice also exhibited increased production of interleukin 4, tumor necrosis factor, CCL3, CCL4, and CCL5, and a notable infiltration of T cells, macrophages, natural killer cells and NKT cells into the liver, among which were cells expressing the chemokine receptor CCR1 (which can also bind to CCL3 and CCL5). The researchers tried blocking CCR5 ligands with antibodies to see whether the hepatitis would improve. Blocking CCL5 significantly reduced serum ALT levels and hepatic mononuclear cell infiltration, whereas blocking CCL3 and CCL4 had no effect. Thus, it appears that the absence of CCR5-expressing cells can result in increased levels of circulating CCL5, potentially exacerbating immune-mediated liver damage. This study was published in the journal *Hepatology* (*Hepatology* 42:854-862, 2005), <http://www3.interscience.wiley.com/cgi-bin/abstract/112093931>.

Human Knockouts

While much of the mice data sounds disconcerting, it remains unclear whether the experience with mice bears any relation to what might happen to humans receiving a CCR5 inhibitor. The closest human equivalents to CCR5 knockout mice are certain rare individuals who possess a genetic mutation that prevents the expression of functional

CCR5 receptors. This mutation is dubbed CCR5 delta32; when inherited from both parents, a person lacks functional CCR5 on his/her cells and is said to be 'homozygous' for the mutation. If a person inherits the mutation from only one parent, he or she displays reduced CCR5 levels on his/her cells and is said to be 'heterozygous' for the mutation. People homozygous for the CCR5 delta32 are highly resistant to HIV infection, although some cases of such individuals becoming infected with X4-using HIV have been reported. While not protected from HIV infection, individuals who are heterozygous for the CCR5 delta32 mutation appear to have slower disease progression.

To date, most studies have not revealed obvious, serious health problems among delta32 homozygotes or heterozygotes, but the literature on delta32 homozygotes remains relatively sparse. There has been one report that delta32 homozygotes infected with hepatitis C experience less inflammation but more fibrosis (scarring) of the liver, compared to infected individuals who lack the delta32 mutation. Data published previously suggested that delta32 homozygotes are more susceptible to hepatitis C infection and have a diminished response to treatment, but recent studies have contradicted these assertions. The delta32 mutation has also been strongly associated with a disease called primary sclerosing cholangitis (PSC). PSC is a disease involving inflammation and scarring of the bile ducts, which can cause bile to accumulate in the liver, damaging liver cells and leading to cirrhosis.

Perhaps the most dramatic human data come from a very recent study of West Nile Virus (WNV) suggesting that delta32 homozygotes may be more susceptible to symptomatic infection with this mosquito-borne pathogen (*J Exp Med* 203;1:35-40, 2006). The investigation was conducted based on results of a prior study in CCR5 knockout mice demonstrating that the mice experienced exacerbated symptoms as a consequence of reduced T cell trafficking to the brain (*J Exp Med* 202;8:1087-1098, 2005). The human study analyzed two different cohorts of individuals with laboratory

confirmed symptomatic WNV infection. The results found that delta32 homozygotes were significantly overrepresented in both cohorts relative to the expected frequency of the mutation in the population. In one of the two cohorts, delta32 homozygote genotype was also associated with an increased risk of death. The study authors went so far as to conclude: "Our results have important implications regarding the potential safety of CCR5-blocking agents now under development for the treatment of HIV/AIDS. Clinical care of individuals taking these medicines while residing in WNV-endemic areas may mandate strict measures to limit mosquito exposure and a high index of suspicion for symptoms consistent with WNV."

The extent to which any or all of these problems might occur in the setting of pharmacological CCR5 inhibition cannot be known until a larger amount of safety data accumulates on CCR5 inhibitors. It is possible that the redundancy present in the chemokine/chemokine receptor system may allow other receptors to assume the function of CCR5 in delta32 homozygotes, and that something similar may occur in people receiving CCR5 inhibitors. But the safety and toxicity issues associated with CCR5 receptor blocking are significant enough that regulatory authorities are requiring extensive long term follow up—up to 5 years—of individuals participating in clinical trials of CCR5 inhibitors.

Coreceptors and Pathogenesis

Even back when X4-using viruses were characterized as syncytium-inducing, researchers recognized that these viruses became detectable almost exclusively during late stages of disease. Large cohort studies now indicate that around 50% of people progressing to AIDS will show evidence of a shift from R5- to X4-using HIV. Initially, researchers assumed that the emergence of X4 viruses was causing accelerated disease progression in these individuals. This assumption remains one of the focal concerns regarding current trials of CCR5 inhibitors: that these drugs might select for X4 viruses and that X4 viruses might accelerate disease progression.

Recently, however, the supposition that X4 virus causes rapid progression has been questioned. The countervailing hypothesis is that X4 virus emerges as a consequence of the severe T cell depletion seen in advanced disease, perhaps due to the loss of appropriate cellular targets for R5-using HIV (this argument is rehearsed in excruciating mathematical detail in a new paper in the *Journal of Virology*, see J. Virol 80;2:802-9, 2006, <http://jvi.asm.org/cgi/content/abstract/80/2/802>). This line of reasoning is further based on evidence that R5 HIV has a competitive advantage over X4 HIV. The precise nature of this advantage remains uncertain, but a number of possible explanations have emerged:

- R5 HIV makes more virus per infected cell than X4 HIV
- R5 HIV is preferentially taken up by dendritic cells and transferred to CD4 T cells
- X4 HIV is preferentially suppressed by CD8 T cells

There are data that support each of these notions, but as of yet, nothing conclusive. It is fair to say, however, that some of the heightened early concern about the potential danger of tropism shifts has waned. A shift to X4 virus has been reported in several recipients of CCR5 inhibitors, but it does not appear to have harmful clinical consequences. CCR5 inhibitor studies are currently using an assay that attempts to quantify proportions of R5- and X4-using HIV as a screening tool. When applied to recipients of normal HAART regimens, the assay has found that roughly 40-50% of HIV+ people show evidence of X4 virus, though there is no association between X4 presence and disease stage.

CXCR4 Inhibitors

One way to address the concern regarding emergence of X4 virus is to focus pharmacological

inhibitor development on this coreceptor. Since CXCR4 knockout mice cannot be bred, and there are no humans lacking CXCR4 expression (as there are with CCR5), trials of CXCR4 inhibitors are subject to equally rigorous scrutiny for signs of toxicity.

At least one such drug (AMD070) is undergoing clinical evaluation. AMD070 has been tested in a very small group of HIV-infected individuals, and the ACTG is currently sponsoring a larger Phase II trial that has enrolled just four people after more than a year of accruing. Recruitment was recently temporarily stopped due to hepatotoxicity seen in a parallel dog study. Upon announcement of the halting of the ACTG study, Anormed opened a similar study. The FDA is likely to keep a close eye on these studies should they move forward.

Conclusion

The targeting of HIV co-receptors represents an exciting new frontier for antiretroviral therapeutics even as it signals a journey into deep, uncharted waters. There are no therapeutic precedents for inhibiting human chemokine receptors. Until more data become available, it is difficult to predict just how rocky this sail may become. In the meantime, FDA is paying very close attention to safety concerns, including convening a consultative meeting in conjunction with the Forum for Collaborative HIV Research to solicit input from the wider community. This meeting was originally slated for January 18th 2006 but has been rescheduled for May 30, 2006.

For more information about coreceptor inhibitors and other drugs in development, see Rob Camp's 2006 Clinical Pipeline report with links to data at: www.aidsinfonyc.org/tag/tx/pipeline2006b.html

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