

HIV TUBERCULOSIS VIRAL HEPATITIS
DRUGS, DIAGNOSTICS, VACCINES, AND
MICROBICIDES IN DEVELOPMENT

TAG 2009 Pipeline Report



JULY 2009

TREATMENT ACTION GROUP

LEI CHOU, MARK HARRINGTON, BOB HUFF,
RICHARD JEFFERYS, JAVID SYED,
AND CLAIRE WINGFIELD

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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.

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THIS REPORT IS DEDICATED TO

Martin Delaney

1945–2009

Pioneering AIDS treatment activist
Founder, Project Inform

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Introduction

BY MARK HARRINGTON

This year's *Pipeline* report shows, in brief, a lull in anti-HIV drug development, an alarming stasis in hepatitis B treatment research, renewed activity (after a gap of almost 40 years) in TB drug development, agonizing slow and incremental progress in TB diagnostics research, very preliminary human studies of several new TB vaccine candidates, a back-to-basics mood in the HIV vaccine research community, renewed hopes for efficacy in microbicide and pre-exposure prophylaxis, and no dramatic developments in the areas of immune-based therapies or therapeutic vaccines for HIV.

In short, as Thomas Kuhn would have said, this report documents a period of relatively “normal” science, with its incremental steps forward and back, its halting progress, its occasional retreat from a blind alley. There is nothing as dramatic here as the bleak pessimism that enshrouded HIV vaccine research after the STEP study was prematurely terminated in 2007, nor the possibly overhyped HAART 2.0 breakthrough of that same year, when two new *classes* of anti-HIV drugs were introduced. Yet, as Richard Jefferys points out in the conclusion to his chapter herein, the despair of Berlin 1993 preceded the euphoria of the HAART revolution of Vancouver 1996 by just three years. We do not have a crystal ball; nor do we know whence the next breakthrough may come.

Instead, in my view, the greatest challenges to AIDS research this year lie not in the discovery and development of a single new intervention but in the much harder questions of how best to use all the tools at our disposal to prevent and treat HIV. This was put extremely well by Kevin De Cock in his farewell address as the World Health Organization's HIV director at the PEPFAR Implementers' Meeting in Windhoek, Namibia, in June, 2009:

Martin Luther King said that the arc of the moral universe is long but it bends towards justice. If public health is rooted in the science of epidemiology, its philosophic values are equity and social justice. We are entering perilous ethical and political waters, and current practice for poor people of color in the global South will not be judged well by history if it does not evolve with science and practice in the richer North. ... *The world cannot allow a permanently two tiered system of global AIDS treatment with late initiation of outmoded drugs reserved for the South.* Nor can we hide behind lack of knowledge or the attitude of “let's wait and see.” *Equipoise no longer exists in the debate about early or late initiation, and today's questions are “treat how early?” and “with what?”*

It is unacceptable, in view of what is at stake—millions of lives, billions of dollars—that despite over three million people in the world on ART [antiretroviral therapy], we cannot definitively answer the question of when to start treatment. Allocation of Global Fund and PEPFAR resources must be based on evidence. There is ethical as well as medical need for a randomized controlled trial to determine optimal starting criteria in Africa, including assessment of the impact of immediate treatment on tuberculosis incidence. PEPFAR and the Global Fund could resolve these questions once and for all through applied research under field conditions, through a large simple trial, for example, with hard end points such as tuberculosis, AIDS, death. Some argue such a study is not needed because we will never have resources to treat more people earlier with better drugs. This is unpersuasive; rationing of health care is a universal reality but let rationing decisions be made transparently, with the involvement of all stakeholders, based on scientific understanding of cost and benefit.

Despite challenges, political will and science could get us closer to one, or a few, global, once-daily, first line regimens, with the best drugs. . . .

. . . We need imaginative thinking, renewed advocacy, innovative financing, and more efficient implementation. Strong support is required for the different innovative financing mechanisms being explored, and pressure on emerging economies to contribute. We cannot expect one country to carry the world's HIV treatment costs.

In closing, we are again entering uncharted territory in HIV/AIDS, challenged by inadequate prevention, uncertainties around treatment, a widening but incompletely defined role for ART, and increasing inequity. Universal access will slip through our fingers unless we reframe it in the broader context of all health related Millennium Development Goals. Sustainability should be redefined in terms of technical sustainability nationally but financial sustainability at the international level, acknowledging that global health needs global financing. From disjointed prevention and treatment of the past we must move towards more intelligent use of ART for treatment as well as prevention, guided by science, stratified by individual serostatus, with all infected persons knowing their rights to health, including sexual and reproductive health. What else is universal access? *

We need to unify the HIV community around continued scale-up of prevention and treatment toward universal access. At the same time we need to battle those who say that AIDS gets too much money; who pit disease against disease in order to justify cutting or shifting health budgets rather than increasing investment in health so that true universal access can be reached. And we need to continue to insist that research is funded at levels healthy enough to bring us closer to long-term solutions to the pandemic such as a cure and a vaccine.

* Kevin De Cock, "WHO at the 2009 HIV/AIDS Implementers' Meeting," http://www.who.int/hiv/events/implementers2009_kdc/en/index.html; emphasis added.

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Antiretroviral Drug Development in 2009

BY BOB HUFF

Although the HIV drug development pipeline in 2009 appears robust if you go by the numbers—at least a dozen agents are in later stages (phases II and III) of clinical testing—there are no obvious miracle drugs on the horizon and little that seems likely to revolutionize treatment; only the next steps in the evolution of antiretroviral (ARV) therapy. This doesn't mean that incremental benefits cannot be revolutionary for some individuals who depend on ARVs—even a slight improvement in gastrointestinal tolerability or mental clarity, for example, can allow someone to enter the job market, start dating again, or think about starting a family.

As good as current HIV drugs are, they are not ideal and not universally accessible. A new generation of HIV therapy is needed—treatments that are more tolerable, less susceptible to resistance, more forgiving of dosing lapses, and that have well-understood long-term safety profiles. To continue expanding treatment for millions of people in the developing world, add requirements for compact regimens that can be produced and distributed inexpensively and used with minimal monitoring. The world needs better AIDS drugs, but the current ARV pipeline does not reflect that urgency.

Unfortunately, many clinicians in the United States and Europe are content with the current drugs and complacent about the need for developing the next generation of therapy. This means that some drug makers do not hear a compelling demand to make major investments in HIV research. In 2009 there has been consolidation among the big HIV drug makers—Schering with Merck; Pfizer with GlaxoSmithKline—and other companies, such as Roche and Abbott, are backing away from new research in the field. The 2009 pipeline chart is half populated by offerings from small pharmaceutical or biotech companies with drugs aimed at specialized, or niche markets at best.

What's in the Pipeline?

A Better Booster?

It could be that the drugs in the AIDS pipeline with the most potential for bringing dramatic change are not anti-HIV drugs at all. Rather, they are new agents that slow the metabolism, boost blood levels, and improve the potency of certain ARVs. As of

Antiretroviral Drugs in Development, 2009

Agent	Class	Sponsor	Status
Rilpivirine (TMC278)	NNRTI	Tibotec	Phase III
Vicriviroc	CCR5 antagonist	Schering	Phase III
Elvitegravir	Integrase inhibitor	Gilead	Phase III
Apricitabine (ATC)*	NRTI	Avexa	Phase II/III
Amdoxovir (DAPD)	NRTI (prodrug)	RFS Pharma	Phase II
Bevirimat*	Maturation inhibitor	Myriad	Phase II
TNX-355*	CD4 blocker	Biogen Idec	Phase II
UK-453,061*	NNRTI	Pfizer	Phase II
IDX889*	NNRTI	Idenix/GSK	Phase II
GSK1349572*	Integrase inhibitor	GSK/Shionogi	Phase II
GSK1265744	Integrase inhibitor	GSK/Shionogi	Phase IIa
PRO 140*	CCR5 Blocker	Progenics	Phase II
Ibalizumab*	CD4 Blocker	TaiMed	Phase II
GS 9350	PK Booster	Gilead	Phase II

* Potential use against extensively drug resistant HIV?

now the only pharmacokinetic (PK) enhancer on the market is ritonavir (Norvir) from Abbott Laboratories. Originally approved as an HIV protease inhibitor, the drug's metabolic inhibiting side effect turned out to be more useful than its antiviral properties, and it became an essential ingredient in Abbott's popular coformulated protease inhibitor Kaletra. However, ritonavir use has been associated with increased blood lipid levels and diarrhea. There are high expectations that new, specifically designed boosters from Gilead, Tibotec, Sequoia, and Pfizer will come with fewer unwelcome side effects. Abundant boosters should also make possible a greater variety of combination tablets and all-in-one regimens, as more companies team up to pack three drugs (plus a booster) into a single daily pill. Convenient, tolerable drugs make treatment easier to start and stick with, which leads to better long-term outcomes. With a trend to prescribing earlier in the disease and a widening campaign to test and treat tens of thousands of people who are infected but undiagnosed, these small advances can help make lifelong therapy a more palatable prospect.

Gilead's GS 9350 booster is farthest along on this path. The drug is getting its first trial as part of an all-in-one regimen containing Gilead's integrase inhibitor, elvitegravir, plus the company's tenofovir and emtricitabine (Truvada) pressed into a single pill. The combo is being studied in a treatment-naive population with approval anticipated

by 2011. Gilead has said it intends to make the new booster available as a stand-alone product and is willing to license the drug to other companies for use in coformulated products. Tibotec's underutilized protease inhibitor (PI) darunavir could get a big boost in the market if it can be bundled with a proprietary PK enhancer. Tibotec also has a new protease inhibitor in early clinical trials that is giving the home team booster a shot. The start-up company Sequoia, with its expertise in protease inhibitors, is another natural in this field. Even a generic version of saquinavir, a first-generation PI, may be a viable treatment if it is mated with a better booster; the drug goes off patent in 2010.

It is not clear how Pfizer can capitalize on its booster candidate, since none of its current HIV drugs requires boosting and the company has recently shifted most of its ARV research and marketing efforts to a new, HIV-focused joint venture with GlaxoSmithKline (GSK). GSK does have a PI on the market that requires boosting, and it may see an opportunity for synergy there. As for Abbott, the original booster maker, after raising the price of Norvir by 400% in 2003 and having indicated that it doesn't anticipate developing a follow-on to surpass Kaletra, the company seems (like so many others) to have shifted its focus to the search for a breakthrough hepatitis C antiviral treatment, content to let the maturing market for HIV drugs play itself out. After many years of delay, Abbott is preparing to release a new heat-stable tablet formulation of Norvir.

Spare Me

Nucleoside reverse transcriptase inhibitors (NRTIs, or "nukes") were the first class of antiretroviral drugs developed. The drugs work by stopping HIV from successfully copying its genetic material into DNA. But NRTIs can affect similar natural processes in the body, and they have been implicated in both severe and subtle toxicities. AZT, approved in 1987, remains a useful drug today, although newer and less toxic NRTIs now dominate the market.

A "nuke sparing" strategy to forgo the use of NRTIs is currently in vogue and is getting a test in several trials that marry a reliable protease inhibitor with a powerful but more resistance-prone integrase inhibitor. A few small studies have begun to report successful results. A preliminary analysis from a trial of Kaletra plus raltegravir versus Kaletra plus the NRTI-combination Truvada showed faster viral suppression using raltegravir but, oddly, slightly increased triglyceride and cholesterol levels in the NRTI sparing arm. Longer-term results from this and other studies are expected in 2010. Other variations include a clever pairing of raltegravir with twice-daily unboosted atazanavir—sparing both NRTIs and ritonavir.

While there are a few new NRTIs in development, it is not clear how even the most promising ones—like apricitabine (ATC), from the Australian company Avexa—are

likely to upset the treatment landscape. ATC has a unique resistance profile that overcomes the M184V mutation associated with lamivudine and emtricitabine resistance and it could represent an important salvage alternative. The drug recently completed a 96-week phase IIb study with no ATC resistance seen in a small number of patients, and is now enrolling a large phase III trial comparing ATC to lamivudine in treatment-experienced patients. However, the current dose of ATC is 800 mg twice daily, which will be limiting in terms of convenience and utility as a first-line drug.

Amdoxovir (DAPD), from RFS Pharma, a Georgia company started by serial NRTI entrepreneur Raymond F. Schinazi, has progressed to the phase II stage, though no currently enrolling studies are apparent. The drug has appeared on pipeline charts since 2002—enrolling just a few hundred people during that time, mainly in short-term, 10- to 15-day studies. Amdoxovir is expected to have activity against lamivudine-resistant HIV, though so far there is scant data in treatment-experienced patients to support that.

Aside from whether the nuke-sparing strategy is practical or not, such trials might discover some interesting facts about HIV and HIV therapy. Maybe nukes will be found essential for preventing neurological symptoms and improving response rates. Maybe the DNA chain terminators will be implicated in the apparent premature aging of people with HIV (*see* box, “Premature Aging and HIV?”). Since these effects may be subtle and the trials to investigate them will not be very large, we may not find any clear answers, and choosing to use nukes will remain a part of the art of medicine as doctors stick with what they know.

Integrase in the House

If the easy HIV targets (HIV’s enzymes, reverse transcriptase and protease) seem temporarily played out, second-generation targets are still getting a lot of attention. Integrase inhibition attacks HIV’s third enzyme (integrase), and with raltegravir the concept has ascended to the main stage.

Merck’s wonder drug raltegravir (Isentress) impresses because it clearly lowers viral load faster than efavirenz and Kaletra. But what’s not clear is how meaningful this talent is, since by 48 weeks suppression rates for these drugs seem comparable. The current theory is that all effective ARV regimens halt viral replication equally well, but because integrase inhibitors protect cells from getting infected in the first place there is less excess virus produced and viral loads fall faster.

Premature Aging and HIV?

There is a growing concern that people with HIV are showing signs of aging prematurely—even when their virus is under control. Rates of cardiovascular problems, diabetes, bone frailty, and cognitive and neurological problems are increased in people who delay therapy too long, but increased incidence of these is also suspected in some who are treated and have no evident viral replication.

Chronic immune activation and inflammation may be responsible for some of the emerging non-AIDS disease seen in people with HIV. But stress, sleep loss, smoking, and genetics could also contribute. Because the epidemiology and pathogenesis of these fairly rare clinical events are poorly understood, it is difficult to identify any single cause for premature aging other than HIV. There may be multiple factors.

Four theories:

1. **Early injury.** If HIV causes irreparable damage to the immune balance within days of infection by wiping out a significant population of T cells in the gut and elsewhere, it is possible that a key part of an immune regulatory mechanism has been destroyed. Trials of treatment during acute HIV infection, and studies of people who become infected despite taking drugs for pre-exposure prophylaxis, may illuminate the impact of moderating the early damage.

2. **Ongoing insult.** Unsuppressed viral replication could cause ongoing damage to the body due to chronic immune activation and inflammation. It is possible that bacteria entering the body from the damaged gut contribute to systemic immune activation. Immune deficiency could be an end-stage outcome after years of accumulated damage. The large Strategic Timing of Antiretroviral Treatment trial of initiating treatment earlier versus later in the disease plans to track inflammation markers, which may shed light on the impact of arresting the damage sooner.

3. **Low-level toxicity due to the release of HIV proteins despite suppression of replication.** HIV proteins are released from viral reservoirs periodically (blips) even when the drugs are working. If some of these proteins (Tat, Vpr) cause errant signaling to the immune system, then improper immune responses may be sustained even in the absence of viable virus. Treatments to purge viral reservoirs might help turn down the production of these toxic proteins.

4. **The drugs.** Even though the current generation of ARV drugs are highly effective and relatively tolerable, very-long-term effects are not known. For example, almost everyone on therapy takes lamivudine or emtricitabine, yet there are few comparative safety data from randomized trials that might reveal subtle toxicities attributable to these ubiquitous drugs. NRTI-sparing studies now underway might help reveal if these drugs are associated with any of the wide range of symptoms of premature aging increasingly recognized in people with HIV.

Some unsettling news about raltegravir resistance came from a study in which people who were suppressed on Kaletra were switched to the integrase inhibitor to take advantage of its better lipid profile. Unfortunately, some of them had virologic breakthroughs after switching, likely due to an unmasking of underlying NRTI resistance. Besides underlining the durability of protease inhibitors, this reveals the fragility of raltegravir—and possibly of other agents in the class—if they are not fully supported in the regimen by active companion drugs.

The critical quality determining raltegravir resistance may be the “on” time during which an integrase inhibitor stays attached to the integrase enzyme in the preintegration complex. If the “on” time is longer than the life of the integration complex, the drug will be effective; but if a mutation in integrase shortens that time, the risk of viral breakthrough increases. If this proves to be the mechanism of resistance to integrase inhibitors, then perhaps more frequent dosing—inconvenient as that may be—could offer a way to rescue viral suppression using the same drug. Merck is developing a second-generation integrase inhibitor that purportedly has not only a longer “on” time but can be dosed only once a day.

Gilead’s elvitegravir is moving forward in phase III trials and could be approved by 2010 in a solo formulation. Though it enjoys the advantage of once-daily dosing, the drug will require boosting, at first by ritonavir and then later by Gilead’s proprietary GS 9350 booster. Elvitegravir is expected to come into its own when a boosted combination regimen in a single pill is ready for approval in 2011 or 2012. Gilead’s QUAD study of the four-in-one combo has already begun enrollment.

GSK has completed extensive drug-to-drug interaction studies on its integrase inhibitor candidate, the Shionogi-discovered GSK1349572, but no larger phase II trial has begun yet. Now GSK appears to be starting a similar development program for a follow-up Shionogi drug, GSK1265744. Besides all the usual qualities required for success, to truly shine GSK’s integrase inhibitor must demonstrate activity against virus that is resistant to raltegravir. New details about GSK1349572 will be reported at the International AIDS Society meeting in Cape Town, South Africa in July, 2009.

The “Non-Nukes”

Rilpivirine, a nonnucleoside reverse transcriptase inhibitor (NNRTI) from Tibotec, has nearly everything in its favor: it is comparable to efavirenz without causing sleep disturbances or increased blood lipids; it is a once-daily drug; and it has low-milligram dosing, which could be ideal for use in low-cost regimens for the developing world. But heart rhythm abnormalities (QT prolongation) seen at higher doses resulted in Tibotec choosing (at the U.S. Food and Drug Administration’s insistence, no doubt) the lowest dose studied in the phase II clinical trial. Unfortunately, this dose was not the natural first choice, and people on the 25 mg dose had more early virologic failures than those

on the 75 mg dose, though suppression rates were equivalent at the end of the trial. The large phase III trials underway will definitively reveal if the lower dose is adequate.

Idenix has partnered with GSK to develop IDX889, an NNRTI expected to be active against virus resistant to the approved NNRTIs as well as to rilpivirine. Pfizer's UK-453,061 is in a phase II trial for treatment-experienced people with resistance mutations to first-generation NNRTIs and is up against efavirenz in another trial as first-line therapy. Between the integrase inhibitors and these NNRTIs, the GSK/Pfizer joint venture is starting to look fat. With other NNRTI compounds from Ardea in earlier stages of development, there may be life left in this class yet.

CCR5 Agonistes

With enfuvirtide and maraviroc, entry inhibition has been proven in two different ways, but each approach has been hobbled by inconveniences unrelated to efficacy— injection and the need for an expensive screening assay, respectively.

One the three drugs currently in advanced phase III trials is Schering's CCR5 antagonist vicriviroc, which, if approved, would become the second drug in the class after Pfizer's 2007 maraviroc. Unfortunately—despite being a very effective and well-tolerated drug—maraviroc has been a bust in the marketplace, mainly because it came into the world in the shadow of raltegravir, and because before it could be prescribed, an expensive tropism test with a four- to five-week turnaround time was required to determine if a patient's virus was susceptible to the drug. By the time vicriviroc arrives, however, cheaper and easier-to-use tropism tests may be available. These drugs are relatively immune to resistance, and they seem to have unparalleled penetration into the brain. If there are advantages to these qualities, they have yet to be worked out. There is also a tantalizing observation that some people who gain no virologic benefit from maraviroc (because their virus is not susceptible) still experience an increase in CD4 cell count while on the drug. Schering likely shares Pfizer's hope that CCR5 blockers exert beneficial effects on functional immunity independent of their antiviral properties, but both companies will have to wait for much more data before making claims. In its phase III trial for first-line use, Schering has adopted the NRTI-sparing model, pitting vicriviroc plus boosted atazanavir against atazanavir plus Truvada. Vicriviroc is dosed at only 30 mg per day when paired with ritonavir.

Newer Targets

Other potential drugs that restrict HIV entry, such as TaiMed's CD4 blocker ibalizumab (acquired from Tanox) and Progenics' anti-CCR5 agent PRO140, are monoclonal antibodies (mAbs). Both will require infusion directly into the

blood since they can't be taken orally, though TaiMed is said to be working on an alternative route of administration. While infused mAbs may effectively stay in the blood for up to a month, which could be a boon for people with adherence problems, nothing beats the simplicity of a single daily tablet (ideally in unit dose packaging—like birth control pills—to boost adherence; *see* www.unitdose.org).

Maturation inhibitors would block a final stage in viral assembly, leaving newly formed viral particles inactive. Bevirimat was the first candidate in this class, though it had a long and tortured path in the hands of its initial maker Panacos. The class is getting a second chance at Myriad, a small Salt Lake City, Utah pharmaceutical company that has acquired bevirimat and will be developing it, backed up by two of its in-house antimaturation drugs. A big limitation is that a fairly common naturally occurring mutation in the HIV protease enzyme renders the virus resistant to bevirimat. A genotype test will likely be required to identify those whose HIV will be susceptible to the drug.

Bevirimat, ibalizumab, and apricitabine are virtually the only hope for people who desperately need at least two new drugs from new classes to overcome HIV with resistance to every other available drug. Such deep salvage patients are rare in 2009, but their need is no less great. Some are veteran volunteers of clinical trials and carry resistant strains as evidence of long treatment histories and one too many placebos. New access mechanisms are needed that will allow doctors to pair up two or more experimental drugs earlier than has heretofore been possible.

Conceptual Therapies: The Blue Yonder

Recent studies of the dependencies of HIV replication on the human cellular machinery have produced an impressive list of proteins that could be potential drug targets. The trick will be to find which human proteins are uniquely required by the virus and which can be blocked without shutting down an essential function of the cell and causing serious side effects. Research is also needed on how to interrupt transitory protein-to-protein interactions that are far less specific and less critical than enzyme reactions for the life of the virus.

Some human gene products would act as natural antivirals if they weren't switched off by one of HIV's so called accessory proteins. For example, APOBEC3G would cause deleterious mutations in HIV DNA when the virus replicated if not for Vif, a small HIV protein that inactivates APOBEC3G. The trick would be to find a small molecule drug that can deactivate Vif and let the body do its own housekeeping on viral interlopers.

Integration is the point of no return in the HIV life cycle at which the viral genome is brought into an infected cell's nucleus and made a permanent guest of the host's

DNA. Raltegravir blocks this stage by preventing the viral DNA from finishing the final stitch. A host protein called LEDGF/p75 is thought to help the HIV integration complex find the right place in the cell's genome at which to join the host DNA. Some have speculated that blocking LEDGF/p75 would be an effective strategy for blocking HIV replication. Chinese researchers, as well as scientists at Schering and CellVir have been exploring the potential for blocking LEDGF/p75.

Conclusion

Though the ARV pipeline in 2009 seems abundant, the drugs most likely to make it to market appear more like successors than revolutionaries. And the candidates in phase II and earlier stages cannot be counted on to be with us the next time this annual pipeline report comes out. The pharmaceutical industry will eventually turn its attention back to HIV—hopefully before too many people develop resistance, side effects, or give up on therapy. As long as HIV requires lifelong treatment, people with HIV all over the world deserve treatments they can live with for a long, long time.

Hepatitis B Drug Development in 2009

BY LEI CHOU

Despite rising worldwide rates of liver cancer and end-stage liver disease due to the hepatitis B virus (HBV), drug development for HBV has come to a virtual standstill over the past year. It is a disturbing situation, given that one quarter of the estimated 400 million people living with chronic HBV worldwide and up to 2 million people in the United States are at risk of HBV-related serious liver disease without treatment. One reason could be a perception on the part of drug makers that a sizable future market for HBV treatments will not materialize—perhaps due to a reduction in new infections owing to the universal vaccination of infants and effective prevention of mother-to-child transmission. A lack of surveillance systems and large-scale screening programs to reliably define HBV epidemiology has likely contributed to the uncertainty. The Centers for Disease Control and Prevention (CDC) currently have no chronic HBV surveillance program, and the recently expanded HBV screening recommendations were not accompanied by a funding increase.

Yet, market factors aside, scientific barriers to new drug development remain a major challenge; these barriers include the inherent difficulty in eradicating HBV infection, the lack of an efficient viral replication model for testing new drug candidates, and the need to avoid nucleoside drug resistance and toxicity. The first two of these barriers is unlikely to be breached anytime soon due to the severe lack of NIH investment—just 0.1% of the total NIH funding in 2007 was spent on HBV research. With the demise of clevudine in 2009 due to its toxicity, there are currently no new HBV investigational compounds in late-phase trials; meanwhile, several promising oral agents have apparently been put into deep freeze.

Amid this scientific vacuum, the unmet medical needs of people living with chronic HBV are growing. There are currently six FDA-approved HBV drugs belonging to just two classes. Pegylated interferon can provide stable disease remission in only one-third of people willing to undergo a full year of weekly injections and tolerate its severe side effects, with less than 10% able to attain a functional “cure.” The remaining five oral antivirals all belong to the nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) class, four of which have reduced efficacy due to prevalent multidrug resistance mutations, particularly in people who were put on sequential HBV monotherapy during the last decade as each of these drugs came on the market.

While tenofovir, the current front runner, has not been compromised to date by clinically significant resistance mutations in two years of clinical trials data, some studies have observed a reduced treatment response in people who are adefovir experienced. Unfortunately, the FDA did not require tenofovir's maker Gilead to conduct a separate dose finding study for HBV, resulting in concerns for potential renal toxicity in the long term, especially in people with liver damage and in Asians with lower body weights—two significant populations in chronic HBV.

Additionally, recent studies have highlighted the need for better treatment options for people coinfecting with HIV and HBV. Despite effective HIV and HBV viral suppression achieved through combination therapy, coinfecting people are still at higher risk for liver-related morbidity and mortality. The lack of access to tenofovir-containing first-line treatment in developing countries in Asia and Africa, where HBV infection is endemic, will likely compound this crisis with widespread HBV multidrug resistance and subsequent treatment failures.

This barren landscape has left doctors and people living with HBV weighing their remaining treatment options amid some big unanswered questions: When should treatment begin, and how long should it continue? While current treatment guidelines recommend starting therapy based on HBV viral load and liver inflammation (as indicated by the surrogate marker alanine aminotransferase, or ALT), no large-scale studies have validated the effectiveness of this approach in preventing cirrhosis or liver cancer over the long term. The optimal duration of treatment is also uncertain, depending on an individual's HBeAg status (Hepatitis B “e” Antigen; *see* box), how long he or she has been infected, and how long effective viral suppressive therapy has been given. Too early initiation of treatment is inhibited by concerns about the development of drug resistance, potential long-term toxicities, and the high cost of therapy.

It is this last point—the undesirability of costly lifelong therapy with oral antivirals—that is fueling ongoing research into immune-based therapies, primarily in Asia. HBV therapeutic vaccines have been investigated since the mid-1990s, mainly through small pilot studies that never advanced to larger trials in people with chronic HBV. Recent advances in HBV immunology, however, have shown that vaccine-induced immune control is theoretically achievable. There are at least four vaccine candidates currently being evaluated in phase I studies.

Another promising development is the establishment of the Hepatitis B Clinical Research Network, funded through the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) branch of the National Institutes of Health (NIH). Still in its start-up phase, the network plans this year to launch a national observational database to advance the understanding of HBV natural history and to conduct clinical trials to investigate optimal treatment strategies.

TAG's second annual HBV pipeline report documents some recent research setbacks, discusses the surviving experimental agents and therapeutic vaccines in development, and provides an update on the Hepatitis B Clinical Research Network.

HBV Clinical Research Terms and Endpoints

HBeAg (Hepatitis B “e” Antigen): There are two main types of chronic HBV disease, based on the presence or absence of HBeAg, a protein produced during the HBV replication process.

People who are HBeAg-positive are in the early phase of chronic disease. HBeAg seroconversion to negative, with development of HBe antibodies and undetectable viral load, is a treatment endpoint equivalent to stable remission. Some people may be able to stop treatment after a period of effective viral suppression.

People who are HBeAg-negative but with a detectable viral load likely have developed HBV core mutations, which allow viral replication without producing HBeAg. Chronic HBeAg negative disease is seen in the later phase of chronic infection—primarily people older than 35 who were infected at birth. These people will need to stay on treatment even with effective viral suppression, since most will experience viral rebound if treatment is stopped.

HBV DNA (Viral Load): Large-scale observational studies done in Asia have shown a clear connection between high HBV viral load and development of serious liver disease. Undetectable HBV DNA is a major treatment goal. People with a detectable viral load after one year of treatment have a higher risk of developing drug resistance.

ALT (Alanine Aminotransferase): A surrogate marker of liver inflammation, elevated ALT is an indicator of liver injury caused by immune activation. ALT levels usually fall within the normal range following effective viral suppression. Some studies have shown this correlates with a halt or reversal of liver damage.

HBsAg (Hepatitis B Surface Antigen): a small protein on the surface of HBV, HBsAg is an indicator of chronic HBV infection. HBsAg seroconversion to negative, with development of HBs antibodies and undetectable viral load, is closest to a sustained cure of chronic HBV, although this is rarely achieved with current treatment options.

Experimental HBV Agents in Development, 2009

Agent	Class	Sponsor	Status
Truvada (emtricitabine coformulated with tenofovir)	Dual NRTI	Gilead Sciences	Phases II/IV
LB80380	NRTI	LG Life Sciences	Phase IIa
Interferon gamma 1b (Actimmune)	Immunomodulator	InterMune	Phase II
Thymosin alpha (Zadaxin)	Immunomodulator	SciClone Pharmaceuticals	Phase IV
DNA vaccine pCMV52.S	Therapeutic vaccine	French National Agency for Research on AIDS and Viral Hepatitis (ANRS)	Phases I/II
DNA vaccine (HB-110)	Therapeutic vaccine	Genexine	Phase I
Hepatitis B vaccine (Synthesized peptide PA-ε44)	Therapeutic vaccine	Chongqing Jiachen Biotechnology	Phase I
HBV DNA plasmid pdpSC18 vaccine	Therapeutic vaccine	PowderMed/Pfizer	Phase I

Recent Research Setbacks

Clevudine

Pharmasset voluntarily halted its phase III clevudine registration trials in April 2009 following reports of 80 cases of myopathy (muscle weakness), some severe, from postmarket use in South Korea. An oral antiviral already approved in South Korea and the Philippines, clevudine was the farthest along in the HBV development pipeline. The U.S. Food and Drug Administration (FDA) had required Pharmasset to conduct 48-week trials for its approval. In this case the FDA saved the day, since reported cases of severe myopathy developed after 32 weeks on treatment. Disturbingly, the drug was approved by South Korean regulators with only 24-week data—before the onset of symptoms.

Thought to be caused by mitochondrial toxicity, a well-known side effect of the nucleoside analog class, one reported case of myopathy was so severe that the patient could not stand up without assistance. Fortunately, all of the 80 reported cases of myopathy resolved after stopping clevudine treatment, but it took up to 16 weeks for some patients to fully recover.

Another trial in France using clevudine in combination with tenofovir was also halted. Bukwang, the maker of clevudine in South Korea, is still conducting trials in Asia. It is not clear if South Korean regulators will take any action based on this information or if other countries will approve the drug.

Combination Therapy with Telbivudine and Pegylated Interferon

Another notable setback occurred last year with the use of combination therapy. Although limited data to date have not shown whether combination therapy with two oral antivirals or an antiviral combined with pegylated interferon improve treatment response, studies were done using older drugs that are less potent than the newly approved antivirals.

Novartis conducted a study using telbivudine (Tyzeka), one of the newer approved antivirals, in combination with pegylated interferon (Pegasys). Novartis halted the study in 2008 after increased incidence of peripheral neuropathy was observed in the combination arm. Peripheral neuropathy related serious adverse events developed between two and six months in 19% of participants in the combination arm versus 4% in the telbivudine-only arm, and nonserious adverse events developed in 16% in the combination arm versus 5% in the telbivudine-only arm. This disappointing development led to a telbivudine drug label change and a physician warning letter issued by the FDA in January 2009.

It is not clear what caused the increase in incidence of peripheral neuropathy, another well-known side effect of the nucleoside class related to mitochondrial toxicity, or if combinations using other more potent oral antivirals and pegylated interferon would have similar results. Other trials are currently investigating different strategies including lead-ins or add-ons of various drug combinations.

Oral Antivirals

Emtricitabine/Tenofovir (Truvada)

Emtricitabine plus tenofovir (nucleoside and nucleotide analogs, respectively; coformulated as Truvada), are in phase II and IV trials using the same fixed-dose combination pill approved for HIV. Gilead is anticipating submission for approval as a combination therapy for an indication in people with drug-resistant HBV, including people who had suboptimal response on adefovir monotherapy or documented lamivudine resistance. Trials in people coinfecting with HIV or with decompensated liver cirrhosis, or in post-liver transplant patients (groups that are at higher risk of developing HBV drug resistance) are also underway.

LB80380

The only other nucleoside analog with an active development program, LB80380, from LG Life Sciences, is slated to start enrollment in its phase IIb trial during the third quarter of 2009 in South Korea. The company is still looking for a new development partner in the United States and Europe.

Immunomodulators

Pegylated interferon alfa 2b (Pegasys) is an approved treatment for chronic hepatitis B. Despite severe side effects and low efficacy, it is the agent most capable of inducing HbsAg (Hepatitis B Surface Antigen; *see* box) clearance and therefore “curing” HBV infection. This is thought to be due to both the immune modulating and antiviral properties of interferon. In the quest to boost response rates, researchers are looking at combining pegylated interferon with other immunomodulators. Two such combinations are currently in early clinical trials.

Interferon Gamma 1b (Actimmune)

Interferon gamma 1b is a synthetically manufactured form of human interferon gamma, an immunomodulator that has been shown in animal studies to play a key role in hepatitis B viral clearance. It is an approved treatment for two hereditary immune disorders mainly seen in children: chronic granulomatous disease and malignant osteopetrosis.

A small, phase II, 30-day safety and efficacy study is currently underway. Sponsored by InterMune, the maker of interferon gamma 1b, and Huntington Medical Research Institutes in California, this three-arm study will compare interferon gamma 1b versus adefovir versus both in combination.

This trial will use daily injections of 200 micrograms of interferon gamma 1b—nearly ten times higher than that used for its approved indications (50 microgram injections three times a week). There are potential safety issues at this higher dose as evidenced by the long list of exclusion criteria for this study. In earlier trials, cardiac and neurological side effects were reported in volunteers with these preexisting conditions using a 250 microgram daily dosing. Results are expected in late 2009.

Thymosin alpha1 (Zadaxin)

Thymosin alpha1 is a synthetic version of a substance that is produced naturally by the thymus. It has been approved in over 30 countries to treat chronic HBV, but not in the United States. It caused fewer side effects than interferon in previous studies, but there are conflicting results in treatment response rates.

SciClone, the maker of thymosin alpha1, is conducting a phase IV trial in HBsAg-positive Korean volunteers using thymosin alpha 1 in combination with pegylated interferon for three months, followed by nine months of pegylated interferon alone. A previous study showed a 70% response rate in HBsAg negative volunteers using 6 months of thymosin alpha1 in combination with 12 months of standard interferon, as compared to 20% response rate with standard interferon alone. Results are expected in 2009. U.S. development plans are unclear.

HBV Compounds in Deep Freeze

Nitazoxanide (Alinia)

Nitazoxanide is an FDA-approved antiprotozoal agent used to treat intestinal parasites that has activity against hepatitis B and C. However, a planned phase II trial comparing nitazoxanide with entecavir for the treatment of HBV did not materialize in the past year. It is not clear if the maker Romark is pursuing further development for HBV. The drug is in phase III trials in combination with pegylated interferon and ribavirin to treat hepatitis C.

Bay 41-4109

Bayer's Bay 41-4109 is a heteroaryldihydropyrimidine (HAP) that inhibits HBV assembly by interrupting viral capsid formation. No news has been heard about this compound since it made a splash in 2001, although it remains listed as an investigational agent in Bayer's annual report.

NUC B1000

Nucleonics' NUC B1000 is an RNA interference-based gene therapy designed to destroy HBV RNA inside infected liver cells. Nucleonics faced financial trouble in 2008 after a series of legal battles, and has halted its phase I development after the company was put up for sale. The fate of this compound is uncertain.

Therapeutic HBV Vaccines

One of the puzzling characteristics of chronic HBV infection is the state of immune tolerance, where there is no immune response despite ongoing viral replication. Since liver damage is not caused by HBV itself but by the immune response to infected liver cells, people can live in a state of immune tolerance for decades without developing any significant liver damage. At the same time, a majority of healthy

adults can clear acute HBV infection through a combination of adaptive (antibody) and cellular (CD4/CD8 T cells) immune responses without sustaining liver damage.

This paradox has led researchers to identify specific types of immune response that can induce viral clearance without destroying infected liver cells in the process. This understanding is driving research on therapeutic vaccines for chronic HBV infection—some in combination with oral antivirals. The most advanced candidates are discussed below.

The French National Agency for Research on AIDS and Viral Hepatitis (ANRS) is conducting a phase I/phase II trial of the HBV Naked DNA vaccine pCMVS2.S in people who are willing to undergo a treatment interruption for at least one year. The researchers are investigating if transient T-cell responses—such as those elicited by the vaccine in a previous study—will be sufficient to maintain viral control in people with undetectable HBV achieved through oral antiviral therapy. The study is still enrolling patients and is slated for completion in late 2010.

Genexine has launched a phase I study in Korea using a mixed plasmid DNA (HB-110) vaccine combined with adefovir. This small study is looking for a response to dose-escalated injections of the vaccine given at 2-week intervals for 22 weeks plus adefovir given for one year in HBeAg-positive people. The trial is expected to be completed in 2009.

Chongqing Jiachen Biotechnology in China recently opened enrollment in its phase I dose-finding trial using Hepatitis B vaccine (Synthesized Peptide ePA-44). This study will compare two different doses of the vaccine in a series of six injections in HBeAg-positive volunteers.

PowderMed's phase I HBV DNA Plasmid pdpSC18 vaccine trial has been completed, but results have not been published to date. The company was purchased by Pfizer in 2007.

The therapeutic vaccine field has suffered some setbacks due to lack of enrollment. Emergent Biosolution's phase II trial of its HBV core antigen vaccine ceased enrollment in 2008 due to recruitment difficulties. According to the company's annual report, new international sites are being sought where hepatitis B treatment is not yet widely available. A combination trial using lamivudine and the preventive HBV vaccine Engerix B in Senegal sponsored by GlaxoSmithKline and the French National Agency for Research on AIDS and Viral Hepatitis was also terminated this year due to poor enrollment.

The Hepatitis B Clinical Research Network

Established by the NIDDK in October 2008, the Hepatitis B Clinical Research Network is composed of 13 leading HBV research centers throughout the United States and Canada, including sites in Alaska, Hawaii, Maryland, Massachusetts, Michigan, Minnesota, Missouri, North Carolina, Ontario, Pennsylvania, Texas, Virginia, and Washington. The network is funded through a \$45 million NIH grant to be spent over a seven-year period, and it is seeking industry participation in provision of drugs and diagnostics to supplement the initial funding level. It has established a website: <http://www.hepbnet.org>.

At the center of this endeavor is the creation of an observational cohort that will enroll 1,000 people at every phase of chronic HBV disease in order to address some of the knowledge gaps in its natural history and pathogenesis, assess long-term treatment outcomes and side effects, and identify better biomarkers for tracking HBV disease progression. Ancillary immunological and virological studies using stored samples from the cohort are also being planned. In addition, the network will conduct clinical trials in order to investigate various treatment strategies and combinations. According to the network's steering committee cochair, Michael Fried, MD, these trials are tentatively slated to start around the third and fourth quarters of 2009.

A major limitation of industry-sponsored trials has been the lack of long-term data, and this is compounded by trial participant attrition. One potential solution can be found in the experience of the AIDS Clinical Trials Group (ACTG). Community participation in every aspect of the ACTG was a groundbreaking initiative. From protocol design and participation in data safety and monitoring boards to development of informed consent forms and patient outreach and education, meaningful community involvement has been shown to improve trial recruitment and retention by increasing volunteer motivation and acceptance of difficult trial requirements. Given this rare publicly funded opportunity to advance our knowledge of chronic HBV disease, investigators of the Hepatitis B Clinical Research Network should vigorously engage those with the greatest stake in the network's success: people living with chronic HBV.

Tuberculosis Treatments

BY CLAIRE WINGFIELD

Current treatment regimens for drug-susceptible tuberculosis (TB) are effective. The most commonly used first-line regimen has a 95% cure rate.* Yet TB killed almost two million people in 2007, and it is the leading cause of death among people with HIV, accounting for 25% of all HIV deaths during that year. Even though the current treatment regimens have good bactericidal activity—in other words are effective at killing *Mycobacterium tuberculosis* (MTB), the germ that causes TB—they are not easy regimens to take consistently due to high pill burden, toxic side effects, long duration of treatment, and drug-to-drug interactions with medications for other conditions. Therefore, there is an urgent need to develop, validate, and approve more effective and more tolerable treatments for drug-susceptible and drug-resistant TB—particularly for children and for people with HIV.

Current Anti-Tuberculosis Drugs

Group	Drugs (Abbreviation)
Group 1 – First-line oral anti-TB drugs	isoniazid (H or INH); rifampicin (R or RIF); ethambutol (E or EMB); pyrazinamide (Z or PZA); rifabutin (Rfb)
Group 2 – Injectable anti-TB drugs	streptomycin (S or SMP); kanamycin (Km); amikacin (Am); capreomycin (Cm)
Group 3 – Fluoroquinolones	ofloxacin (Ofx); levofloxacin (Lfx); moxifloxacin (Mfx or moxi)
Group 4 – Oral bacteriostatic second-line anti-TB drugs	ethionamide (Eto); prothionamide (Pto); cycloserine (Cs); terizidone (Trd); P-aminosalicylic acid (PAS);
Group 5 – Anti-TB drugs with unclear efficacy for MDR-TB treatment (not recommended by the WHO for routine use in MDR-TB patients)	clofazimine (Cfz); linezolid (Lzd); amoxicillin/clavulanate (Amx/Clv); thioactezone (Thz); clarithromycin (Clr); imipenem (Ipm)

Source: Adapted from **WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis, Emergency Update 2008**.

While there are currently more new compounds being investigated to treat TB than there have been for decades, there are still too few sponsors and too few resources dedicated to moving these products through the drug development pipeline. Since there has been no new class of drugs approved for treating TB in over 40 years, there is limited experience in conducting TB drug registration trials meeting modern scientific and regulatory standards. To maximize resources and avoid repeating the

*Two months of isoniazid (H)/rifampicin (R)/pyrazinamide (Z)/ethambutol (E), followed by four months of isoniazid (H) and rifampicin (R).

mistakes of others, there is a need for collaboration and cooperation among industry, foundations, research institutions, governments and community—within the TB world and beyond—to bring these much-needed products to market and to encourage the development of newer compounds. At the same time, greater focus should be directed toward optimizing the use of current treatments for both drug-susceptible and drug-resistant TB.

The emergence of drug-resistant TB in every corner of the world highlights the need for treatments that are not only effective in children and adults but are easily adhered to, well tolerated, and can be safely dosed with other medications—particularly antiretrovirals (ARVs).

Pediatrics

Children account for more than 15% of the global TB disease burden and possibly an even greater proportion of deaths due to lack of diagnosis and proper treatment. Children are often treated presumptively because obtaining a bacteriological diagnosis is almost impossible in infants and young children due to their paucibacillary loads and the difficulty in getting a useful sample. Because there have been very few clinical trials evaluating the currently available TB drugs in children, there are very little data on how best to treat pediatric TB, and it is believed that children are often underdosed or overdosed. Children, particularly those five and under, are at increased risk for TB mortality, and there is an urgent need to figure out how to incorporate these young people into drug trials. There are certainly lessons to be learned from the fields of HIV and cancer, where pediatric clinical trials have been conducted for many years, and there is a slowly growing movement in the TB research community to conduct more pediatric treatment trials. Unfortunately, only two clinical trials included in this report are currently enrolling children, and none of the developers of the novel compounds have developed concrete plans to test their drugs in children.

Latent TB Infection

Approximately one-third of the world's population is infected with TB, meaning that two billion people have been infected with *MTB* but because their immune system has contained the bacteria they have not developed TB disease. Individuals who are latently infected with TB do not have symptoms and are unable to infect others—in fact, many people with latent TB infection (LTBI) may never know that they are

infected with TB. Unfortunately, the most commonly used method to diagnose LTBI, the tuberculin skin test (TST), lacks the sensitivity to give a confirmatory diagnosis in many people with advanced HIV disease and in infants and young children who have been vaccinated with BCG (for more on diagnosing LTBI, *see* “Tuberculosis Diagnostics”).

The risk for developing active TB disease for an HIV-negative person with LTBI is estimated to be about 10% over that person’s lifetime. So if a person was infected with TB when she was 20 years old and lived until she was 60, she would have a one in ten chance of developing TB disease during those 40 years. However if this person is coinfecting with both HIV and TB, her risk for developing active TB disease shoots up to one in ten for *each and every year of her life*.

In 2007, approximately 9.27 million of the 2 billion people who are latently infected with TB developed active disease, with most cases occurring in sub-Saharan Africa, Eastern Europe, Asia, and Latin America—regions where most countries do not offer preventive therapy. This occurs despite the mountain of evidence showing that 6 to 12 months of daily isoniazid preventive therapy (IPT) is highly effective in decreasing the risk for developing TB disease among those with LTBI. Much of the reluctance to implement this treatment is due to fear on the part of health care providers of generating isoniazid resistance, which puts TB patients at risk for developing multidrug-resistant TB (MDR-TB).

Despite these fears, there is a push to make IPT available in HIV care settings, since the risk for developing TB disease is high for people with HIV and increases as immune function decreases. To reduce morbidity and mortality, and demonstrate the usefulness for IPT among people coinfecting with HIV and TB, many ongoing LTBI studies are looking at how best to roll out IPT in high-HIV-prevalence settings.

The Consortium to Respond Effectively to the AIDS/TB Epidemic (CREATE) is evaluating different strategies to improve the uptake and treatment completion of IPT within high-HIV-prevalence settings. CREATE is headquartered at the Johns Hopkins University in the United States, with study sites in Zambia, South Africa, and Brazil, and is funded by the Bill and Melinda Gates Foundation. The THRio study, which is being conducted in collaboration with the Health Secretariat of Rio de Janeiro, Brazil, is evaluating the impact of training health care workers to use the TST to detect LTBI among—and to provide IPT to—people who are accessing HIV care and treatment. The THRio study will be completed in 2010. Current data suggest that those who have initiated IPT have a 90% completion rate.

The Thibela TB study is a CREATE trial that is evaluating the impact of communitywide IPT on TB incidence in a high-TB- and HIV-prevalence setting.

CREATE has partnered with the Aurum Research Institute to enroll almost 70,000 employees of three South African gold mines to be randomized to receive no intervention or to receive active TB case finding and communitywide IPT for all of those without active TB. As of March 2009, over 27,000 mine staff have been enrolled to take nine months of IPT. Adverse reactions have been low, and in the most recent analysis of 13,500 volunteers there were three cases of hepatitis (liver inflammation). Other adverse events among this group included hypersensitivity and peripheral neuropathy, which occurred in 55 and 41 study volunteers, respectively. Adherence to IPT among the first cohort initially peaked at 40–50% but has now improved to 80% in recently enrolled clusters.

Medécins sans Frontières (MSF) and the University of Cape Town have a study underway in Khayelitsha township in Cape Town, South Africa, in which almost 1,300 HIV-positive adults who are eligible for ARVs are being randomized to receive either 12 months of presumptive IPT or no IPT—with no screening for LTBI performed. The aim of the study is to assess the impact of presumptive IPT for people with HIV on the incidence of TB disease as well as on rates of isoniazid resistance. Study completion is expected in 2011.

Another study evaluating IPT among HIV-positive adults is wrapping up and results are expected in December 2009. The U.S. Centers for Disease Control and Prevention (CDC) and the Botswana Ministry of Health randomized 1,995 HIV-positive adults to receive limited IPT (6 months) versus continuous IPT (36 months).

KNCV, a Netherlands-based nongovernmental organization; the Kenya Medical Research Institute; and the Kenya Ministry of Public Health and Sanitation are recruiting for a phase IV open-label, randomized cluster trial at sites across Kenya. The aim of the study is to evaluate the impact of community-based active TB case finding and IPT on TB incidence among child contacts of HIV-positive adults with smear-positive pulmonary TB. The intervention includes “proactive” TB screening in the community and the initiation of communitywide IPT if appropriate. Secondary outcomes are the incidence of adverse events, TB-related symptoms, and measures on the uptake of IPT. Study completion is expected in December 2011.

A phase II/III double blind, randomized, placebo-controlled study funded by the National Institute of Allergy and Infectious Diseases (NIAID) that evaluated the impact of six months of IPT on the incidence of TB disease among HIV-positive children in South Africa completed study follow-up on June 1, 2009. Approximately 450 children, ages three to four months, all had access to ARVs and were prescribed cotrimoxazole treatment (CPT) per World Health Organization (WHO) guidelines. The children were randomized to receive 96 weeks of daily IPT or matching placebo. New enrollments and dosing of enrolled children were stopped by a safety

monitoring board in June 2008 after an interim analysis showed that the study was unlikely to meet its objectives of demonstrating an effect of IPT in reducing TB disease or all causes of mortality in HIV-positive infants when compared to placebo.

Because of the fear of developing isoniazid resistance and the long duration of IPT, a number of clinical trials are exploring alternatives to IPT. However, in many of these studies, taking ARVs is cause for exclusion. As a result, it has been more difficult to recruit HIV-positive volunteers in settings with a higher incidence of LTBI than active disease, such as the United States, where many HIV-positive people initiate ARVs at higher CD4 counts.

The Tuberculosis Trials Consortium (TBTC), with funding from the CDC, is nearing the end of Study 26, which is being conducted at sites in Brazil, Spain, Canada, and the United States to evaluate 12 doses of once-weekly, directly observed isoniazid/rifapentine versus nine months of daily isoniazid. The study reached its goal of enrolling more than 8,000 volunteers, but because of insufficient enrollment of children under 12 years of age and HIV-positive adults, the TBTC has partnered with the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) and the AIDS Clinical Trials Group (ACTG) to increase representation of these populations. Results from a completed substudy of rifapentine pharmacokinetics (PK) in children are not yet available. Hepatotoxicity (liver toxicity) and hypersensitivity substudies also are underway.

The Johns Hopkins University, with funding from NIAID, recently completed a study that randomized HIV-positive, TST-positive South African adults to receive 9 months of isoniazid versus 12 weeks of isoniazid/rifapentine once weekly, versus 12 weeks of isoniazid/rifampicin twice weekly, versus continuous isoniazid (daily isoniazid for the duration of the trial). Study results showed that short-course rifamycin-based regimens and continuous isoniazid were as efficacious as six months of isoniazid; however, development of rifamycin resistance is of concern in both of those arms.

Because most of the trials evaluating alternatives to IPT exclude persons on ARVs there is limited to no drug-to-drug interaction data. While there are some promising studies trying to shorten treatment for LTBI, there is a need for more studies looking at treating LTBI in high-HIV-prevalence settings, and evaluating regimens that may be more durable, more potent, and less likely to generate resistance than IPT. This evidence is vital to allowing national TB programs and AIDS control programs, particularly in settings with high TB prevalence, to begin providing preventive treatment to persons who are latently infected with TB without creating more cases of drug-resistant TB.

Rifapentine

Rifapentine—the last drug licensed by the U.S. Food and Drug Administration (FDA) to treat TB—was approved in 1998. It belongs to a class of drugs called rifamycins, which also includes rifampicin and rifabutin. Rifamycins are some of the most powerful drugs in the TB treatment arsenal and serve as the backbone of first-line treatment regimens. Rifampicin is the most widely used of the rifamycins because of its affordability and effectiveness. Currently, rifapentine is FDA approved only for use in the continuation phase of first-line treatment for HIV-negative patients with drug-susceptible TB. However, the maker of rifapentine, Sanofi-Aventis, has resurrected its TB program and is hoping to expand the indication for the drug and get it listed on the WHO's Essential Medicines List. The rationale for looking at rifapentine in both LTBI and active disease is based on data from mouse models showing rifapentine to be significantly more powerful than rifampicin at lower doses and better tolerated at higher doses. As a result it may help to shorten LTBI treatment and increase the potency of first-line treatment. However, because rifapentine is an inducer of cytochrome p450, it shares rifampicin's drug interactions with ARVs, specifically protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs).

Active TB Disease

Drug-Susceptible TB

Because the standard of care for first-line treatment can cure 95% of drug-susceptible TB cases, it will be difficult to show that a new drug or treatment regimen is superior. In order to do so, one would need to conduct a large-scale study of thousands of volunteers, which would take years and require resources that are not currently available. A topic of much discussion in the TB research community is using noninferiority studies for evaluating treatments for drug-susceptible TB. This study design is based on the premise that a new drug or treatment regimen does not necessarily have to be better than the current standard of care in killing *MTB*, but needs to be as good or almost as good as long as it has some other characteristic(s) that significantly improve(s) treatment completion. Thus, if a new TB treatment regimen is able to cure 90% of cases but has fewer side effects and/or only takes three months, then it may be acceptable as an alternative to the standard of care. One concern shared by regulatory agencies and the scientific community with using noninferiority designs is that by lowering the acceptable cure rate the effectiveness

of treatment declines. If this design is used repeatedly to assess treatments, cure rates may decline to unacceptable levels.

Currently, no novel compounds are being evaluated for treating drug-susceptible TB, where the focus is on shortening treatment. Three existing drugs have moved into phase II and III clinical trials to evaluate their potential for shortening treatment of drug-susceptible TB.

The TBTC's Study 29 is a phase II randomized, open-label study evaluating the safety and efficacy of daily rifapentine in place of rifampicin during the intensive phase of first-line treatment in adults with smear-positive pulmonary TB at sites in the United States, Canada, South Africa, Uganda, Spain, and Brazil. It is expected that enrollment will be completed by early 2010, with final study follow-up completed by September 2010. HIV-positive adults are eligible for enrollment as long as they are not taking ARVs. Serum samples are being collected to investigate biomarkers for assessing the efficacy of study drugs and hypersensitivity to study drugs. A substudy is also underway to evaluate the PK behavior of rifapentine and rifampicin in 60 patients. A single, timed serum sample from a large subset of volunteers in the rifapentine arm is being collected to evaluate the PK of rifapentine in the broader population.

The Johns Hopkins University, University of Cape Town (UCT), and the UCT Lung Institute with funding from the FDA will be evaluating the safety and efficacy of two doses of rifapentine in the intensive phase in adults with CD4 counts above 200. Volunteers will be randomized to receive 450 mg of rifapentine along with isoniazid/pyrazinamide/ethambutol versus 600 mg of rifapentine plus isoniazid/pyrazinamide/ethambutol versus the control of 600 mg of rifampicin plus isoniazid/pyrazinamide/ethambutol for 8 weeks, and will be followed for 12 months after completing the experimental phase. This study has not yet begun enrollment.

The Global Alliance for TB Drug Development (TB Alliance) in collaboration with Bayer HealthCare and a number of research institutions is conducting the REMox trial. This is a phase III trial evaluating whether using moxifloxacin in place of ethambutol or isoniazid is effective in shortening first-line treatment from six to four months. Previous phase II studies showed that using moxifloxacin in place of ethambutol or isoniazid during the first two months of treatment gave similar or better sputum smear conversion rates and resulted in faster time to sputum culture conversion. It is believed that these endpoints are good indicators of the potential for shortening treatment. Recruitment is underway at sites in South Africa, Zambia, and Tanzania. Additional sites in Africa, Asia, and Latin America are expected to begin enrolling in the second half of 2009. It is expected that the last patient follow-up will be complete in mid-2012, with final results later that year.

Fluoroquinolones

Moxifloxacin and gatifloxacin, two drugs farthest along in the pipeline for shortening first-line treatment, are of a class of drugs called fluoroquinolones. These drugs are broad-based antibiotics, meaning that they are used to treat a number of bacterial infections and are not specific to TB. Fluoroquinolones are among the most powerful drugs in any second-line treatment regimen for MDR-TB. Because they are highly cross-resistant with one another, some in the field question whether fluoroquinolones should be reserved for treating drug-resistant TB and not used as part of first-line treatment. The main argument in favor of using these drugs in first-line treatment is that they have shown good potency against replicating and persistent strains of *MTB*, and it is believed that these nonreplicating or slowly replicating mycobacteria are the cause of the long duration of TB treatment. Resistance to fluoroquinolones requires multiple mutations, making it more difficult for loss of susceptibility to occur. However, drugs in this class are widely used and available over the counter in many parts of the world, particularly in Eastern Europe, which has the highest rates of MDR- and extensively drug-resistant TB (XDR) in the world. Thus it is likely that a high rate of fluoroquinolone resistance already exists in the general population, and therefore in the circulating *MTB* population.

The OFLOTUB consortium, an international collaboration of ten research institutions and organizations from Europe and Africa, and the WHO's Special Programme for Research and Training in Tropical Diseases (TDR), have completed enrollment for a phase III study evaluating gatifloxacin-containing regimens for shortening first-line treatment. Study volunteers are being followed for 18 months to monitor for failure and relapse. Gatifloxacin has shown the potential to cause dysglycemia (abnormal glucose levels) in previous studies. In order to address this issue, the eligibility criteria for this study were restricted to reduce the chance of enrolling volunteers with increased risk for developing this condition. It is expected that the initial safety and efficacy analysis will be conducted one year after treatment completion (likely in mid-2010), and will be followed by a final two-year analysis.

The Johns Hopkins University in collaboration with the Federal University of Rio de Janeiro is set to begin enrollment in a phase II open-label 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 2010.

Novel Compounds

With five new compounds with novel mechanisms of action in clinical trials, the TB drug pipeline is fuller than it has been in decades. Despite this promising news, the fate of many of these compounds is questionable due to lack of resources to adequately evaluate them. Industry, foundations, research institutions, governments, and regulatory agencies need to work together to bring these much-needed products to market and to conduct phase IV postmarketing studies. It is unclear whether there are enough funds for phase III studies of any of these new compounds. Unfortunately, the cost of large-scale clinical trials for TB are huge due to a number of unique aspects such as nonroutine microbiology (solid and liquid culture methods and drug susceptibility testing), complying with regulatory monitoring requirements in resource limited settings with underdeveloped research infrastructure, and the length of follow-up required to show efficacy (the lack of reliable surrogate makers requires longer follow-up to show efficacy for both drug-susceptible and drug-resistant TB). Based on these factors and costs of previous trials, it is estimated that the cost per volunteer will range from US\$15,000–20,000 for a phase III study.

Experimental TB Drugs with Novel Mechanisms of Action

Agent	Class	Sponsor	Status	Indication
TMC-207	Diarylquinolone	Tibotec	Phase II	MDR TB
OPC-67683	Nitroimidazole	Otsuka	Phase II	MDR TB
PA-824	Nitroimidazole	TB Alliance	Phase II	DS-TB
SQ 109	Diamine	Sequella	Phase I/II	DS-TB
PNU-100480	Oxazolidinone	Pfizer	Phase I	

Sequella is currently evaluating SQ 109 at 75 mg and 150 mg doses given daily for 14 days, and 150 mg daily for five days with additional doses on days 9 and 14 in healthy volunteers. Pending discussion with the FDA, Sequella is hoping to add a group to receive 300 mg daily for 14 days. The European and Developing Countries Clinical Trials Partnership (EDCTP) has funded Sequella via the Pan African Consortium for Evaluating Anti-tuberculosis Agents (PanACEA) for an early bactericidal activity (EBA) study, synergy studies, and a phase II/III double-blind, active-control efficacy study of SQ 109 for drug-susceptible TB. In total, around 600 patients will be enrolled in these studies, which will begin in mid- to late 2010. If results are promising and resources are available, Sequella intends to evaluate SQ 109 in drug-resistant TB.

Drug Resistance

With multi- and extensive-drug resistance on the rise throughout the world via primary transmission and as the result of inadequate TB treatment, it is encouraging to see Otsuka, Tibotec, Pfizer, NIAID, and the TBTC conducting trials to improve treatment for MDR-/XDR-TB. At best, cure rates of up to 70% have been seen for MDR-TB, but this is hardly the norm and is likely closer to 50% for MDR-TB and no higher than 30% for XDR-TB. To further complicate this problem there is growing concern about the impact of other forms of drug-resistant TB. There is little to no evidence on how best to treat people with monoresistance (resistance to only one TB drug) and polyresistance (resistance to two or more TB drugs but not, specifically, isoniazid and rifampicin). There has been a call for studies to assess how prevalent drug resistance is and what its impact is on treatment. For example, what is the best treatment regimen for someone with TB who is resistant to pyrazinamide, an important drug in first- and second-line treatment because of its sterilizing effect on *MTB*?

The TB Alliance recently completed a phase IIa EBA study evaluating four doses (200 mg, 600 mg, 1000 mg, and 1200 mg) of its nitroimidazole drug PA-824 and found it to be safe and well tolerated. The EBA seen from days 0–2 and the extended EBA (measured over 14 days) were equivalent, meaning that the drug seems to be equally as active (at these doses) in the first 2 days as over the entire 14-day period. The TB Alliance is planning to evaluate PA-824 in both drug-susceptible and drug-resistant TB, if funding allows. Clinical studies are currently on hold while the FDA reviews recent data suggesting that the drug may have the potential to cause cataracts. PA-824 caused cataracts in rats at high doses but did not have the same effect in monkeys or humans. Because previously tested doses demonstrated equivalent activity, recruitment is expected to begin in the summer of 2009—assuming the clinical hold will be lifted—for a new EBA study to explore lower doses and identify a dose to move forward into subsequent trials. This phase II, low-dose, extended EBA study is planned for once-daily doses of 50 to 200 mg/day for 14 days. Phase III funding for this compound is not yet guaranteed.

PA-824 belongs to the same class of drugs as another up-and-coming novel TB treatment compound, Otsuka Pharmaceuticals' OPC-67683. Both are nitroimidazoles and are believed to have the same mechanism of action, and

therefore it is anticipated that strains resistant to either of these two drugs will also be resistant to the other, although potentially at different drug concentrations. For this reason it is likely that only one of these two drugs would ever be included in a regimen—similar to the situation with fluoroquinolones.

Otsuka is evaluating OPC-67683 in 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 in Eastern Europe, East and Southeast Asia, South America, and the United States. Full enrollment of 430 volunteers is expected by the end of 2009. 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 completion. All patients who have completed the double-blind portion will be eligible to enroll in an open-label study of OPC-67683 for six months. Otsuka is planning studies of drug-to-drug interactions with ARVs to allow for the safe inclusion of people with HIV in future trials of OPC-67683. Plans for phase III studies are still in early stages and no definitive decisions will be made until the data from phase II are available.

Tibotec Pharmaceuticals, a subsidiary of Johnson & Johnson, is currently enrolling for the second stage of a phase II study of its diarylquinolone, TMC-207. This compound is active against drug-susceptible and drug-resistant TB, and, as first in its class, is not cross-resistant with any other TB drugs. Tibotec is evaluating TMC-207 for an indication to treat MDR-TB. Results from an interim analysis of stage one of the study were promising, and found that the addition of TMC-207 to standard background therapy (SBT) for MDR-TB resulted in faster culture conversion and a higher number of culture conversions at the end of the eight-week treatment. In the second stage of this study, 150 volunteers are being randomized to receive either TMC-207 plus SBT or placebo plus SBT for 24 weeks, then continued on SBT alone for up to 18 months, and followed for an additional 6 months once treatment is completed.

The final results of the entire phase II study will not be available until the two-year follow-up is complete. However, a primary analysis of stage two will be conducted when all patients in that stage have been treated for six months. That analysis will include first-stage long-term follow-up results available to date. If results from the second stage are as promising as they were in the first, it may be reasonable for Tibotec to consider seeking accelerated approval for TMC-207 for treatment of MDR-TB given the dire need for effective therapy. This approval would be conditional on phase III studies being conducted to prove clinical efficacy. Plans for phase III are still under consideration, as it is unclear what will be required by regulatory agencies to consider TMC-207 for accelerated approval and/or traditional approval.

Tibotec and the TB Alliance recently announced that the Alliance has been granted a royalty-free license to develop TMC-207 for drug-susceptible TB. The TB Alliance will be evaluating the compound as part of a shortened first-line treatment regimen. Because TMC-207 drug levels are reduced when dosed with rifamycins, more data are needed to determine how best to use TMC-207 for treatment of drug-susceptible TB. Additionally, Tibotec and the TB Alliance will be collaborating on a drug discovery program to identify new compounds for TB treatment. Under the terms of the agreement, the Alliance will own the rights to any new compound through a royalty-free license.

The Role of Antibiotics in Background Therapy

In all of the current trials evaluating treatment for MDR-/XDR-TB, volunteers are being given placebo or a study drug plus standard or optimized background therapy (SBT or OBT). This begs the question, what is the difference between these two background regimens? The answer ranges from study to study and country to country based on which drugs are available. Ideally, any background regimen for drug-resistant TB will contain at least four drugs that the person is sensitive to, based on drug susceptibility testing (DST). Because DST results are often not available when a volunteer begins treatment, the background regimen may initially be limited to the available second- and third-line drugs on the WHO Essential Medicines List.

Broad-Based Antibiotics Being Evaluated for Treatment of Drug-Resistant TB

Agent	Class	Sponsor	Status	Indication
Metronidazole	Nitroimidazole	Pfizer (U.S.)/ Sanofi-Aventis (global)	Phase II	MDR-TB
Linezolid	Oxazolidinone	Pfizer	Phase II	MDR-/XDR-TB

Metronidazole is a broad-based antibiotic that is being evaluated for treatment of MDR-TB. Previous data show metronidazole is active against *MTB* in an anaerobic environment (one with little to no oxygen). It is believed that bacilli that can survive in this environment contribute to the long duration of TB treatment. In Korea, NIAID is sponsoring a phase II randomized, double-blind controlled trial comparing metronidazole plus SBT in volunteers with confirmed MDR-TB to placebo plus SBT.

Linezolid is an antibiotic that has been used off label in treating drug-resistant TB for years, but has not been approved for a TB indication. Because of its cost and toxicity it has been considered a drug of last resort. Linezolid can cause anemia (which stops with drug discontinuation) and irreversible peripheral neuropathy. However, given the dearth of treatments available to people with XDR-TB, two single-site studies are evaluating the efficacy, tolerability, and safety of lower doses of linezolid.

The TBTC's LiMiT study (TBTC Study 30) is a phase II, double-blind, placebo-controlled pilot study evaluating the safety and tolerability of low-dose, limited-duration linezolid in volunteers with confirmed MDR-/XDR-TB in Durban, South Africa. After the 16-week study treatment period, volunteers with limited treatment options, particularly those with XDR-TB, will have the option of taking linezolid on an open-label basis. In addition to evaluating the safety and tolerability of linezolid, another goal of TBTC Study 30 is to evaluate how best to conduct a clinical trial among people with drug-resistant TB. It is important to note that this study is not adequately powered to fully assess the efficacy of linezolid. A substudy will be evaluating the PK of linezolid and ofloxacin, a fluoroquinolone used in treating MDR-TB, in 40 of the 64 volunteers. A second substudy will evaluate blood and microbiological biomarkers for assessing treatment success.

The other linezolid study is being funded by NIAID and is about to begin recruitment in South Korea. It is a phase II study evaluating the efficacy and tolerability of two different doses of linezolid in volunteers with confirmed XDR-TB. Volunteers will be randomized to add 600 mg linezolid to a regimen or to wait two months to add 600 mg linezolid. After two consecutive negative sputum smears or at least four months of linezolid (whichever comes first), subjects will be randomized to either stay on 600 mg or dose-reduce to 300 mg once daily. HIV-positive volunteers are excluded from this study.

Pfizer has recently begun phase I testing of its novel compound PNU-100480 for a TB indication. Mouse studies suggest that this linezolid derivative has more bactericidal activity than linezolid despite lower exposure, and when combined with isoniazid and rifampicin, PNU-100480 showed significantly more reduction in colony forming units (CFUs) than linezolid.

In TAG's 2008 Pipeline Report, it was mentioned that Lupin Pharmaceuticals had a novel TB compound, Sudoterb/LL3858, in phase I clinical trials; however, it is unclear whether the company is continuing to pursue this compound.

Conclusion

There is an urgent need to develop, validate, and approve better, more effective, and more tolerable treatments for drug-susceptible and drug-resistant TB, particularly among populations bearing the highest burden of morbidity and mortality. It is encouraging to see a pipeline containing five novel compounds, and many more in preclinical studies, but it is important to acknowledge the challenges that must be overcome before these become useful treatments. We need new drugs as well as new regimens that are more effective, reduce the duration of treatment, and increase cure rates for drug-susceptible and drug-resistant TB. Clinical trial sponsors, designers, and regulators need to come together to invest in building research capacity and to formulate innovative strategies to develop and approve new drugs and new regimens in parallel, rather than sequential, trials. In conjunction with the development of novel drugs, current treatments for both drug-susceptible and drug-resistant TB need to be better understood so that their use may be optimized.

Because of the undue burden of TB morbidity and mortality borne by people with HIV and by infants and young children, it is imperative and ethical for these populations to be included in clinical trials of any new TB drugs, regimens, or treatment strategies. There also needs to be a review of the regulatory requirements for TB drug trials with the aim of expediting the process without sacrificing the safety of volunteers. In the same vein, endpoints used for trials of treatments for drug-susceptible and drug-resistant TB need to be reevaluated, and new surrogate markers developed to better assess the efficacy of treatment. Multi-experimental drug studies of new regimens for drug-susceptible and drug-resistant TB need to be conducted prior to individual drug approval, but these should not delay the clinical trial process, particularly for effective treatments for MDR-/XDR-TB.

Since no new class of drugs has been approved to treat TB in over 40 years, there is limited experience in conducting TB drug registration trials that meet modern scientific and regulatory standards. Therefore, in order to achieve the goal of ridding the world of TB by 2050, industry, regulatory bodies, research institutions, funders, and communities need to commit the necessary resources, to be innovative, to be collaborative, and to act with a sense of urgency in improving current TB treatment regimens and developing new drugs that offer the opportunity to shorten first-line TB treatment, improve cure rates, and shorten the duration of treatment for drug-resistant TB.

Tuberculosis Diagnostics

BY JAVID SYED

Introduction

The lack of accurate and accessible diagnostic tools for tuberculosis (TB) is the major stumbling block holding back better TB control efforts worldwide. The methods commonly used to detect and characterize TB are generally inadequate, but they are especially ineffective in people with HIV, infants, and people with drug resistant TB—three groups at greater risk of death due to TB.

The most commonly used test to diagnose TB is the sputum smear test. Robert Koch invented this test about 125 years ago when he discovered that *Mycobacterium tuberculosis* (*MTB*) was the cause of the disease. The smear test requires that sputum coughed up by a person with TB is smeared on a slide, stained, and visually identified under a microscope. The presence of the rod-shaped *MTB* bacteria will diagnose a person as being smear-positive and as having TB disease. However, this test not only routinely fails to identify about 50% of TB cases but is especially ineffective in people with HIV and in children, who are more likely to have smear-negative TB. Because no *MTB* are detected on the sputum smear despite the patient having TB disease in the lungs, smear negative TB must be diagnosed using culture.” Additionally, TB

Sensitivity and Specificity

These are two important measures of the accuracy of a diagnostic test.

Sensitivity measures the proportion of people with a disease that are correctly identified by a diagnostic tool as having the condition. **Low sensitivity** means that the diagnostic tool is less likely to catch everyone tested who has the given condition. These false negatives will lead to patients not being treated for their condition—patients who might be at risk of transmitting the infection to others and of becoming sick and dying.

Specificity measures the proportion of people without a disease that are correctly diagnosed. **Low specificity** means that people without a condition may be wrongly diagnosed as having it. These false positive cases will thus run the risk of patients being wrongly treated for a condition they do not have.

An accurate test will have both a high sensitivity and a high specificity.

that causes disease outside of the lungs (extrapulmonary TB) cannot be diagnosed by the sputum smear test. Despite these limitations sputum smear microscopy remains a key element in TB control efforts. This is in part because the test is inexpensive and does not require sophisticated technology.

The culture test is another common method for diagnosing TB disease. This test involves growing the bacteria in a media that provides nutrients, usually the solid Lowenstein-Jensen (LJ) media. If TB grows from a sample, then the patient is diagnosed as culture positive and the person has TB disease. The culture test is close to 100% sensitive and specific, and can also be used to diagnose extrapulmonary TB. However, *MTB* grows very slowly, going through one binary-fission cycle every 24 hours. Therefore, it can take four to eight weeks for a sample to grow sufficiently to be identified. Additionally, the technology and biosafety measures required in a laboratory to keep workers safe from the growing live TB culture means that these tests cannot be decentralized easily. In a variation of the test, anti-TB medications can be mixed into the media so that growth or lack of growth can provide information about the bacteria's susceptibility to various drugs. However, drug susceptibility tests (DST) in solid media can take another four weeks.

There are other tools used to assess TB infection or disease. Though they may not be sufficient on their own to confirm a diagnosis, these tests are often used by care providers in combinations to detect TB and start treatment. The symptom screen is one such simple, low-cost tool that diagnoses the disease by looking for common symptoms (persistent cough that lasts for more than two weeks, weight loss, night sweats, fatigue, and blood in sputum).

The Tuberculin Skin Test (TST) can detect TB infection, even in the absence of disease. This commonly used test involves injecting fragments of the *MTB* cell wall under the skin and measuring the swelling of the hard bump or induration that occurs at the injection site after 72 hours. The presence and size of the induration is caused by the strength of a person's immune response to the TB proteins. Someone who has never been exposed to TB should not have a response. However, since the TB vaccine Bacillus Calmette-Guérin (BCG) includes TB cell wall fragments similar to those contained in the TST, people who have been vaccinated may have a false-positive reaction. As the test depends on normally functioning immune responses, people with low immunity, including people with HIV, are routinely misdiagnosed via TST. The test is not able to distinguish between latent TB infection and active TB disease.

Chest X-ray is commonly used to detect lesions in the lung that are characteristic of current or past TB disease. Abnormalities in the lungs that are visible on X-rays

provide information that a health care worker can use in conjunction with other tools such as the symptom screen and the TST to diagnose TB.

The specific choice and order in which health care workers use tools to come to a diagnostic decision is called an algorithm. As there is no one TB test ideal for use in health posts in low resource settings where most TB patients seek testing and treatment for TB, the improvements offered by new tools are assessed by their additive benefit to existing diagnostic algorithms.

TB is primarily a disease of the poor, and 95% of people with TB live in developing countries. In these resource-constrained settings, the utility of a diagnostic is limited by the infrastructure required to implement it; an inaccessible technology will ultimately not be a useful tool for saving lives.

What Does the Pipeline Contain?

TAG's 2009 pipeline report for TB diagnostics focuses on tools and strategies that are likely to be considered by the Strategic and Technical Group on TB (STAG-TB) at the World Health Organization (WHO) in the next three years.

Unfortunately, since TAG's *2008 Pipeline Report*, no new technological breakthroughs have occurred in TB diagnostics and no new tools have entered the pipeline. The pipeline contains products that are being studied for use in high-TB-burden settings.

To understand the potential impact of tools in the pipeline, the tools are divided into three levels of the health system where they are most likely to be used—the health post, peripheral laboratory, and reference laboratory levels. These health systems serve about 60%, 25%, and 15% of total people in need of TB services, respectively. However, as was mentioned in TAG's 2008 pipeline report on TB diagnostics, this, too, is country specific. For instance, in Kenya only 18% of public health facilities had the capacity to carry out the relatively simple smear microscopy test. Additionally, the basic infrastructure available at any health system level, such as regular supply of electricity and water, varies from one country to another and can severely impact a tool's accuracy and use.

Strategic and Technical Group on TB (STAG-TB) Approval and WHO Policy Guidance

The WHO convenes its STAG-TB annually. The STAG-TB is made up of researchers, national TB program representatives, implementation agencies and TB advocates. The STAG-TB considers recommendations from expert committees convened to assess data on topics such as new tools or policy issues relevant for TB control. The STAG-TB takes into account the recommendations from the expert committee and makes its own recommendations to the WHO. Though these recommendations are nonbinding, the WHO will usually issue guidance on the use of a tool or strategy in high-TB-burden settings based on an endorsement from the STAG-TB.

The WHO recommendations focus on particular strategies and methods, and not on specific commercialized products. For instance, the WHO will recommend the use of liquid media to culture and diagnose TB in high-burden settings, but will not specifically recommend Becton Dickinson's product MGIT (mycobacteria growth indicator tubes), even though most of the data on liquid media culture were based on the MGIT. This leaves open the possibility for new tools that use the same diagnostic methodology to be considered for STAG-TB approval again.

In order for a tool to be brought to the STAG-TB for consideration, it needs a champion who, through a funded and focused product development plan, can standardize and validate the method and present a portfolio for STAG-TB's review. A number of diagnostics techniques currently used in many laboratories (such as bleach and filter methods to improve smear microscopy) need to be standardized and evaluated but currently do not have a clear development plan. Without a systematic approach, it is not clear how these products will be considered for WHO recommendation in high-TB-burden settings.

Though the WHO recommendation is an important endorsement of a tool, it is not essential for its uptake. Currently, some countries use tools that have not yet been approved by the WHO, and other tools that have been endorsed by the WHO have not been adopted by many high-TB-burden countries. However, in the absence of strong regulatory agencies in most developing countries, the WHO recommendations do provide the most rigorous process for evaluating the utility of a diagnostic tool.

This year, prior to the STAG-TB, an expert meeting will be held to analyze data on approaches to improve sputum smear microscopy, noncommercial culture methods, and mycobacteriophage tests.

Technologies Appropriate for Health Posts

Health posts are the most basic level of health service facilities and serve nearly 60% of people in need of TB services. Health posts typically have minimal to no infrastructure and few trained health care workers. Diagnostic tools used in health post settings must be robust, should not depend on a regular supply of power or water, should resist contamination, and be usable with minimal training.

MPT-64 Skin Patch Test

Sequella's TB Patch test is a skin test being developed to detect active TB. The test uses the MPT-64 TB antigen, contained in a Band-Aid-like skin patch. When the patch is applied, the MPT-64 suspension is absorbed through the skin. In people with TB infection, a reddish immune reaction will appear at the site of exposure within three to four days (the skin reaction is being optimized for easier readout on darker skin). It is not yet clear whether the test can distinguish between latent and active TB. The MPT-64 antigen is specific to *MTB* and cross-reactions to antigens from BCG vaccine formulations are not expected. Initial data found no false positives in BCG-vaccinated, TST-positive persons without active TB disease. A positive reaction is therefore thought to be an accurate indication of TB infection. Sequella is conducting field utility studies in the Philippines. Marketing approval is predicted in 2011.

Advantages: The patch test does not require sophisticated laboratory capacity, electricity, running water, or injectables, and can be used in health post settings. It does not have a false-positive reaction to most BCG antigens and may be an improvement over the TST for detecting TB infection.

Limitations: There are scant data available on the patch test. Some issues were reported about the readability of the test result on darker skin. The ability of the test to distinguish between active TB and TB infection needs to be validated. The test needs to be studied for use in people with HIV and children. The four-day waiting period for a skin reaction to develop is still too long, and might not be appropriate for a point-of-care diagnostic. More data are needed on potential dermatologic side effects caused by the patch.

TB Diagnostic Tests or Processes in the Pipeline, 2009

Name of Test or Process	Sponsor/ Developer	Technology	Potential Application	Anticipated Time for WHO Approval for High-TB-Burden Settings
Health Posts				
MPT-64 skin patch	Sequella	Antibody production to MPT-64 exposure	Detects TB infection and disease	2011
Peripheral Laboratories				
Front-loaded smear microscopy*	Special Programme for Research and Training in Tropical Diseases (TDR)	Conduct same-day sputum smears	Detects TB	2009
LED adaptor for existing microscopes	LW Scientific, FRAEN	Fluorescent microscopy	Detects TB	2009
Primo Star iLED microscopy	Zeiss Microimaging, Foundation for Innovative New Diagnostics (FIND)	Fluorescent microscopy	Detects TB	2009
Sputum digestion process by sodium hypochlorite (bleach)*	TDR	Sputum processing with bleach for microscopy	Improves yield of microscopy	2010
Filter concentration*	Academic laboratories	Concentrates sputum to improve microscopy yield	Improves yield of microscopy	2010
Fluorescent vital dye staining*	Academic laboratories, MSF, Epicentre	Stains only live TB bacteria	Detects live TB	2010
Eiken	Eiken Chemical, FIND	Nucleic acid amplification	Detects TB	2010
Lipoarabinomannin (LAM) antigen test	Inverness-Chemogen, TBDiaDirect, FIND	Detects the LAM antigen in urine	Detects TB infection and disease	2011

Name of Test or Process	Sponsor/ Developer	Technology	Potential Application	Anticipated Time for WHO Approval for High-TB-Burden Settings
Reference Laboratories				
Microscopic observation drug susceptibility (MODS)* **	Academic laboratories	Inverted light microscopy that detects growing TB	Detects TB/drug susceptibility testing (DST)	2009
Thin layer agar (TLA) *	Academic laboratories, FIND	Solid media culture and light microscopy to detect growth	Detects TB/DST for rifampicin, isoniazid, and fluoroquinolones	2009
Nitrate reductase assay (NRA)*	Academic laboratories	Solid media; TB growth causes color change	DST	2009
Colorimetric DST*	Academic laboratories	Solid culture; TB growth causes color change	DST	2009
FASTPlaque	Biotec Laboratories	Phage based DST for rifampicin on solid culture	DST	2010
MPT64** detection	Tauns, Standard Diagnostics, FIND	Lateral flow technology uses antibodies to detect presence of MTB	Speciation	2010
MTB/RIF	Cepheid, University of Medicine and Dentistry of New Jersey, FIND	Nucleic acid amplification of TB DNA	Detects TB/ DST for rifampicin	2010
Urinary nucleic acid amplification*	University College London, Spaxen, FIND	Nucleic acid amplification	TB detection	2011
QuantIFERON-TB Gold test	Cellestis	Interferon-gamma release assay	Detects latent TB infection	2009
T-SPOT.TB	Oxford Immunotec	Interferon-gamma release assay	Detects latent TB infection	2009

* Tests/diagnostic systems or methods that are not standardized products but are promising processes to detect TB cases or drug resistance, some of which may be developed into products that are standardized in quality, process, and of assured performance.

** The WHO already approved the general diagnostic strategy that these tools utilize. MODS in liquid culture and MPT-64 are rapid speciation tests. These are new products that fall under previously approved diagnostic methods and are currently being validated for use in high-TB-burden settings.

Point-of-Care Dipstick Tests

Antigen/antibody dipstick test: A point-of-care (POC) dipstick-style test that can accurately detect TB is urgently needed. Clarifying which TB antigens or antibodies can be used as biomarkers for detecting TB disease will be a major first step toward realizing an inexpensive and convenient dipstick test and revolutionizing TB diagnosis in the field. Besides validating the biomarkers, technologies for detecting them must also be assessed. Important work is being done by a number of academic and research institutions to identify host biomarkers that are predictive of disease or immunity. The Public Health Research Institute, the Foundation for Innovative New Diagnostics (FIND), and other partners have screened the entire TB proteome to identify and purify 19 priority target proteins. Validation studies of these proteins are being conducted. Similar biomarker identification work is being carried out at the Max Planck Institute, the London School of Hygiene and Tropical Medicine, and New York University, among others. Despite these efforts, it is unlikely that a TB POC dipstick will be developed within the next three years. Current efforts to identify and assess antigens and technology platforms are underfunded and uncoordinated.

In order to galvanize activity toward developing a POC dipstick for TB and to come up with specifications for it, TAG, Médecins sans Frontières (MSF), and Partners in Health organized a meeting on March 17–18, 2009. The meeting brought together 34 participants, including laboratory workers, doctors, TB patients and their advocates, and researchers, as well as experts from implementation and technical agencies. The meeting built upon the results of an expert opinion survey conducted by MSF of 30 TB clinicians, researchers, and national TB program staff from 17 countries asked to identify gaps in TB diagnostics and propose minimal requirements for a POC TB test. The Paris meeting participants came up with the following POC test specifications:

Specifications for Point of Care Tests

Criteria	Minimum Specifications Required
Medical decision	Treatment initiation
Sensitivity—adults (regardless of HIV status)	Pulmonary TB: Smear positive, culture positive: 95% Smear negative, culture positive: 60–80% (no agreement on a minimum) (Detection of extrapulmonary TB preferred but not required)
Sensitivity—children (regardless of HIV status)	80% compared to culture of any specimen and 60% of probable TB (noting the lack of a gold standard)
Sensitivity— extrapulmonary TB (regardless of HIV status)	80% compared to culture of any specimen and 60% of probable TB (noting the lack of a gold standard)
Specificity	Adults: 95% compared to culture Children: 95% compared to culture 90% for culture negative, probable TB (noting the lack of a gold standard)
Time to results	Maximum 3 hours (patient must same day results, desirable would be <15 minutes)

Besides developing the above specifications, the meeting participants also recommended the following actions to expedite the development of a POC dipstick for TB:

- Perform systematic, early evaluation as promising biomarkers emerge.
- Assess and document the adequacy and accessibility of existing specimen banks.
- Establish a clearinghouse with open access to information and regular, rigorous evaluation of progress in different areas (biomarkers, platforms).
- Increase investment in TB diagnostics research and development at least fourfold, as well as creating new funding mechanisms, including a prize fund for a TB POC test.

For further information on the Paris TB POC test meeting, go to http://www.msfaaccess.org/TB_POC_Parismeeting/.

Technologies Appropriate for Peripheral Laboratories

Peripheral laboratories are basic laboratories that can be found in district hospitals and TB clinics. They have trained laboratory technicians and adequate infrastructure to carry out microscopy and other tests that do not require a high level of biosafety containment.

Front-Loaded Smear Microscopy

In 2007 the WHO recommended that countries with well-functioning, quality-assured laboratories could reduce the number of patient sputum samples obtained and analyzed from three to two, and that one positive sputum with a lower bacillary load per sample is sufficient to diagnose a case of TB. This created the opportunity to obtain both samples on the same day and to diagnose a smear-positive case during one clinic visit, thereby reducing the number of clinic visits required and reducing the burden on health care workers and laboratory technicians, as well as people seeking TB services. Despite these recommendations, people with TB symptoms are often asked to return the next day to provide a morning sputum sample. The front-loaded smear microscopy technique simply takes the second sputum sample one hour after the first, thus eliminating the need for another visit the next morning.

The WHO-based Special Programme for Research and Training in Tropical Diseases (TDR) provided evidence to validate front-loading through a study of 923 symptomatic persons with chronic cough. The study collected sputum samples using the traditional method of collection in which the first sample is taken on the first clinic visit, the second one is taken the next morning, and a third is taken after the morning sample. In the study, an additional sputum sample was collected one hour after the first sample. The yield of TB cases identified using the front-loaded technique compared with the traditional method was similar. Data from front-loaded smear microscopy studies will be considered by STAG-TB later and might be recommended by the WHO in 2009.

Advantages: Front-loaded smear microscopy offers the significant convenience of same-day results for persons with symptoms, which should help prevent people from dropping out of the diagnostic pathway.

Limitations: This technique does not improve the poor sensitivity of smear microscopy, which misses about 50% of TB cases.

Optimizing Microscopy: Bleach and Filter Concentration

Two simple techniques have been proposed to improve the sensitivity of sputum smear microscopy. Bleach (sodium hypochlorite) digests sputum. Theoretically, the bacilli in digested sputum would be easier to stain and detect. Filter concentration

has been proposed to reduce the fluid content of a sputum sample, thereby concentrating the bacilli. Unfortunately, data from studies of these techniques are inconsistent. The techniques have not been standardized and a focused effort is needed to validate their use in high-TB-burden settings. Initial attempts to standardize the use of bleach in improving accuracy of microscopy did not show promise. Final results from a study will be available later this year.

Advantages: If effective, these could be cheap and easily accessible ways to improve microscopy.

Limitations: No definitive data are available, and initial assessment on bleach is not very promising. No product development plan has been funded to standardize and validate these methods.

Optimizing Microscopy: Fluorescent Microscopy

MTB stained with acid-fast fluorochrome dye fluoresces when exposed to an intense light source. Fluorescing *MTB* samples are much easier to identify than samples viewed with a conventional light microscope, and laboratory technicians can spend less time on each sample to make a diagnosis. However, the expensive mercury vapor lamp and dark room required for fluorescent microscopy are not always available. The development of low-cost, ultrabright light emitting diodes (LEDs) has created the potential for using inexpensive LED-lit microscopes for fluorescent microscopy (FM).

Though FM increases the efficiency of microscopy, there is likely less impact on its sensitivity to detect *MTB*. Studies have produced varied results in improvement of sensitivity—from none to about 30%. Increases in sensitivity may depend on the TB burden in a population and may improve as technicians gain experience with FM.

There are efforts underway to make FM-capable microscopes less expensive and battery operated. Some companies are developing adaptors or other techniques that can allow conventional microscopes to perform FM. FIND has partnered with Zeiss Microimaging to develop the Primo iLED, which uses reflected blue light for fluorescence detection and allows the microscopist to switch between fluorescent and bright field microscopy. The device is being validated in developing countries. The Italian company FRAEN makes adaptors that can transmit LED light through a microscope slide. LW Scientific makes equipment to adapt existing microscopes through use of reflected LED light. TDR and the International Union Against TB and Lung Disease are currently validating the utility of these LED-capable microscopes for use in high-TB-burden regions. Data for LED-based approaches will be submitted to the WHO for approval in late 2009.

Advantages: FM reduces the burden on laboratory workers and could potentially improve the sensitivity of smear microscopy by an average of 10%. FM can produce a fourfold increase in speed of TB detection.

Limitations: FM requires more training of the laboratory worker than initially expected, according to one researcher. The results of increased accuracy of microscopy due to FM are varied, and currently there is no strategy for the quality control of FM.

Optimizing Microscopy: Vital Dye Staining

Fluoresceine diacetate (FDA) stain is used to identify live bacteria in a sample. Enzymatic activity in viable cells breaks down the dye and releases its fluorescing properties. Dead cells will not fluoresce. Epicentre and MSF in Thailand are currently studying the technique as a substitute for *MTB* culture for identifying living bacilli in smear-positive patients undergoing TB treatment. Initial data showed poor performance of FDA stain and attempts are being made to standardize and simplify the technique to improve outcomes. Final results will be available by the end of 2009.

Advantages: The ability of vital dye staining to identify viable bacteria could be very useful for early detection of true treatment failures. These cases can then be examined for drug resistance. Vital dye staining requires less technology and technician expertise than culturing and is much quicker.

Limitations: Scant published data are available on vital dye staining and available data have shown mixed results. More research is needed to standardize vital dye staining and validate its utility.

LAM Urine Antigen Detection Test

The mycobacterial protein lipoarabinomannin (LAM) is excreted in urine and can be detected to diagnose TB infection and possibly TB disease. Inverness-Chemogen is developing a test using an enzyme-linked immunosorbent assay (ELISA) in which an antibody embedded in the test's surface binds with LAM. The antibody is linked to an enzyme that changes color when a reagent is added. The color change indicates the presence of LAM and therefore of TB infection and perhaps disease.

Initial data showed that the test had 79% sensitivity in detecting mycobacterial LAM among people with HIV who were also smear- and culture-positive. For all others sensitivity was much lower, ranging from 42% in HIV-negative, smear-positive patients to an overall 28% sensitivity for smear-negative, culture-positive patients. Other studies found even lower sensitivity. The test, Clearview TB LAM ELISA, is being marketed by Inverness-Chemogen for use in detecting TB among

people with HIV. However, its utility is unclear until its performance is improved in smear-negative, culture-positive cases. FIND is working with academic partners to improve LAM reagents. Feasibility studies in South Africa and Zimbabwe are underway, and additional published data are anticipated in 2009.

Advantages: The LAM test can give results within three hours. The ability to detect TB antigens in urine would be a significant improvement in diagnostics, since urine samples are much easier to obtain than sputum, and unlike blood samples, do not require needles. LAM antigens might also be able to identify cases of extrapulmonary TB.

Limitations: LAM's low sensitivity, especially among smear-negative, culture-positive cases, is a significant impediment to the technique's utility, since these cases are far less likely to be detected by smear tests. The technical demands of an ELISA test will limit its use in health post settings.

Eiken LAMP Nucleic Acid Amplification Test

The loop mediated isothermal method of nucleic acid amplification (LAMP) can amplify DNA in a sample without using heating and cooling cycles that other nucleic acid amplification tests (NAATs) require. The LAMP test from Eiken is conducted at one temperature (65 degrees Celsius). As reported in TAG's 2008 pipeline report on TB diagnostics, initial results from feasibility studies conducted in Peru, Tanzania, and Bangladesh had shown it to be 98% sensitive and 99% specific in smear-positive, culture-positive cases. Its sensitivity in smear-negative, culture-positive cases was less than 50%. FIND has been involved in the development of this test over the last two years and further data are anticipated by the end of 2009.

Advantages: The test is easier to conduct than other NAATs, as it doesn't require heating and cooling cycles. Laboratory technicians with no prior NAAT experience can be trained to perform a LAMP test within one week. The test can be used on sputum samples or culture, and the results can be read by the naked eye under ultraviolet light.

Limitations: Data on sensitivity of the current format of the test are not available. A previous version of the test had low sensitivity in smear-negative, culture-positive cases, required electricity, and was not appropriate for peripheral health post settings.

Immune-Based Tests for Latent TB

Interferon-gamma (IFN-gamma) is produced by the immune system when exposed to antigens it recognizes from prior exposures. The IFN-gamma release assays (IGRA) expose a blood sample to *MTB* antigens then measure the production of

IFN-gamma, if any. The presence of IFN-gamma indicates TB infection. Antigens in the IGRA tests are not present in the BCG vaccine, so false-positive results should not occur in BCG vaccinated people without TB.

QuantIFERON Gold Test

Produced by Cellestis, the QuantiFERON Gold (QFT Gold) Test is approved by the U.S. Centers for Disease Control and Prevention (CDC) for use wherever the TST is used. The QFT Gold in a Tube (QFT GIT) is a version of this test with a coating of *MTB* antigen in a test tube. The blood sample is collected in the tube, and IFN-gamma is assayed after the tubes have been incubated for 16 to 24 hours at 37 degrees Celsius. The tubes need to be incubated within 16 hours of blood collection. The presence of IFN-gamma is measured by an ELISA test. The tube version of QFT is easier to use and requires fewer sample processing steps than QFT Gold.

Since there is no definitive test for latent TB infection, sensitivity of QFT GIT is estimated by looking at QFT GIT positivity rates in people who have culture-confirmed TB disease, and specificity by examining rates of positivity among individuals with no known risk for acquiring TB (such as U.S.-born individuals who had not been vaccinated with BCG). The test's package insert claims its sensitivity is 89%, when considering only valid tests (excluding any indeterminate tests), and its specificity is 99% based on unpublished data. The rate of agreement between QFT and TST results in studies published in peer-reviewed articles are varied.

Trials are being conducted in high-TB-burden countries to examine the utility of a QFT Gold test result in predicting the risk of developing TB disease and in improving the diagnosis of TB in children, people with HIV, and smear-positive and -negative TB cases. ZAMSTAR is one such TB/HIV focused research project, and has a nested study that will follow 2,400 household contacts of TB patients over two years to study the incidence of TB in persons with a positive and negative QFT GIT and TST results. Though final data are not yet available, initial results from implementing the QFT GIT test showed a 14.6% rate of indeterminate results. These indeterminate results were primarily due to errors made during sample preparation and due to power outages. Attempts to improve the readability of the tests are being made through laboratory worker trainings.

Advantages: Unlike the TST, the QFT Gold test will not have a false positive due to previous BCG vaccination nor will it run the risk of boosting a positive reaction because it is a noninvasive test. Test results are available within 24 hours. The QFT does not require the patient to return to the clinic for a reading of the test results.

Limitations: The QFT Gold test needs to be run on the blood sample within 16 hours of sample collection. The sample preparation requires trained laboratory staff. The test also requires a stable power supply, and this is not always available in peripheral laboratories in resource-poor settings. Results of the test, including its specificity and sensitivity, need to be interpreted in the context of the TB burden of the setting where the test is being conducted. The test cannot distinguish between latent and active TB.

T-SPOT.TB Test

Made by Oxford Immunotec, the T-SPOT.TB test works on the same principle as QFT GIT. Immune cells are exposed to *MTB* antigens and incubated overnight. IFN-gamma production by T cells responding to *MTB* antigens are detected by a simplified ELISPOT method. The T-SPOT test requires significant sample processing; blood is centrifuged to extract the mononuclear cells and the cells must be counted. Sample processing must occur within eight hours of collection. The package insert for the product claims a sensitivity of 98% and specificity of 100% based on 83 samples from culture-confirmed TB patients and 93 samples from people with low risk of TB infection.

Peer-reviewed studies suggest that T-SPOT.TB has the potential to surpass the utility of the TST in people with HIV, although validation in people with fewer than 200 CD4 cells is needed.

Advantages: The T-SPOT has low potential for false positives due to reactions with BCG or most environmental mycobacterial antigens.

Limitations: Sample processing must be initiated within eight hours of collection, which will limit the utility of T-SPOT.TB in most high-burden peripheral settings. The test cannot distinguish between latent and active TB.

Technologies Appropriate for Reference Laboratories

Reference laboratories have access to sophisticated technology, the highest level of biosafety, and trained laboratory technicians. They are located in the capitals or major cities and have access to reliable infrastructure, and a regular supply of power and clean water.

Noncommercial Culture Methods: MODS

The Microscopic Observation Drug Susceptibility (MODS) assay is a method of detecting drug-resistant *MTB* in a liquid media culture. *MTB* growth can be detected by observing its characteristic cordlike shape under an inverted light microscope. The

MODS assay provides results in 7–21 days. Some studies of MODS have shown 92% sensitivity and 99.5% specificity for detecting MDR TB. Another study found MODS 96.7% sensitive and 78.4% specific for isoniazid resistance and 96% sensitive and 82.9% specific for rifampicin resistance. Though the WHO already recommends the use of liquid culture for TB detection and DST, the MODS method of liquid culture will be considered for WHO endorsement in November 2009.

PATH and Tulip Diagnostics are close to producing a MODS prototype that will further standardize the method and evaluate it in field settings. There is also an attempt to develop an inverted light microscope costing less than \$60; current costs range from \$500 to \$6,000.

Advantages: Developed by academic laboratories, the MODS assay is an open-source technique with nearly all of its components nonproprietary. The test gives results in 7–21 days, comparable to much more expensive proprietary liquid media culture systems such as the MGIT.

Limitations: The inverted light microscope required by MODS is not available in all laboratories. Though it provides results in a relatively short time, a week to three weeks is still too long. MODS has mainly been used to diagnose TB and DST in people not yet on treatment. Its potential for DST in patients who are on treatment and failing treatment needs to be examined.

Noncommercial Culture Methods: Thin Layer Agar

The Thin Layer Agar (TLA) test is basically MODS for solid media. *MTB* is grown on a thin layer of solid media and the growth is observed under a microscope. Multisite studies have shown its sensitivity to be 93%. Another study that showed its sensitivity to be lower (76%) showed its specificity to be 99%. The time to results for diagnosing TB is about 10 to 16 days. A petri dish format of TLA has the significant advantage of also being able to do DST concurrently. Research is being conducted in Peru that looks at TLA plates that have a plain LJ quadrant to detect drug-susceptible TB and three other quadrants containing rifampicin, ciprofloxacin, and isoniazid to detect resistant strains. The observation of TB growth on any of the quadrants with anti-TB medication will indicate resistant strains. FIND is involved in this effort as well. The WHO's-STAG TB will consider TLA data this year.

Advantages: Time to diagnose TB is ten days—significantly shorter than that of other solid media methods, which can take up to four weeks. TLA time to diagnosis is comparable to liquid, is much cheaper than automated liquid media systems, and is less prone to contamination. Since the plate can be sealed permanently once the specimen is smeared, a high level of laboratory biosafety is not required. TLA is also quite inexpensive, less than \$2 per test.

Limitations: Ten to sixteen days for results is still quite long. TLA systems require carbon dioxide incubators, which are not available in all laboratories. They also require electricity and infrastructure, which will prevent their use in peripheral health posts in resource-poor settings.

Noncommercial Culture Methods: The NRA/Griess Method

The Nitrate Reductase Assay (NRA), also known as the Griess Method, works by mixing a clear reagent into a LJ culture tube along with an anti-TB drug, inoculating with the specimen, and incubating. If *MTB* growth causes a color change in the drug-containing tube that is darker than the control tube with no drugs, the sample is considered to be resistant. Average time to results is ten days.

A meta-analysis of 28 studies determined the NRA has a sensitivity and specificity of greater than 94% for rifampicin and 92% for isoniazid. There is also some evidence for testing drug sensitivity to all first-line TB drugs, with a 98% concordance between NRA and LJ media. The NRA is in use in public laboratories in Peru.

Advantages: This test is much less expensive and relatively fast compared to solid media culture tests, which can take up to four weeks to detect TB and another four weeks for DST.

Limitations: Though faster than solid media DST, the NRA method still takes too long to produce results. It has been mostly studied on culture specimens and its utility on direct sputum needs to be further validated to reduce cost and time to results.

Noncommercial Culture Methods: Colorimetric DST

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is a yellow salt that changes to blue as TB grows in the media. Resazurin, which is blue, turns pink in the presence of viable *MTB*. Colorimetric methods developed by academic laboratories use these changes in color to indicate presence of viable *MTB*. A culture specimen is introduced into media containing either rifampicin or isoniazid and one of the colorimetric indicators. The tube is then incubated overnight. The growth of drug-resistant TB is indicated by change in the color of the media. A meta-analysis of 18 colorimetric DST studies showed a high degree of accuracy in detecting rifampicin and isoniazid resistance. Their sensitivity and specificity ranged between 89% and 100%. The WHO will consider the utility of colorimetric DST tests for use in high-TB-burden settings later this year.

Advantages: The overnight time to result, high accuracy, and relative low cost are all advantages over culture methods most frequently used to perform DST.

Limitations: This method still requires a culture specimen and requires biosafety infrastructure.

Mycobacteriophage Test

Bacteriophages are parasites that can infect and grow in *MTB*. They can be used to detect rifampicin resistance in a smear-positive sample without having to wait four weeks to visually see *MTB* colonies growing on the culture media. To detect rifampicin resistance, the smear-positive sample is infected with the mycobacteriophage. The sample is then washed with a solution that kills all the bacteriophage outside of the *MTB*. The *MTB* is then grown in rifampicin-containing media. If the *MTB* remain viable, the bacteriophages grow inside it, destroying the cell resulting in spots in the media. These spots indicate rifampicin resistance.

The FASTPlaque-Response test developed by Biotec Laboratories is a phage-based test that can diagnose rifampicin resistance in two days. Its sensitivity and specificity has been seen to be greater than 95% in some peer reviewed reports of studies conducted in high-burden settings. However, high levels of contamination nearing 40% are a concern. The WHO will analyze the utility of the phage-based test for high-TB-burden resource-constrained settings later this year.

Advantages: This test can identify rifampicin resistance directly from a smear-positive specimen saving the time and cost of culturing TB. It provides a result in two days.

Limitations: Bacteriophage tests require biosafety precautions, a sterile environment, supply of electricity, and a laboratory infrastructure that will limit their use to reference laboratories. They are only accurate in smear-positive samples. High levels of contamination and unreadable results may further limit this test's usefulness.

Speciation Tests

In 2007, the WHO endorsed the use of liquid culture methods to detect TB in high-burden settings. These methods require a speciation test to further distinguish *Mycobacteria tuberculosis* complex from non-*MTB* species, some of which also cause disease. Therefore, in 2007, the WHO also endorsed the use of rapid speciation tests.

MPT-64 Test

The MPT-64 Test is a rapid speciation test that determines the type of mycobacteria present. It uses a strip containing an antibody for MPT-64, a protein highly specific for *MTB* complex and present in unheated media growing *M. tuberculosis* and some *M. bovis* strains. The presence of MPT-64 distinguishes between *MTB* complex and non-TB mycobacteria (NTM) species. The Capilia test from Tauns and the

TB Ag MPT64 test from Standard Diagnostics, both of which FIND is helping to evaluate, are two lateral flow assays using MPT-64 antibodies. The culture specimen moves through the lateral assay and antibodies embedded in the test strip react to the presence of MPT-64 to create a visual line that is read to detect *MTB*. Both tests have a sensitivity of over 90% and specificity of 100%. These tests will likely be ready for use in high-burden-setting public health sites in 2010.

Advantages: Unlike the other speciation methods, such as nucleic acid amplification tests, these tests provide results within 15 minutes and are simpler.

Limitations: While these tests are simple, they require culture samples and biosafety equipment.

Nucleic Acid Amplification Tests

Using polymerase chain reaction (PCR) to amplify nucleic material, nucleic acid amplification tests (NAATs) are able to detect very small amounts of DNA in a sample. Enzymes break the amplified DNA at specific sites and the fragments are run through a gel where characteristic patterns are formed corresponding to various strains of TB, including drug-resistant strains. In 2008, the WHO's STAG-TB approved the use of two NAATs, the Hain MDR TB Plus and the INNO-Lipa, for use in high-TB-burden settings.

NAAT: Xpert MTB/RIF

Cepheid is developing a NAAT in partnership with FIND and the University of Medicine and Dentistry of New Jersey. This is a closed system test that detects *MTB* and rifampicin resistance in a cartridge that contains all the reagents needed for the test. TB DNA is amplified, concentrated, and detected through a fully automated mechanism. The machine uses ultrasound to break apart the DNA and then microfluidic technology to wash and concentrate the DNA fragments. The machine detects *MTB* and rifampicin resistance in less than two hours. Rifampicin resistance genes and mutations are identified through fluorescent beacon molecules that attach themselves to the genes in the test cartridge. Being a closed-system test, it does not require a laboratory worker to process samples or add reagents. The Xpert MTB/RIF test was registered for use in Europe in April 2009.

The Xpert MTB/RIF test is still being studied for use in district hospitals in high-burden settings for detection of *MTB* and rifampicin resistance. Initial data from Latvia and Peru show its sensitivity to be 81% in smear-negative, culture-positive specimens and 99% in smear-positive specimens. Initial results from the same study conducted by FIND indicated that the Xpert MTB/RIF was 100% sensitive and specific in detecting rifampicin resistant strains. Data from another multicenter

study also showed that the Xpert MTB/RIF detected more than 90% of all TB cases regardless of HIV status when compared to the gold standard of culture. The Xpert MTB/RIF is likely to be reviewed at the STAG-TB in 2010.

Advantages: The Xpert MTB/RIF can be used on raw samples, unlike other PCR tests that require a processed sample and a DNA extraction step. The test can provide results in less than two hours. The test's high degree of accuracy, even in smear-negative specimens, will be especially useful in people with HIV and in children, who have greater proportions of smear-negative TB. The self-contained system eliminates the need for reagent or specimen handling or high levels of biosafety infrastructure. This simple test will reduce time needed to train laboratory workers. The GeneXpert system, which is used by the postal service to detect anthrax, is a multidisease platform.

Limitations: The Xpert MTB/RIF technology requires electricity and infrastructure that will likely not be available consistently in district hospitals in resource-constrained high-TB-burden settings. Its relatively high cost (\$25/test) plus the cost of the instrument will also be a barrier to widespread use.

MTB DNA Amplification from Urine

MTB DNA fragments can be detected in the urine of patients and can be amplified by PCR to diagnose TB. Initial attempts through varied PCR methods have shown sensitivities ranging from 40% to 100% with specificity near 100%. This test's sensitivity was highest in people with HIV who had smear-positive TB. No new data on this test has emerged in the past year. FIND, the University College London, and Spaxen are still attempting to optimize the test.

Advantages: Urine is an easier, less invasive sample to obtain. Its high specificity in all TB patients, its high sensitivity among people with HIV with smear-positive TB, and its potential use in identifying extrapulmonary TB cases are assets.

Limitations: The test's antigens need to be optimized and its sensitivity needs to be improved, especially in patients with smear-negative TB.

Conclusion

People infected and affected by TB ideally need a single safe and easy-to-use diagnostic tool that provides accurate results during a single clinic visit, diagnosing smear positive, negative, and extrapulmonary TB. This tool also needs to be effective in children and people with HIV. Optimally, this tool would also perform DST; otherwise, a second tool will be needed to do so in a manner that is fast and accurate

to ensure that people are being put on appropriate treatments. Despite these needs being well documented, it is clear that they will not be met in a technology suitable for use at the health post level within the next three to five years.

With a current level of global investment in TB diagnostics at about \$42 million in 2007, it is no surprise that TB diagnostics research and development efforts are merely limping along a path of incremental improvements. The most exciting improvements in the pipeline use technologies that will never be appropriate for use at health post levels and are unlikely to contribute to major improvements in clinical outcomes for most people living with TB. Though it is important to optimize the current technologies and reap these low-hanging fruit to improve the state of TB diagnostics in the short run, they are not what are needed to truly revolutionize TB care.

In addition to the required increase in dedicated investment for TB diagnostics, there is also a need for increased investment in basic TB science. Though we have been working on TB for more than 125 years, it is a travesty that we currently do not have a good biomarker that can predict the risk for disease, immunity, or cure. The focus on basic science needs to happen in a way that balances investigator-initiated efforts with more directed efforts that bring together the best minds in the field to work in unity to solve this problem with a sense of urgency. Currently there is a dearth of coordination in the efforts to identify biomarkers and develop platform technologies appropriate for a point-of-care dipstick. The Bill and Melinda Gates Foundation (through its Grand Challenge Grants) and FIND (through its work on screening the TB proteome), along with other academic institutions, are involved in these efforts. But we need public funders of TB research, such as the U.S. National Institutes of Health, to play a greater leadership role in catalyzing this effort. Only through focused leadership and advocacy on the part of all of us—researchers and funders, as well as people affected by TB—will we be able to prevent the needless deaths of the nearly two million people who perish each year due to TB.

Tuberculosis Vaccines

BY CLAIRE WINGFIELD

Introduction

The Bacille Calmette-Guérin (BCG) vaccine is the most widely administered vaccine in the world, and it saves the lives of an estimated 40,000 children per year. The BCG vaccine is a live attenuated vaccine—a weakened version of *Mycobacterium bovis* (*M. bovis*), a germ that can cause a tuberculosis-like disease in cows and humans. After several disastrous attempts at trying to develop a tuberculosis (TB) vaccine using different types of mycobacteria, Drs. Albert Calmette and Camille Guérin discovered that *M. bovis*, after having been artificially cultured for over 11 years, was so weakened that it was unable to cause disease in humans—yet could trigger an immune response that provides protection against *Mycobacterium tuberculosis* (MTB). Unfortunately this protection appears to last only until late adolescence, and protects only against two forms of TB disease that occur outside of the lungs: meningeal TB (which affects the lining of the brain) and miliary TB (which is disseminated throughout the body).

Another limitation of the BCG vaccine is that it can trigger a negative immune response in HIV-positive infants. It appears that some infants with a confirmed HIV diagnosis who have been vaccinated with BCG develop BCGitis (also known as BCGosis), a severe immune reaction that is not common but potentially fatal. As a result, the World Health Organization no longer recommends BCG vaccination for infants with a confirmed HIV diagnosis.

Because BCG confers such limited protection, a few academic and research institutions and pharmaceutical companies are working on developing a more effective vaccine that can provide lifetime protection against all forms of TB in all populations. The TB vaccine research community hopes to have a safe vaccine available at a reasonable cost by 2015. There are approximately 40 vaccine constructs (collections of immunogenic proteins) currently in preclinical studies and 10 in clinical trials. These constructs face a number of challenges in moving forward through the development pipeline, one of the largest being a funding gap of more than US\$1 billion. As with developing any new product, for each drug, diagnostic test, or vaccine that is successful there are thousands that have failed for one reason or another. Therefore, while it is encouraging to see such a robust pipeline of vaccine constructs, given the burden of disease, the dearth of options, and lack of resources available one must be cautiously optimistic and question whether it is reasonable to think that a new TB vaccine will be ready for the market in six years.

Of the ten vaccine candidates in clinical studies, six have entered into phase I safety studies, three are being evaluated for immunogenicity (the ability of a vaccine to induce an immune response and/or the extent to which it induces an immune response) in phase II studies, and one has recently completed a phase III efficacy study. It should be noted that the phase III construct might require further investigation; therefore it is possible that one of the constructs in phase II may progress through the pipeline sooner, assuming that phase II results are promising and phase III studies are adequately resourced.

The Aeras Global TB Vaccine Foundation (Aeras) is a public-and-private product development partnership currently funded by the Bill and Melinda Gates Foundation; the Mary Lynn Richardson Fund; the governments of Denmark, the Netherlands, and Norway; and the State of Maryland in the United States. Aeras is guiding many of the current vaccine constructs through clinical development and has six vaccines in or about to enter phase I or II clinical trials. As part of its strategy to prepare the vaccine field for large-scale phase III studies, Aeras is conducting epidemiological studies in India, Kenya, South Africa, and Uganda to establish the rate of acquisition of TB infection among infants, children, and adults to determine the number of volunteers needed to demonstrate the efficacy of a new vaccine in reducing TB incidence. In each of these countries, as well as in Cambodia and Mozambique, Aeras is also developing clinical trials sites to ensure that the research infrastructure is ready for phase III studies when a new construct is ready and fully funded. Aeras has also built an internal facility to manufacture its vaccines for wider distribution, in order to ensure quality and consistency across vaccine batches.

TB Vaccine Candidates

All of the constructs that have entered into phase II studies are prime boost vaccines. This means that they were designed to boost the efficacy, potency, and durability of a priming vaccine. The premise behind prime boost vaccines is that infants who have been vaccinated with a priming vaccine would be given a boosting vaccine weeks, months, or years later to strengthen the immune response induced by the priming vaccine, by increasing the number or broadening the activity of TB-specific immune cells. All of the prime boost vaccine candidates in clinical trials are being evaluated as BCG boosters. Given BCG's limitations, it is important to note that newer versions of the BCG vaccine (referred to as recombinant BCG, or r-BCG) that are more potent, more durable, and have greater utility than the current BCG are in development.

TB Vaccine Candidates in Development, 2009

Agent	Type	Description	Sponsor	Status
AERAS-402/Crucell Ad35	Prime boost	Adenoviral vector	Crucell N.V./Aeras	Phase II
MVA85A/AERAS-485	Prime boost	MVA vector	University of Oxford	Phase II
GSK M72	Prime boost	Recombinant protein	GSK Biologicals /Aeras	Phase II
<i>Mycobacterium vaccae</i>	Prime boost	Heat killed NTM	SR Pharma	Phase III (recently completed)

Note: All candidates currently evaluated as BCG boosters

AERAS-402/Crucell Ad35

AERAS-402/Crucell Ad35 from Crucell NV and Aeras is a replication-deficient adenovirus (Ad35) that serves as a vector for DNA expressing TB antigens 85A, 85B, and 10.4. Adenoviruses are potent inducers of CD8 cell responses, which are considered important for developing an effective vaccine-induced immune response. For this construct, adenovirus 35 (Ad35) has been modified to include specific *MTB* antigens in order to trigger an immune response to TB. A series of phase I studies in adults was conducted in the United States and South Africa to evaluate the immune response to an adenoviral vector vaccine after priming with BCG. The results demonstrated TB antigen specific CD4 and CD8 responses in BCG-naïve and BCG-primed volunteers after receiving AERAS-402/Crucell Ad35. Aeras has begun a phase II clinical trial in South African adults who have been exposed to TB.

Adenoviral vectors have been used in investigational vaccines for a number of diseases—including HIV, herpes, and rabies—because they can be easily modified to deliver genetic material from an organism to trigger an immune response, and because they are potent inducers of CD8 cell responses. Some concern has been raised about using adenoviral vectors after evidence from the Merck/HIV Vaccine Trials Network STEP Study suggested that the adenovirus 5 (Ad5) may have increased susceptibility to HIV infection (*see more on this in “Immune-Based Therapies and Preventive Technologies”*) for a small subgroup of study volunteers. Since the discontinuation of this trial the data have been heavily scrutinized, and it appears that the enhancement effect of the Ad5 on HIV infection is transient and the result of a rare synergistic effect among volunteers who tested positive for Ad5 antibodies and were also uncircumcised. There is no evidence to suggest that Ad35 will have any impact on susceptibility to HIV infection. For its part, Aeras convened

its Safety Monitoring Committee and several additional experts to evaluate any possible risk to volunteers from AERAS-402/Crucell Ad35. The committee found no reason to stop trials of this construct. However, to avoid any possible risk of increased susceptibility to HIV infection, Aeras initiated the following additional safety precautions:

- Exclude all individuals at high risk of acquiring HIV
- Inform all individuals of the Merck data and its potential implications
- Counsel all volunteers not to engage in high-risk behavior
- Monitor all volunteers for acquisition of HIV

MVA85A/AERAS-485

The University of Oxford's MVA85A/AERAS-485 is a recombinant attenuated (non-disease-causing) version of the vaccinia virus (cowpox) combined with TB antigen 85A. The antigen 85A stimulates a strong TB-specific immune cell response, thereby boosting the immune recognition of TB initiated by BCG. Aeras, in partnership with the Oxford-Emergent Tuberculosis Consortium, the University of Cape Town, and the Wellcome Trust have begun a phase IIb proof-of-concept study of MVA85A/AERAS-485 that will be led by the South African Tuberculosis Vaccine Initiative. This clinical trial is evaluating the safety, immunogenicity, and efficacy of MVA85A/AERAS-485 in approximately 2,800 BCG-vaccinated children under one year of age.

GSK M72

GSK Biologicals, a subsidiary of GlaxoSmithKline, is developing a protein subunit vaccine that induces an immune response to TB protein M72. It is the only candidate in a BCG prime boost regimen thus far to offer better protection than BCG in the long-term primate model. GSK Biologicals in partnership with Aeras, recently completed phase II clinical trials of the M72 vaccine in Belgium, South Africa, and the Philippines. The construct is well-tolerated and immunogenic. Phase II studies of M72 in HIV-positive Swiss volunteers are ongoing in partnership with TBVAC.

Mycobacterium vaccae

Mycobacterium vaccae (*M. vaccae*) is a heat-killed non-TB mycobacterium (NTM) that was originally evaluated as an immunotherapeutic vaccine to strengthen the immune system of people already infected with TB with the aim of preventing disease progression or improving the impact of treatment. TAG's 2008 Pipeline Report discussed the Dar Dar study, a phase III clinical trial evaluating *M. vaccae* as

a BCG booster vaccine among people with HIV for the prevention of disseminated TB. The study, a collaboration between the Dartmouth School of Medicine in the United States and Muhimbili University in Tanzania, randomized 2,000 HIV-positive adults with an identifiable BCG scar and CD4 counts over 200 to receive either five inoculations of *M. vaccae* or placebo over a 12-month period. The primary endpoint of disseminated TB disease was observed in 13 volunteers in the control arm and in 7 volunteers in the experimental arm, and the secondary endpoint of culture-confirmed TB disease (disseminated and pulmonary) was observed in 52 in the placebo arm and 33 in the *M. vaccae* arm. Based on these results investigators believe that *M. vaccae* may have utility as a vaccine in early HIV infection to prevent the future development of pulmonary and disseminated TB disease. Aeras's external Vaccine Selection Advisory Committee (VSAC) has reviewed the Dar Dar data. Based on the VSAC's recommendation, Aeras is considering preliminary work on manufacturing *M. vaccae*.

Conclusion

Despite the fact that it is the most widely used vaccine in the world, BCG has not had a significant impact on the growing TB pandemic. There is an urgent need for new, safe, effective, and affordable vaccines to protect against all forms of TB; to prevent TB in children, adolescents and adults; and to be safe enough for use in people with HIV. With almost 50 vaccine candidates in the preclinical and clinical pipeline, there is much reason to be optimistic that the science exists to create a better, more durable, and more potent TB vaccine. But if funding levels remain inadequate, the resources to properly evaluate, validate, and manufacture these constructs may not materialize. There is overwhelming agreement that a safe, tolerable, easy-to-administer vaccine that provides lifetime protection against all forms of TB infection and disease in all populations and age groups is key to reaching the goal of eliminating TB by 2050. However, there are currently few who are willing to pay for the research and development required. Thanks to a small number of public and private donors—most notably, the Bill and Melinda Gates Foundation—initial investments are filling the vaccine pipeline after years of neglect. But much more is needed to keep it filled with viable candidates and keep them moving toward approval.

Immune-Based Therapies and Preventive Technologies Pipeline

BY RICHARD JEFFERYS

Introduction

In 2009, immune-based therapies and biomedical preventive technologies retain the benighted distinction of lacking any approved precedents. The failure of Merck's T cell-based HIV vaccine in 2007 has left the field parched, and none of the candidates that remain in human trials are likely to be efficacious, let alone efficacious enough to be considered for FDA approval. Clinical vaccine research has now shifted toward a "discovery" mentality, with current trials hoping to obtain data that can inform and improve the design of next-generation candidates. In parallel, an increased emphasis has been placed on preclinical (laboratory) work—particularly efforts aiming to solve the persistent problem of inducing broadly neutralizing antibodies against HIV. At least two pharmaceutical companies—Merck and Wyeth—have shuttered their clinical HIV vaccine research programs.

Microbicide research, after suffering the slings and arrows of multiple product failures in recent years, finally received a welcome filip in 2009. At the 16th Conference on Retroviruses and Opportunistic Infections (CROI) in February, Salim Abdool Karim revealed that PRO2000 gel had shown a glimmer of protective efficacy in a phase IIb trial. Women using the gel had a 30% reduced risk of HIV acquisition, which represented a strong trend but just failed to reach statistical significance. Results from a much larger phase III trial are anticipated by the end of 2009.

The biomedical approach to HIV prevention generating the most optimism is pre-exposure prophylaxis (PrEP). The first efficacy results from ongoing trials are anticipated within the next year. Currently, tenofovir and tenofovir/FTC (Truvada) are the only drugs under study, but discussions are beginning about the use of other agents, such as the integrase inhibitor raltegravir (Isentress). Preliminary research looking at the safety of intermittent rather than continuous PrEP is also getting underway, although at the moment no efficacy trials of intermittent PrEP are planned.

Immune-based therapies (IBT) for HIV continue to haunt a sort of developmental twilight zone. This year saw the sad denouement of interleukin-2 (IL-2), the hardy perennial of HIV IBTs, when results of two large trials—ESPRIT and SILCAAT—

showed absolutely no clinical benefit to the intervention. Although IL-2 increased peripheral CD4 counts numerically, it appears that the mechanism by which this occurred did not equate to a commensurate increase in functional immunity. Despite IL-2's failure, there is still a potential need for effective IBTs. Recently published studies have made clear that most individuals with low CD4 T-cell counts at the time of starting antiretroviral therapy (ART) do not regain normal T-cell levels even after seven or more years of treatment. Data from cohort studies indicate that these individuals remain at elevated risk for clinical disease (both AIDS-defining and "non-AIDS" events). Taken together, these findings argue that the discovery and development of IBTs with the potential to enhance and accelerate immune reconstitution should be a research priority.

Another possible goal for IBTs—and some experimental gene therapies—is improving control of HIV replication in the absence of ART in order to reduce or even eliminate dependence on drug treatments. While a number of candidates continue to pursue this goal, an ominous cloud has appeared on the horizon in the form of inflammation. Specifically, viral load levels correlate with immune activation, which in turn is linked to inflammation, and recent studies—particularly the Strategies for the Management of AntiRetroviral Therapy (SMART) trial—have revealed that elevated levels of inflammation are associated with the development of a wide spectrum of illnesses and mortality in people with HIV. Standard methods for measuring the impact of an IBT during an ART interruption—CD4 T cell counts and viral load levels—do not fully capture the potential negative impact on health of inflammation, which increases in parallel with viral load. The upshot is that even an IBT that maintained CD4 counts and lowered viral load during ART interruption might turn out to be inferior to continuous ART in terms of preserving health due to the potential for inflammation-related illness. This concern now hangs over all clinical trials involving ART interruptions.

Recent studies have also shown that even untreated individuals with undetectable viral loads (so-called elite controllers) have levels of immune activation and inflammation that are significantly higher than comparable uninfected individuals. The level of immune activation in elite controllers correlates with a slow but progressive loss of CD4 T cells. These new data suggest that if the goal is to create a truly nonprogressing disease course, an IBT would have to induce an extraordinarily strict containment of HIV replication.

Preventive Vaccines

Over the course of 2008 and in early 2009, additional details emerged from STEP, the efficacy trial of Merck's HIV vaccine candidate that was halted in September 2007. As described in TAG's *2008 Pipeline Report*, the vaccine did not prevent infection or reduce HIV viral load in study participants who became infected. Yet worse, in a subset of study participants, susceptibility to HIV infection was increased. In terms of the lack of efficacy, analyses of the data have highlighted the following potential contributors:

- The magnitude of the T-cell responses against HIV created by the vaccine were around tenfold lower than the HIV-specific T-cell responses that are typically observed in long-term nonprogressors.
- The breadth of the T-cell responses was poor. Although there are multiple protein fragments (epitopes) in each HIV protein, recipients of the Merck vaccine developed CD8 T-cell responses to an average of just one epitope from each protein in the vaccine (Gag, Pol, and Nef).
- Only around one-third of vaccine recipients developed both CD4 and CD8 T-cell responses against HIV; evidence strongly argues that a balanced response involving both subsets is needed.
- Vaccine-induced CD8 T-cell responses were not capable of efficiently killing HIV-infected CD4 T cells in laboratory tests. In contrast, HIV-specific CD8 T cells from long-term nonprogressors kill HIV-infected cells very effectively in these tests.

Since almost all the HIV vaccine candidates remaining in the pipeline aim to induce HIV-specific T-cell responses, these findings from STEP may offer insight into the parameters that need to be improved in order to achieve a better outcome. It is worth stressing, however, that it is profoundly uncertain whether a solely T cell-based vaccine can achieve enough of a benefit to attain licensure. As mentioned in the introduction, even the robust immunological control of HIV replication observed in elite controllers is typically associated with significantly elevated levels of immune activation and inflammation, which can eventually lead to disease progression. Elevated levels of inflammation in elite controllers have also recently been reported to be associated with markers of increased risk for arteriosclerosis. So while a T cell-based vaccine that lowered postinfection viral load might conceivably have a public health benefit by reducing risk of onward transmission, and perhaps an individual health benefit by delaying the need for ART, it is clear that such a vaccine would be at best a stopgap until a completely protective product could be developed.

HIV Preventive Vaccines Pipeline, 2009

Agent	Type	Sponsor	Status
ALVAC vCP1521	Canarypox vector encoding: HIV-1 CRF01_AE <i>env</i> , clade B <i>gag</i> , the protease-encoding portion of the <i>pol</i> gene and a synthetic polypeptide encompassing several known CD8 T-cell epitopes from the Nef and Pol proteins.	Sanofi Pasteur	Phase III (results anticipated Sept. 2009) Phase I (in infants)
AIDSVAX B/E (booster only)	Recombinant gp120 envelope protein.	VaxGen	Phase III in combination with ALVAC vCP1521 (results anticipated Sept. 2009)
VRC-HIVDNA016-00-VP + VRC-HIVADV014-00-VP	Prime: Six separate DNA plasmids containing <i>gag</i> , <i>pol</i> , and <i>nef</i> genes from HIV-1 clade B, and <i>env</i> genes from clades A, B, and C. Boost: Adenovirus serotype 5 vectors including <i>gag/pol</i> genes from HIV-1 clade B and <i>env</i> genes from clades A, B, and C.	National Institutes of Health (NIH) Vaccine Research Center/ GenVec/Vical	HVTN 505
pGAZ/IS7 DNA MVA/HIV62	DNA prime and MVA booster vaccines including <i>gag</i> , <i>pol</i> , and <i>env</i> genes from HIV-1 clade B.	National Institute of Allergy and Infectious Diseases (NIAID), Geovax	Phase IIA
ISS P-001	Recombinant Tat protein from HIV-1 clade B.	Istituto Superiore di Sanità, Rome; Excell	Phase IIA
LIPO-5	Five lipopeptides containing CTL epitopes (from Gag, Pol, and Nef proteins).	French Agence Nationale de Recherche sur le Sida et le hepatitis (ANRS), Aventis	Phase II
HIVIS 03 DNA-MVA prime boost HIV-1 vaccine candidate	Prime: HIVIS DNA including <i>env</i> (A, B, C), <i>gag</i> (A, B), reverse transcriptase (B), <i>rev</i> (B). Boost: MVA-CMDR including <i>env</i> (E), <i>gag</i> (A), <i>pol</i> (E).	Karolinska Institute, SMI, Vecura, USMHRP	Phase I/II
DNA-C + NYVAC-C	Prime: DNA vaccine including clade C <i>env</i> , <i>gag</i> , <i>pol</i> , and <i>nef</i> . Boost: NYVAC-C attenuated vaccinia vector including clade C <i>env</i> , <i>gag</i> , <i>pol</i> , and <i>nef</i> .	EuroVacc Foundation, GENEART	Phase I/II
PolyEnv1 EnvDNA	Vaccinia viruses including 23 different <i>env</i> genes and DNA vaccine with multiple <i>env</i> genes.	St. Jude Children's Research Hospital	Phase I

Agent	Type	Sponsor	Status
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 <i>env</i> and <i>gag</i> genes ; ADVAX p/n-t includes HIV-1 subtype C <i>pol</i> and <i>nef-tat</i> . Administered by Ichor Trigrif™ 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 <i>gag</i> , reverse transcriptase, integrase and <i>nef</i> genes and the other including HIV-1 subtype A <i>env</i> (gp140).	IAVI, University of Rochester	Phase I
Ad26.ENVA.01	Prototype adenovirus serotype 26 vector including the HIV-1 subtype A <i>env</i> 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 <i>env</i> .	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 <i>env</i> , <i>gag</i> , <i>nef-tat</i> and <i>pol</i> genes from HIV-1 subtype C. Boost: MVA vector encoding <i>env</i> , <i>gag</i> , <i>tat-rev</i> , and <i>nef</i> -reverse transcriptase genes from HIV-1 subtype C.	Indian Council of Medical Research, IAVI, Aaron Diamond AIDS Research Center	Phase I

Scientists have also been trying to understand the enhanced susceptibility observed in some Merck vaccine recipients. The blame appears to lie with the attenuated adenovirus serotype 5 (Ad5) vector that was used as a vehicle for delivering HIV proteins, but the effect was largely restricted to a subset of trial participants: uncircumcised gay men with preexisting antibodies to Ad5 (many people have been exposed to Ad5 in its natural form, which can cause a bad cold). The leading hypothesis is that vaccination with the Ad5 vector increased the numbers of HIV target cells in the foreskin, leading to an increased risk of infection for uncircumcised insertive partners. Long-term follow-up of STEP participants indicates that the effect was transient, as the difference in risk of acquisition between vaccine and placebo recipients has waned over time.

Chasing Complete Protection

Scientists continue to work on strategies that might completely protect against HIV infection, and some signs of progress have been reported in the past year. One new idea is to use vaccine vectors in a manner more akin to gene therapy; rather than delivering HIV proteins designed to elicit an immune response, the vectors include genes that directly manufacture neutralizing antibodies (a handful of such antibodies have been isolated over the years, but no one has been able to elicit them with conventional vaccines). In a test in monkeys, this strategy was very effective at preventing simian immunodeficiency virus (SIV) infection, but a number of hurdles need to be cleared before it can be considered for human testing. The Swiss company Mymetics has been focusing on inducing antibodies that may be especially good at inhibiting the transport of HIV across mucosal surfaces. In a study presented earlier this year, a vaccine that induced these antibodies 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). 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.

Although it has traditionally been assumed that T cell–based vaccines cannot completely protect against infection, researcher Louis Picker published data early in 2009 that challenge this assumption. Picker studied cytomegalovirus as a vaccine vector in the SIV model, and reported that 4 out of 12 immunized animals were able to resist a mucosal virus challenge despite the absence of neutralizing antibodies. (While protecting only one-third of the animals may seem meager, it is unusual to see any protection from infection with solely T cell–based vaccines.) Additional studies are now being conducted to try and elucidate the exact nature of the T-cell response associated with this salutary outcome, in hopes of informing the design of a similar vaccine for human trials.

ALVAC from Sanofi Pasteur is an HIV vaccine candidate that uses a bird virus called canarypox as a vector. ALVAC induces persistent HIV-specific CD8 T-cell responses in just 10–20% of recipients, leading to considerable skepticism about its potential efficacy. A version of ALVAC is undergoing an efficacy evaluation in Thailand in a 16,000-person trial initiated by researchers affiliated with the U.S. Military HIV Research Program. The trial is fully enrolled and a Data Safety Monitoring Board review in July 2007 determined that no safety issues had emerged and that the study could progress to completion. Results are anticipated in the third week of September 2009.

Evaluating T-Cell Immunogenicity

ELISpot (Enzyme-Linked ImmunoSpot) is a test that measures the ability of T cells (CD4, CD8, or both) to make cytokines when exposed to a given antigen. T cells are first exposed to the antigen; then antibodies that bind to a specific cytokine are introduced 6 to 24 hours later. The cells are chemically treated so that any antibodies bound to cytokine-producing cells are stained blue and can be counted. (These cells are called *spot-forming cells*). Background cytokine production (i.e., production that occurs without any antigen stimulation) can be a problem, and must be subtracted to get an idea of how many T cells were specifically responding to the antigen. The readout for ELISpot assays is usually production of the cytokine interferon gamma but this is increasingly viewed as inadequate for capturing the full magnitude and functionality of a vaccine-induced T-cell response.

Intracellular Cytokine Staining (ICS) also measures the ability of T cells (CD4, CD8, or both) to make cytokines when exposed to a given antigen. Unlike ELISpot, this test employs a substance that traps the cytokine within the T cell, allowing easier identification of the precise type of T cell that is making a given cytokine. Initially, the cytokine most commonly measured in ICS assays was interferon gamma. Over the past few years there has been an explosion in the use of ICS combined with multiparameter flow cytometry to assess expression of multiple cytokines, chemokines, and other functional markers (particularly CD107a, a marker of a T cell's cell-killing ability). T cells capable of exerting multiple functions have been dubbed *polyfunctional cells*.

Proliferation and Cell Killing: Because there are limitations to both the ELISpot and ICS assays, new methods are being developed to evaluate the ability of T cells to proliferate (an important correlate of immunity in many animal models) and also to kill HIV-infected cells in the lab.

Adenovirus vectors continue to be studied despite the failure of Merck's Ad5-based candidate, largely due to their potency as CD8 T-cell immunogens. Due to concerns over the use of Ad5 vectors in people with preexisting immunity to the virus, several of the vaccine candidates that have entered the pipeline over the last year are adenovirus-based constructs specifically designed to circumvent this problem; examples include vectors using Ad26, Ad35, and Ad48 serotypes that are less common in nature (in the case of Ad48, just the outer part of the virus has been used to make an Ad48-Ad5 hybrid vector that is impervious to the effects of anti-Ad5 antibodies).

The results of the STEP trial also drastically altered plans for a trial of an Ad5-based candidate developed by the National Institutes of Health's Vaccine Research Center (VRC). The VRC regimen involves three shots of a DNA vaccine followed by one shot of the Ad5 vector, encoding the same HIV antigens as the Merck vaccine (Gag, Pol, and Nef) plus three different Env proteins from clades A, B, and C. The vaccine was originally going to be studied in an international trial involving several thousand people, but the Ad5 safety issues documented in STEP halted those plans. The vaccine will now be evaluated in a 1,350-person trial dubbed HVTN 505, with enrollment restricted to circumcised gay men who lack anti-Ad5 antibodies (i.e., the subgroup in STEP that experienced no Ad5-related enhancement of susceptibility to HIV acquisition). TAG has publicly questioned the rationale for HVTN 505, based on the data indicating that the vaccine offers no advantages over the failed Merck product (*see* table, below).

Merck/VRC Vaccine Immunogenicity Comparison

Merck Ad5 Vaccine, STEP Trial (4 weeks after second or 4 weeks after third immunization), Ad5 antibody titer <18	VRC DNA/Prime Boost Vaccine, HVTN 204 (6 weeks after Ad5 boost), no significant differences based on Ad5 seropositivity
CD4 T-cell responses (any antigen)	
23/52 (44%)	26/57 (45.6%)*
CD8 T-cell responses (any antigen)	
53/59 (90%)	30/56 (53.6%)*
Proportion of recipients with both CD4 and CD8 T-cell responses	
31%	Unknown

Note: Data represent the proportion of recipients with T-cell responses capable of making interferon-gamma and/or IL-2 after stimulation with HIV peptides in the ICS assay.

** U.S. stratum only*

Modified Vaccinia Virus Ankara strain (MVA) is an attenuated, nonpathogenic derivative of the cowpox virus. Data suggest that MVA is less effective than Ad5 for inducing CD8 T-cell responses (the best response rate is around 40–50%). The Karolinska Institute and the U.S. Military HIV Research Program are advancing a DNA/MVA prime boost approach into phase II studies. A similar DNA/MVA approach developed by a company called GeoVax is in a phase IIA immunogenicity trial under the aegis of the HIV Vaccine Trials Network. Two other MVA-based HIV vaccine candidates are in human studies; one is manufactured by the Aaron Diamond AIDS Research Center and the other by Therion, a company that has now gone out of business but whose investigational new drug licenses have been transferred to the Division of AIDS at the National Institutes of Health.

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 Eurovacc Foundation. Judith Horwitz at St. Jude Children's Hospital in Memphis, Tennessee, is also employing a vaccinia vector as part of an experimental HIV vaccine regimen that delivers a cocktail of 23 different viral envelope proteins. Horwitz's candidate is one of the only vaccines in the current clinical pipeline that is aiming to induce antibody rather than T-cell responses.

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. However, 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. The Aaron Diamond AIDS Research Center, the International AIDS Vaccine Initiative, and Ichor Medical Systems are currently collaborating on a phase I trial of DNA vaccines administered by this method.

PrEP and Microbicides Pipelines, 2009

Product	Type	Sponsor	Status
PrEP			
Tenofovir (Viread, TDF)	Nucleotide reverse transcriptase inhibitor	Gilead Sciences	Phase III
Truvada (TDF/FTC)	Combined nucleoside and nucleotide reverse transcriptase inhibitors	Gilead Sciences	Phase III
Microbicides			
PRO 2000/5 Gel	Adsorption inhibitor	Indevus Pharmaceutical	Phase III
Tenofovir/PMPA Gel	Reverse transcriptase inhibitor	Gilead Sciences	Phase IIb
Dapivirine (TMC120)	Reverse transcriptase inhibitor	International Partnership for Microbicides (IPM)	Phase I /II
VivaGel (SPL7013 gel)	Entry/fusion inhibitor	Starpharma	Phase I/II
UC-781	Reverse transcriptase inhibitor	Biosyn	Phase I
Combination monoclonal antibodies (C2F5, C2G12, and C4E10)	Neutralizing antibodies	Polymun, European Microbicides Project	Phase I
BufferGel Duet	Combination microbicide and cervical barrier	ReProtect	Phase I

Pre-Exposure Prophylaxis

Pre-exposure 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 in 2010. A separate CDC safety and acceptability study in 400 gay men in the United States was completed this year. 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 becoming available in the next year. The University of Washington has recently launched a trial of tenofovir versus Truvada as PrEP in 3,900 serodiscordant couples in Kenya and Uganda.

Two other PrEP trials are on the verge of opening. The Microbicide Trial Network's (MTN*) VOICE study plans to enroll 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 is slated to conduct a trial of Truvada as PrEP in 3,900 women at sites in Kenya, Malawi, South Africa, and Tanzania.

** TAG's 2008 Pipeline Report noted that the reorganization of the National Institutes of Health's clinical trials apparatus has led to the formation of a specific Microbicides Trial Network (MTN). However, we misidentified the principal investigator of the network; it is led by Sharon Hillier from the University of Pittsburgh School of Medicine. We apologize for the error.*

Microbicides

Microbicides are substances that aim to prevent HIV infection (and possibly other sexually transmitted infections) via topical application to the vaginal or rectal surface prior to sex. As mentioned in the introduction, 2009 saw the first tantalizing hint of efficacy for a candidate HIV microbicide when it was reported that PRO2000 gel reduced the risk of HIV acquisition by approximately 30% in a phase IIb efficacy trial (HPTN 035) in southern Africa. The results represented a strong trend but did not quite attain statistical significance. Encouragingly, however, secondary analyses support the conclusion that the product has some protective effect; for example, efficacy was highest (~78%) among the subgroup of women reporting the most consistent gel use and least consistent condom use. Fortunately, another larger ongoing phase III trial of PRO2000 gel is scheduled to end later this year, and results should demonstrate conclusively whether the approach is efficacious. If the phase IIb results are confirmed, PRO2000 gel would be the first ever biomedical HIV prevention intervention to show significant efficacy against sexual transmission. Although 30% protection may sound unimpressive, there are contexts in which the impact could be significant. As Salim Abdool Karim noted in an informal talk after his CROI presentation, women can be exposed to HIV through unprotected sex while attempting to become pregnant. Because PRO2000 has no contraceptive effect, it could, unlike condoms, offer a means of allowing pregnancy while simultaneously providing some protection against HIV infection.

In 2010, results are anticipated from the first efficacy trial of an antiretroviral-based microbicide. The phase IIb study of tenofovir gel was launched in South Africa in 2007 by a collaboration involving the Centre for the AIDS Programme of Research in South Africa (CAPRISA), Family Health International, the United States Agency for

International Development (USAID), LIFElab, and CONRAD. The study plans to enroll 1,250 women, and data collection is ongoing through December 2009.

Adsorption inhibitors block the binding of HIV to target cells. PRO2000 is the most advanced candidate, with phase III results due later this year. Starpharma's VivaGel (SPL7013 gel) is at an earlier stage of development.

Acid-buffering agents. A key aspect of vaginal health is the maintenance of a low pH by hydrogen peroxide—producing lactobacilli. Several microbicides are designed to maintain the acidity of the vagina, thereby making it inhospitable to viruses like HIV. One such agent, BufferGel, was included in one of the arms of the phase IIb trial of PRO2000 gel, but showed no protective efficacy against HIV or any other sexually transmitted infection. However, it remains in testing as a component of Duet, a pre-coated cervical barrier.

Antiretrovirals. A number of microbicides that have direct antiretroviral effects, including several reverse transcriptase inhibitors, are advancing in human trials. The farthest along is tenofovir gel, which is being studied in an ongoing trial in South Africa and will also be included in the MTN's VOICE study. The reverse transcriptase inhibitor UC-781, originally developed by Uniroyal Chemical and Biosyn, is in a phase I trial sponsored by CONRAD. The International Partnership for Microbicides (IPM) is developing a nonnucleoside reverse transcriptase inhibitor, dapirivine gel (licensed from Tibotec and formerly known as TMC120), which is currently in a phase I/II trial. The Population Council is planning clinical trials of MIV-150, a reverse transcriptase inhibitor, combined with another gel or integrated into a ring. Among a large array of preclinical candidates following on the heels of these compounds are drugs that target attachment and entry of HIV; IPM has licensed the CCR5 inhibitors maraviroc (Selzentry) from Pfizer, CMPD 167 from Merck, the fusion inhibitor L'644 (also from Merck), and the attachment inhibitor BMS-378806 from Bristol-Myers Squibb.

Immune-Based Therapies

Immune-based therapies (IBTs) are a broad category of treatments that aim to produce a therapeutic benefit by affecting the function of the immune system. IBTs can be subdivided into therapies that try to boost the immune response to HIV itself (e.g., therapeutic vaccines), those that may improve immune function and/or clinical health overall (e.g., cytokines like IL-7 and anti-inflammatory approaches) and gene therapies that may alter the makeup of the immune system in ways that ameliorate the harmful effects of HIV. Given the success of combination antiretroviral therapy (ART), the logical focus

for IBTs is addressing the limitations of ART. Of greatest concern are the studies showing that individuals who experience poor CD4 T-cell reconstitution on ART are at an increased risk for not just opportunistic infections but also clinical events that traditionally have not been considered HIV-related, such as liver and kidney disease, cardiovascular problems, and cancers. An IBT capable of improving immune reconstitution could conceivably provide significant clinical benefits to individuals in this situation (which the published literature consistently estimates to be ~5–10% of individuals on ART). As alluded to in the introduction, the failure of IL-2 to show any efficacy does not mean that IBTs with different mechanisms of action are all doomed to the same fate. The incidence of clinical events in the setting of suboptimal immune reconstitution on ART, while relatively low, is nevertheless of sufficient magnitude (in the region of 5% per year) that randomized trials could evaluate the clinical benefit of any promising IBTs in that setting without the need to enroll thousands of people.

There also remains a rationale for studying approaches, such as therapeutic vaccination, that might reduce dependence on ART by allowing safe interruptions of therapy. At one time it was thought that intermittent ART might be a safe and viable treatment strategy, but the SMART trial showed otherwise. SMART was a very large (5,742-person) randomized comparison of intermittent versus continuous ART. The study had to be stopped early because interrupting ART was associated with an increased risk of clinical disease and death, and also an increased risk of cardiac, kidney, and liver problems that heretofore were widely assumed to represent drug toxicities. Although the absolute risk of these events was very low overall in both arms of SMART, the relative risk was doubled in the arm that interrupted ART. Additional analyses of the SMART data indicate that the immune activation and inflammation that accompanied viral load rebounds was the main explanation for the adverse outcomes in the treatment interruption arm. These results have raised the bar for IBTs that aim to reduce use of ART, because they indicate that a strong anti-HIV effect would be needed to render ART interruptions safe. Alternatively, an IBT would need to prevent HIV replication from causing immune activation and inflammation (there are some monkey species that do not develop immune activation despite high SIV viral loads, suggesting that this might be possible in theory, but so far no IBTs in development are aiming for this outcome). The other very important implication of the SMART data is that research involving treatment interruptions needs to be very carefully designed in order to avoid placing participants in harm's way.

An emerging issue in the pathogenesis of HIV infection is immune senescence, which is characterized by the accumulation of dysfunctional memory T-cell populations in the CD4 and the CD8 T-cell pools (and particularly the latter). These dysfunctional cells are characterized by a lack of expression of the costimulatory molecule CD28 and elevated expression of a senescence marker, CD57. A similar phenomenon is seen in the elderly in the absence of HIV infection; in this setting, elevated levels of senescent CD8 T cells

Therapeutic Vaccines Pipeline, 2009

Agent	Type	Sponsor	Status
Vacc-4x	Four synthetic peptides derived from the HIV-1 Gag p24 protein, delivered intradermally with GM-CSF.	Bionor Immuno AS	Phase II
DCV-2	Autologous myeloid dendritic cells pulsed ex vivo with high doses of inactivated autologous HIV-1.	Hospital Clinic of Barcelona	Phase II
CD4-specific T-cell vaccine	Prepared from autologous T cells that proliferate in response to recombinant CD4. These T cells are expanded in vitro by IL-2, then fixed by glutaraldehyde. Each vaccine preparation consists of 10,000 cells suspended in saline and given subcutaneously every three months.	Soroka Medical Center, Israel	Phase II
HIV-1 Tat vaccine (ISS T-002)	Tat protein vaccine at two different doses (7.5 mcg or 30 mcg) in five or three immunizations.	National AIDS Center at the Istituto Superiore di Sanità, Rome	Phase II
AGS-004	Mature dendritic cells coelectroporated with autologous HIV-1 RNA and CD40L RNA.	Argyros Therapeutics	Phase 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 MVA vector encoding <i>gag</i> and multiple CTL epitopes.	Cobra Pharmaceuticals, Impfstoffwerk Dessau-Tornau GmbH (IDT), Oxford University/MRC	Phase I/II
MVA-mBN120B	Multiantigen MVA vector.	Bavarian Nordic	Phase I

Agent	Type	Sponsor	Status
Autologous dendritic cell	Autologous dendritic cells pulsed with conserved HIV-derived peptides.	University of Pittsburgh	Phase I
Multipitope 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
GSK protein HIV vaccine	Recombinant Tat, Nef, and gp120 proteins in AS02A adjuvant.	GlaxoSmithKline, Marcus Altfeld	Phase I
GX-12	Multiantigen + IL-12 DNA vaccine	Genexine, Seoul National University Hospital	Phase I
DC vaccine	Autologous dendritic cells generated using GM-CSF and interferon alpha, loaded with lipopeptides and activated with lipopolysaccharide.	Baylor University, French National Agency for Research on AIDS and Viral Hepatitis	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, pLL15EAM	PENNVAX-B is a cocktail of three expression plasmids. The plasmids include the genes that encode a synthetic HIV-1 envelope protein (pEY2E1-B), Gag (gagCAM02), and Pol (pK2C1). GENEVAX IL-12-4532 is a molecular adjuvant plasmid that contains nucleotide sequences necessary for expression of the human IL-12 protein; pLL15EAM is a plasmid that encodes human IL-15.	University of Pennsylvania, Drexel University	Phase I
GSK HIV vaccine 732461	Gag, Pol, and Nef proteins in proprietary adjuvant.	GlaxoSmithKline	Phase I

designate an “immune risk phenotype” that is associated with frailty, ill health, and earlier mortality. 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. Researchers such as Rita Effros from the University of California–Los Angeles, who is recognized as a pioneer in the field, 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.

Studies of long-term nonprogressors have played a key role in guiding the development of therapies aimed at bolstering the immune response to HIV. As outlined earlier (*see* box, “Evaluating T-Cell Immunogenicity”), recent studies have found that CD4 and CD8 T cells capable of performing multiple functions have advantages over those with more limited ability, such as the production of interferon gamma alone. It must be stressed that no proof exists that these types of T-cell responses are responsible for controlling HIV replication; they may emerge as a consequence of low viral load or they may work alongside other—as yet unknown—factors. For developers of therapeutic vaccines, however, these immunological parameters at least provide some guidance as to the types of immune response their constructs should induce. In addition to T-cell function, recently accumulated data strongly suggest that the targeting of multiple epitopes in HIV’s Gag protein is an important correlate of immunological control of viral replication in infected individuals, indicating that the induction of broad T-cell responses against Gag is an important goal for therapeutic vaccines.

Newcomers to the therapeutic vaccine pipeline in 2009 include two approaches using dendritic cells (described as “nature’s adjuvant” by the immunologist Ralph Steinman) as a vehicle for delivering HIV antigens. One of these dendritic cell vaccines is being developed by Baylor University in Texas, but—unusually—the funding support is coming from the French Agence Nationale de Recherche sur le Sida et le hepatitis (ANRS), rather than the NIH. The University of Pennsylvania is exploring the efficacy of DNA vaccines with cytokine adjuvants (either IL-12 or IL-15), and GSK has launched a phase I trial of a protein-based vaccine in a proprietary adjuvant. Unlike its prior candidate, this GSK construct includes the HIV Gag protein in addition to the Pol and Nef proteins. A biotech company called Thymon has initiated a trial of a therapeutic Tat vaccine at Conant Medical Clinical Research in San Francisco.

Immunomodulator, Cytokine, and Gene Therapy Pipeline, 2009

Agent	Type	Sponsor	Status
Immunomodulators/Cytokines			
Maraviroc (Selzentry)	CCR5 inhibitor.	Pfizer	Phase IV
Chloroquine phosphate	Anti-inflammatory.	ACTG A5258	Phase II
Pegasys (peginterferon alfa-2a)	Cytokine.	NIAID, Hoffmann-La Roche	Phase II
Interleukin-7 (CYT 107)	Cytokine.	Cytheris	Phase I
HLA-B*57 cell transfer	Cell infusion.	NIH Clinical Center	Phase I
Gene Therapies			
OZ1 ribozyme gene therapy	Antiviral ribozyme targeted against the <i>tat</i> 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, introduced into CD4 T cells ex vivo.	EUFETS AG	Phase I
Autologous T-cells genetically modified at the CCR5 gene by zinc finger nucleases SB-728 for HIV (zinc-finger)		University of Pennsylvania, Sangamo Biosciences	Phase I
Combined Anti-HIV RNA-based therapeutics		City of Hope/Beckman Research Institute	Phase I

Anti-inflammatory approaches. The significant associations between inflammatory markers and adverse clinical events that emerged from the SMART trial 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; the outcome measures being looked at are markers of cardiovascular disease risk.

Cell infusion and gene therapies. Several phase I and II studies of gene therapies are ongoing. The broad goal of these approaches is to enhance the ability of 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 *Nature Medicine* 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.

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 zinc finger nucleases, which 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 to high numbers and reinfused into the individual.

M87o is a gene therapy that, once integrated into cells, encodes an HIV entry inhibitor similar to the drug Fuzeon. The approach is now being studied as an additive therapy in individuals with AIDS-related lymphoma who require stem cell transplantation; the M87o gene is added to the stem cells prior to transplantation.

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. The drug was well tolerated. These results suggest that IL-7 may be an appropriate 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 (Selzentry) is an approved antiretroviral drug that works by blocking the interaction between HIV and the chemokine receptor CCR5. Four 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.

One small pilot study involving just nine people has already been conducted, and results were presented at the 2008 Interscience Conference on Antimicrobial Agents and Chemotherapy. Overall, the addition of maraviroc showed no benefit (http://img.thebody.com/confs/icaac2008/slides/H-1247_Paez_SL_poster.pdf), which perhaps calls into question the hypothesis that maraviroc can have an independent effect on CD4 T cell counts in this setting. The ongoing trials, which plan to enroll over 400 participants in total, will be able to answer the question definitively. Another small pilot study at the University of California–Davis plans to evaluate whether maraviroc-containing regimens are better at restoring gut CD4 T cells than standard ART.

Conclusion

Although the immune-based therapies and preventive technologies pipeline has yet to produce an effective product, it is far from dry. And while these areas of research present stern challenges, it is worth remembering that clouds of doom enshrouded the antiretroviral field at the Berlin AIDS conference in 1993, only to be dispelled by the HAART revolution that occurred just three years later. The coming year promises crucial results from trials of PrEP and PRO2000, offering hope that the landscape for these research fields will soon be considerably more fertile.

Resources

<p>AIDS Clinical Trials Group http://www.aactg.org/</p>	<p>HIV Vaccine Trials Network http://www.hvtn.org/</p>
<p>AIDS Vaccine Advocacy Coalition (AVAC) http://www.avac.org/</p>	<p>International AIDS Vaccine Initiative Database of AIDS Vaccine Candidates in Clinical Trials http://www.iavireport.org/trials-db/Pages/default.aspx</p>
<p>Alliance for Microbicide Development R & D Database http://www.microbicide.org/cs/mrdd</p>	<p>International Partnership for Microbicides http://www.ipm-microbicides.org/</p>
<p>Community HIV/AIDS Mobilization Project (CHAMP) http://www.champnetwork.org/</p>	<p>NIH/National Library of Medicine Clinical Trials Database http://clinicaltrials.gov/</p>
<p>Global Campaign for Microbicides http://www.global-campaign.org/</p>	

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