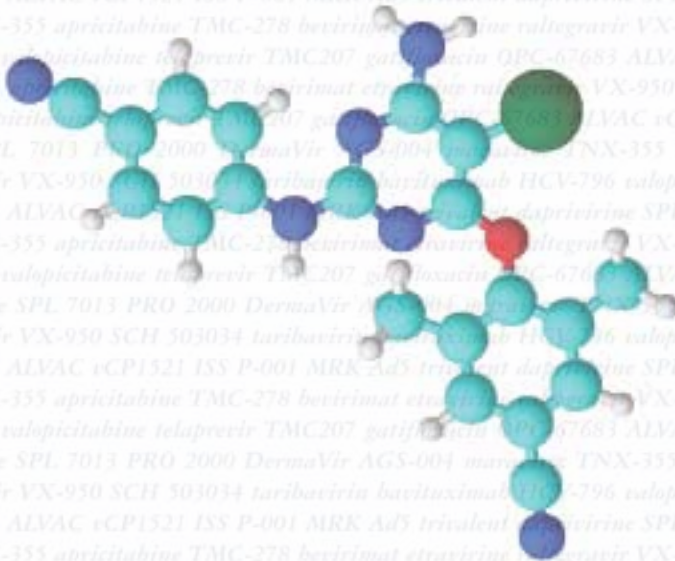


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**HIV, TUBERCULOSIS, AND VIRAL HEPATITIS:  
DRUGS, DIAGNOSTICS, VACCINES, IMMUNE-BASED THERAPIES,  
AND PREVENTIVE TECHNOLOGIES IN DEVELOPMENT**

# TAG 2010 Pipeline Report



**JULY 2010**

**TREATMENT ACTION GROUP**

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## ABOUT TAG

The Treatment Action Group is an independent AIDS research and policy think tank fighting for better treatment, a vaccine, and a cure for AIDS. TAG works to ensure that all people with HIV receive lifesaving treatment, care, and information.

## ABOUT HIV I-BASE

HIV i-Base is a London based HIV treatment activist organization. i-Base work in the UK and internationally to ensure that people living with HIV are actively engaged in their own treatment and medical care and are included policy discussions about HIV treatment recommendations and access.

**i-base**

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THIS REPORT IS DEDICATED TO

## Vuyani Jacobs

a fierce advocate for access to care and treatment for people with HIV, gender equality, and human rights. A dedicated activist, Vuyani's leadership contributed to the success of the Treatment Action Campaign, Médecines Sans Frontières, and Community Health Media Trust. Through his media work, his community organizing, and his compassion he contributed to a world where people could live free from stigma and have access to life-saving information and treatment. He died on 30 June 2010 of meningitis.

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# Introduction

BY MARK HARRINGTON AND SCOTT MORGAN

For the sixth time, Treatment Action Group (TAG), now in collaboration with HIV i-Base (UK), presents the current clinical pipeline for new drugs and vaccines for HIV, hepatitis C virus (HCV), and tuberculosis (TB), along with new sections on the hepatitis B virus (HBV) pipeline and diagnostics for TB and HIV.

Despite the global economic crisis and the erosion of political will to continue scaling up effective, lifesaving, evidence-based preventive and treatment interventions for HIV and its most common coinfections worldwide, TB, HBV and HCV, the scientific outlook is unexpectedly positive. Continuing growth and maturation in the HIV therapeutics market space have not yet led to a visible diminution of efforts by industry to discover and develop new antiretroviral drugs and classes. A pair of antiretroviral drugs approved in 2006 and 2007—the protease inhibitor darunavir (Prezista, Tibotec/Johnson & Johnson) and the first-in-class integrase inhibitor raltegravir (Isentress, Merck)—joined efavirenz (EFV) and boosted atazanavir (ATV) as preferred first-line anti-HIV drugs in combination with tenofovir (TDF)/emtricitabine (3TC) (combined as Truvada) in the U.S. Department of Health & Human Services adult and adolescent HIV treatment guidelines published in December 2009. These advances show that there is a continued market for innovation in HIV treatment and that industry, regulators, and public health authorities agree on how best to study new drugs in order to rank them relative to existing regimens. In the coming years there may be fewer persons experiencing multidrug class failure to participate in earlier phase studies, which means that new trial designs will be needed; thus, the over \$10 billion yearly market for HIV therapy will continue to experience dynamic changes and evolution. Five new compounds and combinations are in advanced phase III studies and expected to be filed for U.S. Food and Drug Administration (FDA) review in 2010–2011: Tibotec’s nonnucleoside reverse transcriptase inhibitor rilpivirine (TMC278); the triple combination with tenofovir/3TC/rilpivirine; Gilead Science’s integrase inhibitor elvitegravir; the novel pharmacokinetic enhancer cobicistat; and the so-called Quad pill containing elvitegravir/cobicistat/tenofovir/3TC. Additional drugs in existing and new classes, the latter including maturation and attachment inhibitors, are in earlier phases of testing.

The global HIV market is estimated to be growing toward over \$16 billion by 2016, around the time when a wave of patent expiries will make it ever more essential for new market entries to possess qualities that are measurably superior to what will then be a much more generic sales-centered market.

This year, in addition to Simon Collins's overview of the adult HIV pipeline, Polly Clayden, also of HIV i-Base (UK), presents an update on a much-neglected area of HIV research, the pediatric antiretroviral pipeline. Shockingly, some of the most critical agents used in adult therapy, such as tenofovir, are still not available for very young infants and children; indeed, the pediatric HIV standard of care globally resembles adult HIV care about ten years ago. This must change, and Clayden's chapter explains what will be required.

In a foretaste of things to come, Clayden also provides a quick overview of global needs in HIV diagnostics, with particular focus on point-of-care diagnostic tests for early infant diagnosis, CD4 counts, and HIV RNA load.

Richard Jefferys once again presents a sweeping overview of the vast areas of the HIV clinical research agenda that have yet to provide a convincing advance in either preventive or therapeutic vaccines, microbicides, immune-based therapies, cytokine treatment, or gene-/cell-based therapies, including a new section on HIV cure and eradication research. Despite the difficulties in these research areas, activity is extensive and the ultimate solution to the pandemic can only come from the development and worldwide distribution of an effective vaccine and a cure for HIV. The vast unmet needs in these portfolios make it even more essential to increase investments in basic and translational science over the coming years.

Lei Chou's overview of the virtual paralysis afflicting HBV research in the past year makes for much more depressing reading. There is no visible drug development for HBV in North America or Europe, with only scanty activity in east Asia, and no clinical trials from the new U.S. National Institutes of Health-funded HBV research network despite almost two years of funding. Relying exclusively on HBV vaccination for the uninfected, public health authorities seem to be consigning the fate of the hundreds of millions of people infected with chronic HBV infection to a very short list of effective drugs to which HBV may well develop pan-resistance before new agents are in the pipeline. The world must move beyond a vaccination-only strategy and focus on saving the lives of the many who have chronic HBV-related disease.

TAG's Hepatitis/HIV Project Director Tracy Swan has been predicting a revolution in HCV treatment since the mid-2000s. This year, her prediction has come measurably closer to reality as phase III results from trials of two HCV protease inhibitors, boceprevir (Merck/Schering Plough) and telaprevir, (Vertex/Tibotec) are expected by the end of 2010. Although both drugs come with added toxicity, boceprevir and telaprevir have considerable promise, offering the potential to significantly increase cure rates for the most difficult to treat genotype 1 infections, and, in some cases, to reduce treatment duration from 12 to 6 months. Farther back in the pipeline but even more promising are combinations of oral, direct-acting HCV antiviral compounds that may render today's



standard of care—based on dauntingly expensive and toxic peginterferon-alpha and ribavirin—obsolete. But these drugs come with new challenges: optimal treatment strategies for the HIV/HCV coinfecting, people with non-genotype 1 infections, and subgroups of treatment-naïve and treatment-experienced people are needed. These drugs must be prescribed properly, and response to treatment must be monitored closely to avoid development of drug resistance. Global access to HCV treatment is limited and will become more so with the addition of expensive new drugs to the standard of care. But if three to six months of all-oral combination therapy can cure HCV, it would become easier to expand access to treatment worldwide, potentially saving hundreds of millions of lives.

While it would be premature to say that a revolution in TB diagnosis, treatment, and prevention is around the corner, it is possible to see a glimmering of hope on the horizon. There are now a handful of new highly sensitive nucleic acid amplification tests able to quickly detect *Mycobacterium tuberculosis* (MTB) itself and mutations associated with drug resistance, which indicate the presence of multi-drug resistant TB (MDR-TB). Although they are still too complex and expensive for use at the health post level, with economies of scale and engineering to make them simpler and more robust, some of these tests may be able to be used at the peripheral laboratory level. This is a significant advance, because more difficult TB diagnosis procedures would no longer be confined to central laboratories. Increasing access to much more rapid diagnostics for MDR-TB will be necessary to optimize use of new TB drugs. Two new drugs from two new classes of compounds—the diarylquinoline TMC207 from Tibotec/Johnson & Johnson and the nitroimidazole OPC-67683 from Otsuka—are likely to be submitted to the FDA and the European Medicines Agency for regulatory approval for treatment of MDR-TB. These drugs could revolutionize the treatment of MDR-TB by making it shorter, safer, more tolerable, and more effective; but the world is not prepared for the advent of two new TB drugs. Inadequate preparation and lack of better diagnostic tools could cause a crisis in which laboratory capacity, human resources, and background second-line TB drug supply are all insufficient to meet increasing demand (currently just 5% of the world's one million cases of MDR-TB are undergoing appropriate treatment).

TAG will continue to report on the developments in research to prevent, treat, and cure HIV, HBV, HCV, and TB. In the meantime, TAG and our comrades in activism around the world are threatened by a new and deadly foe—the global economic crisis and the indifference of the current generation of world leaders.

## **A Shifting Treatment Landscape**

A resurgence of political indifference coupled with a disastrous global economic situation has placed the lives of 33 million people around the world in danger. Only four million people in developing countries are receiving antiretroviral treatment. Compli-

ance with new treatment guidelines recommending initiation of antiretroviral treatment when CD4 cell counts drop below 350 cells/mm<sup>3</sup> places new demands on countries striving to reach universal access targets (generally considered to be 80% coverage of essential prevention, testing, and treatment targets). Ever stronger evidence about the preventive value of reducing communitywide viral load through universal uptake of appropriate antiretroviral therapy (ART) is ignored by policy makers who claim their pockets are empty, even while the financial sector and the automobile and insurance industries have received billion dollar bailouts from overstressed public purses. This section reviews some of the key issues that affect global funding for treatment, the clear and present danger that we are losing ground on HIV treatment scale-up, the promise of treatment as prevention, and a review of the debate about when to initiate antiretroviral therapy.

On May 10, 2010, the *New York Times* front page stated, “At Front Lines, Global War on AIDS is Falling Apart.” This was not news to activists, program managers, political leaders in global health, clinicians in developing countries or people living with HIV/AIDS in those countries. But it was a salutary warning that the mounting global AIDS emergency has fallen off political leaders’ agendas. At least 29 million people who are HIV positive do not have access to necessary treatment. Without it, they will die from AIDS.

Despite evidence that the global HIV epidemic is far from under control, funding for prevention, care, and treatment is flattening at an alarming rate, and shrinking relative to the need as new infections outpace HIV treatment access.

Over the past years the AIDS backlash has been growing. Donor fatigue and changing political fashions have taken their toll. Political commitment for universal access to HIV prevention, care, and treatment has wavered in the shadow of the global economic crisis. The AIDS funding backlash pits activists, health professionals, and policy makers against one another in a circular, “disease versus disease” debate that shifts our focus from the true issues: the U.S. government allocated only 0.3% of its budget to global health initiatives in 2010<sup>1</sup>; as of 2007 only 6 of 53 African countries have met their commitment to the Abuja Declaration of 2001 to allocate a minimum of 15% of their national budgets to health; African heads of state accepting foreign aid for health shift monies to other budget priorities<sup>2</sup>; \$427 million in donor commitments remain unpaid to the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) through 2009<sup>3</sup>; and a \$3 billion shortfall exists for GFATM funding in 2010. More than 2,090 people with HIV in eleven U.S. states are currently on waiting lists for AIDS drug assistance programs<sup>4</sup>; hundreds of thousands more are not yet linked to care.

Continued scale-up of effective evidence-based HIV prevention, treatment, and care programs is required to bring the pandemic under control and put it into reverse, while continuing to support research on a cure and a vaccine to end it forever.

Intensified research to develop a cure and safer, more effective, more tolerable and durable ART regimens is crucial. Exploration into novel targets for new drugs and optimized delivery must continue.

Treatment Action Group and our allies all over the world are working to keep AIDS on the forefront of the global public health agenda. Activists, people living with HIV, clinicians, civil society members, and policy makers must be tenacious if we are to continue to support healthy HIV treatment and prevention programs and keep the development of a preventive vaccine and a cure for HIV on the global research and policy agenda. We must continue to pressure the G8 to fulfill its 2005 commitment to universal access and we must hold African heads of state accountable to the Abuja Declaration on Health.

**Surviving the AIDS backlash.** We must not allow health economists or political leaders to pit disease groups against one another. We must take what we have learned from ART scale-up, which is the largest public health response in history, and apply those lessons to strengthening health systems as a whole. We must not scrap over meager allotments—0.3% of the entire U.S. budget to fund global health initiatives—but pressure our governments to expand their commitment to global health and lead other donor countries in responding to the global AIDS epidemic.

**AIDS is still an emergency** for the more than 16 million people who immediately need but cannot access lifesaving medicines. If the U.S. President's Emergency Plan for AIDS Relief is to transition from an emergency response to a sustainable program, it must be adequately funded to make that transition. But we must move out of the emergency stage first—not through rhetoric, but with evidence that we have started to reverse the spread of HIV. The pandemic, not the programmatic response, must be reversed.

**Support crucial research such as the Strategic Timing of Antiretroviral Treatment (START) study and thoroughly explore the potential of treatment as prevention.** Treatment decisions and prevention policy need to be based on evidence from randomized controlled trials. We need research that optimizes treatment benefits by balancing when to start with potential long-term side effects. We need research to strengthen the evidence base for continued scale-up. We also need better first-line regimens for potentially earlier initiation of ART.

A disastrous obsession with fiscal austerity has spread among the developed countries like an unstoppable airborne infectious virus. Millions of people's lives are at risk because global leaders refuse to meet their commitments to scale up HIV, TB, malaria, immunization, and maternal and child health programs and to reverse global poverty by the year 2015. The emergency posed by AIDS funding freezes in the United States and around the world presents a challenge to front-line providers, researchers, policy

makers, government officials, industry, and treatment activists—and most of all, people living with AIDS. We must work together as never before as a unified force to fight for human rights, public health, and social justice as truly achievable goals through universal access. We must redouble our efforts to carry out research that will end the epidemic as we continue to save lives now with effective prevention and treatment interventions.

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# The Antiretroviral Pipeline

BY SIMON COLLINS

## Introduction

Current HIV drugs are sufficiently safe, potent, and effective for recent modeling studies to suggest that, for someone newly infected with HIV, life expectancy should be approaching that of an HIV-negative person.<sup>1,2</sup> This is dependent on access to care and treatment, and such models usually exclude complications such as hepatitis C or tuberculosis coinfection or ongoing drug use. Therefore, any review of pipeline drugs must acknowledge that these assumptions for normalizing life expectancy currently apply to a minority of patients globally unless universal access and uptake can be achieved.

At levels of viral suppression to <50 copies/ml, HIV evolution is not slowed, but stopped,<sup>3</sup> with the evidence suggesting that residual virus is more likely to originate from latently infected resting cells than from ongoing replication in a part of the body not reached by treatment.<sup>4</sup> Treatment is more effective than people realize. Recent studies using more sensitive viral load tests suggest that perhaps more than 50% of people suppressed to <50 copies/ml generally have HIV RNA <5 copies/ml.<sup>5,6</sup> At these low levels, further intensification has no additional impact on viral load in plasma or in sanctuary sites such as the central nervous system.<sup>7,8</sup> New drugs and classes need to be designed to increase flexibility to adherence with the potential for new delivery methods and smaller molecular weight formulations to reduce costs. So the bar for new drugs is set higher, but this, by definition, has always been the case.

Virological failure rates are low. In some settings, rates of viral failure in people on stable suppression therapy are less than 5% annually,<sup>9</sup> supported by pharmacokinetic profiles that allow the maintenance of therapeutic levels of some drugs after the strict dosing time.<sup>10</sup>

However, rates for switching HIV treatment due to toxicity or tolerability are significantly higher, showing that tolerability is still clinically significant. Other examples of unmet antiretroviral (ARV) need include combinations that:

- treat people with multidrug resistance
- protect against or reverse central fat accumulation

- do not increase the risk of metabolic complications (lipids, glucose, bone)
- increase CD4 counts for the approximately 10% of people who respond virologically but not immunologically

New therapies active against multidrug-resistant (MDR) HIV are needed and will be lifesaving. The absolute number of people each year who become unable to construct a combination that includes at least two active drugs is currently low, estimated at perhaps 1,500 people annually in the United States. This means that a growing number of people now have resistance to five classes of ARVs. Globally, resistance to only three classes may reduce or eliminate further treatment options because of the more limited formulary in many developing countries.

For these MDR patients to benefit from treatment advances, flexibility in and new approaches to trial design are required. For example, trials need to allow people who do not have the required number of active drugs for an optimized background regimen in phase III studies to be able to use more than one unlicensed compound in a research setting.<sup>11</sup> The risk:benefit ratio for an MDR treatment is different from one developed for treatment-naïve patients. The broadest indication for any ARV is a more lucrative market. However, using orphan-drug designation for MDR HIV might generate sufficient financial incentives to develop lifesaving drugs for this special population. Antiviral efficacy has far greater priority for an MDR treatment than do formulation, adherence, or convenience of dosing.

This is an area where renewed activist focus on treatments for experienced patients is needed.

Many of the newer drugs and classes are not yet widely available in developing countries, where barriers to market entry include, at the highest level, lack of World Health Organization (WHO) inclusion on the essential medicines list or in the 2009 WHO ARV guidelines, and at the country level lack of registration, regulatory capacity, high prices, and lack of clarity on how best to use these newer agents.

Treatments in developed countries are much safer and more protective than was previously assumed.<sup>12</sup> If people are to start treatment earlier, the need exists for them to become safer still. The limitations of current ARV use include late diagnosis, unequal access to treatment, and complications related to social stigma including drug and alcohol use and discrimination based on gender and sexual orientation.

Within the last year, ARV treatment has achieved a more deserved and central position in prevention, as potentially among the most effective biomedical prevention strategies

(see the chapter on Immune-Based Therapies and Preventive Technologies Pipeline). However, the ideals of universal access to treatment, and widespread use of treatment at high CD4 cell counts, is in stark contrast to the current reality in which the median CD4 count for patients in developed countries upon diagnosis remains  $<250$  copies/mm<sup>3</sup> and is significantly lower in most resource-poor settings.<sup>13</sup>

Finally, any discussion of ARVs in the context of earlier treatment involves the question of when to start therapy. This raises the importance of accurate data on both the benefits and the risks of treatment in order to define a CD4 threshold for when to start, acknowledging that absolute CD4 counts are imperfect surrogate markers. This is currently the focus of the National Institute of Allergy and Infectious Diseases–funded START study that will randomize 4,000 patients with a CD4 count  $>500$  copies/mm<sup>3</sup> to either immediate HIV treatment or to defer initiation until the CD4 count reaches 350 cells/mm<sup>3</sup>.

Notably, large randomized studies also provide the opportunity to study the pathology of HIV disease and its interaction with treatment, other illnesses, and age-related morbidity. With ARV treatment options unlikely to change radically in the next few years, this is a stable and opportune time for such research.<sup>14</sup>

We need new drugs. The antiretroviral pipeline in 2010 that is detailed below, focusing predominantly on compounds in phase II or phase III development or with in vivo data on virologic activity, looks surprisingly strong. Many of these compounds will be active against MDR HIV. However, the development of some compounds with potential activity has been suspended due, at least in part, to financial reluctance from investors.

The global demand for alternatives to lifelong treatment, compounded by economics that are beginning to cap treatment programs both in the United States and internationally, is discussed more fully in the introduction to this report and has reinvigorated the urgency for strategies focused on a cure (see the chapter on Immune-Based Therapies and Preventive Technologies Pipeline).

## Activity since 2008

There are no guarantees in drug development, even for compounds that complete phase III studies. Predicting pipeline development is a similar process. It is therefore perhaps most significant that none of the ARVs that were listed as pipeline compounds in TAG's *2008 Pipeline Report* have been approved as new treatment. The only new ARV to be approved by the U.S. Food and Drug Administration (FDA) since 2008 for sale in the United States has been a Meltrex formulation of the protease inhibitor ritonavir in Janu-

ary 2010. The principal advance this has is heat stability, no longer requiring refrigerated storage. However, the time taken for this development attracted criticism for Abbott, the drug's producer, since it came five years after the same compound had been coformulated with lopinavir in a heat-stable version of Kaletra.<sup>15</sup>

Of seven compounds listed in 2008, only two (rilpivirine and elvitegravir) continue in phase III studies; one (TNX-355) has remained in tentative phase II and four (vicriviroc, bevirimat, apricitabine and amdoxovir) have been put on hold or discontinued (see table 1).

**Table 1. The Status of Pipeline Compounds from the TAG 2008 Report**

Compound	Class	Status	Comment
Rilpivirine	NNRTI	Phase III	Submission 3Q 2010
Elvitegravir	Integrase	Phase III	Phase III complete 2011/12
Ibalizumab (TNX-355)	CD4 mAb	Phase IIb	CD4-specific monoclonal antibody (mAb)
Vicriviroc	CCR5	Discontinued (Ph 3)	On hold; efficacy vs. placebo
Bevirimat	Maturation	Phase Iib	Development suspended
Apricitabine	RTI	Discontinued (phase 2)	No financial backing for phase III studies
Amdoxovir	RTI	Discontinued (phase 2)	No recent data published on ongoing studies

*Notes:* NNRTI = nonnucleoside reverse transcriptase inhibitor; mAb = monoclonal antibody; RTI = reverse transcriptase inhibitor.

In the unfortunately named Victor-E phase III studies, the CCR5-inhibitor vicriviroc, when added to optimized background therapy, failed to show benefit compared to placebo in treatment-experienced patients. While the issue of the strength of background therapy has been discussed, darunavir and/or raltegravir were only used by 25–40% of people and etravirine was not available. Approximately 65% of the participants had >3 active drugs (defined by baseline genotypic sensitivity) and only 30–40% of the participants had two or fewer active drugs. At least some of the differences in antiviral activity of vicriviroc compared to placebo were likely to be obscured by an uneven distribution of baseline resistance in the active compared to control arms. This difference was not sufficiently promising for Merck, which acquired vicriviroc when the company bought Schering Plough in 2009, to continue development in experienced patients.<sup>16</sup> However, phase II studies in treatment-naïve patients are ongoing.<sup>17</sup>

Bevirimat (now MPC-4326 with Myriad, and formerly PA-457 with Panacos) is a maturation inhibitor that has had a long development history dating back to a ten-day monotherapy activity study back in 2005.<sup>18</sup> While further efficacy data have not been presented,



a phase IIb dose-finding study in treatment-experienced patients was started in November 2009.<sup>19</sup> The drug has reduced activity in around one-third of treatment-naive patients with HIV subtype B due to naturally occurring polymorphisms in the Gag cleavage site and greater levels were expected with subtype C. In protease-resistant patients the proportion of non-responders was estimated at 45%.<sup>20</sup> Disappointingly, in June 2010, Myriad announced in a press release that development was now suspended, stating financial pressure and a decision to focus on oncology.<sup>21</sup> This can only have been hastened by commentary three months earlier in the journal *AIDS* suggesting “future development should be abandoned.”<sup>22</sup> From an activist perspective this is alarming because conversely the molecule potentially retained activity for 55% of people with multiple drug resistance.

Apricitabine (AVX754, formerly Shire SPD754), a cytidine analogue similar to 3TC, with activity against M184V resistance, with or without additional thymidine or other nucleoside analogue mutations (either TAM pathway, L74V etc), and no further development of mutations following 21 days monotherapy. In the phase 2 AVX-201 study apricitabine, showed viral load reductions of  $-0.7$  log for people with three or more TAMs,<sup>23</sup> but has since failed to move into phase III. The Australian biotech company Avexa announced the end of the development program in a press release in May 2010, explaining its inability to find an investment partner to back further development on the limited market for twice-daily medication.<sup>24</sup> This is another missed opportunity for MDR options.

Finally, the development of amdoxovir (DAPD), a purine nucleoside analogue with activity against M184V and other reverse transcriptase inhibitor-associated mutations, has maintained a low profile, perhaps because its potential to reduce resistance to AZT when used in the same combination currently has reduced applicability now that AZT is less commonly recommended in Western countries. The last presentation on the development of this compound was at the Conference of Retroviruses and Opportunistic Infections (CROI) 2008, published this year, that reported  $-2.0$  log reduction in viral load after 10 days of 500 mg amdoxovir plus 200 mg AZT, showing synergistic activity compared to monotherapy.<sup>25,26</sup> Until 2004, amdoxovir was in development by Gilead, under license from Emory University and the University of Georgia Research Foundation, who acquired the compound when they bought Triangle Pharmaceutical in 2003.

The positive news is that both rilpivirine and elvitegravir are proceeding in phase III studies, while TNX-355 (ibalizumab, now TMB-355 with TaiMed) currently has ongoing studies but they are moving slowly.

## The ARV Pipeline in 2010

Fortunately, newer compounds expand the 2010 pipeline (see table 2), which does not include new formulations of existing drugs such as the extended-release nevirapine that may be submitted later this year or the once-daily formulation or indication for raltegravir.

Of the compounds in phase III development, rilpivirine (formally TMC-278) is likely to be submitted to the FDA this year, and potentially an additional fixed-dose combination (FDC) of rilpivirine/tenofovir/FTC.

Rilpivirine is a nonnucleoside reverse transcriptase inhibitor (NNRTI) from Tibotec that showed short-term activity of <1.2 log in the phase 2a monotherapy study.<sup>27</sup> This year top-line results from two phase III studies in treatment-naïve patients (TMC278-C209 and C215) have already been reported. When used in combination with tenofovir/FTC, rilpivirine is reported to be noninferior compared to efavirenz, based on the primary end-point of viral suppression, <50 copies/ml at week 48. Results are due to be presented at the Eighteenth International AIDS Conference in Vienna in July 2010. These will determine submission to the FDA, which is anticipated in the summer of 2010.<sup>28</sup>

Results for rilpivirine from phase II studies at 96 weeks reported lower rates of side effects including reduced rash, lower central nervous system toxicity, less sleep disturbance, and fewer lipid changes compared to efavirenz. However, grade 3 and 4 side effects and laboratory abnormalities were similar, so while these results are encouraging this is a compound that suggests an improved rather than clean tolerability profile. Early concerns about cardiovascular toxicity (from prolonged QTc intervals, although stabilized), were largely overcome by selection of the 25mg dose.<sup>29</sup> If there is wide interpatient variability in drug levels, the low dose would need to demonstrate the proportion of patients failing to achieve the minimum effective concentration (MEC). Efficacy compared to Atripla (the combination efavirenz/tenofovir/FTC) are also likely to determine uptake and use. With intent-to-treat analysis both efficacy and tolerability contribute to primary analysis and both should be tracked closely when phase III results are presented.

The once-daily low-dose (25mg) formulation supports easier development in FDCs, including the Gilead-led formulation with tenofovir/FTC mentioned above.<sup>30</sup> If current bioequivalence studies are successful, this could see regulatory submission for FDCs before the end of 2010. Additionally, a slow-delivery formulation requiring an injection every four weeks is currently undergoing pharmacokinetic studies, potentially for both postexposure prophylaxis and treatment indications.

The next most promising pipeline compounds are from Gilead, singly or in fixed-dose formulations: an integrase inhibitor (elvitegravir), a pharmacokinetic booster (cobicistat,

previously GS 9350), and the Quad FDC that combines both with tenofovir/FTC. While the four-in-one FDC is the clearly preferred lead, development issues could see earlier submission to regulatory agencies of a coformulated elvitegravir plus cobicistat or even stand-alone cobicistat. Submission for these compounds is unlikely before 2012. Limited data are available on these compounds.

When Quad ( $n=48$ ) was compared to Atripla ( $n=23$ ) in the 236-0104 phase II study, 90% vs 83% (NS: weighted difference +5% 95%CI -11.0% to +21.1%) of patients had an undetectable viral load (<50 copies/ml) at 24 weeks by intent-to-treat, missing=failure analysis (ITT M=F).<sup>31</sup> Patients were treatment-naïve, with no documented resistance and were HBV/HCV negative. Mean age was 35, approximately 90% of participants were Caucasian, baseline CD4 was 389 vs 450 in the Quad vs Atripla groups and 4–6% had an AIDS diagnosis. However, mean baseline viral load was low at <40,000 copies/ml (4.6 log), and only 25% of people had levels >100,000 copies/ml. Patients in the Quad group ( $n = 48$ ) became undetectable more quickly than those on Atripla ( $n = 23$ ) which is likely to be an integrase class effect, as this was also seen with raltegravir, though it has not shown clinical significance so far. After eight weeks, about 80% of people had undetectable viral loads with Quad, compared to about 50% with Atripla. Quad was better tolerated in terms of lack of efavirenz-related side effects (35% vs 57% with any grade 1–4 drug-related adverse event). This was driven by reduced CNS toxicity: abnormal dreams 10% vs. 35%; dizziness 0 vs. 13%; fatigue 8 vs 13%; somnolence 4 vs. 9%). There were three discontinuations in each arm, with one due to adverse events (in the Atripla group). A caution was also reported due to the impact of cobicistat on reducing estimated—but not actual—glomerular filtration rate suggests that a new management algorithm for renal toxicity will need to be developed.

Results from a second phase II study (216-0105), this time comparing the new booster cobicistat ( $n = 50$ ) to ritonavir ( $n = 29$ ) in the same population, each in combination with atazanavir plus tenofovir/FTC, showed limited differences in efficacy or tolerability between the two boosters. Virological responses were 84% vs 86% (ITT M=F; NS: weighted difference -1.9% 95%CI -18.4 to +14.7) in the cobicistat vs. ritonavir groups respectively. Grade 1–4 adverse events occurred in 20 vs 24% with grade 3/4 events in 4% vs. 0 patients. GI tolerability was similar (diarrhea 6% vs 10% but nausea 10% vs. 3%). Similar small median increases were seen in cholesterol, HDL, LDL and triglycerides in each arm but grade 2–4 increases in total cholesterol were higher in with cobicistat (6% vs 0) and in amylase (12% vs. 7%). These are tiny numbers but while data are too limited for a detailed comparison, cobicistat appears to have similar GI, lipid, and cytokine P450 3A4-boosting activity to ritonavir, which is not ideal. Unlike ritonavir, cobicistat has no antiretroviral activity.<sup>32</sup>

These small studies are promising. Quad, elvitegravir, and cobicistat are all currently in larger phase III studies and nothing should be assumed until we see the results. Quad is

also going head-to-head against Atripla (mainly in the United States) and against atazanavir/ritonavir plus tenofovir/FTC in the United States, Europe, South America, and Asia.<sup>33,34</sup> Cobicistat is going head-to-head against ritonavir with atazanavir plus tenofovir/FTC.<sup>35</sup>

While cobicistat may not yet have demonstrated advantages over ritonavir on tolerability and toxicity, it may have the advantage of allowing easier and potentially cheaper coformulated FDCs. If this compound is safe and effective, Gilead will be spared royalty payments to Abbott, and the example with Atripla may be an indication that collaborations could follow with other companies whose drugs require boosting.

In 2008, Sequoia, Tibotec, and Pfizer had booster compounds in early development, but none of these have reported further progress in vivo.

Phase I study results of the Sequoia compound SPI-452 in HIV-negative individuals showed proof-of-concept boosting activity with atazanavir or darunavir; these were presented at CROI 2009.<sup>36</sup>

The booster from Tibotec (TMC558445) completed single and multiple escalation phase I studies to increase darunavir or Tibotec's investigational protease inhibitor TMC310911 in HIV-negative volunteers.<sup>37</sup> For results to have neither been presented nor published indicates that both TMC558445 and TMC310911 are unlikely to advance along the pipeline, at least in the short term.<sup>38</sup>

The pharmacokinetic booster PF-03716539 was one of the compounds coming from Pfizer when it formed a joint venture with GlaxoSmithKline (GSK) in 2009 to form ViiV Healthcare. Although a phase I study in HIV-negative people was completed, the results have not been published or presented, nor other studies listed.

The ViiV pipeline is probably led by the integrase inhibitor GSK1349572, developed by GSK in partnership with Shionogi. Phase IIb dose-ranging results are expected to be presented at the Eighteenth International AIDS conference in Vienna,<sup>39</sup> and Phase IIb studies in integrase-experienced patients are already ongoing.<sup>40</sup> A broad range of drug-to-drug interaction studies, mostly already completed,<sup>41</sup> indicate confidence in GSK1349572, and phase III studies are likely to start enrollment before the end of the year. This compound is a once-daily formulation that does not require pharmacokinetic boosting and has potential for coformulation with abacavir/3TC.

ViiV also has two NNRTI compounds. GSK2248761 is the development name (formerly IDX-12899) for the compound bought by GSK from Idenix. Antiviral activity was shown in results from a seven-day Phase I/IIa dose-finding study in Argentina in 40 treatment-

naïve patients randomized 8:2 to once-daily monotherapy with 800mg, 400mg, 200mg or placebo. All patients switched to 28 days monotherapy or started HAART at the end of the study period. Results were available for all but two patients in each of the 200mg and placebo arms. Viral activity was similar in each of the active drug groups, which saw steady, linear viral load reductions reaching -1.8 log at day eight from mean baseline of approximately 4.3-4.6 log copies/mL.<sup>42</sup> Though further efficacy data have not been presented, a phase IIb dose ranging study is due to start later in 2010. ViiV also acquired UK-453061 (lersivirine) from the joint venture with Pfizer, with even more distant efficacy results (from 2007, but published in 2009). In 48 treatment naïve patients, mean viral load reductions at day 8 of 0.3, 0.8, 1.3 and 1.6 log after receiving 10, 30, 100 and 500 mg twice daily, respectively, and 0.9, 1.7 and 1.8 log after receiving 100, 500 and 750 mg once daily, respectively.<sup>43,44</sup> However, the lersivirine phase IIb studies in naïve patients compared against efavirenz are ongoing and may report results in early 2011.

Drug interaction and/or formulation studies are ongoing in HIV-negative groups. As both compounds showed similar approximate reductions in viral load of at least -1.7 logs following 7-days monotherapy one of these compounds will be prioritized for development, with dual-stage development for the same class unlikely.

As a result of a collaboration with Concert Pharmaceuticals announced last year,<sup>45</sup> GSK is developing a deuterium-based protease inhibitor (CTP-518) that is similar to atazanavir but may not need pharmacokinetic boosting; this is still in preclinical development.

At the end of 2008, interesting results from an NNRTI developed by Ardea Bio (RDEA806) showed viral load reductions of 1.5-2.0 log following seven days of monotherapy in 12 treatment-naïve patients.<sup>46</sup> It is disappointing that nothing further has been heard about this compound, with this most likely due to failure to find a development partner.

Other compounds in phase IIb studies include two entry inhibitors (an attachment inhibitor from Bristol-Myers Squibb (BMS) about which little is known, and a CCR5 inhibitor from Tobira), a long-term development survivor (a monoclonal antibody ibalizumab—which has been listed in every TAG *Pipeline Report* at least since 2004), and a tenofovir-like nucleoside reverse transcriptase inhibitor (from Chimerix).

BMS is currently enrolling HIV-positive patients in a phase II dose-finding study of an attachment inhibitor called BMS-663068, with and without ritonavir, at a single site in Berlin. However, few details have been published from earlier studies or on its mechanism of action.<sup>47</sup>

A dose-finding phase I study of the CCR5 inhibitor (with off-target CCR2 activity) called TBR-652 from Tobira was presented at CROI 2010.<sup>48</sup> These first results in 54 HIV-positive patients produced median viral load reductions of 1.7 log with the 50mg, 75mg, and 150mg doses after ten days of monotherapy. Although baseline viral load was lower in the 150mg group (median 4.0 logs, compared to 4.5 and 4.6 logs in the 50mg and 75mg groups), all patients using the 75mg dose had >1.0 log reductions. Patients were treatment-experienced (though off treatment for at least six weeks), CCR5-naïve and CCR5-positive. No dose-related or serious side effects were reported. Mild side effects (none reported at the 75mg dose) included nausea, diarrhea, headache, and fatigue in greater frequency at the 100 and 150mg doses, although many of these were reported as being in a single patient with a concomitant infection. The compound has a plasma half-life of 35–40 hours, allowing once-daily dosing and although metabolized by CYP and non-CYP pathways is neither an inducer nor inhibitor of CYP P450.

The monoclonal antibody ibalizumab in development with TaiMed Biologics since 2007 (now TMB-355, formerly TNX-355 with Tanox) has had a similarly long development history, and is still listed as having a phase II dose-finding study in treatment-experienced patients. Ibalizumab is given by intravenous infusion every two to four weeks.<sup>49,50</sup> Although there are interesting plans to include ibalizumab in studies with other investigational drugs in people with multiclass resistance, it is unclear whether this will be delayed by a decision to focus on a new formulation.

Finally, in the reverse transcriptase inhibitor class, CMX-157, a prodrug of tenofovir, which has activity against broad RTI-associated resistance at lower dose concentrations,<sup>51</sup> has just entered phase I studies with Chimerix in HIV-negative volunteers.<sup>52</sup> A financial backer for this compound will be needed for the development timeline to quicken, as Chimerix is a small biotech with no other antiretrovirals in development.

**Table 2. Pipeline Compounds in 2010 with Demonstrated Activity in Humans**

Compound	Company	Class	Status	Comment
Rilpivirine	Tibotec	NNRTI	Phase III	FDA submission expected third quarter of 2010.
Rilpivirine/tenofovir/FTC	Tibotec	FDC (NNRTI + Truvada)	Pharmacokinetic equivalence	FDA submission based on equivalence studies is possible before the end of 2010.
Elvitegravir	Gilead	Integrase inhibitor	Phase III	Phase III expected to complete by 2011-12.
Cobicistat	Gilead	Pharmacokinetic enhancer	Phase III	P450 CYP 3A4 inhibitor/ protease inhibitor booster.

<b>Elvitegravir/cobicistat/tenofovir/FTC (Quad)</b>	Gilead	FDC (boosted integrase + tenofovir/FTC)	Phase III	Phase III expected to complete by 2011-12.
<b>GSK1349572</b>	ViiV/ Shionogi	Integrase inhibitor	Phase IIb	Ongoing study in patients with raltegravir resistance. Results expected in July 2010.
<b>GSK2248761 (IDX-12899)</b>	ViiV	NNRTI	Phase II	Second-generation NNRTI, currently in pharmacokinetic studies. Activity showed potency in phase I monotherapy study.
<b>UK-453061 (Iesivirine)</b>	ViiV	NNRTI	Phase II	Viral load reductions of -1.8 log following ten-day monotherapy.
<b>CTP-518</b>	GSK	Protease inhibitor	Phase I	Deuterium-based protease inhibitor.
<b>RDEA806</b>	Ardea Bio	NNRTI	Phase IIa	Viral load reductions of 1.5-2.0 log, but failure to find development partner.
<b>BMS-663068</b>	BMS	Attachment inhibitor	Phase II	Dose-ranging study +/- ritonavir is ongoing.
<b>vicriviroc</b>	MSD	CCR-5 inhibitor (also active against CCR2)	Phase II/III	Treatment-naive patients only. Program for experienced patients has been stopped.
<b>bevirimat (MPC-4326; was PA-457)</b>	Myriad	Maturation inhibitor	Phase IIb	Treatment for experienced patients. Resistance testing required for screening treatment-naive patients.
<b>ibalizumab (TMB-355, was TNX-355)</b>	TaiMed Biologics	CD4 mAb	Phase IIb (by invitation)	Ibalizumab; CD4-specific humanized IgG4 monoclonal antibody administered by intravenous infusion every two weeks or four weeks.
<b>TBR-652</b>	Tobira	CCR5	Phase I	Median -1.7 log reductions after ten-day monotherapy with 75mg, 100mg, and 150mg doses.
<b>CMX-157</b>	Chimerix	NRTI	Phase I	RTI similar to tenofovir, currently in pharmacokinetic studies. Activity showed potency in phase I monotherapy study.
<b>SPI-251</b>	Sequoia	Pharmacokinetic enhancer	Phase II	P450 CYP 3A4 inhibitor/protease inhibitor booster. Boosting data in vivo but no HIV-positive data.
<b>PF-3716539</b>	Pfizer/ ViiV	Pharmacokinetic enhancer	Phase I	P450 CYP 3A4 inhibitor/protease inhibitor booster.
<b>TMC558445</b>	Tibotec	Pharmacokinetic enhancer	Phase I	P450 CYP 3A4 inhibitor/protease inhibitor booster. Development on hold.
<b>TMC-310911</b>	Tibotec	Protease inhibitor	Phase I	Development on hold.

## Other Compounds and Targets in Preclinical Research

New approaches to HIV treatment, mainly in preclinical preliminary studies, include non-antiretroviral targets and approaches, including attempts to target latently infected cells.

Some of these were presented at CROI 2010 included a new class of integrase inhibitor called LEDGINS, unlikely to be cross-resistant to raltegravir or elvitegravir, as they do not bind at the active site. These potential molecules, 2-(quinolin-3-yl) acetic acid derivatives, were designed by rational drug design and identified after screening 200,000 molecules. Two early compounds that could interfere with the assembly and stability of the capsid core are in development at Boehringer Ingelheim.<sup>54</sup>

Now, at least five new types of treatment are the focus of research on how to target latently infected cells. These include cellular restriction factors—human proteins that reduce HIV replication and that can help or block infection—such as tetherin, a protein that blocks HIV release; APOBEC3, an immunity gene that has anti-HIV activity; and TRIM5-alpha, a protein that in some monkeys protects against HIV infection. Gene therapy could perhaps be modified to adapt the related human protein.

A compound in development with Koronis (KP-1461) that had shown interesting results in vitro as a viral decay accelerator failed to show significant activity in vivo in a phase IIa study. Although the mechanism of increasing the error replication rate to a point when the virus becomes unable to sustain further replication is intriguing, this would not impact latently infected cells and, even if successful, implies limited clinical application.

Another new approach in phase I for both treatment-naïve and -experienced patients, including immunological nonresponders, is the compound SB-728 (in development by Sangamo) that is using zinc finger nuclease–modified CD4 cells delivered by infusion to inhibit CCR-5 binding.<sup>55</sup>

## Patent Expiry and Generic Compounds

The next few years will see additional patents expire for many of the earlier ARVs. The ARV pipeline could technically include in Western countries generic FDCs that were prevented by previous patent restrictions.

However, production and approval of generic AZT and ddI did not lead to either the availability of new drugs that were significantly cheaper nor to any widespread shift in



prescription policy, even when some savings could occur. This is reassuring given the poorer tolerability of these earlier RTIs.

In 2009, 3TC and abacavir (both from GSK in 2009, now ViiV) came off patent,<sup>56</sup> with the next in line being saquinavir (Invirase; Roche worldwide) in November 2010, nevirapine (Viramune; Boehringer Ingelheim) in 2011, and combination AZT/3TC (Combivir; ViiV) in 2012.

The balance of safety, efficacy, and certainly convenience remains with more recently approved drugs and more contemporary FDCs, but many of these soon-to-be-available generic options, while not included as preferred choices in treatment guidelines, are still used by at least 10–20% of people.

It is unclear why larger cost reductions have not followed patent expiry, but this may change in the future. Health care systems in Western countries are coming under increasing pressure to include cost as a factor, and the potential for limited treatment choices for poorer patients is a concern that HIV activists will need to counter with an awareness of the data showing the clinical limitations of these choices.

Conversely, generic companies may bring coformulated FDCs to Western countries that have long been available outside the United States.

Over the last year, some companies have left the HIV research field and others have entered it. Several of the largest companies pursued mergers: Roche, although announcing in 2008 that it had ceased HIV research, announced a take over of Genentech for \$47 billion earlier last year, and the company is still active in hepatitis C virus (HCV) therapeutics. GSK created a joint venture with Pfizer to launch the new HIV-specific development company called ViiV (with GSK holding an 85% share and Pfizer 15%, subject to changes in market share), and Merck acquired Schering Plough in a \$41 billion merger in November 2009. ViiV is marketing both GSK and Pfizer's legacy antiretrovirals and developing new ones, while Merck is integrating Schering's HCV and HIV pipelines into its own (with some trimming as appropriate).

Many research companies are investing in generic manufacturing plants in countries where production costs are likely to be lower, blurring the concerns about generic versus brand formulations, as long as each individual drug manufacturing facility has undergone regulatory approval.

## Conclusion

This review should demonstrate reasons for optimism in the ARV pipeline. The effectiveness of current treatment ensures, in the absence of a cure, that HIV-positive people are a growing population and therefore remain a lucrative market for investment. It is worrying when the development of potential treatments are suspended or discontinued and a lack of financial backing is cited as the cause. But drug development is a commercial activity and, like much in industry—pharmaceutical or otherwise—details are obscured when it comes to costs and development plans. The public health aspect of medicine has yet to impact on a wider knowledge of these costs. The necessity to maintain stock prices may drive company press announcements more than accurate details about the activity (or lack thereof) of pipeline compounds. The information in this report is susceptible to these influences, just as trial results only tell a limited story.

The demand for new and better drugs remains high for each stage of treatment management, and the protective impact of antiretrovirals in suppressing viral load and, in turn, reducing infections should drive the need for new drugs as powerfully as it should drive the demand for broader access to care and treatment. In 2010, after more than 25 years of research into treatment and prevention, these two fields are more neatly joined than more people imagined or wanted. The benefits of earlier treatment are plausibly supported by many studies highlighting the potential negative implications of unsuppressed viremia. The shift to treating at higher CD4 counts raises the importance of really long-term tolerability. Treatment needs to be used for up to 40 years for adults, and much longer for children.

The ARV pipeline for resource-limited countries led the world in the availability of fixed-dose generic combinations that were never available in Western countries, but the timeline for access to the latest drugs remains imperiled due to patent restrictions.

The newest pipeline drugs have seen both patient and financial benefits overlap for promising combinations, and this looks set to continue in the immediate future, within and among Western companies.

However, the greatest clinical need—patients with broad class resistance—currently provides a lower financial incentive compared to an ARV approved with a treatment-naïve indication. This shows the need for activist pressure for new regulatory solutions. Fortunately, drugs developed for one group often have the potential to be as effective in the other, but when this isn't the case—and often it won't be—we need to find a way not to lose compounds with lifesaving potential.

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# The HIV Diagnostics Pipeline

BY POLLY CLAYDEN

The effectiveness of antiretroviral treatment delivery was traditionally thought to depend on the use of sophisticated diagnostic tests.

Earlier this year, important findings from the Development of AntiRetroviral Therapy in Africa (DART) trial, conducted in Zimbabwe and Uganda were published, showing impressive survival rates in people receiving routine clinical monitoring alone. This trial randomized patients to receive either clinical monitoring or laboratory (hematology, biochemistry and CD4) plus clinical monitoring. At five years, 87% and 90% of patients were alive in the clinical and laboratory arms respectively.<sup>1</sup>

These results were even more impressive as patients in this trial had a median baseline CD4 count of 86 cells/mm<sup>3</sup>.

The DART investigators concluded that ART could be delivered safely with good quality clinical care, allowing treatment delivery to be decentralized. Despite DART results being occasionally misinterpreted to suggest that this argues for no monitoring at all, the investigators also recommended that there is a role for CD4 testing from the second year on ART to guide the switch to a second-line regimen—and clearly to start treatment. They added that this should encourage accelerated development of simpler, cheaper, point-of-care CD4 tests.

Furthermore, in an accompanying editorial, Andrew Phillips and Joep van Oosterhout argue that, although the authors suggest that the advantage of measuring viral load on survival may have been modest in this cohort, the reduction of transmission of drug-resistant virus, which will undermine the effectiveness of antiretroviral therapy over the longer term may be another reason to measure it. “Therefore development of cheap robust assays for viral load that can easily be used in rural and urban settings is of the highest priority for researchers.”

There are many arguments for the development of affordable point-of-care assays. CD4 measurements are essential for knowing when to initiate ART. CD4 counts also guide the use of opportunistic infection prophylaxis.

Viral load testing is essential to diagnose HIV-infected infants (see next chapter) and to monitor virological suppression in pregnant women to reduce the risk of mother to child transmission. These tests can be used to monitor adherence early on, and better inform decisions about treatment modifications or switches.<sup>3,4</sup>

The next edition of the *Pipeline Report* will include a more detailed survey of diagnostics appropriate to low resource settings. Here we look at the qualities required from a point-of-care test to be useful and two initiatives that appear to be close to emerging from the pipeline.

## Requirements for Point-of-Care Tests

Point-of-care refers to a test that can be conducted in the same facility where a patient receives his/her treatment and other care. In rural areas many patients have to travel vast distances to reach testing facilities and then return to get their results. There is considerable loss to follow up from people who do not return for their test results.

These technologies need to be quick and easy to use and interpret, that work with a finger-prick blood sample or other non-venous sample and have a simple read out. They need to be appropriate to settings without sophisticated laboratories, where electricity and running water cannot be guaranteed. They need to perform in hot, humid or dusty environments and have a long shelf life. They need to be used and maintained by health workers without advanced technical skills.

## The CD4 Initiative Point-of-Care CD4 Test

The CD4 Initiative, at Imperial College, was established in 2005. The Initiative came about through a substantial activist push from Gregg Gonsalves, and a generous grant from the Gates Foundation.<sup>5</sup>

Led by principal investigator Dr Hans-Georg Batz and project manager Dr Stephen Reid, their objective is to develop new, low-cost, rapid point-of-care tests to measure CD4 counts, which are suitable for use in rural areas of low-income countries.

The initiative set out to produce tangible results in four years, which is a very ambitious timescale to develop this type of product.

The project began by establishing a set of predetermined specifications:

- Simple and robust
- Semi-quantitative, minimum cut off of 250 cells/mm<sup>3</sup>
- Stable at 40°C for 12 months
- Quality assurance material to check correct functioning of test
- Use of finger-prick blood/other non-venous blood sample
- Simple to perform, few steps and <2 hours training required



- <30 minutes from patient to result
- Simple read out
- All-in-one kit
- 25 tests performed/person/day
- Target price around \$2 per test
- Customer capital outlay (if any) <\$1,000
- Safe solution for infectious waste materials

Phase I of the project was proof of concept, in which new tests were developed, that can reliably measure CD4 counts in blood. Phase II involved developing prototypes, scale-up and validation. That is, to establish whether the new tests met most, if not all, of the specifications and provided reliable and reproducible results in medium-scale production.

In Phase III the tests will be trialled in resource-poor settings to make sure that specifications are achievable in field conditions. The costs will also be evaluated. The group has completed two rounds of independent verification using samples from clinics in London. Field-testing will start later this year in Malawi and Uganda.

Three prototypes (Beckman Coulter, Burnet Institute and Zyomyx Inc.) were assessed against the gold standard method of measuring CD4 counts called flow cytometry. Flow cytometry is a technique for counting CD4 cells by suspending them in a stream of liquid and passing them by an electronic detector. The machines used to perform these tests are big and expensive and require an uninterrupted supply of electricity and highly trained technicians.

This verification trial used around 150 blood samples from HIV-positive patients in London to see if the same results could be obtained with the prototype tests as with flow cytometry. The results showed that only the test from Zyomyx, Inc compared favorably with flow cytometry. The other two tests failed to correctly identify samples from patients with low CD4 counts with sufficient sensitivity, thus the initiative is proceeding with the Zyomyx Inc., point-of-care CD4 test.

Zyomyx is based in San Francisco. The device is a CD4-gauge “much like a thermometer” and this simple test will be able to provide a quantitative CD4 count from a finger-prick of blood—exceeding the original specification of semi quantitative with a minimum cut-off.

After the field-testing, phase IV of the project will be technology transfer (ideally in the developing world), large-scale production and distribution.

This test can be used for both adults and children with a different measuring range for younger children and infants.

## Point-of-Care Viral Load Test

The Diagnostics Development Unit based at the Department of Haematology, University of Cambridge, and led by Dr Helen Lee, are developing a simple amplification based assay (SAMBA). This is a nucleic test based on visual detection of viral nucleic acid by dipstick designed to detect a broad range of HIV-1 subtypes typical to sub-Saharan Africa.<sup>6</sup>

Like the CD4 Initiative, the group conducted a survey of potential users in resource-limited settings. Again, respondents need tests that are quick and simple and can be performed while patients are at the clinic. The SAMBA needs to be, “simple, robust, inexpensive, stable and self contained with predispensed unit-dose reagents and disposables included and with a design and procedure that comply with biosafety standards”.

Recent data published in the April 2010 supplement of the Journal of Infectious Diseases showed the sensitivity and subtype coverage of the SAMBA test, when tested with 69 samples provided by The Royal London Hospital, to be at least as good as those of commercially available diagnostic tests.

The investigators suggested that the SAMBA system is not restricted to HIV and could also be adapted to detect other infections.

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# The Pediatric Antiretroviral Pipeline

BY POLLY CLAYDEN

This new chapter of the Treatment Action Group's *Pipeline Report* looks at antiretroviral formulations suitable for use in children.

In resource-rich countries, most HIV-positive children are treated early with highly active antiretroviral therapy (HAART) employing three or more antiretroviral drugs (ARVs). As with adults, HAART has changed the course of HIV in children dramatically, and the majority can expect to survive into adulthood.<sup>1,2</sup> Furthermore, identifying women of unknown status in pregnancy, and appropriate care and treatment for HIV-positive mothers, has led to a sharp decline in perinatal infections.<sup>3</sup>

However, UNAIDS estimated in 2008 that there were 2.1 million children living with HIV; among them, 430,000 were newly infected (about 1,200 new infections per day), and 390,000 were in sub-Saharan Africa. The overwhelming majority were infected through mother-to-child transmission (MTCT).<sup>4</sup>

One study of nearly 3,500 children enrolled in perinatal trials in Africa estimated that, without treatment, 35% would die before their first birthday and 53% by the time they reached two years of age. By five years of age, it was deemed likely that 62–89% of these children would die.<sup>5</sup>

A more recent analysis, using pooled data from 12 African studies describing almost 11,000 children born to HIV-positive women, has suggested that by one year of age, an estimated 16% infected through breast-feeding and 44 % perinatally infected children would die.<sup>6</sup>

Unfortunately, the rollout of antiretroviral therapy (ART) to treat children with HIV has been gradual and has lagged behind that of adults. More recently, though, there has been significant progress and in 2008 almost 276,000 children received HAART worldwide, compared to 127,300 in 2006. Despite this increase, every year, new infections are nearly double the number of children who gained access to HAART in 2008.

This chapter looks at all new ARVs in the pipeline for children, with the main focus being products (often new formulations of existing medicines) suitable for use in parts of the world with the greatest and most urgent need. The emphasis is also on infants and younger children, as older children are treated with adult antiretroviral formulations.

## Why Not Avoid Pediatric HIV in the First Place?

That MTCT is almost entirely preventable deserves emphasis. In more richly resourced countries, where use of ARVs in pregnancy and avoidance of breastfeeding are routine, MTCT has been reduced to 1–2%, and new pediatric HIV infections are rare.

In sharp contrast, several areas of unmet prevention, care, and treatment collide to swell the pediatric epidemic in poorly resourced settings. First, in prevention of HIV in women: in 2008, one million women were estimated to have been infected. Second, in prevention of unwanted pregnancies: an alarming proportion of pregnancies have been reported to be unwanted—51%, 74%, 84%, and 93% in studies of HIV-positive women in Cote d’Ivoire, Rwanda, South Africa, and Uganda, respectively.<sup>7,8,9,10</sup> Third, the implementation of prevention of mother to child transmission (PMTCT) interventions has been limited and relied on regimens with poor efficacy. And fourth, few eligible pregnant women receive HAART to treat their own HIV.

This global failure of prevention means that children continue to be infected, and that those children will need treatment with ARVs for life.

## When to Start Children on Antiretroviral Therapy

Following the announcement of early results from the Children with HIV Early Antiretroviral Therapy (CHER) trial—which found that starting antiretroviral therapy (ART) before 12 weeks of age reduced early mortality by a highly significant 75% when compared to starting at CD4 cell percentages less than 25%, or starting it based on clinical symptoms—international guidelines recommended universal ART for children age one year or less.<sup>11,12,13,14</sup>

Furthermore, new World Health Organization (WHO) guidelines recommend universal ART for children up to two years of age in recognition of the high mortality risk and less-frequent monitoring in this age group in resource-limited settings.

Data to guide when to start ART between one and five years of age are scant, and this is reflected in differences in recommendations among guidelines. After five years of age, guidance is similar to that for adults (see tables 1 and 2).

**TABLE 1. WHO 2010 Guidelines When to Start Children on ART**

Age	WHO 2010 Guidelines
<b>Less than 24 months</b>	
	All
<b>24–59 months</b>	
Clinical	Stage 3 or 4
Immunological*	< 25% or < 750
<b>5 years and older</b>	
Clinical	Stage 3 or 4
Immunological	< 350

Note:\*CD4 percentage/absolute CD4 count, cells<sup>3</sup>

**TABLE 2. Guideline Comparison, United States and Europe**  
When to Start Children on ART

Age	US DHHS 2008 Guidelines	PENTA 2009 Guidelines
<b>Less than 12 months</b>		
	All	All
<b>12–35 months</b>		
Clinical	CDC stage B	CDC stage B or C/ WHO stage 3 or 4
Immunological*	< 25%	< 25% or < 1000
<b>36–59 months</b>		
Clinical	CDC stage B	CDC stage B or C/ WHO stage 3 or 4
Immunological	< 25%	< 20% or < 500
<b>5 years and older</b>		
Clinical	CDC stage B or C	CDC stage B or C/ WHO stage 3 or 4
Immunological	<25% or <500	<350

Note:\*CD4 percentage/absolute CD4 count, cells mm<sup>3</sup>; DHHS = U.S. Department of Health and Human Services; PENTA= Paediatric European Network for Treatment of AIDS.

Additionally, the WHO recommends that uninfected, breastfed infants born to HIV-positive women who are not receiving HAART receive antiretroviral prophylaxis to reduce the risk of MTCT during breastfeeding.<sup>15</sup>

## Identifying Children with HIV and Starting Them on Treatment

Although the benefits of early treatment are clear, in reality, when children do start treatment it tends to be late. A literature review of 30 pediatric studies or treatment programs has revealed that children receiving HAART ranged from infants aged two months to adolescents aged 15 years. Five of 26 studies that reported age at HAART initiation, 19 (73%) showed a mean or median age at start of treatment of greater than five years. Only two studies reported a median age at start of treatment of less than two years.<sup>16</sup>

In order to initiate treatment immediately, infants need to be diagnosed at the earliest opportunity. Infants with known HIV exposure—that is, born to mothers in PMTCT programs—should be tested at four to six weeks of age using HIV virologic assays. Any infant at a health facility presenting signs or symptoms that may be an indication for HIV should also be tested. All infants should have their HIV status established upon their first contact with the health system, preferably before six weeks of age. There needs to be an evaluation to determine where and with what symptoms, when infants present at a health facility, it is best to test them in order to identify most infections—for example, those presenting with malnutrition are very frequently HIV-infected.

If virological testing is not available, the WHO recommends presumptive diagnosis in accordance with nationally defined algorithms, and serological tests are used. (It is important to note that infants and children younger than 18 months of age will often test positive on an HIV antibody test even if they are uninfected. This is because of the passive transplacental transfer of maternal HIV antibodies to the infant. Therefore, accurate diagnoses require more expensive and complex virological tests.)

If children are identified as HIV-infected in PMTCT programs this means they will have been infected despite maternal prophylaxis, so they are likely to have resistance to nonnucleoside reverse transcriptase inhibitors (NNRTIs) and a greater proportion are likely to have been infected in utero and have faster disease progression.

Preliminary results from the IMPAACT 1060 study (in which children previously exposed and unexposed to nevirapine were randomized to start treatment with either nevirapine or lopinavir/ritonavir-based regimens) were sufficiently concerning for the study's data safety monitoring board to stop the nevirapine-exposed, nevirapine-initiating arm early.<sup>17</sup>

Infants with NNRTI exposure through PMTCT are usually recommended to begin treatment with a protease inhibitor-based regimen. Unexposed children or those with unknown or less recent exposure will start with an NNRTI-based regimen.

Data are needed to guide ongoing strategies for children starting treatment. Whether children initiated early on treatment can discontinue it later is unclear (and this is also an important question for adolescents who are at risk for treatment non-adherence). There are also questions about the reuse of NNRTIs in nevirapine-exposed children and whether an initial, more potent, regimen could be a useful strategy.

The NEVEREST (nevirapine resistance) studies are looking at switching children who are initiated on lopinavir/ritonavir-based regimens to nevirapine versus remaining on lopinavir/ritonavir. Early findings suggest reuse of nevirapine may be possible in some circumstances.<sup>18</sup>

The ARROW (Antiretroviral Research for Watoto) study is looking at an induction/maintenance strategy: whether there is an advantage to starting with a more potent combination of four drugs for 36 weeks and then maintaining treatment with three drugs, versus continual treatment with three drugs.<sup>19</sup>

The CHER study is continuing to follow children to look at whether after starting early they can stop treatment after one or two years.

The BANA (Botswana/Baylor Antiretroviral Assessment) II and PENTA 11 studies will determine whether children on stable therapy are disadvantaged by taking CD4-guided planned treatment interruptions.<sup>20,21</sup>

## What to Start With?

The WHO's and some national guidelines recommend starting with lopinavir/ritonavir for nevirapine-exposed infants and young children less than two years of age. Unexposed children under three years should receive nevirapine, and those over three years efavirenz. All others (including nevirapine-exposed children) should receive an NNRTI (efavirenz is preferred for children receiving TB treatment unless they are less than three years of age).

Recommended nucleosides are zidovudine plus lamivudine, abacavir plus lamivudine, or stavudine plus lamivudine.

Appropriate formulations are available to facilitate all these combinations, including many generics and fixed-dose combinations (FDCs) pre-qualified by the WHO and/or with tentative approval (for use outside the U.S., particularly for PEPFAR programs) from the U.S. Food and Drug Administration<sup>22,23</sup> see table 3).

**TABLE 3. Antiretroviral Formulations Suitable for Pediatric Use with Tentative Approval from the FDA and/or Pre-qualified by the WHO**

Drug	Formulation and strength	Supplier/applicant	FDA TA	WHO PQ
Abacavir	Oral solution 20mg/ml	GlaxoSmithKline		●
Abacavir sulfate	Oral solution 20mg/ml	Cipla		●
Abacavir sulfate	Tablet 60mg	Aurobindo Pharma	●	
Abacavir sulfate	Oral solution 20mg/ml	Aurobindo Pharma	●	
Abacavir sulfate + lamivudine	Tablet 60mg/30mg	Aurobindo Pharma	●	
Abacavir sulfate	Tablet 60mg	Matrix Laboratories		●
Abacavir sulfate + Lamivudine	Tablet 60mg/30mg	Matrix Laboratories		●
Abacavir sulfate + lamivudine + zidovudine	Tablet 60mg/30mg/60mg	Matrix Laboratories		●
Didanosine	Powder for oral solution 2g	Bristol-Meyers Squibb		●
Didanosine	Tablets 25mg, 50mg, and 100mg	Bristol-Meyers Squibb		●
Didanosine	Delayed release capsules 125mg, 200mg, 250mg, and 400mg	Matrix Laboratories	●	
Didanosine	Delayed release capsules 125mg, 200mg, 250mg, and 400mg	Aurobindo Pharma	●	
Didanosine	Oral solution 10mg/ml	Aurobindo Pharma	●	
Efavirenz	Oral solution 30mg/ml	Merck Sharp & Dohme		●
Efavirenz	Capsules 50mg, and 200mg	Merck Sharp & Dohme		●
Efavirenz	Tablets 50mg, and 200mg	Merck Sharp & Dohme		●
Efavirenz	Capsule 200mg	Ranbaxy		●
Efavirenz	Tablet 200mg	Strides Arcolab	●	●
Efavirenz	Tablets 50mg, 100mg, and 200mg	Matrix Laboratories	●	
Efavirenz	Capsule 200mg	Cipla	●	
Efavirenz	Tablet 100mg	Aurobindo Pharma	●	
Efavirenz	Capsules 50mg, 100mg, and 200mg	Aurobindo Pharma	●	
Lamivudine	Oral solution 10mg/ml	GlaxoSmithKline		●
Lamivudine	Oral solution 10mg/ml	Cipla	●	●
Lamivudine	Oral solution 10mg/ml	Aurobindo	●	
Lamivudine + stavudine	Tablets for oral suspension 60mg/12mg and 30mg/6mg	Cipla	●	
Lamivudine + stavudine + nevirapine	Dispersible tablets 30mg/6mg/50mg and 60mg/12mg/100mg	Cipla	●	●



Lamivudine + zidovudine	Tablet 30mg/60mg	Aurobindo Pharma	●	
Lamivudine + zidovudine	Tablet 30mg/60mg	Matrix Laboratories		●
Lamivudine + nevirapine + zidovudine	Dispersible tablets 30mg/50mg/60mg	Matrix Laboratories		●
Lopinavir/ritonavir	Oral solution 80mg/ml and 20mg/ml	Abbott Laboratories		●
Lopinavir/ritonavir	Capsules 133.3mg/33.3mg	Abbott Laboratories		●
Lopinavir/ritonavir	Tablet 100mg/25mg	Abbott Laboratories		●
Lopinavir/ritonavir	Tablet 100mg/25mg	Matrix Laboratories Ltd		●
Lopinavir/ritonavir	Tablets 100mg/25mg and 200mg/50mg	Aurobindo Pharma Limited	●	
Nelfinavir	Powder for oral solution 50mg/1g	F. Hoffman-La Roche		●
Nevirapine	Oral suspension 10mg/ml	Boehringer Ingelheim		●
Nevirapine	Oral suspension 10mg/ml	Aurobindo Pharma Limited		●
Nevirapine	Tablet for oral suspension 50mg	Aurobindo Pharma Limited	●	
Nevirapine	Oral suspension 50mg/5ml	Aurobindo	●	
Nevirapine	Oral suspension 50mg/5ml	Cipla Limited		●
Ritonavir	Oral solution 80mg/ml	Abbott Laboratories		●
Stavudine	Powder for oral solution 1mg/ml	Bristol-Myers Squibb		●
Stavudine	Capsules 15mg and 20mg	Bristol-Myers Squibb		●
Stavudine	Capsules 20mg	Aspen Pharmacare		●
Stavudine	Powder for oral solution 1mg/ml	Aurobindo Pharma		●
Stavudine	Oral solution 1mg/ml	Aurobindo	●	
Stavudine	Capsules 15mg, 20mg, 30mg, and 40mg	Hetero Drugs Limited	●	
Stavudine	Oral solution 1mg/ml	Cipla Limited	●	
Stavudine	Capsules 15mg and 20mg	Aurobindo Pharma Limited	●	
Stavudine	Capsules 15mg, 20mg, 30mg, and 40mg	Aurobindo	●	
Zidovudine	Capsule 100mg	GlaxoSmithKline		●
Zidovudine	Oral solution 10mg/ml	GlaxoSmithKline		●
Zidovudine	Infusion solution 10mg/ml	GlaxoSmithKline		●
Zidovudine	Tablet 60mg	Aurobindo Pharma Ltd	●	
Zidovudine	Oral solution 50mg/5ml	Cipla Limited	●	●
Zidovudine	Capsule 100mg	Cipla Limited	●	●
Zidovudine	Capsule 100mg	Aurobindo Pharma Limited	●	
Zidovudine	Oral solution 50mg/5ml	Aurobindo	●	
Zidovudine	Oral solution 10mg/ml	CombinoPharm S.L		●
Zidovudine	Tablet 100mg	Matrix Laboratories Limited		●

## Dosing

Pediatric dosing can be complicated. There are often insufficient pharmacokinetic (PK) data to determine target concentrations, and there is wide interpatient variability, particularly in very young children. In industrialized countries providers usually use a body surface area calculation to determine dosing, which is not feasible in many health care settings with less-experienced health workers.

In order to simplify dosing, the WHO has developed weight-band tables offering a single, harmonized dosing schedule.<sup>24</sup>

Notably, manufacturers (particularly generic manufacturers) have produced dosing forms to use with these dosing schedules—including FDCs, such as 200mg tablets of efavirenz scored in two directions (approved) or 600mg scored once on one side and twice on the other (awaiting approval)—to enable accurate division of tablets and, in turn, dosing. Triomune Baby, an FDC from Cipla (stavudine 6 mg/lamivudine 30 mg/nevirapine 50 mg) is suitable for dosing infants down to 3kg and is dispersible in breast milk.

## Formulations

Some, but not all, approved adult ARVs have pediatric formulations for children who are too young to swallow tablets; traditionally these have been liquids or syrups. These formulations are expensive (about six times more costly than solid forms), often require a cold chain, have a short shelf life, and are not easy to store or transport. Cost and logistical barriers have prohibited their widespread use. Besides transport to and storage at the dispensary, the following example illustrates the lack of practicality for the caregiver:

A 10 kg child being treated with standard doses of stavudine, lamivudine, and nevirapine, for whom a 3-month supply of drugs is dispensed at a clinic visit, would require 18 bottles of liquid weighing almost half as much as the child (4.3 kg). For a rural family who may have walked a long distance to reach the clinical centre, this is a significant issue.<sup>25</sup>

For manufacturers, development of liquid formulations is not always as simple as it sounds. Development of a liquid formulation of efavirenz has been besieged by setbacks for years. Efavirenz has potential for oral mucosa irritation; it also has poor aqueous solubility. Early development focused on palatable alternatives to the aqueous suspensions using oily vehicles that were known to mask irritation. The original oral

solution, a suspended sugar solution, was found to have a low level of bacterial contamination; the culprit was confectioner's sugar. A heating step was then incorporated into the process to destroy the bacteria, but this then led to clumping. The current formulation is a sugar-free strawberry mint flavor 30mg/ml solution. It does not provide sufficient drug exposure for children less than three years of age.

It was not possible to formulate tenofovir as a liquid; this formulation would have required huge volume and tasted very nasty. The pediatric formulation will now be a powder of coated granules, which will mask the taste (although anecdotally it may still be fairly unpalatable) but the powder does not dissolve. Nor was it possible for etravirine, which will be a dispersible mini-pill, or atazanavir, which will be a powder.

The extremely unpleasant taste is not uncommon, and taste has been documented as a factor in treatment failure. Conversely, masking taste and developing palatable flavors for children can also be a barrier to creating a successful oral solution.

More recently, manufacturers (notably generic manufacturers) have developed more useful ARV formulations such as crushable mini-pills, scored tablets, dispersible formulations, "sprinkles" and FDCs that can be used by very young children. This has been a very important aspect of pediatric drug development in recent years, and along with the simplified dosing tables has overcome two significant barriers to widespread pediatric treatment (see table 4).

**TABLE 4. Desirable Qualities**

Liquid	Solid (preferred)
Stable at room temperature	Stable at room temperature
Small dose/volume	Crushable, granular or dispersible
Long shelf life	Long shelf life
Suitable dosage forms for convenient weight-band dosing	Scored tablets
Masking of bad taste	Masking of bad taste
Dispensing tools (e.g., syringes) included	

An ARROW substudy looked at the acceptability of tablets and syrups (NNRTI plus two of zidovudine, abacavir, and lamivudine). The children in this substudy received syrups on enrollment and switched to crushable tablets. Eight weeks after switching, 93% of caregivers and 56% of their children (median age at switch, 2.9 years) preferred tablets.<sup>26</sup>

Protease inhibitors, however, have poor bioequivalence with crushed tablets. In a recent study, investigators observed significantly lower exposure with crushed lopinavir/ritonavir compared to the reference product.<sup>27</sup> For young nevirapine-exposed children, lopinavir/ritonavir “sprinkles” are currently being studied in the Children with HIV in Africa—Pharmacokinetics and Acceptability of Simple Antiretroviral Regimens trials.<sup>28</sup>

These trials have looked at or are looking at simplifying antiretroviral regimens—for example, toxicity and adherence/acceptability profiles of new pediatric FDCs that contain abacavir or zidovudine rather than stavudine, and simplification strategies such as once-daily regimens and whether it is necessary to dose-escalate nevirapine. The trials use WHO weight-band tables.

### **Approval of Pediatric Formulations**

There are now considerable incentives and/or penalties from regulatory agencies to ensure that any new drug that may be of benefit to children must be studied in children.

This is mandatory on the part of both the FDA, which also extends six-month patent protection to companies that perform requested pediatric studies (voluntary), and the European Medicines Agency (EMA), which enforces penalties for companies that do not provide a pediatric investigational plan as part of their application (or request a waiver).<sup>29</sup>

Companies must include PK data for all age groups of children, efficacy, tolerability, and differences in side effects. They must have stability and palatability data for formulations and demonstrate that they are able to achieve PK targets associated with efficacy in adults.

Most pediatric development programs take a staggered approach, starting with the older cohorts of children and working down in age. The studies are conducted in children as soon as there are sufficient data from studies in adults.

Applications for generic formulations must demonstrate bioequivalence. A single product needs to be compared to the reference product (innovator). Generic FDCs need to be compared to the individual reference drugs taken together. Preferred bioequivalence studies are randomized, single-dose, two-way crossover studies. Bioequivalence studies need not be done in children.

Dissolution testing is required when evaluating solid or suspension formulations to assure reproducible drug release.

## The Innovator Pipeline

### Nucleotide Reverse Transcriptase Inhibitors

Gilead's tenofovir disoproxil fumarate is one of the most widely used antiretroviral drugs in adults. Development of a pediatric formulation has been slow, and there have been concerns about loss of bone mineral density in children. There has been considerable off-label use with adult tablets in drug-experienced children; the FDA recently gave a new indication for children and adolescents from 12 to 18 years of age. Gilead plans to file the oral powder formulation with regulatory agencies in the second half of 2010.

### Protease Inhibitors

Atazanavir is approved for children over six years of age in capsule formulation. Trials of atazanavir powder are ongoing for younger age groups, with and without ritonavir boosting, within the Bristol-Myers Squibb-supported PACTG 1020 program.

The darunavir oral suspension, boosted with a ritonavir solution, is currently in phase II studies in treatment-experienced children ages three to six, twice daily; there is a waiver for children under three years of age. Tibotec's darunavir is approved for children over six years, and there is a 75mg tablet.

### Nonnucleoside Reverse Transcriptase Inhibitors

Tibotec's etravirine is in the phase II safety and efficacy stage of its pediatric development program using 25mg mini-pills.

Rilpivirine (TMC 278), also manufactured by Tibotec, is currently in pediatric phase I trials that compare the oral availability of three concept formulations: a solution, a suspension, and granules.

### Integrase Inhibitors

Merck's raltegravir has two pediatric formulations, a chewable tablet for children under 12 years old, and granules for children less than two years old. They are being studied in the IMPAACT P1066 study.

Plans for elvitegravir from Gilead include both liquid and solid age-appropriate dosage forms for children. Gilead will also attempt, if the doses of all four drugs are similarly scalable with age/body weight, to make a coformulated quad pill (an FDC with elvitegravir, cobicistat, tenofovir, and emtricitabine) for children able to swallow tablets.

## Pharmacokinetic Enhancers

Gilead's cobicistat (GS-9350) is a heat stable boosting agent that will also be produced as a stand-alone to boost other antiretrovirals as an alternative to ritonavir. Gilead plans to make it available in both age-appropriate liquid and solid forms for children. It is hoped that cobicistat will offer an alternative to ritonavir, which is only available as 100mg or as an unpleasant-tasting liquid.

## CCR5 Receptor Antagonists

Pfizer's maraviroc is currently being evaluated in children 2–18 years of age infected with CCR5-tropic HIV-1. As with adults, this drug is expected to have less clinical utility than other ARVs in children, as it requires an expensive CCR5 tropism assay.

**TABLE 5. The Innovator Pediatric Pipeline**

Drug	Formulation	Comments
Tenofovir	Oral powder formulation	Filing with FDA/EMA second half of 2010
Atazanavir	Oral powder formulation	PACTG 1020: Phase II study of atazanavir and atazanavir/ritonavir powder/capsules in treatment-naïve and treatment-experienced children 3 months–21 years.
Darunavir	Oral suspension 100mg = 1ml	ARIEL: Phase II study in treatment-experienced children 3–6 years. Needs to be boosted with ritonavir.
Etravirine	Solid formulation: 25mg tablet	Phase II safety and efficacy study in children 6–17 years.
Rilpivirine	Concept 1: oral solution free base (10 mg/ml) Concept 2: oral suspension HCl salt (5 mg base eq/ml) Concept 3: oral granules HCl salt (2.5 mg base eq/ml)	Phase I open-label study comparing oral bioavailability of the three concept formulations to the 25mg phase III tablet formulation.
Raltegravir	Chewable tablet Oral granules for suspension	IMPAACT 1066: granules < 2 years; chewable tablets <12 years.
Elvitegravir/ Cobicistat (booster)	Age-appropriate solid and liquid forms in development, separately and coformulated	Pediatric investigational plan to FDA/EMA, second half of 2010. Also development plan for pediatric quad pill.
Maraviroc	Liquid solution (20mg/ml)	A4001031: Children 2–12 years of age infected with CCR5 tropic HIV-1.

## Generic Pipeline/Wish List

Many of the new pediatric formulations will not be available for some time; moreover, some of them may be too expensive or complicated to use in resource-limited settings. WHO and national guidance in resource-limited settings (for both adults and children) is weak beyond second-line therapy. Table 6 lists formulations, either known to be in development by generic companies or will be needed, that will work with dosing according to WHO weight band tables.

**TABLE 6. Pediatric Formulations Needed**

Drug	Formulation (mg)	Comments
<b>Drugs needed for PMTCT</b>		
Nevirapine	Scored tablet 20 mg	Used for infant prophylaxis from 6 weeks onward.
<b>Drugs needed for Pediatric ART</b>		
Lopinavir/ritonavir	Sprinkle 40/10 mg	Heat-stable formulation that will be equivalent to 0.5ml of liquid and used in treating infants and children who are unable to take the pediatric tablet.
Abacavir/lamivudine	Scored adult tablet 300/150 mg	Used in children over 25kg.
Abacavir/lamivudine/nevirapine	Tablet 60/30/50 mg	Triple FDC to align with the dual FDC.
Ritonavir	Sprinkle or tablet 50 mg heat stable	Useful for coadministration with unboosted protease inhibitors and for superboosting when protease inhibitors need to be dosed with rifampicin.
Tenofovir/lamivudine	Tablet 75/75 mg; scored 300/300 mg tablet	
Darunavir	Unclear	Current labeling calls for different ratios of darunavir to ritonavir for different age brackets. It is unclear what the correct ratio should be to produce a coformulated FDC, but this is a priority formulation.
Raltegravir	Unclear	Raltegravir is not yet approved for pediatric use but this is high priority formulation.

Adapted from World Health Organization 2010 Pediatric guidelines Annex E.

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# The Immune-Based Therapies and Preventive Technologies Pipeline

BY RICHARD JEFFERYS

Unlike the other pipelines discussed in this report, there are no approved immune-based therapies or biomedical preventive technologies for HIV—aside from antiretroviral therapy itself—(see 2010 Pipeline Summary) to offer a guidepost for product developers. For those attempting to navigate this uncharted terrain, 2009 proved to be another year of vertiginous ups and downs. In the realm of biomedical prevention, a microbicide candidate (PRO2000 gel) that came within a whisker of showing statistically significant protection in a phase IIb study (Abdool Karim 2009) failed to show any efficacy in a larger, definitive phase III trial (Chisembele 2010). PRO2000 gel was essentially the last in a class of broad-spectrum microbicide candidates and the focus has now shifted to products with more potent and specific anti-HIV effects. Meanwhile, a huge HIV vaccine trial that took place in Thailand involving two ancient candidates (ALVAC and AIDSVAX) surprised everyone by showing a meager (31.2%) but just about statistically significant degree of protection (Rerks-Ngarm 2009). Despite the size of the trial, however, there were relatively few HIV infection endpoints and the confidence intervals—a statistical measure of the uncertainty associated with a result—were vast, raising the specter that the findings may be as illusory as those of the phase IIb PRO2000 gel trial. Nevertheless, after the paucity of good news in HIV vaccine research, and despite the possibility of a statistical fluke, the Thai trial results have been widely hailed as historic. Plans are now afoot to try and reproduce and improve upon them with similar regimens.

Results from the first efficacy trials of preexposure prophylaxis (PrEP) are due to become available later this year, after this report is printed. The antiretrovirals being studied for PrEP are tenofovir and the combination of tenofovir and emtricitabine in a single pill (Truvada). Several other agents are under consideration but, unsurprisingly, PrEP research is currently in a holding pattern awaiting the crucial first efficacy data. Several small studies are evaluating the safety and acceptability of intermittent rather than continuous PrEP, but as yet no efficacy studies are planned.

As in previous years, there remains an imbalance between the need for novel immune-based and gene therapies for HIV and the limited number of candidates trickling sluggishly through the pipeline. Similar to HIV vaccines, the immune-based therapy (IBT) pipeline is prone to tortuous plumbing; even apparently promising candidates often seem to circle back to earlier phase trials rather than progress onward. An example is IL-7, which is widely viewed as the lead CD4 T-cell-boosting candidate: after showing promising results in two phase I trials reported in 2007 (Levy 2007; Sereti 2007), this cytokine therapy was modified to ease its dosing schedule and only recently re-entered phase I testing in its new form. Over the same time period, data have accumulated demonstrating that poor CD4 T-cell reconstitution is a significant risk factor for illness and death in the antiretroviral therapy (ART) era (Marin 2009; Tan 2008; Tuboi 2010). Concern about the overlap between the immunological effects of HIV and aging—particularly depletion of a subset of T cells called naive cells—has renewed interest in boosting the function of the thymus, but there is a striking lack of therapies with any demonstrated potential. IL-7 may have some effect, but appears to preferentially stimulate naive T-cell division rather than enhancing thymic production (Fry 2005). The one approach proven to increase thymus function in people, human growth hormone, is associated with a counterproductive panoply of side effects (Napolitano 2008; Smith 2010).

Beyond general immune-boosting, achievement of a “functional cure”—defined as an absence of detectable HIV replication in the absence of any ongoing treatment—is an ambitious goal of some IBTs and gene therapies. Once almost unimaginable, this possibility has been pushed back into the spotlight by the case of an individual who appears to have been cured of HIV infection after receiving a stem cell transplant while undergoing treatment for a life-threatening cancer (Hütter 2009). The German doctor who performed the transplant, Gero Hütter, smartly sought out a donor with the mutation that abrogates expression of CCR5 (the major HIV coreceptor) on cells. The result was that the individual’s immune system was repopulated with cells highly resistant to HIV infection. More than three years after the procedure, despite a slow and complicated recovery from the cancer and its treatment, the individual remains off ART and lacks any detectable HIV in blood or tissues. The case is being viewed as a “proof of concept” that a cure for HIV is possible, providing a welcome impetus for research efforts in this area.

## In Pursuit of a Cure

A number of other developments have helped pushed the pursuit of a cure back toward the top of the agenda:

- The recognition that ART completely suppresses HIV replication in the majority of individuals has revived interest in strategies aiming to deplete remaining latent viral reservoirs, and several large pharmaceutical companies (including Merck and GILEAD) have acknowledged they now have programs working on latency-reversing strategies.
- Following on from a workshop held in 2008 (co-sponsored with TAG and Project Inform), the non-profit organization amfAR has instituted a targeted program supporting collaborative cure-related research, named the Research Consortium for HIV Eradication (ARCHE).
- An opinion piece published in the journal *Science* last year proposed the establishment of a “collaboratory” to accelerate and streamline cure research (Richman 2009), and the NIH has very recently issued a request for funding applications for a project modeled on this proposal. The project has been named in honor one of the authors of the opinion piece, the AIDS activist and founder of Project Inform, Martin Delaney, who died last year.

In terms of imminent research, David Margolis has received FDA approval for a clinical trial of SAHA, a treatment for cutaneous T cell lymphoma that laboratory studies suggest may be able to prod HIV out of latency (Contreras 2009; Edelstein 2009); however, funding for the study has not yet been secured. The French non-profit ORVACS (Objectif Recherche Vaccin SIDA) is soon launching two trials of reservoir-depleting strategies. One (named Eramune 01) will investigate ART intensification plus IL-7, while the other (Eramune 02) will combine ART intensification and therapeutic immunization with the Vaccine Research Center’s DNA/Ad5 vaccine candidate.

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**Table 1. HIV Vaccines Pipeline 2010**

Product	Type	Manufacturer	Status
ALVAC vCP1521	Canarypox vector including HIV-1 CRF01_AE env, clade B gag, the protease-encoding portion of the pol gene and a synthetic polypeptide encompassing several known CD8 T-cell epitopes from the Nef and Pol proteins.	Sanofi Pasteur	Phase I Phase I (in infants)
AIDSVAX B/E (booster only)	Recombinant gp120 envelope protein.	Global Solutions for Infectious Diseases	Phase I
VRC-HIVDNA016-00-VP + VRC-HIVADV014-00-VP	Prime: Six separate DNA plasmids including gag, pol, and nef genes from HIV-1 clade B, and env genes from clades A, B, and C. Boost: Adenovirus serotype 5 vectors including gag/pol genes from HIV-1 clade B and env genes from clades A, B, and C.	U.S. National Institutes of Health (NIH) Vaccine Research Center/GenVec/Vical	Phase II (HVTN 505)
pGAZ/JS7 DNA MVA/HIV62	DNA prime and MVA booster vaccines including gag, pol and env genes from HIV-1 clade B.	National Institute of Allergy and Infectious Diseases (NIAID)/Geovax	Phase IIa
LIP0-5	Five lipopeptides containing CTL epitopes (from Gag, Pol and Nef proteins).	Agence Nationale de Recherche sur le Sida et le hepatitis (ANRS)	Phase II
HIVIS 03 DNA-MVA prime-boost HIV-1 vaccine candidate	Prime: HIVIS DNA including env (A, B, C), gag (A, B), reverse transcriptase (B), rev (B). Boost: MVA-CMDR including env (E), gag (A), pol (E).	Karolinska Institute/Swedish Institute for Infectious Disease Control (SMI)/Vecura/U.S. Military HIV Research Program	Phase I/II
DNA-C + NYVAC-C	Prime: DNA vaccine including clade C env, gag, pol, nef. Boost: NYVAC-C attenuated vaccinia vector including clade C env, gag, pol, nef.	The Collaboration for AIDS Vaccine Discovery/GENEART/Sanofi Pasteur	Phase I/II
PolyEnv1 EnvDNA	Vaccinia viruses including 23 different env genes and DNA vaccine with multiple env genes.	St. Jude Children's Research Hospital	Phase I
VICHREPOL	Chimeric recombinant protein comprised of C-terminal p17, full p24, and immunoreactive fragment of gp41 with polyoxidonium adjuvant.	Moscow Institute of Immunology/Russian Federation Ministry of Education and Science	Phase I
ADVAX e/g ADVAX p/n-t	Two DNA constructs: ADVAX e/g includes HIV-1 subtype C env and gag genes; ADVAX p/n-t includes HIV-1 subtype C pol and nef-tat. Administered by Ichor TrigrifTM electroporation.	Aaron Diamond AIDS Research Center/International AIDS Vaccine Initiative (IAVI)/Ichor Medical Systems	Phase I
GSK HIV vaccine 732461	Gag, Pol, and Nef proteins in proprietary adjuvant.	GlaxoSmithKline	Phase I
Ad35-GRIN/ENV	Two adenovirus serotype 35 vectors, one including HIV-1 subtype A gag, reverse transcriptase, integrase and nef genes and the other including HIV-1 subtype A env (gp140).	IAVI/University of Rochester	Phase I

Ad26.ENVA.01	Prototype adenovirus serotype 26 vector including the HIV-1 subtype A env gene.	NIAID/Crucell	Phase I
Ad5HVR48.ENVA.01	Prototype hybrid adenovirus vector consisting of a backbone of serotype 5 with the Hexon protein from serotype 48. Includes HIV-1 subtype A env.	NIAID/Crucell	Phase I
rAd35 VRC-HIVADV027-00-VP	Adenovirus serotype 35 vector.	NIH Vaccine Research Center/HIV Vaccine Trials Network	Phase I
ADVAX + TBC-M4	Prime: DNA vaccine including env, gag, nef-tat and pol genes from HIV-1 subtype C. Boost: MVA vector encoding env, gag, tat-rev, and nef-reverse transcriptase genes from HIV-1 subtype C.	Indian Council of Medical Research/IAVI/Aaron Diamond AIDS Research Center	Phase I
DNA + Tiantian vaccinia vector	DNA and recombinant Tiantian vaccinia strain vectors encoding gag, pol and env genes from HIV-1 CN54	Chinese Center for Disease Control and Prevention/National Vaccine and Serum Institute/Peking Union Medical College	Phase I
MVA.HIVA	MVA vector encoding a synthetic copy of a major part of HIV's gag gene and 25 CD8 T cell epitopes.	University of Oxford/Medical Research Council/University of Nairobi/Kenya AIDS Vaccine Initiative/Impfstoffwerk Dessau-Tornau (IDT) GmbH	Phase I in infants born to HIV-infected (PedVacc002) and HIV-uninfected mothers (PedVacc001)
MYM-V101	Virosome-based vaccine designed to induce mucosal IgA antibody responses to HIV-1 Env	Mymetics Corporation	Phase I

The HIV vaccine field has been deluged by disappointment over the years, but September 2009 saw the first sliver of encouragement emerge from Thailand. A huge 16,402-person efficacy trial of two candidates widely viewed as en route to the scrap heap, ALVAC and AIDSVAX, reported evidence of 31.2% efficacy in protecting against HIV infection, a result that just scraped across the boundary of statistical significance. In raw numbers, this represented 51 infections among the 8,192 people randomized to receive the vaccines and 74 infections among the 8,198 placebo recipients (5 participants were excluded from these analyses after being found to have been HIV infected at baseline). However, it is important to note that the statistical robustness of a trial result derives not just from the total sample size but is crucially dependent on the number of endpoints that occur, which in this case was very few relative to the overall number of participants. The upshot is that the confidence intervals around the 31.2% reduction in risk of HIV infection are extremely wide, ranging from 1.1% to 52.1%. In statistical terms, the confidence interval represents the range of possible outcomes if the experiment were to be repeated. From a glass-half-full perspective, it may be considered encouraging that the efficacy of the vaccines might have been as high

as 52.1%. But given the poor track record of the candidates involved, the glass-half-empty view inevitably must consider that a large swathe of the numerical territory between 1.1% and 52.1% is indistinguishable from the efficacy of the vaccines being zero.

Beyond the borderline nature of the findings, there was some controversy at the time of the initial announcement by the trial's sponsors—the U.S. Military HIV Research Program, the Thai Ministry of Health and the U.S. National Institutes of Allergy and Infectious Diseases (NIAID). Rumors abounded that the statistically significant result was undermined by other unreported analyses. The publication of the data in the *New England Journal of Medicine* a few weeks later set these concerns to rest, as the issue turned out to be related to the strictest “intent-to-treat” (ITT) approach, under which the five individuals found to be HIV infected at baseline had to be included. Using this method the result no longer reached statistical significance, but given that exclusion of people who were infected prior to receipt of any vaccine is both logical and standard in these trials there was no reason for this to be controversial. The fact that so few infection endpoints could affect significance in this way does, however, highlight the statistical fragility of the main result (which is described as the “modified ITT” analysis).

But even if the Thai trial had produced a statistical trend toward efficacy rather than achieving significance, it would have been important for the vaccine field to follow up, and this is indeed what is occurring. Data from participants will be mined in the hope of revealing vaccine-induced immune responses associated with reduced risk of acquiring HIV, known as correlates of immunity. While discovering correlates would be a huge advance, the marginal efficacy and limited numbers of samples may stymie the effort. Currently, the main theories point to some type of antibody-mediated effect, either relating to binding antibodies or antibody-dependent cellular cytotoxicity (ADCC) wherein nonneutralizing antibody responses promote killing of virus-infected cells. The reasoning behind these theories is partly based on evidence of a short-term protective effect in the trial; after the first year, infection rates in the vaccine and placebo groups were very similar, and this observation tracks with binding antibody responses, which peaked in magnitude after the last vaccine booster at six months and declined precipitously thereafter (Michael 2010). Evidence of ADCC was observed in the majority of recipients of the vaccine regimen in earlier trials (Karnasuta 2005). Less likely candidates for immune correlates include HIV-specific CD4 T-cell responses, detected in around 50–90% of vaccinees (as measured by lymphoproliferation to p24 or gp120 antigens) and HIV-specific CD8 T-cell responses that, while detected in around 20% of recipients in prior studies (Nitayaphan 2004), were essentially undetectable in the analyses reported in the *New England Journal of Medicine* paper (Rerks-Ngarm 2009). The lack of CD8 T cell responses is consistent with the failure of vaccination to measurably affect post-infection viral load among the trial participants who became infected.

## The Thai Trial Follow-Up

The main stakeholders in HIV vaccine research have outlined a variety of plans for new studies informed by the Thai trial outcome. Future research needs to address and try and disentangle several interrelated issues:

### The Population

The vast majority of the Thai study population were heterosexuals at very low risk for HIV infection, and the entry criteria limited the age range of participants to between 18 and 30. This contrasts with prior trials, which have focused on higher-risk gay men and intravenous drug users as well as recruiting from a broader age range. The extent to which the study population may have affected the Thai trial outcome is unclear, and needs to be addressed. Additional population-specific factors potentially relevant to efforts to replicate or build upon the results elsewhere include circulating HIV strains, routes of HIV exposure, other prevalent sexually transmitted diseases, and co-infections (such as tuberculosis, helminths, and hepatitis B & C).

### The Vaccine Constructs

Contrary to a number of erroneous media stories published when the results were first announced, Sanofi Pasteur's ALVAC vector was not previously tested for efficacy. The vector is made of a bird virus that is harmless to humans called canarypox, and it is not known if there might be unique aspects of this virus that could contribute to immune protection against HIV. ALVAC was not previously regarded as very promising because it only provokes weak immune responses to the HIV antigens it contains. Now it will be necessary to study whether similar poxvirus vectors that appear better at inducing immune responses (such as MVA and NYVAC) have superior or inferior protective efficacy compared to ALVAC. According to a letter sent to collaborators on April 23, 2010, Sanofi Pasteur is in the process of producing additional doses of ALVAC for research purposes. New supplies of vCP1521 are expected to become available in the fall.

The vaccine used as a booster in the Thai trial, AIDSVAX, consists of two recombinant HIV Env proteins from clades B and CRF01\_AE and had previously failed to show any efficacy when tested alone in two large trials involving high-risk gay men and intravenous drug users (Flynn 2005; Pitisuttithum 2006). It is impossible to know what role, if any, AIDSVAX played in the trial outcome because there was no comparison with ALVAC alone. One principal investigator, Nelson Michael, has spoken of the need to "deconvolute" the contribution of the two constructs. A planned US-based phase III trial that would have compared ALVAC alone to ALVAC+AIDSVAX did not take place because a prespecified level of immunogenicity was not achieved in phase II (Russell



2007). The company that manufactured AIDSVAX, VaxGen, no longer exists and the rights are held by a non-profit organization called Global Solutions to Infectious Diseases (GSID). Currently planned studies are using existing lots of AIDSVAX and GSID is not being funded to produce additional supplies, at least as yet.

The HIV Vaccine Trials Network (HVTN) has developed a proposal for “sequential adaptive design phase IIb trials” that are intended for the higher-incidence setting of South Africa. The population would be high-risk heterosexual people, with each trial enrolling 1,500 per arm. Based on an annual HIV infection rate of 4%, the HVTN calculates that a non-working vaccine could be identified and discarded within 20 months, and by 24 months an efficacy signal would be detectable. Around 50 HIV infection endpoints would be enough to know if a vaccine wasn't working, while 80 would be sufficient to show evidence of efficacy (a 40% or greater reduction in risk of infection). A variety of DNA, poxvirus vector, and protein prime-boost combinations are under consideration for these trials, including DNA+NYVAC+gp120, NYVAC+NYVAC+gp120 and ALVAC+ALVAC+gp120. The exact types of inserts and envelope boosts are still under discussion.

The U.S. Military HIV Research Program (USMHRP) is looking to shed more light on the Thai trial results by conducting new, detailed phase I immunogenicity studies of the vaccine regimen, particularly focusing on mucosal immune responses. Another plan is to boost a small subset of the original trial participants (~100) with another dose of ALVAC or AIDSVAX, or a combination of the two. The USMHRP also has additional candidates waiting in the wings, including a DNA prime MVA boost approach (Earl 2009; Gudmundsdotter 2009).

The Collaboration for AIDS Vaccine Discovery (CAVD), supported by the Bill and Melinda Gates Foundation, is developing a clade C DNA prime/NYVAC boost regimen that has shown immunogenicity in early phase trials (Harari 2008; McCormack 2008). A replication-competent version of the NYVAC vector is also under consideration (vectors currently being studied cannot replicate in humans). The current plan is for an efficacy trial to be conducted in Africa starting in 2013 or 2014.

A DNA prime/adenovirus serotype 5 (Ad5) boost approach developed by the Vaccine Research Center (VRC) at NIAID is being evaluated in an ongoing phase IIb trial designated HVTN 505. The main goal is to assess the effect of the vaccines on viral load levels among recipients who become infected. The Thai trial results provoked some discussion as to whether the size of HVTN 505 should be increased in order to find out if the vaccines could reduce risk of acquiring HIV infection, but this has not occurred. The DNA/Ad5 combination has demonstrated the ability to induce HIV-specific CD4 and CD8 T-cell responses, along with binding (but mostly nonneutralizing) antibodies (Koup 2010). However, whatever the outcome of HVTN 505, Ad5 vectors will not be developed fur-

ther due to evidence suggesting they may increase risk of HIV infection among people with preexisting antibody responses against adenovirus serotype 5 (which many people have been exposed to in its natural form, as it is a common cause of bad colds) (Buchbinder 2008). To try and circumvent this problem, the VRC is developing vectors based on adenovirus serotypes that are far less common in nature, Ad26 and Ad28.

Overall, the major repercussion of the Thai trial results has been the re-prioritization of prevention of HIV infection as the major goal for vaccines. This is a significant shift from the previous focus on slowing post-infection disease progression using candidates that only induce T-cell responses against HIV.

## The Antibody Revival

HIV's mutating, sugar-clustered outer envelope presents a daunting obstacle to antibody-mediated neutralization. Up until last year, only a few rare antibodies capable of broadly neutralizing a diverse array of primary HIV isolates had been identified. These antibodies were cloned from HIV-infected people and, while they are unable to retard disease progression in the setting of chronic infection, it is hoped they could protect an uninfected person if similar antibodies could be induced by vaccination. Reverse engineering a vaccine from an antibody is not easy, however, and only limited progress has been reported to date (Walker 2010). The year 2009 saw notable advances in this area with the discovery of several new broadly neutralizing antibodies (Corti 2010; Walker 2009; Wu 2010). These newer antibodies are far more potent than prior candidates, meaning they exert their inhibitory effect at lower concentrations. While efforts are ongoing to uncover their precise targets on HIV (Kwong 2010; Pejchal 2010), many stakeholders in vaccine research are considering conducting a trial in which a combination of the antibodies would be passively infused in order to test their efficacy at preventing HIV infection.

The International AIDS Vaccine Initiative (IAVI) is planning a phase I trial using adeno-associated virus (AAV) in a manner more akin to gene therapy than traditional vaccination: the AAV vector will encode a gene that persistently manufactures neutralizing antibodies after injection, an approach that has shown promise in macaques challenged with simian immunodeficiency virus (SIV) (Johnson 2009).

## Vaccine Approaches in Human Trials

The majority of HIV vaccine candidates in trials represent variations on the prime-boost theme, in which one vaccine is used to initiate immune responses to HIV antigens and a second boosts the responses to higher levels. The goal is to create "memory" immune re-

sponses specifically targeting HIV, including CD4 T cells (also called helper cells), CD8 T cells (known as killer T cells due to their primary role of killing infected cells) and B cells that act as factories for the production of antibodies. Exactly which components of HIV represent the best targets for immune responses remains uncertain, so many different possibilities are being studied. Although it has been argued that the viral envelope makes a poor target for immune responses (Kiepiela 2007), the Thai trial results have been interpreted as a strong counterargument, and most vaccines in trials include an envelope antigen or antigens. A key issue identified after the failure of Merck's HIV vaccine candidate is the need to improve the breadth of immune responses. T cells and B cells specifically recognize fragments of viral proteins called epitopes, and HIV contains hundreds of potential epitope targets. However, recipients of the Merck vaccine showed CD8 T cell responses against only three epitopes on average (McElrath 2008). To try and address this problem, researchers have developed a new type of antigen called a mosaic which has improved the breadth of epitope targeting in macaque studies (Barouch 2010). Vaccines containing mosaic HIV antigens are expected to enter human studies soon.

## **ALVAC**

Prior to the results of the Thai trial, a plethora of different versions of the ALVAC canarypox vector had been studied in phase I and II trials involving well over a thousand volunteers. The construct used in Thailand, vCP1521, contains the gene encoding the gp120 protein from a virus code named 92TH023 isolated from a Thai individual in Bangkok in the early 1990s. The virus was originally designated as belonging to subtype E, but it has since been recognized that this subtype is largely a circulating recombinant form now known by the name CRF01\_AE. The vCP1521 vector also contains a portion of the gp41 protein from the first HIV ever isolated, LAI, which belongs to subtype B. The other two antigens encoded by the ALVAC vector are Gag and protease, also derived from LAI.

## **Adenovirus Vectors**

Controversy persists regarding evidence that Merck's Ad5 HIV vaccine candidate enhanced susceptibility to HIV infection among study participants with preexisting antibodies against Ad5. A series of analyses conducted by Susan Buchbinder and colleagues considering the impact of multiple variables on the infection rate was unable to rule out an independent contribution of Ad5 vaccination, but the enhancement effect was almost exclusively seen in circumcised men with antibodies against Ad5, whose main risk factor for acquiring HIV infection was insertive anal sex (Robertson 2008). One hypothesis put forward to explain the results was that the vaccine activated Ad5-specific CD4 T cells in a way that provided more targets for HIV infection. Two studies subsequently argued against this possibility by showing that Ad5-specific CD4 T-cell

levels—as measured by cytokine production—were not linked to baseline Ad5 antibody status (Hutnick 2009; O'Brien 2009). But a UK-based group has since reported that Ad5-specific CD4 T-cell responses measured by their ability to proliferate do correlate with Ad5 antibody levels, and these CD4 T cells become more susceptible to HIV after stimulation and display markers associated with homing to mucosal tissues (Benlahrech 2009). These researchers argue that there is a link between the extent of prior exposure to natural Ad5 infection and the likelihood of generating mucosal-homing Ad5-specific CD4 T cells in response to Ad5 vaccination. New data has revealed that natural adenovirus infection can be remarkably persistent, with 75% of a small sample of HIV-positive Peruvian men who have sex with men showing detectable virus DNA in rectal swabs (Curlin 2010). More studies are needed to address this concern, which may also apply to other adenovirus vectors because adenovirus-specific CD4 T-cell responses cross-react with multiple different serotypes. A number of alternate adenovirus serotype HIV vaccines are in trials, including Ad26, Ad35, and Ad5HVR48 (the latter uses a backbone of Ad5 but the major antibody target, the hexon protein, is from the rarer Ad48 serotype).

### **Modified Vaccinia Virus Ankara Strain**

The Modified Vaccinia Virus Ankara strain (MVA) is an attenuated, nonpathogenic derivative of the cowpox virus. The Karolinska Institute and the USMHRP are advancing a DNA/MVA prime-boost approach into phase II studies. Published studies indicate the approach induces HIV-specific CD4 and CD8 T-cell responses in the majority of volunteers (About 2010; Sandström 2008). A similar DNA/MVA approach developed by a company called GeoVax is in a phase IIa immunogenicity trial under the aegis of the HVTN.

### **Vaccinia-based Vectors**

NYVAC is a highly attenuated derivative of the Copenhagen strain of vaccinia virus being studied as an HIV vaccine vector by the CAVD. The vector is being manufactured by Sanofi Pasteur. Judith Hurwitz at St. Jude Children's Hospital in Memphis, Tennessee, is employing a vaccinia vector as part of an experimental HIV vaccine regimen that delivers a cocktail of 23 different viral envelope proteins (Sealy 2009).

### **DNA Vaccines**

DNA vaccines represent one of the simplest approaches to vaccination. They consist of DNA sequences encoding protein antigens and typically contain little in the way of extraneous components. Despite encouraging initial results in mice, DNA vaccines have proven poorly immunogenic in people. One promising approach for improving the immune response to DNA vaccines is called electroporation, which involves using

a special wand to deliver a brief electrical charge to the muscle into which the vaccine is being injected. The electricity opens transient pores in local cell membranes, allowing the DNA easier access to the cell's nucleus, where it produces vaccine-encoded antigens. Electroporation also attracts inflammatory cells—including antigen-presenting dendritic cells—to the immunization site. Preliminary results from a phase I trial conducted by the Aaron Diamond AIDS Research Center, the International AIDS Vaccine Initiative, and Ichor Medical Systems suggest that the approach may be able to improve the magnitude, breadth and rate of response to DNA immunization (Vasan 2009).

### **MYM-V101**

One of relatively few vaccines not following the DNA or vector model is a candidate developed by the Swiss company Mymetics. The approach involves components from the HIV envelope encased in a mimic of the viral membrane called a “virosome.” The intent is not the induction of traditional neutralizing antibodies, but rather antibodies that can inhibit the transport of HIV across mucosal surfaces. In a study presented at CROI last year, the vaccine completely protected monkeys from a hybrid SIV/HIV virus called SHIV162p3 (which, unlike prior simian-human immunodeficiency viruses, includes the envelope from an R5-using primary HIV isolate) (Bomsel 2009). Mymetics is now working with animal model expert Chris Miller at the University of California–San Diego to establish whether these results can be independently confirmed. Phase I human trials are also underway.

## **Preexposure Prophylaxis**

Preexposure prophylaxis (PrEP) is the prophylactic use of antiretroviral drugs to prevent HIV infection. Currently two drugs are being evaluated in phase II and III studies as PrEP: the nucleotide reverse transcriptase inhibitor tenofovir (Viread) and a combination pill called Truvada, which contains tenofovir and the nucleoside reverse transcriptase inhibitor emtricitabine (Emtriva).

The U.S. Centers for Disease Control and Prevention (CDC) is sponsoring two ongoing PrEP efficacy trials: a study among 2,400 injection drug users in Thailand is evaluating tenofovir alone, while a study in Botswana is looking at Truvada in a population of 2,000 heterosexual men and women. Results from these trials are anticipated later in 2010. An NIH-sponsored efficacy trial of Truvada as PrEP in high-risk gay men in Brazil, Ecuador, Peru, South Africa, Thailand, and the United States—which underwent a long period of community consultation, planning, and preparation—is now well underway, with interim results possibly also becoming available before the end of 2010.

More recently initiated trials include a comparison of tenofovir to Truvada as PrEP in 3,900 serodiscordant couples, being conducted by the University of Washington in Kenya and Uganda. The Microbicide Trial Network's VOICE study is enrolling 4,200 African women and will compare three strategies: oral PrEP, using tenofovir or Truvada, versus a tenofovir-containing vaginal microbicide gel. Family Health International has launched a trial of Truvada as PrEP in 3,900 women at sites in Kenya, Malawi, South Africa, and Tanzania. Finally, a smaller pilot study looking at the acceptability and feasibility of PrEP among men who have sex with men aged 18–22 is taking place in Chicago under the sponsorship of the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

**Table 2. PrEP and Microbicides Pipeline 2010**

Product	Type	Manufacturer	Status
Tenofovir/PMPA gel	Reverse transcriptase inhibitor	Gilead Sciences	Phase IIb
Dapivirine (TMC120) gel	Reverse transcriptase inhibitor	International Partnership for Microbicides	Phase I/II
Dapivirine (TMC120) vaginal ring	Reverse transcriptase inhibitor	International Partnership for Microbicides	Phase I/II
VivaGel (SPL7013 gel)	Entry/fusion inhibitor	Starpharma	Phases I/II
UC-781	Reverse transcriptase inhibitor	Biosyn	Phase I
BufferGel Duet	Combination microbicide and cervical barrier	ReProtect	Phase I
Tenofovir (Viread, TDF)	Nucleotide reverse transcriptase inhibitor	Gilead Sciences	Phase III
Truvada (FTC, TDF)	Combined nucleoside and nucleotide reverse transcriptase inhibitors	Gilead Sciences	Phase III

## Microbicides

Microbicides are substances that aim to prevent HIV infection via application to the vagina or rectum prior to sex. As mentioned in the introduction (and covered in last year's *Pipeline Report*), a presentation by Salim Abdool Karim at the 2009 Conference of Retroviruses and Opportunistic Infections (CROI) generated a great deal of excitement because it suggested that PRO2000 gel might have been mildly efficacious at preventing HIV infection in a trial among high-risk South African women (Abdool Karim 2009). In a comparison with a control group that received no gel, PRO2000 appeared to reduce

risk by 33% with a p-value of 0.06 (the standard threshold for significance is 0.05). A CROI audience member noted that a comparison between the PRO2000 gel group and a combination of two control groups in the trial (one that received no gel and another that received a placebo gel) would push the result into significance. To his credit, Abdool Karim explicitly resisted this illegitimate statistical maneuver because combining the control groups was not part of the prespecified plan.

Abdool Karim's caution proved prescient when the results of a far larger phase III efficacy trial of PRO2000 gel (which enrolled close to 10,000 women) were announced at the end of 2009. HIV infection rates were essentially identical between placebo and active gel recipients (Chisembele 2010). While disappointing, the outcome has reinforced a shift in microbicide research toward antiretroviral-based products. Efficacy results from an important first test of this approach—a phase IIb study of a gel form of the reverse transcriptase inhibitor tenofovir (Viread)—are expected to be released at the International AIDS Conference in Vienna on July 21, 2010. The trial started in South Africa in 2007 and is sponsored by a collaboration involving the Centre for the AIDS Programme of Research in South Africa, Family Health International, the United States Agency for International Development, LIFElab, and CONRAD.

A number of other antiretroviral microbicides are advancing in human trials. The reverse transcriptase inhibitor UC-781, originally developed by Uniroyal Chemical and Biosyn, is in a phase I trial sponsored by CONRAD (Schwartz 2008). UC-781 is also being explored for potential rectal use, with encouraging results from a phase I trial published recently (Ventuneac 2010).

The International Partnership for Microbicides (IPM) is developing a nonnucleoside reverse transcriptase inhibitor, dapirivine gel (licensed from Tibotec and formerly known as TMC120), that is currently in phase I/II trials. The IPM has pioneered studies of novel delivery methods, and results indicate that dapirivine can be safely delivered via a matrix intravaginal ring (Nel 2009).

The push toward long-acting delivery methods for microbicides reflects longstanding concerns about the usability and efficacy of topically applied candidates. Adherence to the use of coitally dependent products has been reported to be an issue in some trials (Skoler-Karpoff 2008), and even if adherence is optimal there is evidence that the physical act of sex may affect the genital-tract distribution of a topical microbicide and thereby reduce its ability to inhibit HIV infection (Keller 2010).

## Immune-Based Therapies

The designation *immune-based therapy* encompasses a broad range of approaches that aim to produce health benefits by affecting the function of the immune system. IBTs can be subdivided into candidates that seek to improve immune function and/or clinical health overall (e.g., cytokines like IL-7 and anti-inflammatory approaches), those that try to enhance the immune response to HIV itself (e.g., therapeutic vaccines), and gene therapies that alter the makeup of the immune system in ways intended to ameliorate the harmful effects of HIV (or perhaps even shut down the virus completely).

Suppression of viral replication by ART is associated with a huge reduction in risk of illness and death, closing the life expectancy gap between HIV-positive people and their HIV-negative counterparts (Antiretroviral Therapy Cohort Collaboration 2008, 2009; Lohse 2007; van Sighem 2010). However, in most studies, a gap still exists. Furthermore, poor CD4 recovery despite viral load control and persistently elevated levels of immune activation and inflammation on ART are associated with an increased risk of morbidity and mortality (Kuller 2008; Rodger 2009). These findings suggest that IBTs capable of enhancing immune reconstitution and/or reducing residual immune activation and inflammation could provide significant health benefits to a subset of people with HIV on ART. Unfortunately however, there remains a dearth of candidates in the pipeline.

An interrelated concern is the association between HIV infection and immune senescence, which is characterized by the accumulation of dysfunctional memory T-cell populations in the CD4 and the CD8 T-cell pools—particularly the latter. These dysfunctional cells are stubbornly resistant to apoptosis (cell death), produce large amounts of proinflammatory cytokines and are characterized by lack of cell surface expression of the costimulatory molecule CD28 and elevated expression of a senescence marker, CD57 (Effros 2005). A similar phenomenon is seen in the elderly in the absence of HIV infection; in this setting, elevated levels of senescent CD8 T cells designate an “immune risk phenotype” that is associated with frailty, ill health, and earlier mortality (Larbi 2008). A number of recent studies suggest that people with HIV may face similar issues at a younger age due to an acceleration of immune senescence (Desai 2010). Researchers such as Rita Effros from the University of California–Los Angeles are working on strategies aiming to reverse senescence and/or eliminate senescent cells, but they are as yet only at the preclinical stages of development (Fauce 2008). Another important immunological consequence of both aging and HIV infection is the decline in thymus function and resultant diminution of naive T cells (Schacker 2010) and, as stated in the introduction, researchers continue to look for approaches that may halt or reverse this process.



**Table 3. Therapeutic Vaccines Pipeline 2010**

Product	Type	Manufacturer	Status
DCV-2	Autologous myeloid dendritic cells pulsed ex vivo with high doses of inactivated autologous HIV-1.	Hospital Clinic of Barcelona	Phase II
HIV-1 Tat vaccine (ISS T-002)	Tat protein vaccine at two different doses (7.5 micrograms or 30 micrograms) in five or three immunizations.	National AIDS Center at the Istituto Superiore di Sanità, Rome	Phase II
AGS-004	Mature dendritic cells electroporated with autologous HIV-1 RNA and CD40L RNA.	Argyros Therapeutics	Phase I/II
DermaVir patch (LC002)	DNA expressing all HIV proteins except integrase formulated to a mannosilated particle to target antigen-presenting cells.	Genetic Immunity	Phase II
Autologous HIV-1 ApB DC vaccine	Autologous dendritic cells pulsed with autologous, inactivated HIV-infected apoptotic cells.	University of Pittsburgh	Phase I/II
DNA/MVA	DNA vaccine and an MVA vector encoding HIV-1 gag and multiple CTL epitopes.	Cobra Pharmaceuticals/Impfstoffwerk Dessau-Tornau/University of Oxford/UK Medical Research Council	Phase I/II
Thymon Universal Tat Immunogen (TUTI-16)	A synthetic Tat lipopeptide vaccine administered by subcutaneous injection.	Thymon	Phase I/II
MVA-mBNI20B	Multiantigen MVA vector.	Bavarian Nordic	Phase I
Autologous dendritic cell HIV vaccine	Autologous dendritic cells pulsed with conserved HIV-derived peptides.	University of Pittsburgh	Phase I
Multipeptide DNA	Twenty-one CTL epitopes and proprietary, non-HIV derived "universal" CD4 T-cell epitope.	Pharmexa-Epimmune	Phase I
Tat vaccine	Recombinant protein.	Sanofi Pasteur	Phase I
DC vaccine	Autologous dendritic cells generated using GM-CSF and interferon alpha, loaded with lipopeptides and activated with lipopolysaccharide.	Baylor University/Agence Nationale de Recherche sur le Sida et le hepatitis (ANRS)	Phase I

mRNA-transfected autologous dendritic cells	Dendritic cells transfected with vectors encoding consensus HIV-1 Gag and Nef sequences.	Massachusetts General Hospital	Phase I
PENNVAX-B biological: GENEVAX IL-12-4532, pIL15EAM	PENNVAX-B is a DNA vaccine that encodes a synthetic HIV-1 envelope protein (pEY2E1-B), Gag (gagCAM02), and Pol (pK2C1). GENEVAX IL-12-4532 and pIL15EAM are DNA adjuvants encoding the cytokines IL-12 and IL-15.	University of Pennsylvania/Drexel University	Phase I
GSK HIV Vaccine 732462	p24-RT-Nef-p17 fusion protein in proprietary adjuvant AS01B.	GlaxoSmithKline	Phase I
HIV-v	Lyophilised mixture of polypeptide T-cell epitope sequences	PepTcell	Phase I
PENNVAX™-B (Gag, Pol, Env) + Electroporation	DNA vaccine encoding gag, pol, and env genes of HIV-1 + electroporation	VGX Pharmaceuticals/University of Pennsylvania	Phase I
AFO-18	18 peptides representing CD8 and CD4 epitopes mainly on HIV-1 in an adjuvant (CAF01)	Statens Serum Institut, Ministry of the Interior and Health, Denmark/ European and Developing Countries Clinical Trials Partnership	Phase I
MVA.HIVconsv	MVA vector	University of Oxford, Medical Research Council	Phase I
GTU-Multi-HIV B clade vaccine	Multi-antigen DNA vaccine being studied in combination with IL-2, GM-CSF and growth hormone in a study called Novel Interventions in HIV-1	Imperial College London/Medical Research Council	Phase I
DCVax Plus Poly ICLC	Vaccine consisting of a fusion protein containing a human monoclonal antibody specific for the dendritic cell receptor, DEC-205 (CD205), and the HIV gag p24 protein, plus poly ICLC (Hiltonol) adjuvant.	Rockefeller University	Phase I

**Table 4. Cytokine, Immunomodulator, and Gene Therapy Pipeline 2010**

Product	Type	Manufacturer	Status
Maraviroc (Selzentry)	CCR5 inhibitor	Pfizer	Phase IV
Chloroquine phosphate	Anti-inflammatory		Phase II
Pegasys (peginterferon alfa-2a)	Cytokine	NIAID/Hoffmann-La Roche	Phase II
Interleukin-7 (CYT 107)	Cytokine	Cytheris	Phase II
HLA-B*57 cell transfer	Cell infusion	NIH Clinical Center	Phase I
TXA127	Bone marrow stimulant, angiotensin 1-7	Tarix Pharmaceuticals	Phase I
Mesalamine (5-aminosalicylic acid)	Oral anti-inflammatory drug approved for the treatment of inflammatory bowel disease.	University of California–San Francisco/Salix Pharmaceuticals	Phase IV
OZ1 ribozyme gene therapy	Antiviral ribozyme targeted against the tat gene, introduced into CD4 T cells via stem cells.	Johnson & Johnson	Phase II
VRX496	Lentiviral vector encoding antiretroviral antisense, introduced into CD4 T cells ex vivo.	VIRxSYS	Phase II
HGTV43	Vector encoding antiretroviral antisense, introduced into CD4 T cells ex vivo.	Enzo Biochem	Phase II
M87o	Entry inhibitor gene encoded by a lentiviral vector.	EUFETS AG	Phase I
SB-728	Autologous T-cells genetically modified at the CCR5 gene by zinc finger nucleases.	University of Pennsylvania/Sangamo Biosciences	Phase I
Combined anti-HIV RNA-based therapeutics	Three antiviral genes introduced into stem cells ex vivo.	City of Hope/Beckman Research Institute/Benitec Limited	Phase I
Redirected high affinity Gag-specific autologous T cells for HIV gene therapy	Gene therapy that introduces an HIV-specific T-cell receptor into CD8 T cells and re-infuses them.	University of Pennsylvania	Phase I

## **Therapeutic Vaccines**

A bewildering array of therapeutic vaccine candidates continues to undergo testing. The large body of evidence suggesting that HIV-specific CD4 and CD8 cells play a key role in individuals who control viral replication in the absence of ART has prompted this research. (Hersperger 2010; Saag 2010). For the most part, the hope is that therapeutic vaccines may be able to induce the maturation of new HIV-specific T-cell responses while viral load is suppressed by ART, and these responses may be better equipped to battle HIV when ART is subsequently interrupted.

Another strategy being employed is the immunization of HIV-positive people prior to any significant CD4 T-cell decline, with the aim of delaying the need for ART. Two new studies of peptide-based therapeutic vaccines, HIV-v and AFO-18, are taking this tack. However, it is unclear if a peptide vaccine can induce useful new T-cell responses in a setting where there is already abundant presentation of natural HIV antigens due to ongoing viral replication.

One possibility receiving little attention is that therapeutic vaccination might reduce HIV-induced inflammation by bolstering virus-specific T cell responses. This may seem counterintuitive, but decreased immune activation was been reported many years ago in a trial of Jonas Salk's now discontinued whole-killed therapeutic HIV vaccine (Fernandez-Cruz 2002).

Several therapeutic vaccine candidates are based on dendritic cells, which researcher Ralph Steinman has dubbed "nature's adjuvant." Steinman's own laboratory has recently entered the fray with a phase I trial of a vaccine that specifically targets dendritic cells via a receptor called DEC-205 (Nchinda 2010).

## **Anti-inflammatory Approaches**

The associations between inflammatory markers and adverse clinical events have bolstered the rationale for studying approaches that might reduce immune activation in people with HIV infection. The malaria drug chloroquine phosphate is being studied for both direct anti-HIV and anti-inflammatory effects. Aspirin and pentoxifylline are also being studied in combination with ART, but not to assess their impact on HIV progression; instead, the outcome measures being looked at are markers of cardiovascular disease risk. Encouraging results from the phase I trial of pentoxifylline have been published (Gupta 2010), leading to the opening of a larger phase II study.

## Mesalamine

Mesalamine is an oral anti-inflammatory drug that acts particularly on the cells of the gut (Iacucci 2010), and the U.S. Food and Drug Administration has approved it for the treatment of ulcerative colitis, proctitis, and proctosigmoiditis. Researchers at the University of California–San Francisco are conducting a small study to ascertain if mesalamine can reduce inflammation levels in HIV-positive people on ART. The study is motivated by evidence that leakage of normally friendly gut bacteria into systemic circulation (microbial translocation) contributes to immune activation in HIV infection (Brenchley 2006) and is associated with poor immune reconstitution on ART (Marchetti 2008).

## Cell Infusion and Gene Therapies

Several phase I and II studies of gene therapies are ongoing. The broad goal of these approaches is to enable CD4 T cells to resist HIV infection. Results from the phase II trial of Johnson & Johnson's OZ-1 anti-Tat gene therapy were published in 2009; the product failed to meet the primary endpoint of significantly reducing viral load during an ART interruption but several exploratory analysis suggested that there may have been a mild antiviral effect (Mitsuyasu 2009).

Carl June's research group has launched a novel study in which CD4 T cells are sampled and manipulated in the laboratory so that they can no longer express the CCR5 coreceptor. This is achieved using a zinc finger nuclease technology developed and manufactured by Sangamo Biosciences. The zinc finger nucleases act like biological scissors and snip out the CCR5 gene from the CD4 T cells' DNA. The CCR5-negative CD4 T cells are then expanded and reinfused into the individual. Interest in this approach has been piqued by the case of the apparently cured individual cited in the introduction (Hütter 2009). June gave an update on the status of the trial at a Keystone HIV pathogenesis meeting in January 2010: the research is proceeding slowly due to careful safety evaluations and so far only one person has been infused. No concerns have arisen and preliminary evidence suggests that the CCR5-negative CD4 T cells are persisting and expanding slightly *in vivo*, representing 2.1% of peripheral blood CD4 cells at 140 days of follow up (June 2010). June's research group is also evaluating a different gene therapy that modifies CD8 T cells *ex vivo*, equipping them with a T cell receptor (TCR) that is particularly adept at recognizing HIV-infected cells (Varela-Rohena 2008). The souped-up CD8 T cells are then expanded and re-infused back into the individual. The ultimate goal is to combine the CD4 and CD8 T cell gene therapy approaches in order to bolster the ability of both subsets to deal with HIV.

Just prior to this report going to press, researcher John Rossi published results from a phase I trial of a combined gene therapy approach in HIV-infected individuals undergoing hematopoietic stem cell (HSC) transplantation for AIDS-related lymphoma (DiGiusto 2010).

Genes encoding three different anti-HIV RNA molecules were introduced into a subset of transplanted HSCs in four individuals, and long-term persistence in multiple cell lineages was demonstrated, albeit at very low levels. Although no therapeutic effect could be demonstrated, the study has been hailed as proof that the concept is feasible.

A similar protocol is being employed by researchers developing M87o, a gene therapy that encodes an HIV entry inhibitor similar to the drug Fuzeon (van Lunzen 2007). A phase I trial is being performed in individuals with AIDS-related lymphoma who require stem cell transplantation, and the M87o gene is added to a subset of the stem cells prior to the procedure.

## **IL-7**

IL-7 is a cytokine that plays a key role in T-cell development and naive and memory T-cell proliferation and survival. Results from two phase I trials of IL-7 in people with HIV reported substantial increases in CD4 and CD8 T-cell counts even at the lowest dose studied (Levy 2007; Sereti 2007). The drug was well tolerated. These results suggest that IL-7 may be an excellent candidate for studies in people with inadequate immune reconstitution despite ART. A new glycosylated form of IL-7 that allows less frequent dosing is now in phase I trials. The manufacturer is a French company called Cytheris.

## **Maraviroc**

Maraviroc is an approved antiretroviral drug (marketed under the name Selzentry) that works by blocking the interaction between HIV and the chemokine receptor CCR5. Five clinical trials are evaluating whether adding maraviroc can increase CD4 T-cell counts in people on ART with poor CD4 T-cell recovery despite prolonged viral load suppression. Researchers from the U.S.-based AIDS Clinical Trials Group recently presented results of a study addressing this question and, while receipt of maraviroc was associated with declines in immune activation markers, there was no significant CD4 T-cell increase compared to standard ART (Wilkin 2010).

## **TXA127**

TXA127 is one of the very few IBTs being studied in individuals with poor CD4 T-cell reconstitution despite viral load suppression by ART. The other name for the drug is angiotensin 1-7 and it has been shown to stimulate bone marrow production of hematopoietic progenitor cells in animal models (Heringer-Walther 2009). These progenitor cells give rise to multiple lineages of immune cells, including CD4 T cells, hence the rationale for study in HIV. A small phase I/II trial has reported a reduction in the incidence of low blood cells after chemotherapy for breast cancer (Rodgers 2006).

## Conclusion

The Thai results have boosted flagging hopes for HIV vaccine research, but it will be some time before it is known whether they can be repeated in other settings. The apparent success of the trial has shone on a spotlight on the shortsightedness of the design which, as the investigators have acknowledged, made the relative contributions of the two components impossible to disentangle. TAG drew attention to this obvious issue when the trial first began (Jefferys 2004). From a policy perspective this is problematic: on the one hand the urgent need for an HIV vaccine is emphasized to the public to promote support for research funding, while on the other investigators unabashedly propose repeating a huge, lengthy trial in order to “deconvolute” the results. In the future, trials must be designed in a way that precludes the contribution of the individual vaccine components becoming convoluted in the first place. The PreP and microbicide fields are united in anticipation of imminent efficacy trial results. The findings from these trials will be crucial in determining the next steps in these research areas. Immune-based therapies continue to appeal on paper while struggling in the real world. But renewed attention to the importance of translational (bench-to-bedside) research and some encouraging signs from the cancer field offer hope that breakthroughs are possible. Ultimately, it is to be hoped that the reinvigorated research effort into curing HIV infection, along with a successful sterilizing vaccine, will render these pipelines moot.

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# Hepatitis B Drugs in Development

BY LEI CHOU

## Introduction

After a burst of hepatitis B virus (HBV) drug discovery and development over the last decade, the year 2010 saw the development pipeline dry up. While several currently available drugs are highly effective at controlling viral replication and keeping the disease in check, only about one-third of people are able to reach a key treatment goal: sustained viral control while safely off treatment. Life-long suppressive therapy with antiviral drugs appears to be the HBV treatment model for the near future for most people with chronic hepatitis B.

Although antiviral drugs can control HBV, drug resistance may develop over time, leaving people with no treatment options. This is of particular concern as resistance can develop faster for people coinfecting with HIV and HBV. Long-term toxicity is likely to be an issue. These concerns will not be resolved until there is a robust drug pipeline. Advances in basic science are needed to better understand HBV pathogenesis, identify new drug targets, and to stop HBV's destructive track.

While overall cancer rates are on the decline nationally, liver cancer is bucking this trend, driven primarily by chronic viral hepatitis. A recent Centers for Disease Control and Prevention (CDC) publication reported that liver cancer is on the rise: between 2001 and 2006, the incidence of liver cancer increased at an annual rate of 3.5 percent, representing over 45,000 reported cases in the same period. Asians, Pacific Islanders, and African Americans have the highest rates of increase (CDC 2010).

HBV treatment can prevent the development of life-threatening complications, such as cirrhosis and liver cancer. But in the U.S., an estimated 5.3 million people with chronic viral hepatitis (hepatitis B and C) are over 50% percent of the undiagnosed. The challenge is to screen, diagnose, monitor, and treat people in a timely matter, before they develop serious complications. HBV treatment may prevent these complications, not treat them. Sadly, U.S. federal investment in research, testing, surveillance, and publicly funded treatment programs is grossly inadequate at present.

According to the National Viral Hepatitis Roundtable (NVHR), the proposed 2011 annual budget for CDC's Division of Viral Hepatitis is only \$21 million, just two

percent of the overall budget for the National Center for HIV/AIDS, Viral Hepatitis, Sexually Transmitted Disease, and Tuberculosis Prevention (NCHHSTP). Shockingly, this is less than the Division's budget of \$25 million from ten years ago (NVHR 2010).

Key provisions in the new healthcare reform law passed in March could help this dire situation. The insurance industry's discriminatory practice of withholding coverage from people with pre-existing conditions will be banned by 2014. This means that people with chronic HBV can finally gain access to what will hopefully be affordable healthcare. However, implementation of the new legislation is expected to be an uphill battle, and will take a few years.

A recently released Institute of Medicine report (Colvin 2010) highlighted the lack of provider awareness about HBV contributes to the problem. Doctors often do not identify at risk people for screening, nor do they consult current guidelines on how and when to treat chronic HBV. According to one survey, 44 percent of primary care providers did not know HBV can be controlled with treatment.

Hopefully, the gradual expansion of access to health care will drive demand for new and better HBV treatment, and provide more financial incentives for the drug industry to invest in discovery and development of new compounds. In the meantime, there are *no* investigational new drugs in late stage development in the U.S. for HBV. Instead, the glimmer of hope—for what it's worth—is coming from a handful of immune-based therapies, although these are all in early stage development.

**Table 1. HBV Experimental Agents in the Pipeline**

Agent	Manufacturer	Stage of Development	Class
<b>Oral Antivirals</b>			
Emtricitabine (Truvada, in coformulation with tenofovir)	Gilead	Phase III/IV	NRTI
Clevudine	Bukwang/ Eisai	Phase III	NRTI
LB80380	LG Life Sciences	Phase IIb	NRTI
MIV-210 (Lagocidovir valactate)	Medivir/Daewoong	Phase II	NRTI
Simvastatin	University of Oklahoma Health Sciences Center/VA Medical Center	Phase I	3-hydroxyl-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor
Bay 41-4109	AiCuris	Pre-clinical	heteroaryl-dihydropyrimidine
<b>Immune-based</b>			
Thymosin alpha (zadaxin)	SciClone Pharmaceuticals	Phase IV	Immunomodulator

Interferon gamma 1b (Actimmune)	InterMune	Phase II	Immunomodulator
CYT107 (recombinant human interleukin-7)	Cytheris	Phase I/IIa	Immunomodulator
DNA vaccine pCMV52.S	ANRS (French Agency for Research on AIDS and Viral Hepatitis)	Phase I/II	Therapeutic vaccine
DNA vaccine (HB-110)	Genexine	Phase I	Therapeutic vaccine
Hepatitis B vaccine (Synthesized peptide PA-44)	Chongqing Jiachen Biotechnology	Phase I	Therapeutic vaccine
HBV DNA plasmid pdpSC18 vaccine	PowderMed/Pfizer	Phase I	Therapeutic vaccine
DV-601	Dynavax	Phase I	Therapeutic vaccine
Hepilisav	Dynavax	Phase III	Preventive vaccine

## Oral Antivirals

Access to new brand-name drugs in Asia is limited by their prohibitive cost, an option only available in higher-income countries. The need for new drugs with better potency and higher resistance barriers are particularly pressing in the region, since lamivudine monotherapy has been the primary treatment for many years due to the availability of low-cost generics, and the drug's weak resistance profile has contributed to wide-spread lamivudine resistance in people with chronic HBV. Although hepatitis B is endemic in Asia, there are only two oral antivirals in late stage development in China and South Korea.

### Clevudine

Despite Pharmasset's halting U.S. development of clevudine due to post-market reports of severe myopathy (muscle weakness) last year, the South Korean drug maker Bukwang is forging ahead with development and marketing plans for clevudine in Asia. Studies in South Korea and a phase III trial in China are underway. Bukwang's Asian partner, Eisai Pharmaceuticals, has launched clevudine (brand name: Revovir) in the Philippines, with pending licensing plans for India, Indonesia, Malaysia, and Thailand. It will be up to doctors and regulatory authorities in these countries to keep a close eye on reports of myopathy, since the condition can develop after eight months of clevudine use, and people must stop taking it to avoid potentially fatal consequences. In a rapid communication published in *Hepatology* (Seok 2009), the main symptom of clevudine induced myopathy was slowly progressive muscular weakness in the limbs over several months. The condition can take up to four months to resolve after clevudine is stopped.

## LB80380

LG Life Sciences published data from its safety and efficacy study of the new nucleotide analog LB80380 in *Hepatology* (Yuen 2010). The small study done in treatment-experienced Chinese volunteers with lamivudine-resistant chronic HBV showed potency comparable to entecavir and tenofovir, the two leading antivirals on the market. No significant drug-related toxicities were seen in this 12-week dose-finding study. A phase II study in treatment-naïve volunteers is enrolling in South Korea and Hong Kong.

Tenofovir is the first-line treatment for lamivudine-resistant HBV in the U.S. and Western Europe. Resistance to tenofovir has not yet been characterized three years after the drug's approval to treat HBV, but second-line drugs for people who become resistant to tenofovir may be needed in the future. Although LB80380 may be useful as a second-line drug for people with lamivudine resistance, an *in vitro* study revealed that LB80380 has only partial efficacy against adefovir and potential tenofovir resistant HBV strains. Development plans for the United States and Europe are unclear.

## Emtricitabine/Tenofovir

This Gilead workhorse HIV combination pill is being studied in several ongoing HBV trials in different patient populations. Truvada is being studied in HIV/HBV coinfecting people with a detectable HBV viral load despite current treatment, both in combination with pegylated interferon, and in a head-to-head comparison with entecavir. Trials are also underway in people with decompensated liver cirrhosis and liver transplant recipients.

## Lagociclovir Valactate (MIV-210)

The Swedish drug maker Medivir has out-licensed MIV-210 to South Korean Daewoong Pharmaceutical to advance its development in Asia this year. This drug was initially developed for HIV and failed. Now it's enjoying a second chance as an HBV treatment. MIV-210 has shown *in vivo* activity against lamivudine-resistant HBV strains in an animal model study done nearly ten years ago. Daewoong plans to launch a phase II study in South Korea.

## Simvastatin

The University of Oklahoma and the U.S. Veteran's Administration are conducting a phase I proof-of-concept study looking at the cholesterol lowering agent simvastatin, after *in vitro* data showed anti-HBV activity and synergistic activity when combined with approved HBV drugs (Bader 2010). The small three-arm study will compare simvastatin

alone vs. simvastatin with either tenofovir or entecavir. Since the drug is available as a generic, it will radically change the current HBV market if proven effective on its own.

#### Bay 41-4109

Bayer's Bay 41-4109 is a heteroaryldihydropyrimidine (HAP) that inhibits HBV assembly by interrupting viral capsid formation. The drug has recently resurfaced in an investor report from the German company AiCuris, a Bayer spin-off drug discovery company. Clinical development plans are unclear. Since it is the only experimental candidate that targets a different part of the viral life cycle than drugs currently on the market, the compound—if it is ever developed—could be used to treat drug-resistant HBV.

### Immune-based Therapies

#### CYT107

A new entrant from the immune-based front is French company Cytheris' recombinant human Interleukin-7 (CYT107). This immunomodulator is also being studied for HIV and hepatitis C. The CONVERT study will compare CYT107 with the HBV preventive vaccine GenHevac B versus CYT107 alone in HBeAg negative patients on stable tenofovir or entecavir treatment. This phase I/IIa study is enrolling volunteers in France and Italy. The hope is that the agent will stimulate the immune system and restore immune response to HBV so that people do not have to stay on life-long antiviral therapy.

Several other immunomodulators and therapeutic vaccine candidates are currently in early phase development; however no study data have been published in the past year. For detailed descriptions of these agents, please see TAG's *2009 Pipeline Report*.

**Interferon gamma 1b (Actimmune)**—A phase II study of this chemically manufactured form of human interferon gamma has not yet opened for enrollment.

**Thymosin alpha1 (Zadaxin)**—A phase IV trial of this synthetic version of a substance produced naturally by the thymus is still active in South Korea.

**HBV naked DNA vaccine pCMVS2.S HBV**—A phase I/II study is still ongoing with completion expected in late 2010.

**Mixed plasmid DNA (HB-110) vaccine**—A phase I study combining the vaccine with adefovir is still enrolling patients in Korea.



**Hepatitis B vaccine (synthesized peptide PA-44)**—A phase I study in China is still ongoing.

**HBV DNA plasmid pdpSC18 vaccine**—A phase I study has been completed but no data are published as yet.

**DV-601**—A phase I study is open and enrolling patients using this therapeutic vaccine being developed by Dynavax in Europe.

## Preventive Vaccine

### Heplisav

Dynavax successfully resuscitated this preventive vaccine after a series of setbacks. After the U.S. Food and Drug Administration (FDA) placed a clinical hold on the development of Haplisav in 2008 due to a case of vasculitis (inflammation of the blood vessels), a potentially deadly autoimmune condition also known as Wegener's granulomatosis, Merck pulled out as a development partner. Dynavax was able to convince the FDA to release the hold by redesigning its development to target the vaccine for subsets of people with immune deficiencies that render currently approved HBV vaccine ineffective, altering the risk-versus-benefit equation to favor development. The company has since launched two large-scale phase III studies, one in people with end-stage renal disease, and the other in people over the age of 40. While this vaccine could be helpful to some HIV-positive people who do not achieve protective immunity with currently approved vaccines, no study in HIV-positive people is being planned. The vaccine will require only two shots within one-month, compared to the current standard of three shots in six months. It has demonstrated non-inferiority with GSK's Engerix-B vaccine, and data from the two phase III studies are expected in mid-2011.

## Hepatitis B Clinical Research Network

The anticipated opening of the Hepatitis B Clinical Research Network's first trials at the end of 2009 did not materialize. Reportedly, the delay is due to complex contract negotiations with drug and diagnostic companies regarding provision of drugs and funding. This delay is disappointing, as several of the Network's planned trials will address many research questions critical to our understanding of chronic HBV disease progression, as well as optimal treatment strategies with currently available drugs. TAG hopes the negotiations will be completed quickly so as not to impact the

duration of these studies, since the network is nearing the start of the third year in the seven-year grant from the U.S. National Institutes of Health (NIH) without enrolling a single patient.

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# The Hepatitis C Treatment Pipeline

BY TRACY SWAN

## Dedicated to Dr. Roy Arad

Although curable, hepatitis C virus (HCV) has been described by the World Health Organization (WHO) as a “viral time bomb” due to both its prevalence (3% of the world’s population, or 170 million people, have been infected) and potential for causing serious, life-threatening complications (WHO 2010). Up to 130 million people have chronic hepatitis C, and 20 to 30% of them—between 13 and 19.5 million people—will develop cirrhosis if untreated or unsuccessfully treated. People with cirrhosis are at risk for liver cancer (hepatocellular carcinoma; HCC) and liver failure. In fact, more than 365,000 people die each year from these HCV complications (Perz 2006).

Worldwide, an estimated 4–5 million people are coinfecting with HIV and hepatitis C (Alter 2006). They need more effective and tolerable HCV treatment. In places where people have access to antiretroviral therapy, end-stage liver disease from HCV coinfection has become a leading cause of death among HIV-positive people (Weber 2006). This is because HIV accelerates HCV progression and increases the likelihood of complications: HIV doubles the risk of cirrhosis, and immunodeficiency increases the risk of HCC (Clifford 2008; Graham 2001). Unfortunately, HCV treatment with the current standard of care (SOC) is less effective for coinfecting people than their HCV mono-infected counterparts (Carrat 2004; Chung 2004; Torriani 2004).

## Introduction

Approximately half of the people who undergo hepatitis C treatment are cured. In the near future more people with hepatitis C will be cured, some in half the time required now. Scientific advances and keen pharmaceutical interest have led to a flurry of HCV drug development; more than thirty drugs have entered clinical trials. Sales of HCV drugs, which have been plummeting in the U.S., are expected to increase from \$2.3 billion to \$4.5 billion by 2017 as new drugs enter the marketplace. The U.S. (\$1.9 billion), and the E.U. (\$1.7 billion) will be major consumers (Datamonitor 2009).

Oral drugs (known as *direct-acting antivirals*, or DAAs) that specifically target certain steps in the hepatitis C virus life cycle are in late-stage development. In 2011, the U.S. Food and Drug Administration (FDA) approval of two HCV protease inhibitors, boceprevir and telaprevir, is expected. But pegylated interferon (also known as *peginterferon*) and ribavirin—the current standard of care for hepatitis C—will remain as the therapeutic backbone for the first few generations of HCV drugs.

Peginterferon and ribavirin work by killing infected cells (immunologic effect) and protecting new cells from hepatitis C by preventing HCV replication (antiviral effect). Nobody knows whether a combination of DAAs will cure HCV by preventing the virus from reproducing (an approach that has been successful for treating, but not eradicating, HIV). Peginterferon (or another therapy that stimulates the immune response to HCV) may still be required to cure HCV.

Everyone would like to be rid of interferon. It is a huge barrier to HCV treatment access, uptake, and completion because of its cost (~\$25,000 per year), medical contraindications, and many side effects. Even when HCV treatment is readily available at no cost, tolerability is a problem: only one out of 56 people who received HCV treatment through the Veteran's Administration completed their regimen (Butt 2009).

Hopefully, DAA combinations will become the standard of care. By 2013, results from a trio of groundbreaking trials will be available. These studies combine two DAAs, with or without peginterferon and ribavirin. Study populations and drugs differ (in treatment-naïve people, a protease inhibitor/non-nucleoside polymerase inhibitor combination; in prior null responders, a protease inhibitor plus an NS5a inhibitor), but if successful, these trials will provide initial proof-of-concept for peginterferon-free regimens.

In the meantime, results from the first phase III study of a DAA (telaprevir, an HCV protease inhibitor) plus SOC were reported in May 2010, and others are nearing completion. Several ongoing triple therapy trials—adding a single DAA to SOC—are exploring treatment strategies and duration, and evaluating early predictors of successful treatment. Quad trials—two DAAs plus SOC—will soon be underway as well.

The biggest limitation to DAAs is the emergence or development of drug resistance. Drug resistance means that an organism—such as HCV—is able to grow or reproduce despite presence of levels of a drug that would normally stop it from doing so. HCV makes billions of copies of itself each day. They are not identical; some individual virus particles (*virions*) have structural changes (*mutations*). Some mutations may allow the virus to escape from drug pressure, leading to drug resistance. In fact, resistance to one or more DAA classes has already been detected in people who have never used these drugs (Kuntzen 2008; Legrand-Abraham 2009).

HCV treatment strategies must continue to evolve in order to forestall drug resistance and meet the needs of different populations. Some people cannot use peginterferon and ribavirin, and it is ineffective for ~50%, leaving many unsuccessfully treated people (see box: Terms for HCV Treatment Response by Population and Time Point). But adding a single DAA to SOC will not work for all treatment-experienced people.

So far, it is clear that adding a DAA to SOC on therapy is most likely to work for people who relapsed or experienced viral breakthrough. Adding a single drug is less likely to work for people who have HCV that is not responsive to peginterferon, as is the case with treatment nonresponders and null responders. Using two or more DAAs may be effective and lower the risk of drug resistance for non- and null responders, but more research is needed to determine retreatment strategies for these groups.

### Terms for HCV Treatment Response by Population and Time Point

#### Population

*Relapse* means that HCV became—and remained—undetectable during treatment, but reappeared within weeks to months after finishing it.

*Viral breakthrough* means that HCV reemerged after becoming undetectable during treatment.

*Non-response* means that the hepatitis C viral load drops by two logs (99%) but does not ever become undetectable during treatment.

*Null response* means that hepatitis C viral load drops by less than one log (10%) after four weeks of treatment, and drops by less than two logs (99%) drop after 12 weeks of treatment.

#### Time Point

*Very rapid virological response* (vRVR) is a new term, used to indicate that HCV RNA has become undetectable after 14 days of treatment.

*Rapid virological response* (RVR) means that HCV cannot be detected in the blood after four weeks of treatment. RVR is a significant milestone in response-guided therapy because it predicts *sustained virological response* (see below) in ~90% of cases—regardless of HIV status, but a person can still be cured in the absence of RVR.

*Sustained virological response* (SVR) means that no HCV is detectable in a person's bloodstream six months after completion of treatment. SVR is durable, and linked to reductions in liver-related morbidity and mortality; HCV is cured.

*Extended rapid virologic response (eRVR)* is a newly coined term indicating that HCV RNA has become undetectable after 4 weeks of treatment and remains undetectable at week 12.

*Partial early virological response (pEVR)* means that HCV RNA has dropped by at least two logs (99%).

*Complete early virological response (cEVR)* means that HCV RNA is undetectable after 12 weeks of treatment. SVR is more likely for people who have a cEVR than people who have a pEVR. Although an early virological response cannot predict who will be cured, it does indicate who will not be cured if they remain on treatment. Since SVR is extremely unlikely in people who don't have a pEVR or cEVR, HCV treatment is usually discontinued at this point. Sometimes this is called an *early stopping rule*.

*End-of-treatment response (EOT)* means that HCV viral load is undetectable at the end of HCV treatment.

*SVR-12* means that HCV remains undetectable 12 weeks after completion of treatment. Although it has not been prospectively validated (meaning that researchers have found this to be true by looking back at trial results rather than planning in advance to see if it is true), SVR-12 is a good predictor of SVR because relapse usually occurs within a few weeks after treatment completion.

## HCV Treatment: Population-specific Issues

Hopefully, DAAs will be safe and effective for HIV/HCV coinfecting people, since SOC is less effective for HIV/HCV coinfecting people than for people with HCV monoinfection (see Table 1: HCV Treatment Outcomes, by Population). Coinfecting people usually have higher HCV viral loads (HCV RNA) than people with HCV alone. A less effective backbone and a high hepatitis C viral load increase the risk for drug resistance, so coinfecting people may require treatment with more than one DAA. But DAAs may interact with some antiretroviral agents, complicating treatment of both viruses. Coinfecting people and their medical providers are awaiting results from a pair of ongoing DAA studies in HIV/HCV coinfecting people.

The safety and efficacy of DAAs have yet to be explored—or have not been adequately explored—in other key populations. No studies have been initiated in transplant candidates and recipients, despite the urgent need for such studies. Only a small proportion of people with cirrhosis have been enrolled in DAA trials to date.

Enrollment of African Americans, Latinos, and Latinas has been inadequate. Although they constitute the highest-prevalence population, people who use drugs are usually excluded from clinical trials, even when they are ready and willing to participate.

**Table 1. HCV Treatment Outcomes, by Population**

Treatment with peginterferon plus ribavirin (weight-based or flat dosing) for 24–72 weeks; HCV genotype 1 unless indicated

<b>International Registration Trials: HCV Monoinfection (reference)</b>			
<b>Study and Date</b>	<b>Source</b>	<b>Population</b>	<b>SVR</b>
Fried, 2002; Manns 2001	Clinical trial	HCV genotype 1	42–44%
<b>International Trials: HIV/HCV Coinfection</b>			
<b>Study and Date</b>	<b>Source</b>	<b>Population</b>	<b>SVR</b>
Carrat 2004 (Europe) Chung, 2004 (U.S.) Laguno, 2004 (Europe) Torriani 2004 (international)	Clinical trial	HIV/HCV genotype 1	Carrat: 21% Chung: 14% Laguno: 38% Torriani: 29%
<b>Clinical Practice: U.S. and Non-U.S.</b>			
<b>Study and Date</b>	<b>Source</b>	<b>Population</b>	<b>SVR</b>
Borroni 2008	Non-U.S. clinical practice (Italy)	HCV genotype 1	46%
Feuerstadt 2009	U.S.-based faculty practice (FP) and clinic (C)	HCV genotype 1 56% Hispanic, 27% African American, 9% Caucasian, 8% other	Overall: 14% FP: 27% C: 15%
Gheorghie 2007	Non-U.S. clinical practice (Romania)	HCV genotype 1	55.9%
Jacobson 2007	U.S. clinical trial (community and academic setting)	Genotype 1; fixed-dose ribavirin (FDR) vs. weight-based ribavirin (WBR)	FDR: 28.9% (overall) vs. 10.1% (African American) WBR: 34% (overall) vs. 20.7% (African American)
Lee 2006	Non-U.S. clinical practice (Canada)	Cirrhosis vs. noncirrhotic, HCV genotype 1	34% (cirrhotic) vs. 41% (noncirrhotic)
<b>African Americans: Clinical Trials and Clinical Practice</b>			
<b>Study and Date</b>	<b>Source</b>	<b>Population</b>	<b>SVR</b>
Conjeevaram 2006	Clinical trial	African American, HCV genotype 1	28% (vs. 52% among Caucasians)

Jeffers 2004	Clinical trial	African American, HCV genotype 1	26% (vs. 39% among Caucasians)
Muir 2004	Clinical trial	African American, HCV genotype 1	19% (vs. 52% among Caucasians)
Satapathy 2010	Clinical practice, retrospective review	African American, HCV genotype 1	16.1%
Srivastava 2005	Clinical practice	African American, HCV genotype 1	19% (vs. 24% among Caucasians)
<b>Latino Populations: Clinical Trials and Clinical Practice</b>			
<b>Study and Date</b>	<b>Source</b>	<b>Population</b>	<b>SVR</b>
Rodriguez-Torres 2009	Clinical trial	Latino, HCV genotype 1	34% (vs. 49% among Caucasians)
Satapathy 2010	Clinical practice, retrospective review	Latino, HCV genotype 1	13.7%
Yu 2009	Clinical practice, retrospective review	Latino and Caucasian, HCV genotypes 2 and 3	65.9% (vs. 87.3% among Caucasians)
<b>Asian Population: Clinical Trial</b>			
<b>Study and Date</b>	<b>Source</b>	<b>Population</b>	<b>SVR</b>
Liu 2008	Clinical trial	Asian, HCV genotype 1	76% (after 48 weeks of treatment)
<b>Prior Relapse/Nonresponse to Standard or Peginterferon plus RBV</b>			
<b>Study and Date</b>	<b>Source</b>	<b>Population</b>	<b>SVR</b>
Berg 2006	Clinical trial	Relapse after 24 weeks of peginterferon/RBV	51% (retreated for 48 weeks)
Sagir 2007	Clinical practice	Nonresponders to standard interferon/ribavirin	4%
Scotto 2008	Clinical trial	Nonresponders to standard interferon/ribavirin	-19%
Yoshida 2009	Clinical practice	HCV genotype 1, prior relapse/nonresponse to peginterferon plus RBV	65% (prior relapse) 17% (prior nonresponse)
<b>People with Cirrhosis</b>			
<b>Study and Date</b>	<b>Source</b>	<b>Population</b>	<b>SVR</b>
Lee 2006	Clinical practice	People with bridging fibrosis and cirrhosis (stages F3 and F4)	34%
Di Marco 2007	Clinical trial	People with cirrhosis and portal hypertension (low-dose peginterferon and low-dose ribavirin)	11.3%



Iacobellis 2007	Open-label, single-arm study	People with decompensated cirrhosis; 24 weeks of treatment (low-dose peginterferon; standard-dose ribavirin)	7%
Iacobellis 2009	Open-label, single-arm study	People with decompensated cirrhosis; 48 weeks of treatment (standard-dose peginterferon and ribavirin)	16%
Transplant Recipients			
Study and Date	Source	Population	SVR
Hanouneh 2008	Clinical practice	Transplant recipients; full-dose peginterferon and ribavirin	23%
Lodato 2008	Clinical trial; response-guided, open-label study	Transplant recipients; 48 weeks of low-dose peginterferon and standard-dose ribavirin	26%
Zimmermann 2007	Open-label study	Transplant recipients, genotype not specified	19%
Injection Drug Users (IDUs)			
Study and Date	Source	Population	SVR
Bruggmann 2008	Clinical Practice	Active IDU; HCV genotype not specified	69.3% (versus 59.8% among control group of non-users)
Hellard 2009	Meta-analysis	Active IDUs; genotype not specified	Median 54% Range 18.1 to 94.1%

## HCV Treatment Access

Patent protection of peginterferon extends until 2016 (Peg-Intron®) or 2017 (Pegasys®) in the United States. The high cost of peginterferon drastically limits access to HCV treatment; it is unavailable to most of the world's 130 million chronically infected people. According to *Viral Hepatitis: Global Policy*, a 2010 report from the World Hepatitis Alliance, over 80% of low income countries want assistance to improve access to HCV (and hepatitis B) treatment.

Lack of access to HCV treatment is unacceptable. Pharmaceutical companies can remedy this situation. They have an opportunity to save millions of lives while generating unanticipated revenue and goodwill. Global access to peginterferon and DAAs can—and ought to be—facilitated by these and other measures:

- adopting a high-volume, low-profit strategy for low and middle income countries

- registering HCV treatments in all countries
- granting licenses to generic manufacturers supplying low- and middle-income countries.

## Moving Forward: HCV Drug Development

Making sense of the flood of data from HCV trials is difficult. New acronyms appear after each scientific meeting (see box: Terms for HCV Treatment Response by Population and Time Point); HCV treatment duration and strategy vary according to the characteristics of each drug and the populations it is studied in; and trial designs are becoming more complex. Interim reporting (at 4 and 12 weeks) and incomplete data (from press releases, posters, and brief presentations at conferences) add to the confusion. For example, in May 2010, Vertex issued a press release with results from ADVANCE, an international phase III trial of triple combination therapy (telaprevir plus SOC) in treatment-naïve people with HCV genotype 1. They reported an overall SVR of 75% (after 12 weeks of a telaprevir-based regimen plus SOC) without specifying treatment duration. A closer look at the data revealed that SVR for short-course treatment (24 versus 48 weeks) dropped to ~52%, still a significant improvement over SOC.

Ongoing studies are exploring DAA combination studies, shorter-course treatment, and response-guided therapy. Boehringer-Ingelheim, Bristol-Myers Squibb (BMS), Gilead, and Vertex have launched multidrug studies; these are proceeding in parallel with trials adding a single DAA to standard of care. Abbott, Anadys, Idenix, Merck, and Pharmasset have drugs from different classes in clinical development, but have yet to announce combination studies.

A tantalizing glimpse of an interferon-free future comes from Roche/Genentech's pioneering INFORM-1, a two-week proof-of-concept study combining danoprevir, an HCV protease inhibitor, with RG7128, an HCV polymerase inhibitor. The two drug combination worked well in treatment-naïve and treatment-experienced study participants with HCV genotype 1. INFORM-3, a longer combination study, has been delayed by a serious safety issue—elevated liver enzyme levels in some people who got the highest dose (900 mg) of danoprevir (in a different trial); this was resolved when the drug was stopped. Results from a study of ritonavir-boosted danoprevir (meaning that another drug, ritonavir, is used to keep danoprevir in the bloodstream longer to make it more effective, with a lower pill burden and frequency of dosing) will determine the optimal dose for future studies.

Meanwhile, four studies combining DAAs (with or without peginterferon and ribavirin) have begun. For more information on the Boehringer-Ingelheim study, go to [www.clinicaltrials.gov](http://www.clinicaltrials.gov).

- BMS has launched a study combining an HCV protease inhibitor (BMS-650032) with a first-in-class NS5a inhibitor (BMS-790052) with or without SOC, in prior null responders with HCV genotype 1.
- Gilead has opened a 28-day, two-arm study of GS-9256 (an HCV protease inhibitor plus GS-9190 (a non-nucleoside polymerase inhibitor), with and without ribavirin, followed by SOC in treatment-naïve people with HCV genotype 1.
- Vertex is combining telaprevir (an HCV protease inhibitor) with VX-222 (an HCV polymerase inhibitor) in treatment-naïve people with HCV genotype 1. Depending on randomization and early treatment response, participants will receive dual DAAs (followed by SOC if indicated) or quad therapy (DAAs plus SOC).

## Characteristics of the Class: HCV Protease Inhibitors

HCV-specific protease inhibitors will be the first DAA class available. This family of drugs has been used for more than a decade to treat HIV (in combination with other antiretroviral drugs). Protease inhibitors block cleaving of viral proteins (which would otherwise be reassembled into new virus particles) in the same way that inserting something between the blades of a scissor prevents them from cutting.

The first generation, Merck/Schering Plough's boceprevir and Vertex/Tibotec's telaprevir, are in phase III; barring unforeseen circumstances, approval is expected in early 2011. Although treatment strategies and durations differ (see Table 2: Dueling HCV Protease Inhibitors), adding one of these drugs to SOC has significantly boosted SVR among people with HCV genotype 1.

**Table 2. Dueling HCV Protease Inhibitors**

Drug	Dosing/ Pill Burden	SVR in Treatment-naïve People*	Duration of Treatment	Strategy	Drawbacks
Boceprevir	3 times daily, 12 pills/day	54–56% (no lead-in vs. lead-in; 24–28 weeks of treatment) 67–75% (no lead-in vs. lead-in; 44–48 weeks of treatment)	24–48 weeks 44–48 weeks	Triple therapy, or after a 4-week lead-in with SOC	Anemia; epoetin alfa used by ~50% in phase II; long treatment duration *Lack of data in treatment-experienced people; results of phase II trial difficult to interpret due to protocol amendments
Telaprevir	q.8h (every eight hours); or possibly q.12 h (every 12 hours)  6 pills/day (for q.8h regimen)	52–61% after 24 weeks 75% (48 weeks) *The same 24-week regimen was also effective for treatment- experienced people: overall, 51% Prior nonresponders: 31% Prior viral breakthrough: 57%. Prior relapse: 69%	8–12 weeks of triple therapy followed by 12–16 weeks of SOC alone (24 weeks total)	Triple therapy	Rash (which can be severe), anemia, itchy skin, nausea, vomiting, diarrhea

Sources: Berg 2010; Kwo 2009; McHutchison 2009, 2010; Vertex 2010.

Adherence to these drugs will be crucial, since resistance to an HCV protease inhibitor—or to the entire class (cross-resistance)—can develop or emerge within days. Adherence to the first generation of HCV protease inhibitors is likely to be challenging: ribavirin is taken twice daily; boceprevir and telaprevir need to be taken three times a day—although a study comparing twice-daily to thrice-daily dosing of telaprevir reported that efficacy was equivalent (Marcellin 2009). Pill count ranges from 6 (telaprevir) to 12 (boceprevir) per day, not including ribavirin.

Known side effects of HCV protease inhibitors include anemia, rash, anal itching and hemorrhoids, fatigue, nausea, vomiting, diarrhea, dysgeusia (bad taste in the mouth or changes in taste), headaches, dizziness, jaundice, and elevated alanine aminotransferase (ALT) and bilirubin.

**Table 3. HCV Protease Inhibitors in Development**

Agent/Sponsor	Status	Comments
ABT-450 Abbott	Phase I/II; HCV genotype 1, treatment-naive	Currently being studied with low-dose ritonavir
ACH-1625 Achillon	Phase Ib; HCV genotype 1, treatment-naive and treatment-experienced	Once-daily dosing will be explored in future trials
BI 201355 Boehringer Ingelheim	Phase II; HCV genotype 1, treatment-naive and treatment-experienced	May be a once-daily drug
BMS-650032 Bristol-Myers Squibb	Phase II; HCV genotypes 1 and 4, treatment-naive	Genotype 4 and people with cirrhosis added in phase IIb
BMS 824393 Bristol-Myers Squibb	Phase II (opening in July 2010); HCV genotype 1, treatment-naive	
Boceprevir Merck/Schering-Plough	Phase III; HCV genotype 1, treatment-naive and treatment-experienced	Used 3 times daily; large pill burden (12/day); anemia is common side effect; likely to be approved by 2011
CTS 1027 Conatus	Phase II; HCV genotype 1, null responders	24-week study with SOC
Danoprevir ITMN-191/RG 7227 Intermune/Genentech/Roche	Phase II; HCV genotype 1	Has been studied with RG 7128, a nucleoside polymerase inhibitor; dose-limiting liver toxicity was resolved with ritonavir boosting
GS 9256 Gilead Sciences	Phase II	Being studied in combination with GS 9190, a non-nucleoside HCV polymerase inhibitor
MK 5172 Merck	Phase I; HCV genotypes 1 and 3, males only	Demonstrated activity against resistant virus in lab studies and chimps
IDX 320 Idenix	Phase I; healthy volunteers	
TMC 435350 Tibotec	Phase IIa; HCV genotype 1, treatment-naive and treatment-experienced	Favorable dosing (possibly once daily); preliminary data suggests efficacy in treatment experienced
Telaprevir Vertex/Tibotec	Phase III; HCV genotypes 1, 2, 3, and 4, treatment-naive and treatment-experienced	Approval expected by 2011
Vaniprevir (MK 7009) Merck	Phase II; HCV genotype 1, treatment-experienced	A phase II trial in treatment-naive people with HCV genotype 1 is slated to open in August 2010
VX 985 Vertex	Phase I	

## Characteristics of the Class: HCV Polymerase Inhibitors

Nucleoside, nucleotide, and non-nucleoside polymerase inhibitors have been part of combination HIV treatment for years. Now, analogues of those drugs, made specifically for HCV, are in development. Nucleoside and nucleotide polymerase inhibitors are imperfect copies of nucleotides that insert themselves into hepatitis C RNA. Since they are faulty, other nucleotides cannot attach themselves; in other words, nucleoside and nucleotide polymerase inhibitors cause viral dead ends. Non-nucleoside polymerase inhibitors interfere with HCV replication by binding to the hepatitis C polymerase and preventing viral replication—it's as if the virus is a car trying to park in a space that just got too small for it.

Some nucleoside/nucleotide polymerase inhibitors have already been discontinued for toxicity, but other candidates in this promising class are moving forward. If these are safe, effective, and tolerable, nucleoside/nucleotide polymerase inhibitors are likely to become the backbone of HCV treatment, since they are active across genotypes and have a high genetic barrier to resistance (meaning that resistance to this family of drugs is less likely to develop than resistance to protease inhibitors and non-nucleoside polymerase inhibitors).

So far, the hepatitis C non-nucleoside polymerase inhibitors in development are active only against HCV genotype 1, and resistance develops quickly. In fact, mutations that confer resistance to non-nucleoside polymerase inhibitors have already been detected in people who have never taken these drugs (Dryer 2009).

It may be possible to combine non-nucleoside polymerase inhibitors, since the HCV polymerase has at least four binding sites.

Side effects reported in trials of nucleoside/tide and non-nucleoside polymerase inhibitors include nausea, vomiting, diarrhea, fever, weakness, flatulence, chills, headache, fatigue, and rash.

**Table 4. HCV Polymerase Inhibitors in Development**

<b>Non-nucleoside Polymerase Inhibitors</b>		
<b>Agent/Sponsor</b>	<b>Status</b>	<b>Comments</b>
ABT-333 Abbott	Phases I /II; HCV genotype 1, healthy volunteers and treatment-naive	
ABT-072 Abbott	Phases I /II; HCV genotype 1, healthy volunteers and treatment-naive	
ANA 598 Anadys	Phase II; HCV genotype 1, treatment-naive	Twice-daily dosing
BI-207127 Boehringer Ingelheim	Phase I; HCV genotype 1, treatment-naive and treatment experienced	Dosing is q.8 h
BMS 791325 Bristol-Myers Squibb	Phase I/II	
GS 9190 Gilead Sciences	Phase II; HCV genotype 1, treatment-naive	Being studied with SOC and in a combination trial with GS 9256, an HCV protease inhibitor
IDX 375 Idenix	Phase I; healthy volunteers	Possibly once- or twice-daily dosing
PF-00868554/Filibuvir Pfizer	Phase II; HCV genotype 1, treatment-naive	
VCH-222 Vertex	Phase II; HCV genotype 1, treatment-naive	
VCH-759 Vertex	Phase II; HCV genotype 1, treatment-naive	
<b>Nucleoside/Nucleotide Polymerase Inhibitors</b>		
<b>Agent/Sponsor</b>	<b>Status</b>	<b>Comments</b>
IDX 184 (nucleotide) Idenix	Phase IIa; HCV genotype 1, treatment-naive	Once-daily dosing
PSI 7977 (nucleotide) Pharmasset	Phase IIa; HCV genotype 1, treatment-naive	Once-daily dosing
RG 7128 (nucleoside) Roche/Genentech/Pharmasset	Phase II; HCV genotypes 1 and 4, treatment-naive; also studied in 20 prior nonresponders with HCV genotypes 2 and 3	Twice-daily dosing

## Characteristics of the Class: NS5a Inhibitors

NS5a inhibitors may have cross-genotype activity, can be used in combination with DAAs from other classes, and are likely to be effective in people who have developed resistance to other DAA classes.

BMS's first-in-class NS5a inhibitor demonstrated impressive potency after a single 100mg dose. Longer-term data on this drug, although promising, are limited to 12 weeks.

Side effect profile is unclear so far, aside from reports of headache.

**Table 5. NS5a Inhibitors in Development**

Non-nucleoside Polymerase Inhibitors		
Agent/Sponsor	Status	Comments
A-831 Arrows Therapeutics	Phase I	
BMS 790052 Bristol-Myers Squibb	Phase II; HCV genotype 1, treatment-naive and treatment-experienced	Studied in treatment-naive people (including people with cirrhosis) with SOC; also being studied in combination with BMS 650032 (protease inhibitor), plus or minus SOC, in null responders
CF-102 CAN-FITE	Phase I/II; HCV genotype 1	Also studied as a treatment for liver cancer
PPI-461 Presidio	Phase I; healthy volunteers	

## HCV Antivirals

Several antiviral agents, including cyclophilin inhibitors, silymarin, an NS4b inhibitor, an HCV entry inhibitor, a serine C-palmitoyltransferase inhibitor, are in development; more detail is available in TAG's upcoming *Hepatitis C Pipeline Report*.



## Nitazoxanide

Nitazoxanide (Alinia®), was approved in 2002, to treat diarrhea from two intestinal parasites (*Cryptosporidium parvum* and *Giardia lamblia*). Since then, it has been studied as a treatment for HCV genotypes 1 and 4 with SOC. Initially, nitazoxanide generated significant excitement, but SVR rates have been unimpressive so far, with the exception of a small Egyptian study in people with HCV genotype 4 (See Table 6: Nitazoxanide and SVR).

Nitazoxanide (NTZ) monotherapy is being studied to prevent post-transplant HCV recurrence, and in combination with SOC in HIV/HCV coinfecting people who have genotype 1 and have never been treated for hepatitis C.

**Table 6. Nitazoxanide and SVR**

Study	Population	SVR	Comments
STEALTH C-1; 12 weeks of NTZ, followed by 36 weeks of SOC or 12 weeks of NTZ, followed by 36 weeks of peginterferon vs. SOC	HCV genotype 4	61% NTZ + PEG 79% NTZ + SOC 50% SOC	In genotype 4, SVR ranges from 43 to 70% with SOC
STEALTH C-2; 4 weeks of NTZ or placebo, followed by 48 weeks of triple therapy (SOC+ NTZ or placebo)	HCV genotype 1, 80% null responders and nonresponders	7% (NTZ + SOC) vs. 0% (SOC + placebo)	Missing data on response to prior treatment in 20%
STEALTH C-3 4 weeks of NTZ or placebo, followed by 48 weeks of triple therapy (SOC+ NTZ or placebo)	HCV genotype 1, treatment -naive	44% (NTZ + SOC) 32% (placebo + SOC)	

Sources: Antaki 2009; Bacon 2010; Rossignol 2009; Shiffman 2010.

## Novel Interferons

Although the future of interferon is unclear, some sponsors have gambled on development of novel formulations. These novel formulations offer more convenient dosing, and—perhaps—fewer side effects. Development of delivery devices, such as external pumps or implants, is also underway.

**Table 7. Novel Interferon Formulations in Development**

<b>A Agent/Sponsor</b>	<b>Status</b>	<b>Comments</b>
Albupheron/Zalbin/Joulferon Human Genome Sciences/ Novartis	Phase III; HCV genotypes 1, 2, and 3, treatment-naïve and treatment-experienced	Dosed every two weeks; efficacy equivalent to peginterferon. The future of albupheron is unclear; European regulatory authorities have delayed its approval, although FDA filing is expected in 2010
Locteron interferon Biolex Therapeutics	Phase IIb; HCV genotype 1, treatment-naïve	Dosed every two weeks; may have more favorable side effect profile than peginterferon
PEG Interferon Lambda (PEG-rIL-29) Bristol-Myers Squibb/ ZymoGenetics	Phase II; HCV genotypes 1, 2, 3, and 4, treatment-naïve, with the exception of DAA monotherapy for 2 weeks	So far, side effect profile has been favorable; possibly because PEG-IFN Lambda binds to a unique receptor with less distribution throughout the body than the interferon alfa receptor

Other strategies to stimulate and enhance HCV-specific immune responses are being explored, including therapeutic vaccines, monoclonal antibodies, toll-like receptor agonists and interleukin-7. More detail will be available in TAG's upcoming *Hepatitis C Pipeline Report*.

## TAG Research Recommendations

- **Study drugs in clinically relevant populations prior to approval, such as African Americans and Latinos/-as, people with cirrhosis, current and former injection drug users, people with a history of psychiatric disorders, and HIV/HCV coinfecting persons.**

Often, response rates from HCV clinical trials do not apply to “real-life” populations. HCV treatment safety, efficacy, and tolerability must be characterized in high-prevalence populations, particularly those less responsive to SOC; those at risk for rapid progression of liver disease; and those usually excluded from clinical trials. So far, enrollment of African Americans and Latinos/-as in HCV treatment trials has been disappointing, hovering at approximately 10% (Kwo 2009; McHutchison 2009).

*TAG continues to track and document enrollment of African Americans and Latinos/-as in clinical trials, and pushes for sufficient enrollment of members of these populations in HCV clinical trials (Chou 2009).*

Numerous studies have reported that drug users can be safely and effectively treated with SOC (Bruggmann 2008; Dore 2010; Harris 2010; Hellard 2009). Once they are given

access to ongoing mental health care (including medication, if indicated), people with psychiatric disorders can be safely treated (Martin-Santos 2008; Schaefer 2003). Since depression, mood swings, hypomania, and mania are known side effects of interferon, it is sensible for clinical trials to offer a baseline psychiatric assessment, regular screening for neuropsychiatric side effects, and mental health care during clinical trials to avert treatment discontinuation.

Although HCV is curable, it can be fatal if untreated or unsuccessfully treated. HIV accelerates HCV progression, and SOC is less effective for coinfecting people than those with HCV mono-infection (see Table 1: HCV Treatment Outcomes, by Population). Hepatitis C–associated end-stage liver disease has become a leading cause of death among HIV-positive people in the United States and Western Europe, where HIV treatment is widely available (Weber 2006). Drug interactions between DAAs and HIV drugs may limit use of specific drugs in coinfecting people; this must be fully characterized early in development to facilitate HCV treatment trials—and ultimately, safe and effective use of DAAs in coinfecting people.

*TAG works with other activists, regulatory authorities, researchers, the pharmaceutical industry, harm reduction organizations, and clinicians to advocate for trials in representative populations. TAG has co-organized three multi-stakeholder meetings on HCV drug development for HIV/HCV coinfecting people with the European AIDS Community Advisory Board (ECAB). These meetings paved the way for preapproval HCV treatment trials in HIV/HCV coinfecting people by asking that “Trials of novel HCV therapies in HIV/HCV coinfecting people should begin before approval is granted for their use in HCV mono-infection, once results from Phase 2B studies are known, and there are indications from earlier toxicology, pharmacokinetic and drug–drug interaction studies that the specific agent, or agents under investigation will not have the potential for significant drug–drug interactions, or other toxicities relevant to HIV.” (Sitges Declaration, 2007). The consensus built at these meetings and continuing pressure from activists has paid off: HCV treatment trials in HIV/HCV coinfecting people are now being launched in parallel with phase III. Trials of boceprevir and telaprevir in HIV/HCV coinfecting people are underway.*

- **Develop mechanisms to provide early access to DAA combination therapy for people who are ineligible for clinical trials, and cannot wait for their approval.**

It is unacceptable that people with the most urgent need lack access to potentially life-saving therapies. Although preapproval access to single or multiple DAAs poses medical, administrative, and regulatory challenges, it has been accomplished in HIV and is certainly feasible for HCV. Regulators, industry, physicians, and community members need to address and surmount barriers to early access.

*In Spring 2010, TAG asked regulators and sponsors attending an FDA meeting on preapproval access to DAAs to develop a framework so that sponsors could provide potentially life-saving drugs to high-risk populations without endangering drug development programs.*

• **Study drugs in liver transplant candidates and recipients as soon as it is safe to do so.**

Hepatitis C is the leading indication for liver transplantation, accounting for more than 35% of all liver transplants in the United States (Thuluvath 2010). Survival after transplantation is significantly worsened by recurrent HCV, which is difficult to treat; SOC is often ineffective in or intolerable for transplant candidates and recipients (see Table 1: HCV Treatment Outcomes, by Population).

Despite their desperate need for better HCV treatment, clinical trials of new HCV drugs in transplant candidates and recipients are generally last on the list, lagging until drugs have already been approved. HCV clinical trials in transplant candidates and recipients should be launched prior to approval, and should allow use of other experimental agents—an approach used successfully in HIV research.

Clear regulatory guidance is needed to prod sponsors into launching studies in transplant candidates and recipients, as well as in other high-risk populations. For example, panelists at a 2006 FDA meeting on development of novel agents for HCV treatment recommended that “approval of an effective agent in compensated subjects should not be adversely affected by poor outcomes observed in separate studies of decompensated liver disease” (Sherman 2007).

*TAG continues to work with patients, activist groups, academic, and community-based researchers, regulatory agencies, and the pharmaceutical industry to ensure that new HCV drugs and treatment strategies are studied in people with the greatest need, as soon as it is safe to do so.*

In addition, TAG advocates for:

• **Prioritizing access to single or multiple DAAs for trial participants in the control arm of clinical trials, and those who did not achieve SVR.**

Crossover or rollover study designs provide access to an experimental drug for people in the control arm. This approach should be broadened to include study participants unsuccessfully treated with single or multiple DAAs, providing that virtual monotherapy (a multidrug regimen containing only one active agent) can be avoided. A cross-company registry of treatment-experienced trial participants should be established, and these participants should be prioritized for enrollment into trials of DAAs from novel classes.

- **Continued characterization of resistance to all classes of DAAs.** Further characterization of resistance mutations is needed to optimize HCV treatment with DAAs, although the clinical utility of resistance testing is not clear at present. Further assessment of clinical implications of HCV drug resistance is needed. One way to assess the impact of drug resistance would be to retreat people who acquired drug resistance in monotherapy trials with the same drug, plus SOC.
- **Development of second- and third-line drugs effective against commonly occurring resistance mutations.** Adding a single DAA increases the likelihood of SVR for treatment-experienced people, but is not 100% effective. In fact, ~60% of prior nonresponders did not achieve SVR after retreatment with telaprevir plus SOC in Vertex's PROVE-3 trial (McHutchison 2010). Thus, an increasing population of people resistant to at least one drug, or one class of drugs, is likely. Cross-resistance to HCV protease inhibitors has already been reported. Sponsors should prioritize drugs with a unique resistance profile and a high genetic barrier over "me-too" drugs.
- **Development of drugs with activity against all HCV genotypes. There are at least six HCV genotypes.** Most new HCV drugs were designed to be effective against HCV genotype 1, because it is difficult to cure with peginterferon and ribavirin, and it is predominant in the United States, Western Europe, and Japan (major pharmaceutical markets). But some people, such as current and former injection drug users and recipients of blood and blood products in the early to mid-1980s, are infected with more than one HCV genotype, and may require drugs with cross-genotype coverage (Preston.1995; Silva 2010).

As more people with genotype 1 are cured, and immigration patterns shift, global distribution of HCV genotypes will change. It will not be possible to eradicate HCV without safe and effective drugs for all genotypes.

- **Full characterization of predictors and indicators of response and nonresponse to HCV treatment across populations.** Stopping rules may change as HCV treatment evolves. Reliable predictors of response will motivate people to continue their HCV treatment, and facilitate reimbursement for response-guided therapy. In turn, accurate indicators of nonresponse will lower the risk of resistance, spare people from side effects, and save money.
- **Establishment of a system for HCV treatment strategy trials, to facilitate cross-company collaboration. It is time to scale up HCV research.** The

opportunity to address key clinical questions in the next five to seven years must not be squandered. Sponsors prioritize getting their drugs to market, and the current landscape is highly competitive. But HCV treatment is complex, and a dedicated research network could advance crucial areas—exploration of multi-experimental agent trials, population-specific questions, and development of treatment strategies—that are likely to languish without a public/private research network. This has been a fruitful approach in HIV disease, where policy makers have allocated funds and sponsors have contributed drugs and diagnostics.

In the meantime, regulators, researchers, sponsors, and community members need to continue the dialogue on launching cross-company collaborations.

- **Studying DAAs for HCV Prophylaxis**

There is no postexposure prophylactic strategy for hepatitis C, regardless of exposure type. HCV transmission from occupational exposures ranges from 0.2% to 10% (Corey 2009). Clearly, research on efficacy of DAAs for postexposure prophylaxis for occupational and nonoccupational exposures is warranted.

*TAG works with activist partners domestically and internationally to advocate for access to HCV treatment for all who need it.*

## Additional Resources

Information about clinical trials is available at: <http://www.clinicaltrials.gov> (accessed 4 June 2010).

HCV Advocate offers conference reporting, news, fact sheets, an up-to-date HCV pipeline chart, and other resources at <http://www.hcvadvocate.org> (accessed on 6th June 2010).

HIVandHepatitis.com provides news and conference reports at <http://www.hivandhepatitis.com> (accessed 6 June 2010).

The National AIDS Treatment Advocacy Project provides comprehensive coverage of HCV, HIV, and HBV research, access, treatment, and policy issues at <http://www.natap.org> (accessed 6 June 2010).

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# The Tuberculosis Pipeline Introduction

BY ELEONORA JIMENEZ

Dr. Robert Koch's identification and characterization of *Mycobacterium tuberculosis* (MTB) as the cause of tuberculosis (TB) in 1882 was fundamental in proving the relationship between microorganisms and disease, which revolutionized the study and treatment of infectious diseases. The first randomized control trial—considered the gold standard for clinical trials—evaluated streptomycin for the treatment of TB. By the mid-1980s, six-month combination treatment with four drugs could cure 95% of TB cases. However in the 1980s TB research went into hibernation, and despite all of these significant contributions, the scientific community failed to understand or control MTB. The microscope—used by Dr. Koch to discover MTB—is still the most commonly used diagnostic tool but detects fewer than 19% of all TB cases worldwide. A new class of TB drugs has not been approved in over 40 years; and some of the most powerful current drugs cannot be used with certain anti-HIV treatments. Bacille Calmette Guérin (BCG), the only licensed vaccine for TB disease, is almost 90 years old and offers little to no protection from pulmonary TB. Decades of neglect by funders, scientists, and political leaders has led to the unacceptable situation today where there are more TB cases than ever before. To successfully treat and cure TB disease, we must renew our commitment to use all our resources to accelerate the development of better vaccines, drugs and diagnostic tools.

## Introduction

*Mycobacterium tuberculosis* is a large, complex bacterium that causes tuberculosis disease in humans and other mammals. TB is a highly contagious disease that spreads from person to person when an infectious person discharges TB bacilli (germs) into the air by coughing, which are then inhaled into the lungs by another individual. Risk of infection increases in crowded environments with poor ventilation, and little to no sunlight or ultraviolet light exposure (Escombe 2009).

Approximately 90% of persons infected with TB are able to contain the bacilli for their entire lives and may never even know that they are infected with TB. Once

the TB bacilli enter the lung, the immune system sends cells to contain the bacteria and trap it in immunological prisons called granuloma. When this happens, TB does something that science has yet to fully understand—it changes its diet and stops and/or significantly slows down replication. At this point, TB is in latency. Most people latently infected with TB are able to maintain this state for the rest of their lives.

Despite the ease of transmission and the fact that one third of the world's population—2 billion people—is latently infected with TB, the disease is a disproportionately low priority on the global health agenda, as manifested by the lack of political will, meager funding, and inadequate progress against the disease. The majority of people infected with latent TB infection (LTBI) are able to contain the bacilli from causing symptoms and pose no risk of infecting others. However, annually, approximately 10% of those with LTBI go on to develop active TB disease. In 2008, an estimated 9.4 million people developed active TB disease (WHO 2009). Little is known about what triggers LTBI to progress to active TB disease but once TB is able to break out of its immunological prison, it is considered to have progressed to active TB disease. Children under the age of five, and people who are malnourished and/or immune-compromised are at increased risk of disease progression. In most cases, active TB disease develops in the lungs, but it can also manifest in other parts of the body (extrapulmonary TB)—which is much more common in infants, young children and people with HIV.

TB is a preventable and curable disease. Yet it is a killer, especially for pregnant women, children, people with HIV, and others who are malnourished or suffer from immune suppression. In 2008, TB claimed the lives of 1.82 million people, of which 500,000 occurred among people infected with HIV, making it the leading cause of death for people with HIV (WHO 2009).

These data reveal TB control efforts are failing, and that the Millennium Development Goal (MDG) to halt and reverse the incidence of TB by 2015, and eliminate it as a public health threat by 2050 will not be met. In 2008, the case detection rate for all forms of TB was only 61%, and the treatment success rate for reported TB cases in 2007 reached 86%—the first time this indicator has met the 85% target set by the World Health Assembly in 1991 (WHO 2009).

Despite the availability of drugs to treat and cure up to 95% of drug-susceptible TB cases—control efforts are weakened by concurrent HIV in Africa, soaring multidrug-resistant (MDR) TB rates in the former Soviet Union, and weak health systems almost everywhere.

## Multi-drug resistant (MDR) and Extensively drug-resistant (XDR) TB

Over the past 62 years, MTB has been exposed to single and multiple chemotherapy regimens, allowing MTB strains to evolve when treatment is inadequate, incomplete, intermittent, or inappropriate. Failure to properly treat drug-susceptible TB leads to the emergence of circulating strains of drug resistant TB. Multidrug-resistant (MDR-TB) and extensively drug-resistant TB (XDR-TB) are two types of drug-resistant TB strains defined by the number and types of drugs the TB bacilli are resistant to. MDR-TB is TB bacteria that are resistant to two of the most powerful first-line drugs, isoniazid and rifampicin. XDR-TB is TB bacteria that are resistant to any of the fluoroquinolone drugs (cipro-, gatiflox-, levo-, moxiflox-, or ofloxacin) and any one of the three second-line injectables (amikacin, capreomycin, or kanamycin), as well as isoniazid and rifampicin. Inadequate health and TB control systems facilitate the creation of drug resistant TB because they fail to properly treat drug-susceptible TB. Treatment for drug-susceptible TB normally involves a 6–8 month treatment regimen using four oral TB drugs, but patients regularly face adherence obstacles due to the high pill burden, drug-to-drug interactions, toxic side effects, drug stock outs and/or length of treatment. Consequently, when treatment is inconsistent, inadequate, or interrupted, the TB bacteria begin to mutate, develop resistance to the anti-TB medication, multiply and make the individual sick again.

Treatment for drug-resistant TB is complex and expensive. Diagnosis of MDR or XDR-TB requires sophisticated diagnostic tools, technicians and laboratory capacity, which are limited or non-existent in resource poor regions that need it most. The World Health Organization (WHO) estimates there were 500,000 new cases of MDR-TB in 2007—the highest number of MDR cases ever reported—of which only 30,000 cases were confirmed, and a mere 1% were started on treatment (WHO 2009a).

## TB Diagnostics, Prevention, Treatment and Care Challenges

Sputum smear microscopy, the most commonly used TB diagnostic tool, is over 125 years old. The test involves collecting a sputum sample coughed up from the lungs of a patient suspected of having TB, staining the sample, and identifying the rod-like shaped MTB bacteria under a microscope. Smear-positive TB is a diagnosis confirming the presence of actively replicating TB bacteria in the lungs. Unfortunately, the sputum smear test is not very accurate and at best captures 62% of new smear positive

cases (WHO 2009). The smear test functions particularly poor among children and immune-compromised individuals who have low bacterial load in their sputum, and is unable to diagnose TB outside the lungs or drug resistant TB (WHO, 2009). For instance, among people with HIV, smear microscopy detects only ~ 35% of cases (Corbett 2003), resulting in misdiagnosis and delays in accessing life-saving TB treatment. The HIV pandemic highlights the urgent need to develop an easy to use TB point-of-care diagnostic test that can perform well in health posts that do not have regular access to running water, electricity, or skilled laboratory technicians. This tool would benefit all people with TB that are currently unable to get accurate diagnoses, but will be especially helpful for TB/HIV coinfecting persons and children who are at greater risk for disease and death. In low and middle-income settings, where high HIV-related TB is prevalent, new diagnostic tools for use in health posts can be a major step forward in TB control by detecting more cases of HIV-infection related TB, connecting people to TB care more promptly, decreasing TB transmission in the community, and preventing future cases of drug-resistant TB (Dorman 2010).

In sub-Saharan Africa, where up to 70% of people with TB are HIV positive (WHO 2010b), health workers who suspect TB in the absence of a sputum smear positive test are recommended to use a culture test to confirm pulmonary or extrapulmonary TB disease (Getahun 2010). This test involves a lengthy process where sputum or other clinical samples are collected from a TB patient and placed in a solid or liquid media and left to grow until detectable. If TB grows in the media, the person is said to have a positive culture test (i.e. active TB disease). Since MTB multiplies once every 16–20 hours through a process known as binary fission, a clinician must wait 3–4 weeks to confirm drug-susceptible TB and up to 16 weeks for drug resistant TB strains using solid media. In settings where liquid culture is used, bacterial growth can be observed in 8–11 days or two to four weeks for drug-susceptible and drug-resistant TB, respectively.

Relying on a culture test not only requires time, but a well-resourced laboratory with skilled technicians and good biosafety measures to prevent contamination and protect laboratory personnel from infection. In low and middle-income countries, clinicians sometimes rely on one laboratory to culture TB bacteria for the entire country, leading to further delays and weak quality control (WHO 2009). Over the course of 3–4 weeks, a patient without treatment can infect at least three more individuals (Beresford 2010) and be at increased risk of TB morbidity and mortality.

The most widely administered vaccine in the world is the Bacille Calmette–Guérin, or BCG—the only vaccine licensed for TB. With over 100 million doses administered per year, it is estimated that the lives of over 40,000 children are saved annually. Unfortunately, BCG causes a potentially fatal reaction in HIV-infected infants and children and is therefore not recommended for use in this population. Considering that infants,

children and people with HIV, at any age, are at increased risk for TB disease progression, a new vaccine that is safe and effective for these vulnerable populations is vital to eradicating TB.

First-line TB treatment in people with HIV presents special challenges not seen among HIV-negative persons with TB. Among them are increased length of treatment, increased risk of drug toxicities, and higher pill burden and drug-drug interactions when TB treatment and anti-retroviral (ARV) treatments are taken together (Sterling 2010). Nevertheless, treatment success is possible if patients receive ongoing support and coordinated care, including careful monitoring of clinical outcomes for both TB and HIV.

From a prevention angle, data from clinical trials examining the use of ART before, during and after anti-TB regimens shows that early initiation of antiretroviral treatment (ARV) can greatly reduce the risk of developing active TB disease among people with HIV (Getahun 2007), and improve survival rates for HIV-infected individuals with confirmed active TB disease (Sterling 2010). Along with ARV treatment, a 6-9 month regimen of 300 mg of isoniazid preventative therapy is also recommended by WHO for people with HIV, once active TB disease is ruled out (WHO 2007c).

TB continues to outpace our gravely inadequate current global response efforts. The growing rates of HIV-related TB, MDR and XDR-TB underscore the need for bold leadership to mobilize resources that can address the serious gaps in our TB control efforts. To halt and reverse the incidence of TB by 2050 requires a substantial investment in funds to develop and roll out new TB diagnostic tools, better vaccines, and more tolerable TB treatments for use in resource-constrained settings. This investment is estimated at two billion dollars per year (TAG 2010) to meet research targets around new drugs, vaccines, diagnostics, basic science, applied and operational research. Since TB disproportionately impacts children and people with HIV, these new tools must address research and programmatic challenges to meet the needs of the communities at greatest risk for TB (Chamie 2010). The following chapters discuss in detail the latest developments in TB treatment, vaccines and diagnostics, and outline specific recommendations on how to move the research agenda forward.

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# The Tuberculosis Diagnostic Pipeline

BY JAVID SYED

## Introduction

After the doldrums of 40 years in which tuberculosis (TB) research languished without much funding or focused scientific attention, the last decade has brought a flurry of activity to the field for the development of new tools. The creation of the Stop TB Partnership in 2000 led to the first (2001–2005) and second (2006–2015) global plans to stop tuberculosis, the formation of the New Diagnostics Working Group within the partnership, and in parallel the creation of the Foundation for Innovative New Diagnostics (FIND), a product development partnership focused on new tuberculosis (and more recently, malaria and sleeping sickness) diagnostic development. Through these entities the Stop TB Partnership has aimed to harness new scientific tools and approaches to accelerate the discovery, development, approval, and distribution of new diagnostic technologies that could make diagnosing TB more accurate, faster, and more reliable. Until recently it was expected that there would be off-the-shelf technology approaches that could be easily adapted and scaled to diagnose TB in resource-limited settings—the so-called low-hanging fruit.

### Sensitivity and Specificity

*Sensitivity* is the ability of a diagnostic test to accurately identify the condition it is attempting to diagnose. The lower the sensitivity of the test the greater the chances that people with the condition will not be accurately identified by the test; this can lead to false negative results (in which TB is present but not detected).

*Specificity* is the ability of a test to accurately rule out the condition the test is seeking to diagnose. The lower the specificity the greater the chances that people without a condition will be falsely diagnosed as having it (the test will detect TB, but it is not there). Therefore, low specificity will lead to a greater number of false positive results.

An ideal test will have both—high sensitivity and high specificity.

Ten years later, the low-hanging fruit has yielded a small but flavorful harvest. Many advanced technologies are now ready to launch or are in place in central reference laboratories and in some referral laboratories in tertiary (usually major city hospital) settings. Both “rapid” (2–4 week) liquid culture techniques and nucleic acid amplification tests (which detect *Mycobacterium tuberculosis*, or MTB, DNA sequences) are available if the proper financial and technical support is provided. However, there has as yet been no breakthrough diagnostic test to revolutionize TB diagnostics in peripheral health post or community point-of-care settings. These peripheral health post settings are the most decentralized health care facilities that have inconsistent access to running water, electricity, trained laboratory workers, or any laboratory equipment and yet provide care for the largest number of people with TB. To date, TB is most commonly diagnosed with a 125-year-old sputum-smear microscopy test despite the fact that it has low sensitivity, is unable to diagnose TB disease if the bacterial load in the sputum is low (smear-negative TB), is unable to diagnose TB that is not in the lungs (extrapulmonary TB), can pick up acid-fast bacilli that are not TB, and cannot distinguish between drug-sensitive and drug-resistant TB. FIND estimates that only about 20% of TB cases worldwide are detected with sputum-smear microscopy (World Health Organization 2006). The failure of the most common diagnostic test to pick up more than half of TB cases, and the lack of facilities, meant that in 2008 only 61% of all forms of TB (smear-negative, smear-positive, and extrapulmonary) were reported by national TB programs to the World Health Organization (World Health Organization 2009b). Where programs do not exist no one will receive care, and even when programs do exist, if they rely on sputum-smear microscopy they will miss half the cases overall and much more among children and people with HIV who have higher levels of smear-negative and extrapulmonary TB. Cultivating the TB bacillus on solid or liquid growth media, or TB culture, is still considered the gold standard for diagnosis. Though the culture method is very sensitive, it too can be nonspecific, as nontuberculous mycobacteria will also be detected in culture, and subsequent speciation tests using a lateral flow (dipstick) TB antibody test such as the Tauns test are required to definitively identify the growing organisms as MTB. Culture does detect smear-negative and drug-resistant strains, and can even detect extrapulmonary TB if the right sample is drawn, but it is still far from ideal as it takes an average of up to two weeks to become detectable with rapid liquid culture and four weeks to grow to visible levels on solid media (Dorman 2010). Culture also requires skilled laboratory staff, electricity, and biosafety infrastructure in order to be performed safely and accurately. TB culture testing is not available or accessible for most people with TB who live in low-income countries and access care at health posts.

To address these challenges, from 2007 to 2009, the Strategic and Technical Advisory Group for TB (STAG-TB), the group that advises the World Health Organization

(WHO) on new policies including those for the uptake of new TB diagnostics, has approved at least seven new diagnostic approaches for use in high-TB-burden settings. These tools and strategies address some of the most pressing diagnostic challenges in TB care—the need to improve the sensitivity over sputum-smear microscopy and accelerate identification of TB and drug resistance. Despite the improvements offered by these new tools, the impact on TB control has not yet changed the situation on the ground in many places because most of the newly recommended diagnostics are not simple, robust, or cheap enough to use in the field. Increasingly, there is a recognition that though the rapid and sensitive liquid culture and nucleic acid amplification tests (NAATs) are great and urgently need to be rolled out, the real revolution in TB diagnostics will only occur when tools designed for the higher levels of health systems are complemented by an easy to use point-of-care diagnostic that is sensitive, specific, fast, cheap, robust, and safe (Cruciani 2004; World Health Organization 2007, 2009a, 2009b)

**Table 1. Diagnostic Tests Approved by the WHO, 2007–2009\***

Recommended Approach	Name of Test	Sponsor/ Developer	Technique	Measures	Health Systems in Which Test Is Most Likely to Be Used	Year of WHO Approval
Liquid culture	MGIT	BD Diagnostic Systems	Automated liquid culture	TB growth and drug-resistant TB	Reference laboratories	2007
Rapid speciation test	Capilia test	Taun, Standard Diagnostics, and FIND	Lateral flow technology that uses antibodies to detect MTB	MTB DNA	Reference laboratories	2007
Revised case definition of a sputum-positive pulmonary TB case to at least one TB bacilli in one sputum sample		Special Programme for Research and Training in Tropical Diseases (TDR)	Strategy to increase sensitivity of sputum-smear microscopy	TB bacilli	Peripheral laboratories	2007
Line-probe assays for multi-drug-resistant (MDR) TB	INNO-Lipa	Innogenetics	Line-probe assay that requires culture	Rifampicin resistant mutation in MTB DNA	Peripheral laboratories	2008
	GenoType MTBDRplus	HAIN Lifescience	Line-probe assay that can be done on sputum	Isoniazid- and rifampicin-resistant mutation in MTB DNA		
Front-loaded sputum-smear microscopy		TDR	Strategy to prevent dropouts in the diagnostic process by reducing number of clinic visits needed for sputum-smear microscopy	TB bacilli	Peripheral laboratories	2009

Light-emitting diode (LED) microscopy	LED adaptor for existing microscope	LW Scientific and Fraen	Fluorescent microscopy	TB bacilli	Peripheral laboratories	2009
	Primo Star iLED microscopy	Carl Zeiss Inc. and FIND	Fluorescent microscopy	TB bacilli		
Noncommercial culture and drug susceptibility testing (DST)	Microscopic observation drug susceptibility (MODS)	Academic laboratories	Inverted light microscopy that detects TB growth	TB growth and drug-resistant TB	Reference laboratories	2009
	Nitrate reductase assay	Academic laboratories	Solid culture; TB growth causes color change	TB growth and drug-resistant TB		
	Colorimetric DST	Academic laboratories	Solid culture; TB growth causes color change	TB growth and drug-resistant TB		

\*Sources: World Health Organization 2007, 2008b, 2009b

## What Is in the TB Diagnostic Pipeline?

Products in the diagnostics pipeline attempt to address the most pressing challenges faced by TB control by making diagnostic tools available to the lower rungs of health care systems and by developing new tools that are more sensitive, specific, and faster than currently available tools to confirm TB infection or disease and identify drug-resistant strains. It is disappointing that there are no new tools in development currently targeted at the health post level, where the greatest number of people with suspected or active TB are seen.

After the introduction of a number of new tools and technologies in the last few years, it appears that the low-hanging fruit has nearly all been harvested. None of the diagnostic tools in this year's pipeline are appropriate for use at the health post or the point of care. A number of tools covered in last year's *Pipeline Report*, such as light emitting diode (LED) microscopes and the LED adaptor for diagnosing TB using fluorescent microscopy; the strategy to collect sputum samples on the same day to prevent attrition of TB patients during the diagnostic pathway; and the microscopic observation drug susceptibility (MODS) test to detect TB and drug resistance using an inverted light microscope were recommended by STAG-TB in 2009 for use in TB control programs.

Diagnostic tools or strategies from last year's *Pipeline Report* that were dropped this year include fluorescent vital dye staining, sputum concentration strategies to improve the sensitivity of sputum-smear microscopy, the MPT-64 skin patch test that Sequella was developing for detection of latent TB infection, and the MTB DNA test that FIND is still working on with Spaxen and University College London.

This year we focus on technical approaches to TB diagnosis that are the most likely to be ready for review by the STAG-TB in the coming three years, and for which there is at least some peer-reviewed literature.

The impact of a diagnostic tool in reducing disease and death is defined not only by its sensitivity and specificity but also by the health system level at which it can be used. Therefore, we examine these tools according to where within health systems they will be deployed:

- Health posts with or without consistent sources of water and electricity, trained laboratory workers, or laboratory equipment—these serve 60% of people in search of TB care.
- Peripheral laboratory settings that can conduct sputum-smear microscopy, have some trained staff but limited infrastructure and biosafety systems—these serve about 25% of people needing TB services.
- Reference laboratories, with the most trained staff, highest biosafety levels, and most reliable clean water and electricity supplies, usually capable of carrying out at least TB culture if not NAAT as well—accessible to at most 15% of people (O’Brien 2009).

**Table 2. TB Diagnostic Test or Processes in the Pipeline, 2010**

Name of Test or Process	Sponsor/Developer	Technique	Measures	Estimated Date of WHO Review
<b>Peripheral Laboratories</b>				
Manual loop-mediated isothermal amplification process (LAMP)	Eiken Chemical and FIND	Manual nucleic acid	MTB DNA	2011
Clearview Lipoarabinomannin (LAM) antigen enzyme-linked immunoabsorbent assay (ELISA)	Inverness Medical Innovations	ELISA to detect LAM antigen in urine	MTB LAM antigen	2012
<b>Reference Laboratories</b>				
GeneXpert MTB/RIF	Cepheid, FIND and UMDNJ*	Automated nucleic acid amplification test	MTB DNA, rifampicin resistance sequences	2011
QuantiFERON-TB Gold Test	Cellestis	Interferon-gamma release assay	Immune cell response to latent TB infection	2011
T-SPOT.TB	Oxford Immunotec	Interferon-gamma release assay	Immune cell response to latent TB infection	2011

\*UMDNJ-University of Medicine and Dentistry of New Jersey

## The Health Post Setting

As Treatment Action Group (TAG) has documented in the past five years, despite significant progress at the higher-tech level suitable for reference and some peripheral laboratories, investment in the basic and applied science necessary to discover and develop a true point-of-care test for TB disease is shockingly inadequate. There is no peer-reviewed information available to shed any further light on a tool that is likely to be approved by STAG-TB in the next three years for use at the health post (Harrington 2008, Treatment Action Group 2009).

## Peripheral Laboratories

### The Eiken LAMP Nucleic Acid Test

Sputum or other fluid samples are subjected to a loop-mediated isothermal amplification process (LAMP) to amplify and detect TB DNA for active disease diagnosis. This test is easier than previous NAAT TB tests as it does not require heating or cooling—the LAMP device can amplify DNA at a constant temperature of 65 degrees Celsius. A prototype of this LAMP test developed by Eiken Chemical was studied by FIND in Peru, Bangladesh, and Tanzania. We previously reported in 2009 that LAMP had a sensitivity of 97.7% in culture-positive, smear-positive specimens. The sensitivity was poor (48.8%) in smear-negative, culture-positive specimens (Boehme 2007). Subsequently the test has been redesigned and is currently being studied. Data from the redesigned product may be reviewed by the WHO in 2011.

*Advantages:* The LAMP test is easier to conduct than other NAATs due to its isothermal nature. Sample preparation and test readout takes less than two hours. In feasibility studies, laboratory technicians with no prior experience with NAAT could learn to conduct this test in about one week. No DNA contamination was observed even when the test was conducted in one room without biosafety cabinets.

*Limitations:* This test is manual and requires trained laboratory staff, electricity, and laboratory infrastructure, which will prevent its use below peripheral laboratory settings. The redesigned version of this test needs to be validated to address the lower sensitivity that was previously observed among smear-negative, culture-positive patients.

## Clearview LAM antigen ELISA

Lipoarabinomannin (LAM) is a TB cell wall protein excreted in urine. This test, developed by Inverness Medical Innovations, is currently marketed in an enzyme-linked immunoabsorbent assay (ELISA) format as the Clearview LAM Antigen ELISA. Antibodies to LAM present in the test well bind any protein found in urine and when a reagent is added a color change indicates a positive readout (Inverness Medical Innovations 2010).

Several recent studies have examined this test in settings with high burdens of TB and HIV. One assessment of LAM's utility was nested in a prospective study designed to determine the predictors and causes of death among hospitalized TB suspects. All enrolled patients were given HIV antibody and LAM antigen tests. Of 499 persons enrolled, 422 were HIV-positive. The LAM test was 59% sensitive and 96% specific among all patients with culture-confirmed TB. Among people with HIV the LAM test was 67% sensitive and 95% specific and the LAM test's sensitivity was highest in people with a low number of CD4 cells. When stratified by CD4 levels, sensitivity was 55% for those with CD4 greater than 200; 14% for those between 150 and 200; 56% for those between 100 and 150; 71% for those between 50 and 100; and 85% for those lower than 50. Among the 193 confirmed TB cases in this study LAM alone was more sensitive than sputum-smear microscopy alone (32% vs. 16%), though neither sensitivity was optimal. Both LAM and sputum-smear microscopy together identified 75% of the confirmed TB cases (Shah 2009).

An earlier version of the LAM test was also studied in Harare, Zimbabwe, in those suspected of having TB and in registered TB patients who were recruited from hospital settings and tested for HIV and TB. The HIV prevalence in this study population was 77% and the TB prevalence in enrolled patients regardless of HIV status was 49%. LAM test sensitivity was 52% in HIV-positive and 21% in HIV-negative persons. Compared to the 52% sensitivity of LAM, the 60% sensitivity of the sputum smear was higher for people with TB and HIV. In this study the sensitivity of the test was not stratified by CD4 cell count (Mutetwa 2009).

A third study of LAM was conducted among South African HIV-positive adults who had not been on antiretroviral therapy (ART) and were not diagnosed with TB. Sputum and urine were tested for TB, using fluorescence microscopy and liquid culture for sputum and LAM ELISA for urine. In this study the sensitivity of the sputum smear alone was 14% and did not differ by CD4 cell count, while the LAM test was 38% sensitive. LAM sensitivity was strongly associated with lower CD4 cell counts. Among those with fewer than 50 CD4 cells, LAM was 67% sensitive, while among those with CD4 between 50 and 100 it was 35% sensitive, with just 4% sensitivity in

those with CD4 counts over 100. The combined sensitivity of the sputum smear and LAM was highest at 67%, among those with fewer than 50 CD4 cells (Lawn 2009).

Inverness is developing a rapid LAM antigen test that uses the same lateral flow platform that is in its Determine test kit for HIV-1 and HIV-2. The goal is to detect LAM in unprocessed urine within 20 minutes. No additional information is yet public regarding this approach.

The LAM studies indicate that this test will only be useful in TB/HIV patients with very low CD4 counts—which may be helpful, as this population is especially likely to be sputum-smear negative or have extrapulmonary TB. If LAM testing is implemented, it will be important to define an algorithm by which to optimize its use in the diagnostic pathway. The Tuberculosis Clinical Diagnostics Research Consortium, which was established in 2009 through a seven-year grant to the Johns Hopkins University School of Medicine from the National Institute of Allergy and Infectious Diseases (NIAID), will study the LAM test to determine its feasibility.

*Advantages:* The LAM test provides a result in less than 3 hours' time (compared with 14 days for rapid liquid culture or MODS). The urine test has the advantage of being noninvasive and the sample is easier to collect than sputum. It is most sensitive among HIV-positive TB patients with very low CD4 cell levels, a population at increased risk of being missed by the sputum-smear test because of greater chances of having extrapulmonary and smear-negative TB. When used with sputum-smear microscopy, the combined sensitivity of the two tests appears to provide important data to help rapidly identify patients in urgent need of TB treatment.

*Limitations:* The sensitivity of the test in people who are not HIV-positive or with CD4 counts above 100–200 is quite poor. The current test is in an ELISA format and is not appropriate for use in lower rungs of the health care system.

## The Reference Laboratory Setting

### GeneXpert MTB/RIF

Cepheid is developing the GeneXpert MTB/RIF test in partnership with FIND and the University of Medicine and Dentistry of New Jersey. This closed-system NAAT was initially developed to detect anthrax for the U.S. Postal Service and is now being modified to diagnose TB bacilli and rifampicin-resistant strains. The test cartridge, into which sputum or another fluid sample is administered, contains all the reagents, does not require much sample processing, and is able to detect rifampicin-resistant TB in less than two hours.



Initial results of the test reported in 2009 were very promising, showing its sensitivity for TB detection to be 99.1% in smear-positive and culture-positive specimens and 80% in smear-negative and culture-positive specimens. The high degree of sensitivity of the test, especially in smear-negative and culture-positive specimens, requires that the test is run three times on each sample. Unpublished data presented by FIND at the South African TB Conference in June 2010 showed that when each sample is only tested once the test's sensitivity is 67.2% in smear-negative and culture-positive specimens while the sensitivity in smear-positive cases remains very high at 99%. The specificity of the test was 95.7% in initial studies conducted in Latvia and Peru; its ability to detect rifampicin resistance had 100% sensitivity and 96.7% specificity (O'Brien 2009, Roscigno 2010).

Recent data corroborate the initial data and show the test's high degree of sensitivity and specificity. In this newer study the test was able to detect all 23 commonly occurring rifampicin-resistant strain sequences. The study was conducted in Vietnam in 107 sputum samples of persons suspected of having tuberculosis and demonstrated that the GeneXpert was able to detect all 29 of the smear-positive and culture-positive cases—84.6% of the smear-negative, culture-positive samples that were identified through growth on solid media and 71.7% of smear-negative culture-positive cases grown on both solid and liquid media. The test identified all 25 of the culture-negative samples. The test was able to detect 98.4% of the 55 culture-positive cases among retreatment cases in Uganda and 100% of the 9 rifampicin-resistant cases. In addition to the test performance, the buffer in the test was shown to reduce the viability of the tuberculosis significantly, thereby reducing biohazard concerns (Helb 2010).

*Advantages:* The GeneXpert test addresses some of the biggest challenges in TB diagnostics. It is highly sensitive and specific in smear-positive TB cases as well as in detecting rifampicin-resistant strains. Its sensitivity in smear-negative cases is moderate, at 67.2%, when the test is done only once, and increases to 80% when the test is repeated three times on these specimens. The test requires minimal training of laboratory workers and provides results within two hours. As it is a closed-system test it does not require laboratories with high levels of biosafety and has low contamination concerns.

*Limitations:* The test requires a consistent source of electricity that will limit its use outside of settings where a regular power supply can be guaranteed. Currently the cost per test cartridge is over \$20 and the instrument cost is a whopping \$25,000. These costs will pose significant barriers for its uptake, though it may become cheaper if widely used due to economies of scale.

## Immune-based Tests for Latent TB Infection

### Interferon-Gamma Release Assays

Interferon-gamma release assays (IGRAs) expose a blood sample to TB antigens that are specific for MTB and not found in the Bacille Calmette Guerin (BCG) vaccine or in most environmental mycobacteria. The production of interferon-gamma (IFN-gamma), a protein produced by a primed immune system when it recognizes an antigen it has been previously exposed to, indicates TB infection. The three IGRAs that are available on the market are QUANTIFeron Gold test (QFT-G) and QUANTIFeron Gold in a Tube test (QFT-GIT), both produced by Cellestis, and T-SPOT.*TB* from Oxford Immunotec.

BCG vaccination does not cause false positives in the IGRA, as is often the case with the tuberculin skin test (TST). IGRAs are hoped to be more specific than TSTs in detecting TB infection.

IGRAs have been studied for a variety of diagnostic needs—from predicting risk of progression from latent to active disease to monitoring TB treatment response and TB diagnosis among the immunocompromised. A review article has concluded that the IGRAs were less sensitive in diagnosing latent TB infection (LTBI) where TB burdens were high (69%) compared with settings with low burdens of TB (83%). The pooled specificity of IGRAs was between 93 and 99%. Studies comparing TST and IGRA sensitivity in diagnosing TB infection at lower CD4 cell counts found that IGRAs—and especially T-SPOT.*TB*—were less prone to false negatives due to immune suppression, but that the IGRAs were also affected by low CD4 cell counts. One recent study showed that at CD4 cell counts lower than 100, IGRAs had high rates of indeterminate results. Other studies have shown that IGRAs are not useful in diagnosing TB disease or monitoring TB treatment success. Their utility in predicting risk of progression to active TB disease is unclear (Aabye 2009, Dheda 2009, Hoffman 2010, Lienhardt 2010, Pai 2010).

Some limited cost-effectiveness data suggest that IGRAs are best used after an initial TST to rule out false positive results. On the other hand, it might be cheaper to do a symptom screen for active TB, test for HIV, and administer isoniazid preventive therapy to those who are HIV-positive and without TB symptoms. Putting in two cumbersome and expensive tests would further complicate the diagnostic labyrinth that people in high-TB-burden settings must routinely navigate to get proper treatment. The cost-effectiveness of IGRAs is affected by LTBI prevalence and thus needs to be adjusted to the context in which the tests are being conducted (Pooran 2010).

The WHO convened an expert committee in July 2010 to assess available data and develop recommendations on whether to use IGRAs in detecting TB disease or LTBI. The IGRAs will also be discussed at the WHO STAG-TB meeting in 2010.

### **The QuantiFERON Gold Test and QuantiFERON Gold in a Tube**

Cellestis has developed two versions of the QuantiFERON test (QFT), which has been approved by the U.S. Centers for Disease Control and Prevention for use in place of the TST.

These blood tests require the sample to be processed within 16 hours. The sample is incubated with the TB antigens for 16 to 24 hours and an ELISA measures the presence of IFN-gamma. The QFT-GIT offers an additional benefit over the QFT-G by already containing the TB antigen in the tube and is therefore easier to use (Cellestis 2010).

A meta-analysis has shown that the sensitivity of these tests in detecting TB infection was not very high, at 78% for QFT-G and 70% for QFT-GIT, though the specificity of both of the QFTs was 99% in non-BCG-vaccinated persons and 96% in persons who have been vaccinated with BCG (Pai 2008).

*Advantages:* QFTs are more specific than TSTs in identifying TB infection and provide results in 24 hours, rather than the 72 hours needed for TST and neither QFT-G nor QFT-GIT require a person to come back to the health center to have the test read. However, the 24 hours needed to get a test result do require some system through which the result can be communicated to those diagnosed with TB infection and lead to appropriate follow-up.

*Limitations:* The tests requires samples to be processed within 16 hours on an ELISA format, which prevents them from being used in health facilities at levels below reference laboratories. The tests have moderate sensitivity (70% for QFT-GIT and 78% for QFT-G) when compared to the T-SPOT.TB test (90%). Test accuracy is reduced at lower CD4 cell levels and the time to result is still too long at 24 hours (Pai 2008).

### **The T-SPOT.TB Test**

This IGRA also exposes the sample to TB antigens and measures the presence of IFN-gamma to detect TB infection. Unlike QFT sample preparation, the T-SPOT.TB test requires centrifugation to extract mononuclear cells and these cells must be counted before the test is run. The sample preparation and test must be run within eight hours of collecting the sample (Oxford Immunotec 2010).

*Advantages:* There are some data that suggest that the T-SPOT.*TB* test is more sensitive than the QFTs and is also less likely than the QFTs or TST to be affected by CD4 cell levels. The T-SPOT.*TB* test does not react to prior BCG vaccination or most other environmental mycobacteria (Dheda 2009, Pai 2008).

*Limitations:* In the best case, the test will not be decentralized beyond the peripheral laboratory. Compared to the QFTs, the T-SPOT.*TB* test requires a greater degree of sample processing that will need more laboratory tools and greater level of skill among laboratory staff. The blood sample has to be processed within eight hours of being collected and this presents additional logistical challenges.

## Policy and Research Recommendations

### **Develop clear policy recommendations and build laboratory capacity for rollout, uptake, and impact assessment of new diagnostic TB tools.**

The ultimate goal of introducing new diagnostics is to reduce the burden of disease. Though at least seven new tools have been recommended since 2007, there has not been a significant uptake of these tools in national TB programs. This is likely due to a combination of factors that range from the general conservativeness of the TB community to embrace new tools, to the more legitimate need for laboratory capacity strengthening and clearer guidance that recommends specific tools within diagnostic algorithms appropriate for epidemiologic contexts. For instance, cost-effectiveness data that can show improvements in clinical outcomes will help national TB programs decide on the combination of tools most appropriate for their country's TB epidemiology (Pai 2010).

Recent discussions at a Stop TB Partnership meeting to develop an operational research agenda for the *Global Plan to Stop TB 2006–2015* discussed the need to address these research questions to ensure greater uptake of new tools. The NIAID-funded Tuberculosis Clinical Diagnostics Research Consortium will also be addressing this current gap by conducting accuracy and feasibility studies for diagnostic tools that are in late-stage development for which a proof of concept already exists but has not yet been tested extensively in clinical settings (Federal Business Opportunities 2010). The UNTAID-funded \$87.5 million project Expanding Access to New Diagnostics for TB (EXPAND-TB) also aims to strengthen laboratory capacity in 27 countries by 2013. EXPAND-TB is a collaboration of the Global Laboratory Initiative, FIND, the WHO, and the Stop TB Partnership's Global Drug Facility. These efforts are likely to ensure that the incremental improvements offered by new TB diagnostic tools are implemented to their greatest potential to reduce the burden of disease and death caused by TB.

### **Clarify not only which tools work but also which ones do not work.**

A 2006 report by FIND and the WHO's Special Programme for Research and Training in Tropical Diseases (TDR) estimated that the world annually spent \$1 billion on TB diagnostics. In the absence of strong regulatory oversight of TB diagnostics, the WHO needs to provide clear guidance clarifying not only which tools are effective but also which ones are not useful to ensure funds are not wasted on unvalidated diagnostic tests. As part of this effort, the TDR conducted a systematic review of 19 serological tests in 2008 to examine their utility in diagnosing TB. The review showed that the serological tests were much less specific and sensitive than the sputum-smear test and should not be used for diagnosis. The WHO is convening an expert committee in July 2010 to examine all data available regarding the utility of serological tests to diagnose TB, and STAG-TB will likely provide clarification of what role these tests will or won't play in TB control efforts (World Health Organization 2006, 2008a).

### **Accelerate targeted development of a point-of-care assay for all forms of TB disease.**

New TB diagnostic development has opportunistically taken advantage of tools that were already in late-stage development. Many of the tools recommended by WHO put existing tools into public health facilities of countries with high-TB burden where they were not previously utilized or in lower rungs of the health system. However, the result has been that tools that fill certain critical gaps are only practical at district and referral laboratory settings. There is an increasing recognition that these tools at the higher levels of health systems will only contribute to a revolution in TB diagnostics if they are complemented by a point-of-care (POC) TB diagnostic, such as a dipstick, appropriate for use at the health post setting. However, a clear project-driven plan that is focused on a POC dipstick that has clearly defined minimum specifications has not been articulated. Such a product specification needs to drive a coordinated funding and scientific effort to meet this most urgent gap in TB diagnostics.

To develop such a product specification, TAG, Médecines Sans Frontières (MSF), and Partners in Health convened a group of experts in March 2009 that included basic researchers, product developers, laboratory technicians, activists focused on improving TB care, and TB program implementers. This group developed the minimum specifications required for a TB POC dipstick (Treatment Action Group 2009).

While discussing the TB POC specifications, a number of barriers to developing such a tool were identified. These included the need for specimen banks that have samples relevant for the development of a TB POC test from well-characterized patient populations as well as coordination and collaboration between research efforts to identify

antibodies and antigens to assess a combination appropriate for a POC test. Though a number of specimen banks currently exist, such as those organized by FIND and the TDR, it was not clear whether they were sufficient and accessible. At the same time, though FIND has been conducting a systematic search to validate biomarkers for a TB POC test, a number of basic science researchers think that the antigens and antibodies appropriate for a good first-line POC tool are already known but that efforts between researchers has not been coordinated well enough to identify an appropriate combination.

TAG has been working with partners to bring clarity to the above issues and define a way forward, calling for the NIAID to put out a request for information and ideas or convene a meeting to gather the best information and ideas available for antigens, antibodies, and diagnostic test platforms that could lead to a TB POC test. This open, public, and transparent process would allow for a clear assessment of what is currently known and what is needed to push the field forward—whether it is funding, the need for more basic science, a combination of both, or an entirely new approach.

To help create a well-informed advocacy effort for a TB POC test, TAG, MSF and the TB/HIV Working Group of the Stop TB Partnership are working with researchers based at the Imperial College in London to conduct an independent assessment of what is known about antigens, antibodies, and the technology platforms available and to clarify what infrastructure hurdles need to be addressed to develop a TB POC diagnostic tool. A report from this assessment to be completed in mid-2010 will inform TAG and our partner's future advocacy for a TB POC diagnostic test.

### **Significantly expand research capacity.**

Currently, research being conducted in TB drugs and vaccines does not sufficiently include diagnostic components. Such studies can provide important data on the utility of new TB diagnostic tools. Specimens from the study cohorts, if organized in a well-characterized sample bank, can also be a critical resource for biomarker discovery research. If diagnostic tools that already have proof of concept are incorporated into these studies, valuable data on specificity, sensitivity, and patient-relevant data could be collected that would help inform the WHO's policy making. The sample bank created from the study population can also be used to identify and validate biomarkers that can predict treatment outcomes. Biomarkers that can reliably predict treatment outcome can significantly lower cost and time required for TB drug trials by reducing the time and effort required to perform follow up on study participants for at least 12 months to ensure that the regimen being studied is at least as effective as the existing standard of treatment.

To expand the research capacity for TB, TAG has urged NIAID to expand the mandate of its HIV clinical trial networks and sites to include TB-focused research. In 2009, NIAID announced its intent to expand the focus of the AIDS Adult Clinical Trials Group (ACTG) and the HIV Vaccine Trials Network to include TB and other diseases of importance for public health. TAG and other research activists have responded to the NIAID call for information highlighting the need for research to develop a POC tool for TB.

In June 2010 the National Institutes of Health, the Federal Drug Administration, and the Centers for Disease Control and Prevention, three of the leading U.S. government agencies involved in TB research and programs, organized a meeting to address the need for new TB diagnostics as well as the need to harvest samples from TB drug and vaccine trials to create a well-characterized sample bank that could be used to identify and validate biomarkers that can predict treatment outcomes. Though this initiative is in its inception, TAG commends the leadership of these three U.S. agencies coming together to address the gap in TB diagnostics—a gap that hampers not only TB programs but research. The creation of an accessible sample bank from a well-characterized cohort of study participants will greatly assist the efforts of diagnostic developers whose research priorities are in line with global TB control efforts.

### **Increase funding for TB diagnostics research.**

In 2008 the world spent \$49.7 million on TB diagnostics research and development, which is 10% of the total \$491 million invested in TB research (Treatment Action Group 2010). TAG has tracked the resources invested in TB research and development since 2005. We have consistently advocated for an investment of \$2 billion per year in TB research overall—including not only investment in new tools but also in basic science and operational research—in order to reach the targets set out in the *Global Plan to Stop TB 2006–2015*. Starting in 2006, TAG called attention to the initial inadequacy of the *Global Plan's* research estimates, as they did not sufficiently account for basic science or operational research and were neither evidence-based nor sufficiently ambitious. The Stop TB Partnership is currently in the process of revising the research components of the Global Plan to incorporate basic science, operational research, and evidence-based budgeting for the discovery and development of new tools. This first evidence-based and comprehensive TB research plan will be completed by the end of 2010. TAG will continue to track the resources available for funding and advocate for research funders to better coordinate their portfolios to ensure that there is adequate, growing, and coordinated support for a comprehensive research agenda toward the elimination of tuberculosis.

## Conclusion

The significant efforts of those working in TB diagnostics—most notably the Stop TB Partnership’s New Diagnostics Working Group, FIND, the TDR, and a number of academic laboratories and for-profit companies—have yielded a slew of new tools that have addressed important gaps in the current armamentarium. However, most of these tools have only addressed gaps at the reference and highly skilled peripheral lab levels and have not yet been translated into any significant measurable or reported improvement in TB case detection or treatment outcomes. This is in large part because the tools being developed are not appropriate for peripheral health post settings in low-income countries where most people with TB access care.

In the past decade, most of the efforts to develop diagnostic tools for TB have attempted to take advantage of recent scientific breakthroughs or to modify existing tools to better serve TB care and control efforts. Yet there is a need to move from this opportunistic strategy to a focused strategy driven by end-user-defined product specifications. Without such a focused and coordinated effort supported by researchers, funders, TB program leadership, and activist groups, there is a great danger that we will soon have consumed all the low-hanging fruit without successfully fertilizing the soil for the emergence of a revolutionary new crop of TB diagnostics to prevent, through rapid point of care diagnosis, the nearly 9 million new TB new cases and 2 million TB deaths that occur each year .

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# The Tuberculosis Treatment Pipeline

BY CLAIRE WINGFIELD

## Introduction: A Robust Pipeline, but Uncertain Support

Over the past several years we have seen a level of activity in tuberculosis (TB) drug research that hasn't been witnessed since the heady days of the 1950s and '60s when the introduction of combination therapy and the regulatory approval of rifampin revolutionized TB treatment. Indeed, the advent of combination curative chemotherapy for tuberculosis predated the anti-HIV combination antiretroviral therapy (ART) revolution of 1996 by over forty years. The current TB drug pipeline is the fullest and most promising it has been in 50 years, with 10 drug candidates currently in clinical trials—a level unprecedented since the 1960s. Not all of these drugs are new to TB treatment, as some have been used off-label to treat TB. Yet there are still insufficient data to best guide their use. Other drug candidates consist of new molecules with novel, unique ways of inhibiting or killing the TB bacteria and second-generation drugs with better activity and hopefully better safety profiles than their predecessors.

The enthusiasm inspired by this recent progress must be balanced with a realistic acknowledgment that even with ten drugs in the pipeline—six of them novel—it is not robust enough to achieve the World Health Organization's (WHO's) goal of halving global TB incidence from 1990 levels by 2015, nor the Stop TB Partnership's target of having six new TB drugs approved by then. There are an estimated 2 billion people infected with TB around the world, compared with approximately 33 million infected with HIV (UNAIDS 2009; World Health Organization 2009). Over the past 23 years the U.S. Food and Drug Administration (FDA) has approved 24 new compounds to treat HIV infection. During that same time, the FDA approved just one new drug—rifapentine—to treat TB from an existing class of drugs, the rifamycins.

TB is most common in poor countries and communities; despite over nine million new cases each year, the vast majority of patients are unable to pay for the drugs. Because TB is an airborne infectious bacterium, curing TB disease is a public health responsibility yet governments have not allocated the necessary resources to cover the cost of TB diagnosis and treatment. A major rationale for the 50-year drought in TB drug development—aside from complacency of the fact that the disease was curable with existing, off-patent drugs—was the belief that new TB drugs would never

command the blockbuster sales so beloved of pharmaceutical marketing and planning departments when compared with chronic diseases of the developed world such as cancer, diabetes, or heart disease. Thus, pharmaceutical companies felt limited incentive to invest in developing new treatments for TB, despite the emergence in the early 1990s around the world of deadlier, harder-to-cure forms of drug-resistant TB. But companies may be underestimating the potential for profits from superior new drugs and treatment regimens that could improve cure rates and shorten treatment duration, potentially making TB cures accessible to the world's poorest people even in cases of drug-resistant disease.

## If TB Is Curable Why Are New Drugs Needed?

Despite the fact that first-line treatment is able to cure 95% of all drug-susceptible TB, almost two million people died of TB in 2008; of these, 500,000 were HIV positive (World Health Organization 2009). Of the nine million people newly diagnosed with TB in 2008, only 61% were notified of their status (World Health Organization 2009) meaning that the status of a whopping 39% was not recorded and that many of these people likely were never diagnosed or treated properly. The majority of these cases occurred where TB services are underresourced within poorly functioning health systems. TB control programs put the onus on the person with symptoms to seek out diagnosis. Even when someone—particularly young children and people with HIV—appears for diagnosis at a microscopy center, they may remain undiagnosed because the most commonly used tool, smear microscopy, misses extrapulmonary and smear-negative TB. In many treatment programs, properly diagnosed patients are required to travel to health centers every day to pick up their medications for the entire duration of six to eight months of first-line treatment. This burden can make accessing treatment untenable for many patients and costs time and resources for the patient and the health system. It would be difficult to try to improve on the 95% cure rate for drug-susceptible disease, but a substantially shorter treatment regimen—perhaps ten days to cure (similar to that of other bacterial infections), or even two months—would vastly improve treatment adherence and significantly ease burdens on TB patients and health systems.

The emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) and the rise of TB/HIV coinfection have further complicated clinical management of TB and the delivery of primary health services. Health care providers lack reliable information about which drugs to use and for how long to treat and cure drug-resistant TB. As a result, cure rates only reach 70% for MDR-TB patients in the best-run health systems, and drop to 30% for XDR-TB patients who are HIV negative. For many drug-resistant patients, treatment regimens are based on drug availability, many of them unlicensed for TB. Meanwhile, in some parts of sub-Saharan Africa up to 70% of TB patients are coinfecting with HIV (World Health Organization

2009). One of the most powerful first-line TB drugs, rifampin, interacts with some antiretrovirals (ARVs), specifically nevirapine, the most common component of first-line therapy globally, and boosted protease inhibitors—the backbone of second-line ART. The appropriate substitute TB drug, rifabutin, is not available in most parts of the world, and where it is, it is far too expensive.

### **Antiretroviral Therapy as TB Prevention**

ART has been shown to reduce the incidence of tuberculosis among people with HIV (Lawn 2005; Wood 2009). Since the initiation of ART in the Gugulethu township of Western Cape, South Africa, TB incidence among people on ART decreased by 80%, while TB incidence has remained stable among HIV-negative and HIV-positive individuals not on ART. TB mortality rates among HIV-positive people have been brought down to comparable levels to those of HIV-negative individuals (Middelkoop 2009). This evidence reinforces the WHO's recommendation that all TB/HIV coinfecting persons should be given ART regardless of CD4 cell count. The South African Ministry of Health has shown leadership by revising its ART guidelines to include this recommendation. It is important to remember that ART cannot replace TB treatment for latent infection or active disease because it does not kill the TB bacteria. Rather, ART, by reducing HIV levels, allow the immune system to recover enough to counter tuberculosis and other infectious diseases.

Despite the fact that people with HIV, infants, and young children bear an undue burden of TB morbidity and mortality, they are often excluded from many clinical trials (Burman. 2008; Ma 2010; Marais 2010). As a result there is a dearth of age-specific data on the correct dosing of TB drugs in children, or how best to dose TB drugs and ARVs concurrently. TB treatment researchers have begun to grapple with how to include these “special” populations in clinical studies safely and effectively. One solution has been for different research consortia to work together to increase the enrollment of young children and people with HIV in clinical trials. However, most trials exclude anyone with smear-negative or extrapulmonary TB, ruling out most children and most people with HIV up front. In a sign of progress, however, at least one developer—Tibotec—of a novel TB drug, has submitted a plan to evaluate its compound TMC207 in children.

Pregnant women are also consistently excluded from participation in TB clinical trials despite their high risk for TB morbidity and mortality. Women bear the greatest burden of both TB and HIV during their childbearing years. In fact, TB kills more women in this age group than all maternal cause mortality—maternal death during and shortly after pregnancy—combined: it is estimated that 15% of maternal deaths

are among women coinfecting with TB and HIV (Gupta 2009; Marais 2010; Mofenson and Laughton 2007). These dire facts demand a fundamental shift in trial designs to include pregnant women and women who may become pregnant in drug trials.

## Accelerating Research

Despite these challenges there have been some promising developments within the TB treatment research field over the past year that have the potential to increase research capacity, speed up the development of new treatment strategies and accelerate approval of new regimens:

- The Tuberculosis Trials Consortium (TBTC), a research network funded by the U.S. Centers for Disease Control and Prevention (CDC) has been conducting TB drug trials for over 20 years. The consortium was recently reconfigured to include more international sites with increased capacity to conduct treatment trials for drug-resistant TB and enroll more HIV-positive volunteers and children into studies. The shift to evenly distributing its study sites between U.S.-based and international research institutions and a broader research agenda demonstrates the TBTC's commitment to filling important gaps in our knowledge of the best ways to treat and cure both active TB and latent infection.
- In 2009, Dr. Anthony Fauci, the head of U.S. National Institute of Allergies and Infectious Diseases (NIAID)—the largest public funder of TB research in the world—announced that TB would be integrated as one of the focal areas of its reconfigured HIV clinical trials network system, including the AIDS Clinical Trials Group, the HIV Vaccine Trials Network, and other sites (Fauci 2009). By broadening the scope of the NIAID-supported clinical trials infrastructure to include studies evaluating TB drugs for both monoinfection and HIV coinfection, the TB treatment research field will in short order almost double its capacity to conduct clinical trials in high- and medium-TB-burden settings among geographically and demographically diverse groups.
- In March 2010, the Critical Path to TB Drug Regimens (CPTR) Initiative was launched to accelerate the evaluation and regulatory approval of novel TB treatment regimens. The initiative is a collaborative effort of public- and private-sector stakeholders—including the FDA, the Critical Path Institute, the Alliance for Global TB Drug Development (TB Alliance), the Bill and Melinda Gates Foundation, and several pharmaceutical companies including Anacor, AstraZeneca, Bayer, Novartis, Otsuka, Pfizer, Sanofi-Aventis,

Sequella, and Tibotec/Johnson & Johnson—to identify more efficient ways to study TB drugs in combination to expedite regimen change rather than introducing TB drugs sequentially. The move to regimen development is a paradigm shift for the field and will require regulatory agencies, research institutions, funders, policy makers, and advocates to alter their strategies to work collaboratively to ensure that the efficient testing and approval of new regimens is safe and maximizes resources.

## Getting Back to Basics

A great number of unanswered questions about TB pathogenesis make up some of the biggest challenges to discovering new and better TB treatments. Researchers are unable to explain why only some people progress from latent infection to active disease or why most immunocompetent people remain healthy despite infection. We do not yet fully understand what happens to the TB bacterium when it goes into latency and what—at the molecular or cellular levels—triggers reactivation. These knowledge gaps hamper the development of new TB treatments and highlight our lack of understanding of how the current drugs work alone and in combination to inhibit and kill the bacteria. The spectrum of TB disease needs to be better characterized to identify which drugs or combinations of drugs are potent enough to halt and/or cure each phase of the bacterial life cycle. This knowledge could also lead to the development of better surrogate markers—biological measures that might indicate a treatment effect and predict clinical outcomes—to predict the efficacy of TB drugs in individual patients or in clinical trials.

Starting in the early 1990s, the FDA began to approve HIV drugs based on their effect on surrogate markers such as, at first, changes in CD4 cell levels and finally, after the development of quantitative real-time polymerase chain reaction tests, changes in HIV RNA levels (viral load). These changes allowed for an unprecedented acceleration of clinical trials, paving the way for the combination ART revolution of 1996. However, most approved TB drugs came to market in an era when such techniques did not exist. Reading the earliest randomized clinical trial in humans, of the antibiotic streptomycin for monotherapy of TB in the 1940s, one would think the measures used—including chest X-ray and solid bacterial culture, as well as clinical improvement and relapse—were somewhat primitive, except that now in 2010, some 60 years later, virtually the same tools are used to measure TB drug activity. With TB, there is no way to measure drug activity in real time. Unfortunately for TB patients and researchers, at this time the TB field lacks a test that can predict whether a drug or regimen will result in a stable cure for a patient.

## The Pipeline

### The TB Treatment Pipeline—Drugs in Clinical Trials, July 2010

Agent	Class	Corporate Sponsor	Trial Sponsors	Status	Indication
AZD5847*	Oxazolidinone	AstraZeneca	AstraZeneca	Phase I	
PNU-100480*	Oxazolidinone	Pfizer	Pfizer	Phase I	DR-TB
SQ 109*	Diamine	Sequella	Sequella	Phases I/II	DS-TB/DR-TB
PA-824*	Nitroimidazole		TB Alliance	Phases II	DS-TB
OPC-67683*	Nitroimidazole	Otsuka	Otsuka	Phase II	MDR-TB
TMC207*	Diarylquinolone	Tibotec	TB Alliance	Phase I	DS-TB
		Tibotec	Tibotec/J&J (MDR indication)	Phase II	MDR-TB
Linezolid (LZD)	Oxazolidinone	Pfizer	TBTC	Phase II	MDR/XDR-TB
			NIAD	Phase II	XDR-TB
Rifapentine (RPT)	Rifamycin	Sanofi-Aventis	TBTC	Phase II	DS-TB
			FDA/University of Cape Town/the Johns Hopkins University	Phase II	DS-TB
		Sanofi-Aventis	TBTC	Phase III	LTBI
Moxifloxacin (Moxi)	Fluoroquinolone	Bayer	TB Alliance	Phase III	DS-TB
Gatifloxacin (Gati)	Fluoroquinolone		OFLOTB/TDR	Phase III	DS-TB

Notes: \*Indicates novel drug candidates; LTBI = Latent TB infection; DS-TB = drug-susceptible TB; DR-TB = drug-resistant TB; MDR-TB = multidrug-resistant TB; XDR-TB = extensively drug-resistant TB.

## So What's New?

There are six novel compounds being evaluated in clinical trials. Two of these new compounds could be considered for regulatory approval within the next two years; they are Tibotec Pharmaceuticals' diarylquinolone TMC207 and Otsuka Pharmaceuticals' nitroimidoxazole OPC-67683. Both companies have indicated that they will pursue accelerated regulatory approval based on favorable results in ongoing phase II studies taking place in patients with MDR-TB.

In June 2008, Tibotec (a subsidiary of Johnson & Johnson) published preliminary data from stage 1 of a phase II placebo-controlled, randomized trial showing faster time to culture conversion and a higher number of culture conversions after eight weeks of standard background regimen plus TMC207 in patients with MDR-TB (Diacon 2009). A second stage of the phase II study is comparing six months of placebo or TMC207



plus standard background regimen. Stage 2 patients have recently completed their last dose. There is still follow-up being done with stage 2 patients while they complete their background regimen. An interim analysis of both stages will take place in summer 2010. There is some evidence to suggest that TMC207 may affect ARV levels, so Tibotec has initiated drug-to-drug interaction studies with its compound and boosted protease inhibitors nevirapine and efavirenz. Tibotec is recruiting at multiple sites in Europe, Asia, and Africa for an open-label trial of TMC207 for adults who have smear-positive, confirmed MDR- or XDR-TB, including people with HIV. Tibotec is the only drug developer that has submitted a pediatric development plan to regulatory authorities to guide future clinical studies of TMC207 in children to establish safe and effective dosing based on age and development (McNeeley 2010).

Otsuka has completed enrollment of a phase II, double-blind, randomized controlled study comparing twice-daily doses of 100 mg and 200 mg of OPC-67683 plus optimized background therapy (OBT) to placebo plus OBT in volunteers with confirmed MDR-TB at sites in Europe, Asia, South America, Egypt, and the United States. Volunteers must stay in the hospital for the 56-day treatment period, and then be followed in the community for an additional 28 days after treatment. All patients who complete the double-blind portion will then be eligible to enroll in an open-label study of OPC-67683 for six months. Otsuka is also enrolling a study of patients in Latvia who did not have a meaningful clinical response after being treated for nine months or more with second-line drugs. Otsuka has completed ARV drug-to-drug interaction studies of OPC-67683 with tenofovir and lopinavir/ritonavir; results are not yet public. A study looking at OPC-67683 with efavirenz was recently initiated (Geiter 2010).

Both companies are in early discussions about phase III studies for their drugs, but no definitive decisions will be made until data from phase II are available. However, if the results from the second stage of the TMC207 trial confirm the stage I interim analysis, it is very likely that Tibotec will apply to the FDA and the European Medicines Agency (EMA) for early regulatory approval. The approval would be conditional and would require Tibotec to continue to collect confirmatory post-marketing safety and efficacy data. If conditional approval is granted, TMC207 may be brought to market in 2011. Otsuka may also seek regulatory approval for OPC-67683 after its phase II studies are complete. Many ARVs have been granted accelerated approval, but this has never been done for a TB drug before. There may be unanticipated hurdles for drug sponsors, researchers, activists, and regulatory authorities. The FDA and EMA have been working with sponsors to establish the regulatory requirements to ensure that there are sufficient data to determine the safety and efficacy of new TB drugs after phase II/III clinical studies are complete. There is significant concern whether or not regulators in countries with a high burden of MDR-TB will have the resources to grant accelerated approval or if they will be willing to follow the recommendations of the FDA and the EMA.

PA-824 is a novel compound—a nitroimidazole like Otsuka's OPC-67683—that was licensed by the TB Alliance from the former biotechnology company Chiron. The compound hit a snag last year when the FDA put it on a clinical hold due to preclinical reports of cataracts in animals; however, in July 2009, the FDA released the clinical hold and the next month the TB Alliance initiated a planned, second 14-day early bactericidal activity (EBA) study—a clinical trial assessing the ability of varying doses of a drug to rapidly kill metabolically active TB bacilli. The study evaluated doses of 50, 100, 150 and 200 mg per day over a 14-day period. PA-824 continues to appear safe and well tolerated, with no evidence that the drug causes cataracts in humans. Once the final data of the second EBA study are complete and analyzed, these results will form the basis for choosing a dose of PA-824 to take into later stage clinical trials. The TB Alliance is planning to evaluate PA-824 in novel regimens for both drug-susceptible and drug-resistant TB, if funding allows. The first EBA study of a novel PA-824-containing three-drug combination is being planned for initiation in the second half of 2011 (Seidel 2010).

Following the June 2009 announcement of an agreement between the TB Alliance and Tibotec/Johnson & Johnson granting the TB Alliance rights to develop Tibotec's TMC207 for drug-susceptible TB, the TB Alliance conducted a phase I drug-to-drug interaction study further examining the interaction between this drug and rifamycins (specifically, rifampicin and rifapentine). Results are expected by August 2010. The TB Alliance is also conducting a 14-day EBA study of TMC207 in order to explore the potential for lowering the dose for future clinical trials. Further, the TB Alliance is planning to initiate a 14-day EBA study of a novel drug combination containing TMC207 in the second half of 2010. Finally, Tibotec and the TB Alliance are collaborating on a drug discovery program to identify second-generation diarylquinolines—the same drug class as TMC207. Under the terms of the agreement, the TB Alliance will own the rights to any new compound through a royalty-free license to facilitate lower pricing (Seidel 2010).

## Second-generation Compounds

SQ 109 is a distant cousin of ethambutol—a drug used in first-line treatment to prevent the development of isoniazid-resistant TB—that has and is undergoing multiple EBA studies to determine optimal dosing. The drug's sponsor Sequella is working the Pan African Consortium for Evaluating Anti-tuberculosis Agents (PanACEA) to evaluate SQ 109 for drug-susceptible TB. Phase II/III studies are expected to begin enrollment in 2011. In parallel, Sequella intends to evaluate SQ 109 in drug-resistant TB (Horwith 2010).

Pfizer is nearly finished with a multidose study of its second-generation oxazolidinone PNU-100480 in healthy volunteers. Mouse studies have shown PNU-100480 to have superior activity over linezolid, an earlier oxazolidinone, at lower doses. Pfizer is plan-

ning the first study of PNU-100480 in TB patients and intends to study the compound for drug-resistant TB (Wallis 2010).

AstraZeneca Pharmaceuticals also has a second-generation oxazolidinone, AZT5847, that is currently in phase I safety, tolerability, and PK dose-escalation studies in healthy volunteers. Results are expected later in 2010.

## Latent TB Infection

Each person who is latently infected with TB is a potential future case of TB disease. Therefore it is vital for TB control efforts to prevent the progression of latent TB infection to active disease. Treatment for latent infection remains a conundrum for most national TB programs. The current strategy is to give 6–9 months of isoniazid preventive therapy (IPT). Study after study has shown IPT to be a valuable intervention in reducing the incidence of TB disease but its implementation presents multiple challenges for underresourced programs. IPT is, by its very nature, a therapy for healthy people, thus making the long duration of treatment, side effects, and uncertainty of durability an adherence challenge. But many policy makers and clinicians in high-TB-burden countries have been reluctant to implement IPT as an intervention for fear of missing a diagnosis of active disease and putting people on isoniazid monotherapy. Because young children who are contacts of adult TB cases and people with HIV who are latently infected with TB are at increased risk for TB disease progression and death, failure to implement this intervention can be deadly. A few operational studies and clinical trials are underway to evaluate strategies to make preventive therapy easier to operationalize.

The CDC, in collaboration with the Botswana Ministry of Health, conducted the BO-TUSA trial, which compared 36 months of daily IPT to a standard six-month regimen of IPT in people with HIV for the treatment of latent TB infection. The study demonstrated that continuous IPT significantly reduced the risk of developing TB disease in HIV-positive persons who tested positive for exposure to TB using a tuberculin skin test (TST). In both the 6- and 36-month arms the protective effect of IPT waned 6 months after treatment completion (Samandari 2010)—likely due, in part, to reinfection.

The Consortium to Respond Effectively to the AIDS/TB Epidemic (CREATE) is made up of research institutions based in the United States, Brazil, South Africa, and Zambia that are conducting IPT and intensified case finding studies in high-HIV-prevalence settings. Two of the three studies, THRio and Thibela, are evaluating the provision of IPT to people with HIV in urban clinics in Brazil and to gold mine workers (of any HIV status) in South Africa.

As part of the THRio study, health care workers in 29 HIV clinics in Rio de Janeiro received training to use a TST to detect TB among—and to provide IPT to—people who are accessing HIV care and treatment. Over 18,000 HIV-positive clients have been included in the study, and of these over 1,300 received IPT and more than 80% completed therapy (Eldred 2010). Although the primary outcome data of TB incidence are pending, a baseline study conducted in these clinics revealed that combined ART and IPT is more effective in reducing TB incidence than either used individually (Golub 2007).

In the Thibela study, over 27,000 miners have been screened for TB and more than 24,000 have begun IPT. Trial results are due later this year. In implementing IPT, researchers identified more cases of active TB than had been diagnosed through regular gold mine clinical care. This highlights the benefits of intensified case finding before initiating IPT, and could address some of the concerns raised by policy makers about misdiagnosing active TB disease as latent infection. Adverse events and adherence data will be released in fall 2010 (Eldred 2010).

The TBTC is expecting to complete data analysis this fall on Study 26, which is evaluating a 12-week, once-weekly rifapentine/isoniazid regimen versus 9 months of daily isoniazid. If the study demonstrates that the shorter regimen is superior or at least equivalent to 9 months of IPT, it has the potential to simplify adherence. Two substudies of TBTC Study 26 looked at liver toxicity (hepatotoxicity) and pharmacokinetics—how a drug is absorbed, distributed, metabolized, and eliminated by the body—in children. Pediatric dosing is not always studied as it should be—many sponsors simply extrapolate from adult data, which can be misleading—so the TBTC pediatric pharmacokinetic substudy can be a model of how to include children in TB drug studies.

## Drug-susceptible TB

In light of the fact that first-line TB treatment has a cure rate of 95%, the cost and logistics required to recruit thousands of volunteers and establish hundreds of clinical trial sites to demonstrate statistical improvement from the current standard of care appears unlikely. So how can treatment for drug-susceptible TB be improved upon? Rather than showing superiority over current treatment standards, the aim of current research is to identify a new drug regimen or regimens that will improve treatment success by shortening treatment duration, simplifying dosing schedules, and improving side-effect profiles as well as improving treatment outcomes in pediatric TB and TB/HIV coinfection.

Fluoroquinolones—a class of broad-spectrum antibiotics used to treat a variety of bacterial infections—have been used in the treatment of drug-resistant TB since the 1990s, but are also being evaluated as part of a shortened first-line treatment regimen. There

are two phase III studies looking at fluoroquinolones to shorten treatment for drug-susceptible TB from six months to four months.

The REMox TB trial is being conducted by a collaborative of research institutions including the TB Alliance, Bayer HealthCare, and University College London. Moxifloxacin—a newer fluoroquinolone—is being evaluated to replace either ethambutol or isoniazid as part of a treatment-shortening regimen. The last patient visit will be in the second half of 2012 (Seidel 2010).

The OFLOTUB consortium—a partnership of researchers based in Africa and Europe and the WHO's Special Programme for Research and Training in Tropical Diseases (TDR)—completed follow-up of all volunteers from its study evaluating gatifloxacin as part of a treatment-shortening first-line regimen. Safety and efficacy results are expected to be released by the end of 2010 (Lienhardt 2010).

Several other drug-susceptible studies are comparing rifapentine to rifampin as part of the backbone of first-line treatment. The two drugs are from the same class of drugs, rifamycins, and have good penetration and sterilizing ability—meaning that they are able to kill active *and* slowly reproducing TB bacteria—but both are contraindicated for use with certain commonly used ARVs such as nevirapine and boosted protease inhibitors. Rifampin has been one of the most powerful and widely used TB drugs since the 1970s (Fox 1999). Some preliminary data suggest that rifapentine may be more bactericidal—able to kill TB bacteria—than rifampin at lower doses and is better tolerated at higher doses. Several phase II studies are evaluating the use of rifapentine to shorten first-line treatment.

The TBTC has almost fully enrolled Study 29, which is a phase IIb trial comparing rifapentine to rifampin during the intensive phase—the first two months—of treatment for drug-susceptible TB. The Johns Hopkins University, the University of Cape Town, and the the University of Cape Town Lung Institute began enrolling a safety and efficacy study of two doses of rifapentine during the intensive phase of first-line treatment in TB/HIV coinfecting adults with CD4 counts above 200 as compared to standard of care (Efron 2010). The Johns Hopkins University in collaboration with the Federal University of Rio de Janeiro is conducting a second rifapentine study evaluating the safety and efficacy of rifapentine/moxifloxacin in place of rifampicin/ethambutol during the intensive phase of first-line treatment. Study completion is expected in late 2011 (Efron 2010).

A four-month regimen might become a reality within the next five years, but the challenge will be to get national TB programs to adopt the new shorter regimen(s), train health care workers to implement new treatment guidelines, and build patients' TB

treatment literacy to increase demand. Operational and implementation research needs to be scaled up by national programs to expedite and support the adoption of successful strategies where they are needed most.

## Rethinking Last-Chance Drugs

The TBTC recently completed enrollment in the LiMiT study (also known as TBTC Study 30), which is evaluating the safety and tolerability of low-dose linezolid in volunteers with confirmed MDR- and XDR-TB. Linezolid is an oxazolidinone antibiotic that has been used since the 1990s to treat drug-resistant TB; occasionally it has been used as a last resort for XDR-TB regimens, as it has a nasty side-effect profile including irreversible peripheral neuropathy. The TBTC study is using a lower dose of the drug in hopes that it will be safer and more tolerable.

By May 2010 NIAID enrolled 24 of a target of 40 chronic XDR-TB patients in a study evaluating two doses of linezolid (600 mg and 300 mg). In the study, volunteers failing all treatment for the previous six months are enrolled into two arms—one that starts linezolid immediately and one that delays starting the drug for two months. An interim data analysis for sputum conversion is expected to occur sometime in summer 2010 to determine if it is still ethical to continue the delayed arm (Barry 2010).

NIAID is evaluating a broad-spectrum antibiotic, metronidazole, which has been used but never formally indicated for drug-resistant TB. The Data Safety Monitoring Board (DSMB)—an independent committee that reviews ongoing studies to ensure that study volunteers are not exposed to undue harm—officially recommended closing new enrollment into the study after an excess of adverse events, including peripheral neuropathies and seizures, in volunteers receiving the study drug. Only half the projected enrollment was achieved, and it is uncertain whether there will be enough data to support going forward with a new trial using a lower dose. Data analysis is ongoing, and patients enrolled prior to the DSMB closure will continue to be followed up per protocol (Barry 2010).

## Where Is Lupin?

For the past few years, Treatment Action Group's TB drug pipeline has included Lupin Pharmaceutical's LL-3858. According to the Stop TB Partnership's working group on new TB drugs' website, Lupin is set to begin phase II clinical studies of the compound. No representatives from Lupin have attended any of the numerous TB research meetings to present recent data on the compound. Thus, the future of LL-3858 remains unknown. Considering that the field is moving toward novel regimens that will require

greater collaboration between drug developers and research institutions, Lupin seems to be still waiting on the platform while the train has already left the station.

## Recommendations

Relative to the past 50 years, TB treatment research is making significant progress, but not enough is being done to eliminate TB as a public health threat by 2050.

Pregnant women, children, and people with HIV must be included in clinical trials of new TB drugs and regimens. These groups bear a higher risk for TB death, thus underscoring the need for research that will lead to appropriate treatment and dosing. Their inclusion in clinical trials should be planned from the beginning of the development process and not as add-ons once phase III studies are being initiated.

Investment to build the capacity of activists to understand and advocate for research on new drugs and the uptake of these new tools is critical. The achievements of HIV research activists demonstrate the value and influence patients and affected communities can bring to innovation in research and regulatory processes. Research institutions and local trial sites need to engage community members through community advisory boards and educational workshops, and to develop research and TB literacy materials to increase awareness and acceptance of TB research.

TB and TB/HIV activists need to pay attention to research taking place in their communities and make contact with researchers. Activists need to understand the research process and the potential impact of research findings on clinical care and advocate for the uptake of promising interventions by national programs.

Policy makers must think more innovatively about their national TB guidelines and be unafraid to challenge the status quo. Many TB control programs and protocols rely on evidence dating back decades and do not take into account new data. Ministries of Finance need to ensure that national TB programs are adequately resourced and Ministries of Health must adopt new evidence-based treatment strategies quickly and consistently.

Otsuka and Tibotec have shown that it is possible to conduct drug registration trials in areas where drug-resistant TB is being treated; however, a great deal of additional capacity is needed to navigate the regulatory bodies and sustain local research infrastructure. Because there is limited (or no) experience conducting studies in compliance with the regulatory standards of the International Conference on Harmonisation and the WHO's Good Clinical Practice in many of these countries, sponsors of studies will need to commit sizable resources to strengthen the research infrastructure. This capacity development is not just

for researchers but also for regulatory authorities. Therefore, the governments of high- and medium-TB-burden countries need to adequately resource these regulatory bodies so that they are able to respond to trial sponsors and provide timely feedback.

TB treatment research consistently receives the most funding of any area within TB research—\$174 million in 2008, representing 35% of all monies spent on TB research—yet it is still wholly insufficient to address the gaps to support even current efforts (Treatment Action Group 2010). The field needs more funders to invest in TB research including basic science, which is the foundation for the development of all new tools. More funds are necessary to ensure that there are sufficient resources to conduct phase III and IV studies of the compounds already in the pipeline and support the development of those in discovery and preclinical studies. Likewise, operational research needs to be prioritized by national programs to prepare for the quick adoption of promising new interventions.

The great news is that there are ten compounds, six of which are new drugs, in clinical trials. The bad news is that funding for phase III and IV studies is not guaranteed and the capacity to conduct studies in line with regulatory requirements is limited. Initiatives like the CPTR and the expansion of trial networks are fostering greater collaboration among research institutions. As part of these efforts, building capacity of researchers in high- and medium-TB-burden countries to conduct registration trials and supporting national programs to conduct operational research must be a priority. The research priorities and activities should be driven by the realities on the ground. More than anything, radical change is required from all stakeholders to ensure that TB control is equipped with the necessary tools to prevent, diagnose, and cure TB in all populations.

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# The Tuberculosis Vaccine Pipeline

BY CLAIRE WINGFIELD

A vaccine that can safely and effectively protect infants, children, and adults, regardless of HIV status, against pulmonary and extrapulmonary tuberculosis (TB) will be required if we are ever to eliminate tuberculosis as a global public health threat. A 2009 study on TB incidence using mathematical modeling estimated the impact of new TB vaccines, diagnostics, and treatments found that a novel preexposure vaccine given to infants that was 60% effective would have the most significant impact—reducing TB incidence approximately 80% by 2050 (Abu-Raddad 2009).

## BCG: The Current TB Vaccine Strategy

*Mycobacterium bovis*, descended from *Mycobacterium tuberculosis* (MTB), causes a TB-like disease in cows and humans. Starting in 1908, professors Albert Calmette and Camille Guérin began culturing *M. bovis* in order to weaken the bacteria to the point at which it was unable to cause disease but could stimulate an immune response in humans against MTB. The idea was to train the immune system to produce cells that would fight TB by introducing an attenuated (non-disease-causing) strain of a similar mycobacterium. The smallpox vaccine is, in fact, attenuated cowpox (*vaccinia*). In 1921—after 11 years of attenuation—the first human received the Bacille Calmette-Guérin (BCG) vaccine, and since that time it has become the most widely administered vaccine in the world. BCG provides protection against tuberculous meningitis and military TB in infants and young children up to perhaps five years of age, but its efficacy wanes over time and most vaccine-induced immunity appears to be gone by adolescence; revaccination later in life provides no benefit. It has been hypothesized, but never proven, that the natural presence of non-tuberculosis mycobacteria in tropical environments may decrease the effectiveness of BCG.

BCG's use is even more challenging in high-HIV-prevalence settings because it can cause a severe immune reaction in HIV-infected infants. BCGitis (local infection) or BCGosis (systemic disease) are not well characterized complications of BCG vaccination that cause significant morbidity in multiple organs among HIV-infected infants and young children. The incidence of BCG disease is unclear, but it is a leading cause of death in TB/HIV-coinfected infants in South Africa (Zar 2007).

In addition to the risks for developing BCG disease, evidence suggests that BCG provides little to no protection for HIV-infected infants (Mansoor 2009). The World Health Organization (WHO) revised its guidelines to recommend that infants with a confirmed HIV diagnosis should not receive BCG vaccination. This recommendation is impossible to implement in many high-burden settings because of limited capacity to rapidly confirm diagnosis using HIV RNA testing.

Despite its variability and limitations, it is estimated that the BCG vaccine saves the lives of over 40,000 children annually (of over 100 million vaccinated). This makes the decision of whether or not to vaccinate HIV-exposed children where HIV RNA testing is not available a challenge for both parents and health care providers. Without being able to confirm an HIV diagnosis using RNA testing soon after birth, parents and their health care providers are forced to weigh the risks (BCG disease) versus the benefits (protection against severe forms of pediatric TB) of BCG vaccination.

Policy makers, clinicians, and researchers are struggling with how to use BCG more effectively and safely and what alternative strategies can be used in its place. There are a few vaccines in preclinical and early-phase clinical studies that may be replacements for BCG. But these constructs are years away from efficacy testing. Delaying BCG vaccination by providing isoniazid preventive therapy (IPT) to HIV-exposed infants as preexposure prophylaxis until HIV status can be confirmed, as well as early initiation of antiretroviral treatment (ART), may be ways to address the challenges of BCG in HIV-exposed infants, but each strategy comes with implementation challenges. HIV RNA testing is not part of regular clinical practice in many high-TB-burden countries and it is unclear how long BCG vaccination can be delayed; studies are underway that hope to answer this question. Because it is difficult to bacteriologically confirm TB diagnosis in infants and young children, and the fact that pediatric TB is often an indication that a close adult contact is sick, IPT is a significantly underused strategy due to the fear of promoting isoniazid resistance. While great progress has been made in scaling up access to HIV treatments, a majority of those in need are still waiting for treatment; therefore, getting infants into treatment earlier may be a significant challenge in places where many people have been waiting for years to get ART.

Because BCG is part of the WHO's Expanded Program on Immunisation schedule of vaccines it is administered in conjunction with a host of other vaccines (including those for measles, polio, and tetanus) throughout the world. Little explanation is given to parents about the vaccines, and many people misunderstand the limitations of protection that BCG provides. Some may incorrectly believe that because they were vaccinated against TB that they are protected against all forms of the disease for their entire lifetime. The lack of community understanding of the limitations of BCG is a major obstacle in creating community demand for a newer, better, and safer TB vaccine.

## Challenges for TB Vaccine Research

TB has three phases—infection, latency, and disease—with different host (human) and pathogen (TB bacterium) factors influencing each phase. It is unclear what factors are associated with the establishment of latent infection and reactivation. The interactions among host, environment, and pathogen are dynamic and the contribution of each factor to the persistence of the bacteria is not well understood (Dye and Williams 2010). The TB bacterium exists in different metabolic states depending on whether it is infecting, latent, reactivating, or spreading disease throughout the body. During acute infection the bacterial load is high due to rapid replication. Once the infection becomes latent the bacterial load remains relatively stable and is confined within tubercles or granulomas—immunological prisons—in immunocompetent hosts. Attacking TB in latency likely requires a different mechanism of action than what would be used in early infection. When TB enters latency, it changes its metabolism and gene expression and therefore requires a different vaccine-induced immune response to prevent reactivation (Beresford and Sadoff 2010; Russell 2010).

One of the major challenges of TB vaccine research—in addition to our failure to fully understand the full spectrum of the disease—is our inability to predict the level and durability of vaccine-induced immunity. Defined correlates of immunity—the level of protection provided by vaccines—are critical for measuring vaccine efficacy and getting regulatory approval. There are no validated correlates of protection for TB vaccines (Beresford and Sadoff 2010; Wallis 2010). Because clinical signs and symptoms for TB can be difficult to assess and may not be TB-specific, it is challenging in infants and young children to rely on clinical endpoints for assessing efficacy of a TB vaccine (Hanekom 2010). However, it is certain that until we better understand the disease, clinical endpoints will be required.

Much of the data used to determine which vaccines to test in humans is based on what is observed in animals. There are a variety of models—mouse, guinea pig, rabbit and nonhuman primate—used to assess the impact of experimental drugs and vaccines before testing them in humans. Each has their advantages (e.g., cost, ability to manipulate the animal's immune system) and disadvantages (e.g., generalization to humans). The most commonly used model is the mouse because of its relative cheap cost and, like the guinea pig, it can be inbred to emphasize certain genetic characteristics (Neurmberger 2010). Other models such as the rabbit and nonhuman primates may exhibit a more complete spectrum of TB disease (Beresford and Sadoff 2010; Neurmberger 2010; Russell 2010). But none of these animals exactly replicate TB disease in humans, so extrapolation of the data to humans is limited.

Another major challenge that threatens to delay the approval of a new TB vaccine is the lack of capacity to conduct large-scale phase III efficacy trials (Kaufmann 2010).

Because a vaccine trial must show impact on the population level, thousands of study volunteers are required to demonstrate that vaccination with the experimental vaccine significantly reduces TB incidence in the community and that the reduction is durable. As a result, these studies require large sample sizes and longer follow-up than clinical trials that evaluate new drugs. Conducting these studies is labor and resource intensive and few research institutions have experience conducting studies of this magnitude.

There are efforts to build vaccine site infrastructure, but currently only the South African Tuberculosis Vaccine Initiative has the capacity to carry out phase III vaccine studies (Kaufmann 2010). The Aeras Global Vaccine Foundation (Aeras) and the European and Developing Countries Clinical Trials Partnership are supporting capacity building at sites in Africa and Asia, but it is likely that only one or two sites will be capable of conducting phase III studies before any of the current vaccines in phase II are ready to enter later-stage studies (Hanekom 2010; Kaufmann 2010).

### The TB Pipeline

Current TB vaccine candidates are designed to contain the TB bacillus by enabling the immune system to get a head start when exposed, reducing bacterial load and preventing progression to clinical disease (Kaufmann 2010; Russell 2010). No current TB vaccine candidate is designed to produce sterilizing immunity or, in other words, to prevent infection altogether. Rather, current constructs are designed to stimulate immune cell response to, and memory of, TB to prevent disease.

The vaccine candidates farthest along in the pipeline aim to replace BCG or strengthen BCG-induced immunity. Some “boost” or strengthen the initial immunity induced by BCG (and perhaps eventually a superior BCG alternative) and prevent progression to TB disease. The prime-boost strategy involves an initial immunization with a priming vaccine (currently the only prime being used is BCG but others are in the pipeline) that introduces the immune system to TB. A booster vaccine that broadens and strengthens the TB-specific immune response then follows the prime. There are live mycobacterial vaccines that improve BCG by adding genes or are attenuated MTB strains that have the genes deleted that are responsible for virulence (ability of a pathogen to cause disease (Kaufmann 2010; Russell 2010)). Viral vectored vaccines are viruses modified so that they are unable to cause disease but are recombined to express TB-specific proteins. Immune cells recognize the TB genetic material and mount an immune response. Viral vectors have been used safely and effectively in many vaccines, including hepatitis B and human papilloma virus. The other vaccine strategy that has been evaluated is a therapeutic vaccine that is meant to improve response to TB treatment in people with active TB disease.

While a number of vaccine constructs will be entering phase II studies in the coming year, this year's vaccine pipeline report is focused on constructs that have already entered phase II studies. It is expected that in the next few years this report will grow if TB vaccine research is adequately resourced to enable the conduct of later stage efficacy studies.

## TB Vaccine Constructs in Phase II Clinical Trials

Agent	Strategy	Type	Sponsors	Status
M72	Prime boost	Recombinant protein	GSK Biologicals/Aeras	Phase II
AERAS-402/Crucell Ad35	Prime boost	Viral vector	Crucell N.V./Aeras	Phase IIb
MVA85A/AERAS-485	Prime boost	Viral vector	University of Oxford/Aeras	Phase IIb

GSK Biologicals, a subsidiary of GlaxoSmithKline, is working with Aeras to conduct phase II studies of GSK M72, a recombinant protein vaccine. The vaccine is made up of an adjuvant—a molecule that stimulates an immune response—and two recombinant TB proteins meant to strengthen the immune response to two highly immunogenic fragments of the TB bacillus. To date, GSK has conducted phase I and II trials of the candidate vaccine in TB-naïve, TB-infected, BCG-vaccinated, and HIV-positive adults. Safety and immunogenicity trials have been conducted in the United States, Europe, South Africa, and the Philippines. Early results suggest that the vaccine is clinically well tolerated and produces a measurable immune response. Subsequent clinical trials are now planned for adolescents and infants in TB-endemic regions (Ofori-Anyinam 2010).

AERAS-402/Crucell Ad35 from Crucell NV and Aeras is an adenovirus 35 (Ad35) modified to include specific TB antigens to trigger an immune response. A series of phase I studies have demonstrated TB-specific CD4 and CD8 responses in BCG-naïve and BCG-vaccinated adult volunteers after receiving the vaccine. A phase II clinical trial in adults recently treated for pulmonary TB and a phase I study in infants are ongoing. A phase IIb randomized, placebo-controlled proof-of-concept study in HIV-positive adults with CD4 counts above 350 was recently initiated to evaluate the safety and efficacy of AERAS-402/Crucell Ad35 (Wooley 2010). There is a concern that for individuals who have preexisting antibodies to Ad35 the adenoviral vaccine may be less effective. The prevalence of antibodies varies geographically from approximately 5% to 20% (Hanekom 2010; Kaufmann 2010).

MVA85A/AERAS-485 is a live viral-vectored vaccine that is an attenuated version of the *vaccinia* virus—the cowpox virus that confers immunity to smallpox—combined

with TB antigen 85A. The first infant received a dose in July 2009 as part of a phase IIb proof-of-concept study. This is the first time in over 80 years that a vaccine has been tested for efficacy in infants (Beresford and Sadoff 2010). The trial is comparing MVA85A versus placebo in BCG-vaccinated, HIV-negative infants. The first results are expected in 2012 (McShane 2010).

*Mycobacterium vaccae* is a mycobacterium which has been evaluated as an immunotherapeutic vaccine for people with TB infection. In the 2009 pipeline report, it was reported that Aeras's external Vaccine Selection Advisory Committee had reviewed the data from the Dar Dar study—a trial that evaluated *M. vaccae* in HIV-positive adults who had been vaccinated with BCG—and recommended that Aeras determine if new *M. vaccae* vaccine could be manufactured since the trial depleted the existing supply. Aeras has undertaken some limited process-development work to produce more vaccine and this work is almost complete. At this time Aeras does not have any immediate plans for further involvement (Willingham 2010). The limited data on *M. vaccae* are uninspiring. A 2003 Cochrane Review review concluded that *M. vaccae* provided no immunotherapeutic benefit for people with TB and therefore that no further trials were warranted (de Bruyn 2003). However, evidence from the Dar Dar study has suggested that a multidose *M. vaccae* vaccination was associated with protection against TB disease in people with HIV with CD4 counts above 200 (von Reyn 2010). The Dar Dar study results would need to be confirmed via additional studies before any conclusions could be made about effectiveness (Kaufmann 2010). *M. vaccae* is the only vaccine candidate to make it to phase III, but it appears that there is neither supply of the construct nor any research institution evaluating it at this time.

## What Is Needed?

There are still many unanswered questions about the TB life cycle, the spectrum of TB infection and disease, and the impact of host genetics on the immune response that hamper vaccine development. More attention and resources need to be focused on basic scientific research. This is critical to keeping the pipeline full of new candidates, improving existing prevention tools, and identifying novel strategies to induce safe and durable immunity to TB infection and disease. A clear understanding of the differing characteristics of TB in its latent and disease state could lead to the development of a vaccine construct that could prevent infection and thereby significantly lower future cases of TB disease. The identification and validation of correlates of immunity will be vital to expediting the evaluation of any new vaccine candidate. Without the ability to predict whether a vaccine is able to induce an adequate protective immune response and to measure the quality of that response, massive resources—which are not currently available—will need to be dedicated to conducting long-term, large-

scale epidemiological and efficacy studies that could significantly delay the approval of new vaccines for years. At the same time, a better understanding of the limits of BCG protection would help to identify alternatives to its use in HIV-exposed infants.

In addition to these basic and clinical research questions, a number of operational research issues need to be evaluated. Health systems need to be strengthened to provide access to comprehensive diagnostic and treatment options, including HIV RNA testing and IPT. Implementation research would provide examples of how programs could scale up HIV and TB diagnostic and treatment services to reduce the risk of BCG disease in HIV-infected infants and provide alternatives for the prevention of latent TB infection. These studies could also ensure that HIV-exposed, uninfected infants can benefit from BCG vaccination. For too long vaccine research has been the exclusive domain of immunologists and vaccinologists; social scientists and operational researchers should be included in setting research priorities and providing evidence to policy makers and clinicians on how to implement new vaccines.

In this spirit, researchers need to work collaboratively and share data. Aeras—a nonprofit product development partnership that works with vaccine manufacturers from the private sector to test and bring constructs to licensure—and a consortium of 31 research sites in Europe and Africa called the Tuberculosis Vaccine Initiative are organized to bring different institutions working on vaccine development together. However, this may be challenging for individual researchers or small-scale research institutions conducting basic science research that may not be connected to the broader vaccine research community. In order to build upon one another's work and to avoid overlap, more opportunities for partnership and data sharing must be created.

Vaccine developers and researchers need to collaborate with communities and policy makers to create demand for a better vaccine and improved prevention strategies. Communities and policy makers' understanding of BCG and vaccine research is limited at best. Efforts need to be directed toward increasing the awareness of the vaccine research process, the limitations of BCG, and the need for a new vaccine. Without the support of these stakeholders it is unlikely that any new vaccine will be scaled up rapidly—if at all.

As part of these collaborative activities, experienced researchers and vaccine networks should prioritize building the research capacity and infrastructure in high-TB-burden countries to conduct clinical trials and operational research. This will require regulatory authorities to provide guidance on development pathways to ensure that they are consistent with global regulation. Establishing clear criteria for the evidence set required to license a new vaccine would establish a standard by which all trials must adhere, making the process more efficient and allowing the harmonization of data collected across studies.



Finally, without a great increase in funding, TB vaccine research will stagnate. Not only are more funds needed to support basic science, to conduct efficacy studies and operational research, and to encourage young scientists to take on TB research but there needs to be a diversification of funders. The Bill and Melinda Gates Foundation (BMGF) has consistently accounted for the great majority of funding for TB vaccine research, contributing 61% of all vaccine research and development funding in 2008. In fact, a boost in funding for TB vaccine research in 2008 was almost entirely accounted for by a grant from the BMGF to Aeras (Treatment Action Group 2010). Reliance on one or a few funders may result in donor fatigue and a shrinking pool of institutions and researchers able to contribute to vaccine development. The pool of funding must not only increase; the number of funders also needs to increase to get a newer, better, and safer vaccine that can protect everyone from all forms of TB.

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