
HIV, TUBERCULOSIS, HEPATITIS B, AND HEPATITIS C:
DRUGS, DIAGNOSTICS, VACCINES, AND MICROBICIDES IN
DEVELOPMENT

2008 Pipeline Report

JULY 2008

BY TREATMENT ACTION GROUP

LEI CHOU, MARK HARRINGTON, BOB HUFF,
RICHARD JEFFERYS, TRACY SWAN, 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

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Introduction

Treatment Action Group's annual pipeline report is a review of medical technologies that stand a good chance of benefiting people with HIV within the next few years. It also covers those that may take longer to develop but represent innovation within the field.

This year our report has expanded to cover treatment and preventive vaccines for the hepatitis B virus and diagnostics for tuberculosis. These are natural extensions to our updates on HIV antiretroviral treatment, hepatitis C treatment, drugs and vaccines to treat and prevent tuberculosis, immune-based therapies for HIV, and HIV prevention technologies, including vaccines and microbicides.

In some areas, such as treatment for hepatitis C virus (HCV), the therapy pipeline is bubbling, with over 20 drugs listed in middle to late stages of development. Despite all the activity, no single drug is likely to revolutionize the current, difficult HCV treatment paradigm, though shortened treatment durations and increased rates of successful outcomes may begin to benefit people with HCV within the next few years. Unfortunately, people with HIV are often unnecessarily excluded from HCV research.

Tuberculosis (TB) is treatable and curable, yet it remains the top killer of people with HIV worldwide. A major limitation to broader and more effective TB treatment in the developing world is the lack of a simple and reliable means of diagnosis—and TB is especially hard to diagnose and treat in people with HIV. Hampered by limited investment in the field, the TB diagnostic pipeline mainly contains advances aimed at high-tech national laboratories or adaptations of existing technology that can be used in regional hospitals. There is much less on the horizon for TB diagnostics that can be used in rural settings, where the need is greatest.

TB drug therapy is also undergoing a period of renewed activity after decades of stasis. Five novel TB drugs are in clinical trials, and funding to explore improved treatment strategies with existing drugs has increased, though it lags far behind the need—especially in light of the growing problem of drug-resistant TB. As with HCV, the near-term goals for TB therapy are to reduce treatment times and improve success rates. Better TB treatment options for people with HIV are also a high priority.

Improved TB preventive vaccines offer the promise of cost-effective, wide-scale reductions in future cases of TB, though their impact may be decades away. A few candidates are in human trials, but many questions remain. One obstacle to getting the answers is the uncertainty of future funding for large-scale clinical trials of TB vaccines.

After a flurry of new drug approvals in the past year, the HIV treatment pipeline is slowing. Most gains in antiretroviral therapy over the next several years will likely come from treatment strategy refinements that build on recently approved drugs. Agents in the pipeline are generally expected to offer incremental, yet important,

improvements over existing products. With interest in HIV drug research maturing, the field is ready for investment in a conceptual leap to discover radically new therapies that disable or even cure HIV infection, perhaps by unleashing innate mechanisms of anti-HIV immunity that the virus currently evades.

Interest in developing new treatments to control or eradicate hepatitis B virus (HBV) is slowly increasing, but the field is restricted by gaps in the scientific understanding of the virus. Current treatments—mostly spin-offs from HIV drug research—suppress HBV but, as with HIV, are vulnerable to the emergence of drug resistance. Exploration of strategies to prevent resistance, possibly through combination therapy, is the next frontier for HBV, with novel drugs much farther down the road.

The failure of two leading experimental agents in HIV prevention technologies has created a gloomy outlook for this field, though research is generally well funded and continues apace. An unexpected increase in HIV infections associated with a leading HIV vaccine candidate has stimulated a wrenching reappraisal of research priorities. With much greater understanding of the basic science of HIV needed, it may be said that the HIV vaccine field remains in a toddler state. Yet because no other intervention promises so much for future control of the epidemic, support for HIV vaccine research remains strong. The vaginal microbicide field also saw a leading candidate fail in a late-stage clinical trial. Other, likely stronger, microbicide candidates, are at earlier stages of development.

Research on therapies or vaccines to strengthen or stimulate the adaptive immune system to fight HIV remains the poor stepchild to drug and preventive vaccine science. Immune-based therapy research is closely associated with the scientific investigation of how HIV causes disease, and increased investment in this field may pay off in ways that are not immediately foreseeable. Agents in this pipeline tend to be earlier in development but represent a great variety of experimental and innovative approaches.

Research on new technologies to prevent, treat, and diagnose HIV and its coinfections is progressing in 2008. Some fields, such as HCV, are full of activity as drug sponsors race to be first in the market with a transformative therapy. Hepatitis B research may be the next field to get the attention of the commercial sector. Other fields, such as TB research, appear active because they are catching up after years of neglect, though the gap in the needed investment remains large. Antiretroviral research has made dramatic progress over the past 13 years, but seems to be entering a slow phase as recent advances are consolidated into the standard of care. For over 20 years, a preventive HIV vaccine has seemed perpetually just out of reach, though setbacks like the field has just experienced can create opportunities for new ideas to emerge.

Overall, the 2008 HIV pipeline suggests progress and hope. This report reveals not only the current status of these technologies but also underscores the need for continued and greater investment in making them useful and widely available.

The Antiretroviral Drug Pipeline

BY BOB HUFF

TAG's Antiretroviral (ARV) Pipeline Report is usually a story about what's new and what's coming in the world of experimental HIV drugs—and the story typically ends with FDA approval. In 2008, with only a few new drug candidates in the pipeline, and no approvals likely before 2010 or later, a better story may be what's happening (or not happening) with three drugs approved during the past 12 months.

Merck's raltegravir, licensed in October 2007, seems to be a star that shines brighter day by day. Prior to approval it was an object of giddy speculation by the medical elite, some of whom called it a “wonder drug.” And it enjoyed a glittering debut among people with multidrug-resistant HIV who, for the first time in many years, could assemble a regimen able to durably suppress their virus. Some observers warn, however, that this “golden age” of viral pansuppression may not last as a growing number of individuals on the newer drugs experience loss of viral control due to resistance and require even newer options. Unless the pipeline is refilled, their options may be few.

It may be that some former “salvage patients” even felt comfortable enough with their new, potent regimens to drop the inconvenient injectable Fuzeon—a potentially catastrophic development for Trimeris and Roche, the makers of Fuzeon. When Roche announced in June that it was shifting its viral research efforts to concentrate on hepatitis C, there was speculation that future pipeline reports may increasingly look like obituaries.

Yet for now, some clinical investigators seem content with taking a breather and enjoying the lighter burden in their clinics. One editorial even termed current treatment options (with irony) as “an embarrassment of riches.”* There is a worrisome aspect to this, however, if the complacency expressed by some U.S. clinicians regarding the need for improved first-line regimens manifests as reluctance to participate in clinical trials for treatment-naïve people.

The other important new drug of 2007, Pfizer's maraviroc, approved in August, has not fared as well as raltegravir, and while not a candidate for the obituary column it may one day find itself adrift if Pfizer, like Roche, decides to cast off its involvement with HIV. Sales of maraviroc have been far below expectations, mainly because the

* Bernard Hirschel, M.D., and Alexandra Calmy, M.D. Editorial: Initial Treatment for HIV Infection—An Embarrassment of Riches. *New England Journal of Medicine* 358, no. 20 (2008): 2170–2172.

drug faces formidable barriers to acceptance in clinical practice. Currently, using maraviroc requires an expensive and slow-to-report blood test that indicates baseline viral susceptibility to the drug—and susceptibility rates fall to about 50% in people with longtime infections and lower CD4 counts. Because the assay does not catch everyone who lacks susceptibility, there is a risk of loss of viral control—and possible loss of the rest of the regimen due to resistance if too much burden is placed on maraviroc, though a new version of the assay is more sensitive. There were also worries about the safety unknowns of maraviroc's novel mechanism, which targets the host rather than the virus. Finally, a high state of nervousness at the FDA over the Vioxx drug safety scandal likely contributed to a black box warning about liver damage based on one episode with maraviroc and multiple cases with a different, subsequently discontinued drug in the same class. Nevertheless, for those who benefit, maraviroc works quite well.

An intriguing finding from early clinical trials provides a note of excitement about the use of maraviroc and other treatments in the CCR5 antagonist class. It's been observed that some patients who lacked susceptibility to the drug and obtained no virologic benefit still had paradoxical increases in CD4 counts. Because these drugs attach to CCR5 signaling proteins on CD4 immune cells, there is some speculation that they may have immune modulation activity independent of their antiviral effect. Another explanation may be that the specific suppression of HIV that uses CCR5—even if it is not the dominant strain as measured by viral load—helps protect against CD4 cell loss. If this turns out to be the case, then the need for a susceptibility assay may be jettisoned, as CCR5 antagonists are prescribed to quell a particularly immunotoxic form of HIV—whether it shows up in the viral load or not. Until recently there were five CCR5 blockers under development, though this number was reduced by one when Incyte discontinued its candidate in 2008.

Another less heralded drug approval, in January 2008, was that of the NNRTI etravirine (TMC125), from Tibotec. This drug is active against many—but not all—NNRTI-resistance mutations that arise with efavirenz and nevirapine, and it was approved for treatment-experienced patients. Etravirine followed an unusual development path since it was most always paired in clinical trials with Tibotec's protease inhibitor, darunavir. While offering two experimental agents was a step forward in clinical trial design and provided people who had few treatment options extra protections while in the study, it also meant that there was little data produced on using etravirine in any other context than with darunavir. It turns out that there are a complicated set of interactions when etravirine is combined with several other drugs. Nevertheless, it works well with darunavir, tenofovir, and raltegravir, and combinations from among these may be all anyone really needs. Until recently, neither etravirine nor darunavir had been as widely embraced by clinicians as Tibotec had hoped, although the rising tide of raltegravir is now lifting these boats as an

increasing number of treatment-experienced patients switch to new, fully suppressive regimens. Rilpivirine (TMC 278), another NNRTI from Tibotec being developed as a first-line drug, has finally initiated phase III studies at 25mg/day after a long delay.

With the success of raltegravir (and the disappointment of maraviroc) the gold rush in ARV development has shifted to the integrase inhibitor class. Next in the pipeline is elvitegravir, a candidate from Gilead Sciences with once-daily dosing potential when combined with the pharmacologic booster ritonavir. At first glance, the need for ritonavir seems like a significant drawback (Abbott Laboratories currently only offers a suboptimal and expensive form of the drug, though the long-promised Norvir 2.0 tablet may finally be moving forward), but new thinking about ARV development looks to the regimen, not just the drug. Due to favorable drug interactions between elvitegravir and the Bristol-Myers Squibb (BMS) protease inhibitor atazanavir, Gilead may be well-positioned to offer the first all-in-one NRTI-sparing regimen. A successful cooperative venture between Gilead and BMS resulted in the wildly successful single-pill version of efavirenz, tenofovir, and emtricitabine called Atripla. Since BMS also makes atazanavir, the precedent is in place for a next-generation powerhouse with access to a boosting agent being the main (and still possibly insurmountable) sticking point (for a wild-card pairing that skirts this issue, imagine Kaletra plus raltegravir). Gilead, Pfizer, and Sequoia are said to be working on boosting agents to replace ritonavir.

This brings us to another trend in ARV manufacturing. Due largely to an impasse in the availability of ritonavir for coformulation, the United States may soon fall behind the rest of the world in the variety of ARV combinations available to its citizens. The Indian generic pharmaceutical industry operates under a set of patent laws that protect the process for manufacturing drugs but not the final drug product itself. This means the industry has become skilled at inventing new processes, and as a result has been able to supply ARVs to mass treatment programs in Africa at a cost of under \$200 per year per person. Millions of people are alive today because of low-cost, high-quality Indian-made ARVs made possible by this patent system. However, the system is changing, and Indian generic drug makers may be prevented from manufacturing certain newer drugs such as atazanavir and raltegravir without permission. Yet because the need for ARVs is so great and continues to grow (some plans call for treating ten million additional people within the next few years), patent holders such as Gilead have issued licenses that allow the Indian companies to produce and sell tenofovir in Africa with few restrictions. Looking ahead, the Indian companies say they are planning to produce novel all-in-one regimens of smart, convenient combinations that may never become available in the United States, such as generic ritonavir-boosted atazanavir/tenofovir/lamivudine and even raltegravir/atazanavir/ritonavir.

The Current ARV Pipeline

Of ARVs in phase II or beyond, it is not immediately clear which has the staying power needed to make it to the finish line by 2010. Gilead has the money and experience to move its integrase inhibitor elvitegravir forward, and it has a strategy and a market waiting for it when it emerges. What it doesn't have is heat-stable ritonavir available in 25mg doses (a quarter of the Abbott dose and all that may be required for elvitegravir) or a substitute pharmacologic booster.

After many years of setbacks and missteps, Schering's CCR5 blocker vicriviroc may continue to limp forward, but the rationale for investing in large phase III trials seems slim given the dismal performance of maraviroc during its first year on the market.

Tibotec's rilpivirine could be a very important drug for the developing world due to its compact 25mg dosing, which would make it cheap and easy to put into single-pill regimens. But potency may be an issue, since rilpivirine suppressed HIV at a slower rate than did efavirenz in a head-to-head trial (although by 48 and 96 weeks its performance was equivalent to efavirenz, with fewer side effects). Still, after seeing the unprecedented rapidity with which raltegravir suppresses HIV, there may be a perception that the bar for antiviral activity has been set higher.

Bevirimat, a novel maturation inhibitor, once had its day as a bright and shining newcomer. That luster is now long gone, however, as the drug has suffered problems with formulation, unconvincing trial results, and missteps by an underresourced small pharmaceutical company trying to go it alone. Tiny companies like Panacos must inevitably partner with a larger company if they hope to get a drug through expensive phase III trials. That no big pharmaceutical partner has appeared to take bevirimat forward means that most of them had a look at the drug and decided to pass.

TNX-355 is a promising idea from a small company that may have been lost in a corporate shuffle. The drug is a monoclonal antibody that prevents HIV from attaching to the CD4 receptor on target cells. Its developer, Tanox, was acquired by Genentech, where a restart of clinical development is being mulled. A drawback is the need for infusion, although one dose might last for a full month. The drug would occupy a niche market at best, perhaps for use as postexposure prophylaxis, though new developments in slow-releasing nanoparticles might one day make monthly dosing of entire regimens a reality.

With only one or two drugs in the pipeline that have a chance of emerging by 2010, the outlook for new HIV agents looks bleak. If we seek hope in compounds still in early trials we may not be reassured. Merck undoubtedly has a follow-on integrase inhibitor with once-daily dosing properties; GlaxoSmithKline is moving three integrase candidates forward in parallel, looking for a winner to emerge that can vie for best in class. Others may be in this game too.

A surfeit of CCR5 blockers (where the action used to be) is becoming apparent. Incyte canceled its CCR5 program, though newcomer Tobira has entered the scene with two candidates. Pfizer has a follow-on to maraviroc, but it is hard to imagine the company giving it a fast track after maraviroc's poor showing. A couple of monoclonal antibody CCR5 blockers are on the books at Progenics and Human Genome Sciences, but they are not causing much buzz. Development of a CXCR4 blocker, which might make a nice companion to its CCR5 cousin, has at present been suspended by Genzyme.

New and improved versions of well-established classes, such as NNRTIs and protease inhibitors, may be a safer bet. Pfizer and Boehringer Ingelheim each have an NNRTI in the early pipeline, though whether either of these companies—which have each suffered significant disappointments in the marketplace recently—will stick with them, or even with HIV treatment, remains to be seen. Smaller companies, such as Ardea, are also working on NNRTIs.

The protease inhibitor class may still have some life in it if compounds from Merck and promising newcomer Sequoia gain traction.

Finally, there are a gaggle of NRTI molecules from small companies that have been languishing for several years at early development stages; none seem poised for greatness. The exception may be apricitabine, which is currently challenging lamivudine in a head-to-head trial. Despite the enthusiasm for NRTI-sparing regimens, a good, clean NRTI active against current NRTI mutations might find a happy home in a fixed-dose combination pill from Merck or Gilead.

Table 1: Antiretroviral Pipeline

Agent	Class	Sponsor	Status
Rilpivirine (TMC278)	NNRTI	Tibotec	Phase III
Vicriviroc	CCR5 antagonist	Schering	Phase III
Elvitegravir	Integrase inhibitor	Gilead	Phase II
Bevirimat	Maturation inhibitor	Panacos	Phase II
TNX-355	CD4 blocker	Genentech	Phase II
Apricitabine	NRTI	Avexa	Phase II/III
Amdoxovir	NRTI	RFS Pharma	Phase II

Table 2: ARVs on the Rocks

Agent	Class	Sponsor	Status
AMD11070	CXCR4 blocker	Anormed	Suspended
BMS378806	gp120 blocker	BMS	Discontinued
INCB9741	CCR5 blocker	Incyte	Discontinued
KP-1451	Viral decay accelerator	Koronis	Suspended

Hepatitis B Treatment Pipeline

BY LEI CHOU

Treatment strategies for hepatitis B virus (HBV) are evolving rapidly, moving away from the use of injectable interferon, with its notorious side effects and lackluster efficacy. Potent oral anti-HBV agents have significantly improved treatment responses in recent years. Unfortunately, currently available oral HBV drugs, known as nucleoside analogues, all inhibit viral replication the same way: by terminating reverse transcription. For a period of five years, people in need of treatment were kept on lamivudine, the only approved oral agent at the time, even as multiple viral mutations were identified. Not surprisingly, multiple-drug-resistant HBV has emerged as a major challenge facing newer agents from the same drug class even as they improve in potency, resulting in loss of viral suppression and treatment failure for many people. This has created a critical need for more potent agents with higher genetic barriers to drug resistance than current options offer. After the expected FDA approval of tenofovir in August of this year, the HBV pipeline dries up; no next-generation treatment approach has yet emerged as a candidate to break the drought.

Ten companies have suspended development of experimental candidates for HBV in the last two years.

Table 1: HBV Experimental Compounds Graveyard

Current Development Status	Experimental Candidates
Being developed for HIV; HBV development suspended after phase II	Amdoxovir, Elvucitabine, Racivir
Development stalled pending new business partnership	EHT899, Pradefovir.
Development suspended after phase I	CHB-111 (HepaVaxx), Valtorcitabine
Development suspended after phase II	HepeX-B, UT-231B, HI-8 HBV

The main reason for this dry spell is a lack of attention to the basic science of HBV virology, research that lags far behind our understanding of HIV and hepatitis C. The current picture of HBV viral entry, assembly, and maturation is incomplete at best, while the crystal structure of HBV protease has yet to be elucidated. Without this basic knowledge, the ability to identify new drug targets cannot progress and therapeutic advances such as those seen in HIV and HCV are unlikely to materialize for HBV anytime soon.

The difficulty of HBV eradication complicates matters. HBV deposits its genetic material, covalently closed circular DNA (cccDNA), into the nucleus of infected liver cells, a relatively stable reservoir, where the virus can reactivate once therapy is stopped. Research into therapeutic approaches with more effective cccDNA clearance is ongoing, but treatment for HBV appears to be following the pattern of HIV, with a majority of chronically infected people needing lifelong therapy to maintain viral suppression. This is obviously bad news for patients, both in terms of drug costs and the risk of long-term toxicities. It has, however, finally gotten the attention of some pharmaceutical companies.

The global annual HBV market is currently about \$450 million, primarily from the United States and European Union. It is projected to reach \$1 billion by 2010 (one-tenth the size of the HIV market). With lamivudine as the first oral HBV agent to come to market—a drug already approved and priced for treating HIV—pricing of HBV drugs has followed the HIV model, with the exception that there is no public assistance program for people with chronic HBV who cannot afford the drugs. The lack of an HBV drug reimbursement program (like the AIDS Drug Assistance Program for HIV) in the United States, and restrictions of private insurance, which can deny payment for treatment of preexisting conditions, will limit many people's ability to access these new and expensive medications. Fortunately, generic versions of both lamivudine and tenofovir are becoming increasingly available for low-income countries—a benefit of the global HIV treatment scale-up effort. Advocacy is needed in order for these countries to prioritize HBV treatment, particularly in parts of Asia and Africa where HBV is endemic.

The Scope of the Problem

HBV is a global public health crisis. An estimated 400 million people worldwide are chronically infected, and each year one million people die from HBV-associated liver disease. There are upwards of two million Americans with chronic HBV, according to a recent estimate. If left untreated, about 25% of people with chronic HBV will progress to serious liver disease, including cirrhosis and liver cancer.

HBV is vaccine preventable, and universal vaccination programs have reduced childhood transmission of HBV significantly. But due to the high cost of the vaccine, low-income countries in sub-Saharan Africa, the Indian subcontinent, and Eastern Europe have yet to institute national vaccination programs.

HIV/HBV coinfection is common, since both viruses share blood-borne routes of transmission. Approximately 10% of HIV-positive people are coinfecting with hepatitis B. Coinfected people tend to have higher HBV viral loads and faster HBV disease progression, and have an increased risk for serious liver disease—especially those with low CD4 counts. Given that the majority of HBV drugs are also active

against HIV, the risk of developing both HIV and HBV drug resistance complicates treatment strategies in coinfection. Incidents of HBV reactivation have occurred when people stop or change their HIV regimen, leading to dangerous liver enzyme “flares” that can be fatal. Immune restoration from HIV therapy can also cause liver damage if HBV is not fully suppressed with treatment. The lack of access in developing countries to HIV treatment that includes an effective second-line HBV drug could have serious public health ramifications, as demonstrated by a recently identified HBV mutation strain with the potential to elude HBV screening and to compromise vaccine efficacy.

Recent studies are investigating the use of combination therapy for chronic HBV, following the successful approach in HIV treatment. However, results from several small trials to date have not provided convincing evidence of improved efficacy with combination therapy versus potent monotherapy with a drug with a high resistance barrier, although using more than one drug may delay development of drug resistance to less potent agents. Currently, combination therapy is only recommended by treatment guidelines for people who have developed resistance with monotherapy, and for people at higher risk of developing resistance, such as people with cirrhosis or HIV coinfection.

A few large-scale and long-term combination drug trials are currently underway, with data expected in about four years. In the meantime, some providers are arguing for the use of combination therapy in treatment-naïve people to forestall drug resistance, given HBV’s slow disease progression (measured in decades) and the scarcity of new experimental agents. To demonstrate an advantage over monotherapy and justify the added expense and potential toxicity, combination strategies would need to show improved viral suppression, higher rates of viral clearance, and the potential for shorter duration of treatment. It is clear that the development of new drugs targeting different stages in the HBV viral life cycle will be necessary if higher eradication rates are to be achieved.

Chronic HBV disease manifests in two main pathways distinguished by the presence or absence of HBV e-antigen (HBeAg), a viral protein that can be detected in blood. HBV disease progression and response to treatment differs between people who are HBeAg-positive and those who are HBeAg-negative, as measured by normalization of liver enzyme levels, reductions in viral load (HBV DNA) to undetectable levels, and by the loss of HBV surface antigen (HBsAg), a marker of viral clearance. People who are HBeAg-negative generally have lower viral loads and respond better to treatment, but will experience viral rebound faster after treatment is halted, and are more likely to progress to serious liver disease than people who are HBeAg-positive.

Experimental Oral Agents

Tenofovir and Emtricitabine

Tenofovir is a nucleotide analogue that was approved to treat HIV in 2001 and was recently approved in Europe to treat HBV. Gilead, the maker of tenofovir, is expecting U.S. approval for HBV treatment in August 2008. In two randomized trials, tenofovir demonstrated superior efficacy to adefovir (another Gilead HBV drug) in both HBeAg-positive and -negative, largely treatment-naive people. Complete response—defined as undetectable viral load and improved liver function—was reported in 66.5% of HBeAg-positive trial participants at 48 weeks versus 12.2% of those taking adefovir. Response rates in people with HBeAg-negative disease were higher overall, with 70.8% in the tenofovir arm achieving a complete response versus 48.8% in the adefovir treatment arm.

Table 2: HBV Experimental Agents in the Pipeline

Agent	Class	Sponsor	Status
Tenofovir	Nucleotide	Gilead	Phase III
Emtricitabine (in coformulation with tenofovir)	Nucleoside	Gilead	Phase II
Clevudine	Nucleoside	Pharmasset, Bukwang	Phase III
LB80380	Nucleotide	LG Life Sciences	Suspended
Nitazoxanide	Antiprotozoal	Romark	Phase II
YIC (Yeast-derived immunogenic complex) of HB surface antigen and antibody	Therapeutic vaccine	Beijing Vaccine Institute and Shanghai Medical College, Fudan University	Phase IIb
HBV core antigen vaccine	Therapeutic vaccine	Emergent Biosolutions	Phase II
DNA vaccine pCMV52.S	Therapeutic vaccine	French National Agency for Research on AIDS and Viral Hepatitis	Phase I/II

After 48 weeks, all participants were offered tenofovir. At 72 weeks, around 90% in the HBeAg-negative group had undetectable viral load and about 80% had normal liver function tests. This was compared with an 80% complete response rate in the HBeAg positive group. People who switched from adefovir to tenofovir had a lower rate of normalized liver function (61%). Notably, 5% of treatment responders in the tenofovir arm were able to reach viral clearance, compared to none in people who switched from adefovir to tenofovir. Tenofovir was well tolerated, with the most common side effects including headache, diarrhea, vomiting, abdominal pain, nausea,

abdominal distension, flatulence, ALT increase, and fatigue. Kidney toxicity, a known tenofovir-associated side effect, was not observed in these studies.

Tenofovir and entecavir are currently the most effective HBV drugs for treatment-naive people. Due to cross-resistance between entecavir and lamivudine tenofovir is more effective than entecavir for treatment-experienced people with lamivudine-resistant HBV. Although no tenofovir-specific resistant mutations have been identified to date, there appears to be cross-resistance with adefovir. One European retrospective analysis found that 85% of treatment-experienced patients had undetectable HBV viral load after a year of treatment with tenofovir, but only 30% of those with adefovir resistance became undetectable. While it was widely known that tenofovir was a more potent agent than adefovir—partly due to a difference in dosing because of drug toxicity (10mg for adefovir and 300mg for tenofovir)—Gilead's decision to develop adefovir ahead of tenofovir by six years appears to be unfortunate in hindsight, especially for people who have since developed adefovir-resistant HBV.

Emtricitabine, a nucleoside analogue (currently coformulated with tenofovir as Truvada for HIV treatment), is in phase II trials. It is being studied as part of combination therapy for HBV in people who have detectable viral load on lamivudine or adefovir, and is being compared with tenofovir monotherapy. Early data from week 48 showed there was no difference in response rates: 81% of all participants had an undetectable HBV viral load. In a previous phase III monotherapy study, emtricitabine showed similar efficacy as lamivudine, and a shared weak resistance profile—not surprising given the chemical similarity between these two drugs. Trials in HIV/HBV coinfecting people, and people with decompensated liver cirrhosis are currently underway.

Clevudine

A nucleoside analogue approved in South Korea since 2006, clevudine is currently in phase III clinical trials. It is being compared to adefovir in treatment-naive people. Enrollment is expected to be complete by the end of 2008, and Pharmasset is expected to file for FDA approval around 2010. Data from two previous 24-week placebo-controlled trials showed 59% reaching undetectable viral loads, and 68% with normalization of liver function in HBeAg-positive people. Response rates were higher among HBeAg-negative people; 92% had an undetectable viral load, and 75% had normalized liver function.

Notably, a small and short-term follow-up study demonstrated potential sustained antiviral properties of clevudine posttreatment. Another preclinical study in woodchucks (the animal model of choice for HBV) showed a slower than usual viral rebound post treatment, suggesting an immunomodulatory effect not seen with other oral agents. To conclusively determine this possibility, Pharmasset will conduct

96-week follow-up studies with volunteers from their 48-week phase III registration trials to see if people who achieved a complete response will be able to sustain it six months after stopping 72 weeks of treatment with clevudine. If these trials show positive results, clevudine could potentially shorten treatment duration for some people in the future.

LB80380

This nucleoside analogue is being developed by LG Life Sciences, based in South Korea. According to the company, a phase IIb trial is in the works. LG lost its development partner, Anandys, in 2007 and is currently looking for another partner for development in the United States and Europe. In a phase IIa dose-finding trial, lamivudine-resistant participants were able to reduce their viral load by about 4.0 logs. No serious toxicities were reported during 12 weeks of dosing. Another in vitro study showed LB80380 to be active against multiple HBV mutations that are resistant to drugs currently on the market. It remains to be seen if this potency will hold up clinically, providing a second- or third-line therapy option. Unless LG is successful in securing a new partner soon, and phase III trials return with positive results, this compound will be unlikely to reach market before 2013.

Nitazoxanide

This FDA-approved antiprotozoal agent, used to treat intestinal parasites, is also active against hepatitis B and C. Romark is sponsoring a phase II study comparing nitazoxanide alone versus entecavir versus both drugs in combination. Nitazoxanide caused a stir in 2007 when a phase II hepatitis C trial showed that adding it to pegylated interferon and ribavirin improved treatment responses. Since this is the only oral agent in development that is not a nucleoside/nucleotide, nitazoxanide could be another potential weapon against HBV drug-resistant mutations.

And Two More

Finally, there are two new approaches in early development that are sparking interest. NUC B1000, an RNA interference-based gene therapy designed to destroy HBV RNA inside infected liver cells is in a phase I trial. The sponsor, Nucleonic, faced an earlier setback when a patient died in a different gene therapy trial using the same delivery viral vector, which prompted the FDA to put a halt on similar trials. The study was allowed to move forward following another FDA review and additional patient consent procedures.

Bayer's Bay 41-4109 is a heteroaryldihydropyrimidine (HAP) that inhibits HBV assembly by interrupting viral capsid formation. Preclinical in vitro and animal studies have demonstrated this compound's ability to inhibit viral replication. Hopefully these two compounds will advance successfully, providing additional treatment options for people with chronic HBV in the future.

Therapeutic Vaccines

The hepatitis B virus itself is not liver-toxic; instead, liver damage is caused by the body's own immune system killing HBV-infected liver cells. Since there is already an effective HBV vaccine, and up to 95% of healthy adults can clear HBV in acute infection without treatment, an immune-based approach to treatment would seem to stand a good chance of becoming a cure. The following three approaches are farthest along in development.

Results from a phase IIb study of a recombinant HB surface antigen (HBsAG) and surface antibody (HBIG) therapeutic vaccine from China have been published. The study was done in HBeAg-positive people, using HBeAg seroconversion to negative with detectable HBe antibodies and viral suppression (2.0 log drop in HBV DNA) as endpoints. In this 237-person, three-arm, placebo-controlled trial, a series of six monthly injections were given at two different doses. The results did not reach statistical significance, due to a high response rate in the placebo arm (which contained a vaccine adjuvant aluminum salt known to have some effect on B cell immune activation). Delayed responses (at 44 weeks) were reported in the high-dose arm, with 21.8% achieving HBeAg seroconversion to negative versus 7.7% in the placebo arm, and better viral suppression (37.2% vs. 26.9%). There were 11 hospitalizations due to serious adverse events, defined by the study protocol as increases in ALT (greater than ten times the upper limit of normal) and abnormally elevated bilirubin. These were equally distributed across the three study arms, and all participants recovered. The vaccine is expected to move into phase III with a larger cohort and perhaps a longer treatment period in hopes of getting cleaner results and improved response rates. Though the Chinese researchers are proposing this therapeutic vaccine approach as an alternative to expensive interferon and antiviral therapy, they did not provide data on participant's viral load outcome. Given the clear connection between viral load and the risks of developing liver cancer and cirrhosis shown in a recent observational study, the long-term clinical benefits of this approach are unclear.

Emergent Biosolutions is expanding a phase II study in Europe with an HBV core antigen vaccine after a safety monitoring committee gave it a green light early in 2008. The vaccine is administered in a drinkable form with an HBV core antigen gene inserted in live attenuated *Salmonella* bacteria via recombinant technology. Once inside the body, the salmonella bacteria produces the HBV core antigen within gut macrophages, which induces antigen-specific T cells directed to kill HBV-infected liver cells. The company plans to launch phase III trials in the United States if the vaccine proves to be effective.

The French National Agency for Research on AIDS and Viral Hepatitis is conducting a phase I/II study with a naked DNA vaccine in combination with

antiviral therapy to see if the vaccine can activate the body's immune system to improve treatment response while on therapy—or to delay viral reactivation once off treatment. The vaccine is administered in a series of five injections, with the first three given in 8-week intervals at the start of the 48-week trial, and the last two given 4 weeks apart at the end. A phase I study has shown that the vaccine is safe and can temporarily induce immune responses in people not responding to therapy. The current study is being conducted in France and began enrollment in 2008, with completion expected in 2010.

There are two additional DNA therapeutic vaccines in phase I being studied in Asia. One, being developed by Pfizer and PowderMed, is delivered via a needle-free injection of the vaccine in powder form through the skin. The other vaccine is in development by Genexine, and is being administered in combination with adefovir. Finally, SciClone is conducting a phase IV trial in Korea using thymosin alpha 1 in combination with pegylated interferon for three months, followed by nine months of pegylated interferon alone. Thymosin alpha 1, an immunomodulator, is a synthetic version of a substance that is produced naturally by the thymus. It has been approved in over 30 countries to treat HBV. Development plans for the United States are unclear.

In developed countries, universal HBV vaccination programs have significantly reduced HBV infections over the last 20 years. Newly developed potent oral agents have improved treatment responses, providing further benefit for chronically infected people who can access these expensive drugs. However, without an increase in public investment into basic research, a cure for HBV remains beyond reach. Low-income countries still need help to pay for childhood vaccine programs that have benefited so many in wealthier nations. New therapeutic approaches are needed to stem the tide of drug-resistant HBV. Advocacy efforts can help bring about these needed improvements, as exemplified by the call for a national strategy coming from the activist group, ACT-HBV in Australia. More attention from the World Health Organization toward hepatitis will also aid in this fight.

The Big Picture for Hepatitis C Treatment

Still Nasty after All These Years

BY TRACY SWAN

Despite a bulging pipeline of potential treatments for hepatitis C virus (HCV), the long-heralded shift in the treatment paradigm is still years away. Until new treatments are approved, each new drug candidate must be studied in combination with the current standard of care (SOC), pegylated interferon and ribavirin. Interferon may remain in the mix even after combinations of hepatitis C antiviral agents are available, since it is not clear if antiviral therapy without immune stimulation will be sufficient to eradicate HCV. Given emerging concerns with the new candidates—toxicity, drug-to-drug interactions, resistance, and poor adherence—it is especially disappointing to remain saddled with the same old SOC.

Fortunately, the hepatitis C virus offers drug makers a wide range of potential targets. Antiviral candidates from two classes—protease inhibitors and polymerase inhibitors—are farthest along, followed by a host of agents that block other steps of HCV replication, such as entry, assembly, and maturation. In the meantime, it is reasonable to hope that adding one of these new drugs to the SOC will at least boost response rates and shorten the course of HCV treatment, particularly for people with favorable prognostic factors.

Background

The World Health Organization (WHO) describes hepatitis C as a “viral time bomb.” Globally, 120–170 million people have been infected with HCV; the majority will develop chronic hepatitis C. At least 20% of chronically infected people—if untreated or unsuccessfully treated—will eventually progress to cirrhosis. People with cirrhosis are at risk for liver cancer and liver failure. According to WHO estimates, up to 75% of liver cancer and approximately 65% of liver transplants occurring in the developed world are attributable to chronic HCV infection. The incidence of HCV-related morbidity and mortality is expected to increase sharply by 2020.

Hepatitis C is treatable—and can be eradicated—regardless of HIV status, but the current standard of care has significant limitations in tolerability and efficacy. Hepatitis C is treated for up to 72 weeks with daily ribavirin (pills or capsules) and once-weekly injections of pegylated interferon. These drugs can cause laboratory abnormalities (low platelet counts, anemia, and low white blood cell counts) mild to serious neuropsychiatric side effects, and a constellation of flulike symptoms. Some side effects are treatment limiting unless managed with high-priced adjunctive therapies.

Approximately 50% of those who endure HCV treatment have a sustained virological response (SVR), meaning that no hepatitis C virus is detectable in the bloodstream six months after completion of treatment. SVR—generally regarded as a cure—is also associated with reductions in hepatitis C morbidity and mortality.

HCV treatment uptake in the United States and Europe is low, and no significant increase is expected until combinations of more effective, less toxic drugs become available—particularly for people unlikely to respond to, or unable to tolerate, current HCV treatment. According to Data Monitor, sales of pegylated interferon and ribavirin reached 2.5 billion dollars in the United States and Europe in 2006. Sales are projected to increase by a paltry 1.5 billion dollars by 2010, reflecting limitations of the current treatment. Access to HCV treatment in most low- and middle-income countries is virtually nonexistent.

HIV/HCV Coinfection

Worldwide, an estimated 4–5 million people are coinfecting with HIV and hepatitis C. Hepatitis C is more aggressive in HIV-positive people, with cirrhosis sometimes developing within a decade of infection. HCV treatment is also less effective and its side effects may be more debilitating for HIV-positive people than their HIV-negative counterparts.

End-stage liver disease from HCV coinfection has become the leading cause of non-AIDS-related mortality in countries where antiretroviral therapy (ART) is widely available. It is a sad irony that we have dramatically altered the prognosis for HIV—a currently incurable disease—only to see coinfecting people dying from complications of hepatitis C, a disease that we can cure.

An Urgent, Unmet Need

Better HCV treatment is critical for individuals with poor prognostic factors, such as HCV genotype 1 (predominant among mono- and coinfecting people in the United States and Europe), high pretreatment hepatitis C viral load, advanced liver disease, HIV/HCV coinfection, African Americans, and transplant candidates and recipients. A growing population of treatment-experienced patients will require more effective therapy, or at least something to stave off liver disease progression.

Unfortunately, registration trials for HCV therapies continue to enroll from a rapidly dwindling pool of people with hepatitis C who are otherwise in flawless physical and mental health. These trials are likely to show drugs in the most favorable light, and their results may not be relevant for high-prevalence populations with multiple comorbidities. For example, in the United States, most people with chronic hepatitis C are 40 to 59 years old. Many have other chronic health problems, family responsibilities, and demanding jobs that make it difficult or impossible for them to tolerate SOC, resulting in low rates of treatment uptake and completion.

HCV Drug Development: Research and Treatment Issues

The remarkable successes, complexities, and challenges in HIV research and clinical care offer valuable lessons for HCV. Researchers have much to gain by working more closely with activists and community members. Many bring an often overlooked, practical perspective to areas ranging from drug development to care and treatment delivery systems.

- Trials need to identify therapeutic strategies, as well as approval for a single agent.
- As with HIV, a combination of drugs targeting different steps in the HCV life cycle are needed to forestall resistance. Cross-company collaboration on multiagent trials is needed to advance the field.
- Retreating nonresponders with an identical regimen yields abysmal SVR rates. Future nonresponder studies should offer more than one novel agent, preferably in a factorial design. Until combinations of drugs are available, retreatment trials must be designed so that participants—especially those in the control arm—have the best possible chance for SVR.
- Resistance to HCV protease and polymerase inhibitors develops rapidly. The first generation of HCV protease inhibitors must be taken three times a day, a dosing schedule associated with poor adherence. Proven methods to support adherence should be incorporated into clinical trials and clinical practice.
- Therapeutic advances must be accompanied by health care delivery systems suited to the needs of multiply diagnosed persons. These systems need to be created now to meet current needs and in anticipation of future improvements in HCV treatment.
- It is likely that the standard of care for HCV will become somewhat of a moving target in the coming years. Convening an expert, cross-disciplinary panel to develop and update HCV treatment guidelines will optimize treatment and avert therapeutic chaos.

There are practical and financial reasons for sponsors to conduct registration trials in “real-life” populations. Trials with less restrictive eligibility criteria enroll faster. Regulators recognize the importance of enrolling a sampling of the likely patient population, as do health care payors; a broader indication facilitates drug sales, since access to off-label use of drugs is increasingly limited.

Access

Hepatitis C is a disease that reflects and magnifies global social and economic inequality. Advances in HCV treatment must be available to all who need them, since a majority of people with hepatitis C live in poverty. Limited—or the complete lack of—access to HCV prevention, care, and treatment render new therapeutic improvements virtually useless to millions of people with chronic hepatitis C. It is time to develop a reasonable, high-volume, low-profit global pricing framework for HCV drugs and diagnostics.

Desirable Elements

Ideally, HCV treatment will become more effective, far less toxic, and easier to administer. In the future we can hope for a combination of oral antivirals, preferably coformulated into one pill. In the meantime, therapies to reverse or delay fibrosis progression and tolerable, potent drugs that will cure more people and abbreviate treatment duration will be a welcomed advance.

“Drugable” Targets: HCV Polymerase and Protease Enzymes

As with HIV, hepatitis C treatment requires a combination of drugs that target different steps in the replication cycle. Two enzymes, the NS3-4A serine protease enzyme and the NS5B RNA dependent RNA polymerase enzyme, are primary targets for oral antiviral agents, since both are essential for HCV replication. Unfortunately, HCV’s high replication rate—billions of copies per day—quickly leads to drug resistance, and mutations in HCV’s protease and polymerase domains have already been characterized, both in vitro and in vivo.

The consequences of HCV drug resistance are unknown; it may be a transient phenomenon, since HCV does not integrate into the host cell’s genome. But it is possible that mutations will confer resistance to an agent—or an entire class of agents. Studies of treatment efficacy in people who have developed HCV drug resistance (such as volunteers in phase I monotherapy trials) are needed to help understand clinical implications of HCV drug resistance.

HCV Protease Inhibitors

BILN-2061, a pioneering HCV protease inhibitor from Boehringer Ingelheim, was discontinued due to cardiotoxicity in animals, but not before it demonstrated that HCV protease inhibition was a sound concept. Since then, two candidates—Schering’s boceprevir and Vertex/Tibotec’s telaprevir—have made it to phase III. Both were designed to be active against HCV genotype 1 (telaprevir is being studied in people with genotypes 2, 3, and 4 in trials outside of the United States). So far, major shortcomings associated with hepatitis C protease inhibitors are resistance (which develops quickly), dosing (both drugs must be taken three times a day), and anemia, which seems to be a classwide side effect.

Additive toxicity is a problem, since the SOC treatment is already so difficult to tolerate. But phase II studies have made it abundantly clear that ribavirin is needed to maximize SVR, minimize viral breakthrough, and avoid posttreatment relapse.

Schering and Vertex are using strategic trial designs that accommodate the strengths and weaknesses of each drug. Currently, telaprevir is thought to be more potent—and more toxic—than boceprevir.

Boceprevir

In its phase III studies, Schering is using a four-week lead-in with SOC to drive down HCV RNA before adding boceprevir to the mix. The company believes that this approach will maximize the chance of successful treatment by identifying people most likely to respond to interferon-based therapy. However, the lead-in strategy made no difference in response rates among treatment-naïve people in an earlier study. This strategy may foster resistance, since Schering is initiating boceprevir before week 4 viral load results are available, and people who don’t respond to backbone therapy will receive virtual monotherapy for a few weeks. Unfortunately, week 4 results will be analyzed retrospectively, instead of guiding the duration of therapy; week 8 response rates will inform treatment duration, which ranges from 28 to 48 weeks.

Schering’s RESPOND-2 trial will evaluate 36 and 48 weeks of triple therapy versus SOC in treatment-experienced persons. Results from a phase II trial in treatment-experienced people were disappointing (only 7% to 14% achieved SVR with boceprevir-based therapy) and confusing; a hodgepodge of regimens, doses, and treatment durations made interpretation difficult.

Headache, nausea, fatigue, and anemia—but no rash—have been the most commonly reported adverse events associated with boceprevir.

Telaprevir

The cornerstone of Vertex's development program has been to minimize exposure to telaprevir, and to abbreviate the subsequent course of pegylated interferon and ribavirin due to toxicity (primarily telaprevir-associated rash, severe enough in some cases to warrant hospitalization and/or discontinuation of treatment). Vertex has used a 12-week lead-in with telaprevir plus SOC in their treatment-naïve studies and up to 24 weeks of triple combination therapy in PROVE 3, their treatment-experienced trial. ADVANCE, Vertex's phase III study, is assessing an 8- and 12-week triple combination lead-in followed by different durations of SOC.

PROVE 1 and PROVE 2 (phase II studies of telaprevir in treatment-naïve people) reported impressive efficacy, particularly given the shorter treatment duration. SVR ranged from 61% to 68% after 24 weeks of treatment (12 weeks of telaprevir plus SOC, followed by 12 weeks of SOC). However, real-life results may be far less impressive, since PROVE 1 used early treatment response to guide duration. This approach weeded out people who were less likely to achieve SVR with 24 weeks of treatment. In addition, most participants in these trials had favorable prognostic factors (minimal liver fibrosis, low body mass index, a high proportion (~40%) of female participants, and an almost exclusively Caucasian population (93–99%).

Interim results from PROVE 3 (a phase II study of telaprevir-based therapy in treatment-experienced people) are exciting. SVR-12 (undetectable HCV RNA 12 weeks after completion of treatment, usually indicates SVR) occurred in 41% of people who did not respond to previous HCV treatment, after only 24 weeks of treatment (12 weeks of triple-combination therapy, followed by 12 weeks of SOC). Vertex is planning to launch a phase III study in treatment-experienced persons.

Vertex is expected to file for a treatment-naïve indication by 2011, but if the final results from PROVE 3 reflect a significant breakthrough in retreatment response rates, the company may pursue approval in treatment experienced patients before completion of their phase III program.

TMC 435350

Tibotec and Medivir are codeveloping TMC 435350, which has entered phase II. In Europe, TMC 435350 is being studied in treatment-naïve and treatment-experienced people with hepatitis C genotype 1. A 7-day TMC 435350 or placebo lead-in, with or without SOC, is followed by 21 days of triple therapy. Participants will subsequently receive 24 or 48 weeks of SOC, according to early treatment response.

HCV protease inhibitors from Bristol-Myers Squibb (number unreleased, per company policy), Boehringer Ingelheim (BI 12202), Intermune/Roche (ITMN-191) Merck (MK7009), Tibotec/Medivir (TMC 455350), and Vertex (VX-500) are

in phase I. Abbott/Enanta, Gilead/Achillion, and Vertex each have candidates in preclinical development.

HCV Polymerase Inhibitors

HCV polymerase inhibitors hold great promise, but the class has an Achilles' heel: toxicity, which has already led to the discontinuation of more than one candidate. There are two distinct classes of hepatitis C polymerase inhibitors—nucleosides and nonnucleosides, and each inhibits HCV replication by a different mechanism. Nucleosides are RNA chain terminators, while nonnucleosides cause conformational changes that disable the polymerase enzyme. The nucleoside polymerase inhibitors may be active across HCV genotypes, and resistance to this subclass of polymerase inhibitors is less likely to develop (versus nonnucleosides).

Nonnucleoside polymerase inhibitor candidates from GlaxoSmithKline (GSK625433) and Pfizer (PF-00868554) have entered phase I, and Gilead is resuming development of its nonnucleoside GS9190 after resolving a cardiac safety issue that emerged during phase I. Nucleoside polymerase inhibitors are being codeveloped by Roche/Pharmasset (R7128) and Merck/Isis (MK 0608); these are also in phase I. A host of candidates from Anadys, Arrow, Biocryst, Enanta, Genelabs, Gilead, GlaxoSmithKline, Idenix, Inhibitex, Medivir/Roche, Merck/Metabasis, Migenix, Phenomix, Pfizer, and Tibotec are in preclinical development.

R1626

Roche's HCV nucleoside polymerase inhibitor, R1626, is currently being studied in people with HCV genotype 1. Although the drug seems quite effective, a serious safety issue emerged during the phase IIa study: grade 4 (severe, or life-threatening) neutropenia (an abnormally low count of bacteria-fighting white blood cells called neutrophils). In its ongoing phase IIb study, Roche is exploring lower doses of R1626 with full- or half-dose pegylated interferon plus weight-based ribavirin, hoping to identify a safe and effective regimen.

VCH-759

VCH-759 has been studied in treatment-naïve people with HCV genotype 1. So far the drug appears effective (HCV RNA decreased by more than 1 log regardless of dose, and by more than 2 logs in people who received 800mg two or three times per day). There were no serious adverse events or discontinuations during this ten-day study; the most commonly reported side effects were gastrointestinal in nature.

Other Antiviral Agents

Two antiviral candidates, Arrow's A-831, an internal ribosomal entry site (IRES) inhibitor, and Migenix's celgosivir, an alpha glucosidase inhibitor, have entered phase II.

Cyclophilin B Inhibitors

Cyclophilins bind to cellular proteins that regulate the immune system. They are used to prevent posttransplantation organ rejection, but not all cyclophilin inhibitors have immunosuppressive activity. So far an oral candidate, DEBIO-025, has entered phase II. Laboratory abnormalities (elevated bilirubin and low platelets) were reported; an ongoing phase II trial in treatment experienced-persons with HCV genotype 1 is exploring lower doses.

Antiprotozoal Agents

Nitazoxanide: The Upstart

Avid followers of HCV-specific antiviral agents were surprised by results from a phase II study of nitazoxanide (Alinia), an oral agent approved by FDA in 2002, to treat diarrhea from two intestinal parasites (*Cryptosporidium parvum* and *Giardia lamblia*).

So far, nitazoxanide has been studied in treatment-naïve and treatment-experienced people with hepatitis C genotype 4. Unfortunately, a small sample size, as well as incomplete information on study population (liver histology, baseline viral load, body mass index), adverse events and side effects, and SVR, make it difficult to fully and accurately evaluate these results. A pending trial in the United States, STEALTHC-3, will evaluate a 4-week nitazoxanide lead-in, followed by 48 weeks of triple therapy versus SOC plus placebo in treatment-naïve people with HCV genotype 1. Hopefully, STEALTH-2 will address gaps in data.

Antifibrotics

HCV drug development has focused on viral eradication rather than treating liver disease itself. The aim of antifibrotic agents is to delay or prevent additional liver scarring, an especially useful approach for people who cannot tolerate the current standard of care.

Caspase Inhibitors

GS 9450 and PF-03491390 are caspase inhibitors, which block a crucial step in programmed cell death (called apoptosis), which occurs at higher than normal levels in liver cells of people with chronic hepatitis C and other liver diseases. Inhibiting caspase may lead to decreased fibrosis. Two candidates, Gilead's GS 9450 and Pfizer's PF-03491390, have entered phase II.

MitoQ

MitoQ protects mitochondria (cellular structures that produce energy). A phase II study was launched in February 2007 in New Zealand.

Farglitazar

Farglitazar is a peroxisome proliferator-activated receptor (PPAR) gamma agonist. PPARs control inflammatory responses. An ongoing phase II study in treatment-experienced people is evaluating the antifibrotic activity of Farglitazar.

Ribavirin Substitutes

Taribavirin

Taribavirin, a ribavirin pro-drug formerly known as viramidine, is less anemia-inducing than ribavirin. It went back to the drawing board after disappointing efficacy results from phase III studies. An ongoing phase II study is evaluating the safety and efficacy of weight-based viramidine (versus ribavirin) plus peginterferon alfa-2b in treatment-naïve people with HCV genotype 1 infection.

Immunomodulators

Novel Interferon Formulations

Companies have been working on ways to optimize interferon, since it will likely remain the backbone of HCV treatment for several years. At the least, these offer more convenient dosing; they may also be less toxic.

Albuferon

Albuferon is a formulation of interferon alfa-2b, fused to human albumin, to allow long-term exposure from a single infusion (every two weeks). Phase II results indicate that efficacy is comparable—but not superior—to SOC, though dropout rates were higher in the albuferon arms.

Two related phase III albuferon studies in treatment-naïve people with HCV genotypes 1, 2, and 3 are underway. There has been a dose modification (from 1200µg to 900µg) due to serious pulmonary adverse events.

Albuferon has also been studied in treatment-experienced people with HCV genotype 1; the highest SVR rate (30%) was reported in people given the 900 µg dose with ribavirin.

Locteron-interferon

Locteron-interferon is a continuously released formulation of interferon alfa-2b, currently in phase IIa. Early results are promising, despite a single, unspecified serious adverse event. Mild weakness, joint and muscle pain, and headache were common. More data will be presented at the end of 2008.

Omega Interferon

A lackluster 37% SVR rate from a phase II study has not dissuaded the sponsor, Intarcia, from initiating another phase II study of this compound in treatment-experienced people with HCV genotype 1. They will receive ribavirin plus Omega interferon delivered with a novel twist: the DUROS, an implantable mini-pump.

Therapeutic Vaccines

ChronVac-C

Safety and tolerability of ChronVac-C, a DNA-based therapeutic vaccine, are being studied in treatment-naïve people with HCV genotype 1 and a low hepatitis C viral load. ChronVac-C is administered with a brief electrical pulse (a process called electroporation) that creates temporary pores in cell membranes that allow the vaccine to enter cells. Data are expected in 2009.

GI-5005

GI-5005 is a yeast-based vector expressing hepatitis C NS3 and core proteins, intended to stimulate the immune system to clear HCV-infected cells. An ongoing phase II study of GI-5005 is being conducted in 120 people with HCV genotype 1 who are treatment naïve or nonresponders to previous therapy, comparing 48 weeks of GI-5005 plus SOC versus 48 weeks of SOC.

IC41

IC41 uses synthetic T-cell peptides with a polyarginine adjuvant to prime the immune response. Phase II results were encouraging; IC41 significantly reduced hepatitis C viral load in treatment naïve people with HCV genotype 1. Intercell and Novartis are planning further studies of IC41.

Conclusion

Although interferon- and/or ribavirin-free regimens are years away, shorter-course, more effective HCV treatment is on the horizon. Proof of concept has been established for hepatitis C polymerase and protease inhibitors. Backup compounds and novel approaches to inhibit HCV replication continue to move into clinical development. Fixed-dose combinations of oral antivirals may be available in the future.

In the meantime, there is a precious opportunity to identify optimal treatment strategies during HCV drug development through well-designed clinical trials. Collaboration among hepatitis C and HIV community members, researchers, regulators, and the pharmaceutical industry is necessary for swift and strategic drug development.

Table 1: Experimental HCV Agents in the Pipeline

Class/type	Drug name	Sponsor	Status
Antifibrotic	Farglitazar	GlaxoSmithKline	Phase II
	GS9450	Gilead	Phase II
	MitoQ	Antipodean	Phase II
	Pfizer	PF-3491390	Phase II
Antiprotozoal	Nitazoxanide	Romark	Phase II
Antiviral	A-831	Arrow Therapeutics/Astra Zeneca	Phase II
	MX-3253 (Celgosivir)	Migenix	Phase II
Cyclophillin inhibitor	DEB10-25	Debiopharm	Phase II
Monoclonal antibodies	Civacir	Nabi Biopharmaceuticals/Kedrion	Phase II
Novel interferon formulations	Albupheron	Human Genome Sciences/Novartis	Phase III
	Locteron	Biolex Therapeutics/OctoPlus N.V.	Phase IIa
	Omega Interferon	Intarcia	Phase II
Protease inhibitor	Boceprevir	Schering Plough	Phase III
	Telaprevir	Vertex/Tibotec	Phase III
	TMC435350	Tibotec/Medivir	Phase II
Polymerase inhibitor	R1626	Roche	Phase II
	VCH-759	Virochem	Phase II
Ribavirin substitute	Taribivirin	Valeant	Phase II
Therapeutic vaccine	ChronVac-C	Karolinska Institute/Tripep/Inovio	Phase I/II
	GI- 5005	Globe Immune	Phase II
	IC41	Intercell/Novartis	Phase II

Tuberculosis Treatments

BY CLAIRE WINGFIELD

Table 1: Anti-TB Drugs

Grouping	Drugs (Abbreviation)
Group 1 – First-line oral anti-TB agents	Isoniazid (H or INH); Rifampicin (R or RIF); Ethambutol (E or EMB); Pyrazinamide (Z or PZA)
Group 2 – Injectable anti-TB agents	Streptomycin (S or SMP); Kanamycin (Km); Amikacin (Am); Capreomycin (Cm); Viomycin (Vi)
Group 3 – Fluoroquinolones	Ciprofloxacin (Cfx); Ofloxacin (Ofx); Levofloxacin (Lfx); Moxifloxacin (Mfx or moxi); Gatifloxacin (Gfx or gati)
Group 4 – Oral bacteriostatic second-line anti-TB agents	Ethionamide (Eto); Protonamide (Pto); Cycloserine (Cs); Terizidone (Trd); P-aminosalicylic acid (PAS); Thioacetazone (Th)
Group 5 – Anti-TB agents with unclear efficacy	Clofazimine (Cfz); Amoxicillin/Clavulanate (Amx/Clv); Clarithromycin (Clr); Linezolid (Lzd)

Source: Adapted from WHO Guidelines for the Programmatic Management Of Drug-Resistant Tuberculosis, 2006.

Mycobacterium tuberculosis (MTB) has been infecting humans for thousands of years, and even though TB is preventable and curable, millions of people die from TB disease each year. The last major breakthrough in TB treatment was the discovery over 40 years ago of the rifamycins—a class of drugs that is a cornerstone of current first-line regimens. TB drug development experienced its golden age between 1948 and 1963, when all of the most commonly used first-line anti-TB drugs were discovered, developed, and brought to the world market. Since that time TB treatment research has virtually ground to a halt.

While TB drug development slept, the HIV pandemic grew, setting off a public health nightmare as the two diseases fueled one another. Poorly administered TB programs exacerbated the disaster by failing to follow patients through to treatment completion, and this led to the uncontrolled spread of drug resistant TB throughout the world. The lack of new treatment combinations to cure TB now threatens the gains made by the wide-scale provision of HIV antiretroviral (ARV) treatment in the developing world. Millions of people—both HIV infected and uninfected, children and adults—risk dying from this curable disease.

The founding of the Global Alliance for TB Drug Development in 2000 and the reentry of pharmaceutical companies into TB drug discovery have improved the TB treatment research outlook. However, it is important to remember that as each potential new medicine moves through each phase of the drug pipeline, its chances

for successful approval diminish; therefore it is crucial to keep the pool of novel drug candidates full. Currently there are seven new drug candidates in the TB pipeline: two in phase I, three in phase II, and two in phase III clinical trials. While this is the richest TB treatment pipeline since the 1960s, it is far from sufficient to address the needs of the millions who are infected, those who are sick with TB and drug-resistant TB, and the 90 million who will develop TB disease in the coming decade.

Though some progress has been made moving new treatments closer to the market since TAG's 2007 pipeline report, the need for better drugs remains acute. The characteristics of the ideal drug candidate—one that could revolutionize the TB treatment world—would include:

- decreased duration of treatment
- decreased pill burden
- trouble-free dosing with antiretrovirals (ARVs)
- improved LTBI therapy
- efficacy against multidrug and extensively drug-resistant TB
- the ability to treat TB in infants and children

Improved TB treatment will require a combination of new drugs to make up effective and safe regimens to treat drug-susceptible and drug-resistant disease. They will include treatments that are bactericidal (killing actively reproducing TB organisms) and sterilizing (killing slow-growing persistent and latent TB bacilli); will work in children and adults, as well as in people with HIV infection; and will lack undesirable drug interactions with commonly used anti-HIV drugs. Optimally, effective new TB drugs will come from new drug classes to which MTB has not developed resistance.

Unfortunately, each of the new drugs in the current TB pipeline are associated with side effects—some of limited concern (mild rash), and some more serious (hepatotoxicity). A number of these drugs have interactions with other important medications, such as the metabolic interaction of rifamycins with several HIV antiretrovirals. Few of the current pipeline candidates have yet been tested in people taking anti-HIV medications—despite the fact TB is the leading killer of people living with HIV worldwide.

Children are also typically excluded from TB drug research. Infants and children, particularly those under the age of five and/or those who are HIV-positive, are more likely to develop TB disease immediately after being infected (primary TB disease), and are therefore at much greater risk for rapid disease progression and death than adults. Despite this urgent need, the current pediatric treatment strategy is to use weight-based doses of medications that have been primarily evaluated in adults. None of the current new drug candidates has been tested in children.

Latent TB Infection

Latent TB infection (LTBI) refers to the stage at which an individual has been exposed to and infected by the TB bacilli but his immune system has contained the organism and disease has not developed. Treatment for LTBI entails taking a 6- to 12-month regimen of one or two of the most commonly used first-line drugs. Despite its proven efficacy there is a reluctance to use the most studied TB preventive treatment strategy, isoniazid preventive therapy (IPT), in resource-limited settings because of concerns about drug resistance, missed diagnosis of subclinical active TB disease, and uncertainty about programmatic oversight and patient completion of preventive therapy. Research is underway to evaluate the efficacy, safety, tolerability, and efficiency of monotherapy or dual therapy for treating LTBI with the aim of increasing the uptake of this life saving treatment.

The Consortium to Respond Effectively to the AIDS-TB Epidemic (CREATE), based at the Johns Hopkins University with funding from the Bill and Melinda Gates Foundation, has two large-scale, cluster-randomized studies underway that are measuring the impact of widespread IPT on the incidence of TB disease in high-HIV-prevalence settings. The THRio study, which is being conducted in collaboration with the Health Secretariat of Rio de Janeiro, is designed to determine whether routine IPT can reduce the incidence of TB disease in persons receiving free access to ARVs and HIV care. The study will compare outcomes of patients treated at clinics trained on the use of IPT and tuberculin skin testing (TST) to those treated at clinics that have not received training. This is a phased implementation trial to ensure that eventually each of the 29 government HIV clinics in Rio de Janeiro participating in the study will receive training. An analysis of baseline clinic data suggests that IPT when used in conjunction with ARVs significantly reduces the incidence of TB as compared to either ARV or IPT alone.

The second CREATE trial, the Thibela TB study, is evaluating the impact of IPT on TB incidence in South African gold mines. Because TB and HIV prevalence among miners in South Africa is high, implementing mass IPT could have significant impact on the incidence of TB disease. The Aurum Research Institute in partnership with CREATE is enrolling 68,000 mine staff, including miners and those in administrative and executive positions. Study participants are being randomized by mine shaft site to receive either no intervention or mass IPT. The latter intervention involves screening all staff at the site for active TB disease and treating all those without active TB with IPT. It is expected that mass IPT will result in a significant reduction in TB incidence.

The Thibela and THRio trials are important, both for their size and their potential to increase the adoption and implementation of IPT in many countries, particularly those with high TB/HIV coinfection rates.

Though not on as large a scale as the CREATE studies, a number of other significant studies in phases III and IV are looking at TB preventive therapies among people living with HIV. The U.S. Centers for Disease Control and Prevention (CDC) and the Botswana Ministry of Health are evaluating the impact of continual IPT versus limited IPT (six months) on the incidence of TB disease among people with HIV in a phase IV open-label, randomized, controlled trial.

In the Khayelitsha district of Western Cape South Africa, where over half of the adults with TB disease are also HIV-positive, several academic institutions have partnered with Médecins sans Frontières (MSF) to evaluate initiating IPT among people with HIV without using TST or interferon-gamma release assay (IGRA) to screen for LTBI. This study is randomizing 1,270 people with HIV who are eligible for ARV therapy to be put on either 12 months of IPT or no IPT. Researchers will evaluate the impact of INH on TB incidence as well as on the rate of INH resistance among the populations. Study completion is expected in October 2011.

The TB Research Centre in India, with support from the U.S. Agency for International Development (USAID) is randomizing 650 people with HIV to receive either 6 months of ethambutol (EMB) plus INH or 36 months of INH alone. Study completion is expected in September 2008.

A number of clinical trials are being conducted to determine whether the duration of preventive therapy for TB can be shortened. The CDC-funded Tuberculosis Trials Consortium (TBTC), with sites throughout the United States as well as in Spain and Brazil, is conducting TBTC Study 26 to compare a new regimen of 12 weekly doses of rifapentine (RPT) plus INH with the standard regimen of nine months of INH. Rifapentine is a rifamycin that has a longer half-life than rifampin (RIF). As of January 2008, the trial had enrolled 8,000 participants, although too few people with HIV or children have been enrolled to determine the impact in these populations. In order to boost its numbers, TBTC is partnering with other research consortia to increase enrollment of these key populations. Additionally, several substudies have been added to evaluate pharmacokinetics (PK) of RPT in children (how children metabolize the drugs), hepatotoxicity (liver toxicity) and hypersensitivity reaction. The results of the main study are expected 33 months after the last enrollment, in approximately December 2010.

The National Institute for Allergy and Infectious Diseases (NIAID) is sponsoring two phase III treatment shortening trials for TB preventative regimens among people with HIV. Enrollment has been completed in a 1,148 person study in Soweto, South Africa, being conducted with the Johns Hopkins University, comparing three LTBI regimens against six months of INH among people with HIV with positive TSTs and no signs of active TB. Study participants are randomized to one of four

groups: (1) six months of self-administered daily INH; (2) directly observed therapy (DOT) with RPT/ INH once a week for 12 weeks; (3) DOT RIF/INH twice a week for 12 weeks; or (4) continuous self-administered INH for one to four years (depending on enrollment date). Study completion is expected in June 2009. In another study, 2,000 people with HIV from multiple sites in the United States have been randomized to receive either 2 months of RIF and pyrazinamide (PZA) or 12 months of INH plus vitamin B6.

While none of these studies are evaluating novel drug candidates for the treatment of LTBI, they are important because they are asking strategic questions about how to maximize the use of existing treatments for LTBI and increase its uptake. Unfortunately, despite the fact that IPT is endorsed and recommended by leading health organizations like the World Health Organization (WHO), the CDC, and the American Thoracic Society (ATS), it is not available in places where it is needed most. The aim of these studies is to support and encourage the development and implementation of LTBI treatment policies by national health programs.

Active TB Disease

New Treatment Strategies

Despite the fact that the TB drug pipeline is more robust than it has been in years, there are still far too few potential candidates to choose from. Therefore, studies are underway to find simpler and more efficient and effective ways of using the drugs that are currently available.

The TB Research Centre in India is expected to complete a study in September 2008 that compares the standard first-line treatment regimen of two months of INH/RIF/PZA/EMB, followed by four months of INH/RIF with two months of INH/RIF/PZA/EMB, followed by seven months of INH/RIF in 300 people with HIV with pulmonary and/or extrapulmonary TB.

With the aim of shortening the duration of the current treatment regimen, NIAID is sponsoring a phase III randomized trial comparing two months of INH/RIF/PZA/EMB, followed by four months of INH/RIF with two months of INH/RIF/PZA/EMB, followed by two months of INH/RIF in HIV-negative participants with noncavitary disease in the Philippines, Uganda, and Brazil. Because this study excludes people with HIV and persons with more advanced disease, the application of the results may be limited and would likely have little relevance in settings with high TB and HIV prevalence.

TBTC Study 29, expected to begin enrollment in August 2009, is a phase II open-label trial evaluating the safety and efficacy of high-dose RPT in place of RIF during the intensive phase of TB treatment. The study will be conducted at sites

participating in Study 26 as well as at TBTC sites in Uganda and South Africa. The South African TBTC site will also be conducting a trial that evaluates the utility of low-dose linezolid in treating multidrug-resistant TB (MDR-TB). Linezolid is a broad-spectrum antibiotic, and has been associated with severe side effects. As a result, its use in TB treatment is often limited to difficult-to-treat cases of drug-resistant TB. The LiMiT study (TBTC Study 30) is a phase II double-blind trial that will randomize 64 MDR patients (resistant to at least RIF and INH) to either low-dose linezolid plus optimized background therapy (OBT) or placebo plus OBT for eight weeks followed by OBT for all participants. The expectation is that the lower dose will be effective and more easily tolerated. Results from this study are expected in 2011.

Two other broad-spectrum antibiotics are being evaluated for treating TB disease. The farthest along in the TB drug pipeline are gatifloxacin and moxifloxacin, both from a class of antibiotics known as fluoroquinolones. In a previous phase II study conducted by the OFLOTUB Consortium, it was found that gatifloxacin and moxifloxacin, when included as a replacement for ethambutol (EMB) in the intensive phase of treatment, improved the sterilizing activity of INH/RIF/PZA regimen. The OFLOTUB consortium is made up of 11 organizations and institutions from Europe and Africa, and was created to study gatifloxacin-containing regimens for shortening the course of treatment for drug susceptible TB. Based on the data from the phase II study, the consortium started enrollment in June 2005 for a phase III open-label trial where participants are randomized to either a four-month regimen containing gatifloxacin or to the standard WHO-recommended six-month regimen. Inclusion and exclusion criteria have been tightened to address issues of dysglycemia (abnormal blood glucose levels). In previous studies it was found that this side effect was significantly associated with those over 65 years of age and having prior abnormal blood sugar levels, though it was reversible upon drug discontinuation.

Moxifloxacin is being studied by a number of research institutions. The TBTC, the Global Alliance for TB Drug Development (TB Alliance), and the Food and Drug Administration (FDA) with the Johns Hopkins University have all thrown their hats into the moxifloxacin ring, with varying degrees of success. TBTC Study 28, completed last year, did not show a significant difference in efficacy between moxifloxacin and INH when used in the intensive phase. The Johns Hopkins University, with funding from the FDA, conducted a similar phase II study comparing moxifloxacin to EMB during the intensive phase to shorten treatment duration among HIV-negative and HIV-positive persons. This study found that the moxifloxacin group had about a 20% improvement in two-month smear conversion rates as compared to the EMB arm.

The TB Alliance has partnered with Bayer HealthCare, University College London, British Medical Research Council, the Johns Hopkins University, the TBTC, and the European and Developing Countries Clinical Trials Partnership (EDCTP) to determine whether using moxifloxacin in place of EMB or INH during the intensive phase can shorten treatment from six to four months (reducing the continuation phase to two months with the replacement or addition of moxifloxacin throughout treatment). The REMox TB trial, as it is known, is the largest TB drug registration trial ever initiated, with 2,400 volunteers (800 per arm) expected. This is a phase III, double-blind, controlled trial using a noninferiority design with one year of follow up, and will randomize participants to receive one of three treatments: (1) the standard of two months of INH/RIF/PZA/EMB plus moxifloxacin placebo, followed by four months of INH/RIF (moxifloxacin placebo given for first two months of continuation phase); (2) two months of moxifloxacin plus INH/RIF/PZA plus EMB placebo, followed by two months of INH/RIF/Moxi, and then two months of INH/RIF placebo; or (3) two months of moxifloxacin plus RIF/PZA/EMB plus INH placebo, followed by two months of RIF/Moxi plus INH placebo, and then two months INH/RIF placebo. Enrollment has begun in South Africa and Zambia; and the TB Alliance expects to increase the number of sites to include additional locations in Southern and Eastern Africa, as well as Latin America, Europe, and Asia with the aim of achieving global registration of moxifloxacin as part of first-line TB treatment.

While fluoroquinolones have shown good potency against drug-susceptible and drug-resistant TB, and are generally well tolerated, one of the major drawbacks is the fact that they are highly cross-resistant to one another. So if an individual develops resistance to one of the drugs in this class, none of the other drugs in the class will be useful. Aggravating the concern about fluoroquinolone resistance is the fact that drugs in this class are among the most widely prescribed antibiotics worldwide. A study from Vanderbilt University looked at resistance profiles of newly diagnosed patients from 2002 to 2006 and found fluoroquinolone-resistant TB in 2.5% of newly diagnosed cases in this low-TB-prevalent setting.

Metronidazole is another broad-spectrum antibiotic that is being evaluated for treating drug-resistant TB. NIAID is sponsoring a phase II, double-blind trial in South Korea of 60 HIV-negative participants with confirmed resistance to at least INH and RIF who are randomized to eight weeks of metronidazole plus standard background therapy (SBT) or to placebo plus SBT, after which all participants continue on SBT. The goal of this trial is to evaluate the ability of metronidazole to kill TB bacilli that survive in an anaerobic environment (one with little to no oxygen). These bacilli may be responsible for the need for extended treatment duration in MDR TB.

Table 2: 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 and MDR TB
SQ 109	Diamine	Sequella	Phase I	
Sudoterb/LL3858	Pyrrole	Lupin	Phase I	

Besides the existing antibiotics mentioned above—all of which are either older, approved TB drugs or newer broad-spectrum antibiotics being studied for a TB indication—the TB treatment pipeline includes five drugs with novel mechanisms of action. By definition these new classes of drugs are likely to overcome resistance to approved drugs from older classes.

Tibotec, a subsidiary of Johnson & Johnson, has developed a diarylquinolone compound known as TMC-207 that has shown bactericidal activity for both drug-susceptible and drug-resistant TB. TMC-207 specifically inhibits the proton pump of the adenosine triphosphate (ATP) synthase of MTB, meaning that this compound interrupts the energy supply to the TB organism. Drug-drug interactions with the rifamycins have been noted, therefore making TMC-207 potentially more useful as treatment for drug-resistant TB. More studies are needed on TMC-207's interactions with RIF, RPT, and rifabutin (RBT). Because TMC-207 has a long half-life and stays at therapeutic levels for a long period of time, a dosing regimen is currently being evaluated that allows for a daily loading dose of 400mg for two weeks followed by intermittent lower doses. Recently, Tibotec completed stage I of a phase II, double-blind trial comparing TMC-207 to placebo plus standardized background regimen (SBR) for MDR-TB in South Africa. Forty-seven participants were randomized to two treatment groups. One group received two weeks of daily SBR plus TMC-207 at 400mg followed by TMC-207 at 200mg three times a week for six weeks. The other group received SBR plus placebo for eight weeks. Stage I results are still being analyzed but after reviewing initial data, the data safety monitoring board (DSMB) recommended that stage II of this trial go forward as planned. Stage II will be conducted at sites in South Africa, Latvia, India, Russia, and Peru, and has begun enrollment. This phase will randomize 150 newly diagnosed smear-positive MDR patients into two arms: SBT plus TMC-207 at 400mg per day for the first two weeks and then 200 mg three times a week for the next 22 weeks or to placebo plus SBR for 24 weeks. All participants in both stages discontinued rifamycin use for one week prior to initiating treatment to ensure there was no drug-drug interaction. Completion of this trial is expected in early 2010.

Otsuka recently completed a phase II dosing study of OPC-67683, one of two nitroimidazole drugs that have entered into phase II studies. This open-label trial compared 100mg, 200mg, 300mg, and 400mg daily for 14 days in patients with smear-positive, uncomplicated TB. OPC-67683 has no cytochrome p450 interactions so is not likely to be contraindicated for use with rifamycins. Otsuka is currently recruiting for a phase II, double-blind trial comparing twice-daily doses of 100mg or 200mg of OPC-67683 plus OBT to placebo plus OBT at sites in eight countries.

The other nitroimidazole in clinical studies is PA-824, which was the first new compound that the TB Alliance put into development, after in-licensing the drug from Chiron. Both PA-824 and OPC-67683 are prodrugs, meaning that they only become active molecules after they are metabolized. The mechanism of these drugs is not yet well defined, though it likely involves inhibition of mycolic acid synthesis. Cross-resistance has been observed.

In mice, PA-824 has shown bactericidal and sterilizing activity and works against persistent TB organisms. Its mechanism is fairly specific to MTB, which makes it less likely to be used as a broad-spectrum antibiotic. In phase I studies, PA-824 was well tolerated. Serum creatinine levels were increased, indicating possible kidney toxicity, but based on a renal effects study conducted by the TB Alliance the effect was isolated, reversible, and not clinically significant. A phase IIa early bactericidal activity (EBA) study comparing doses of 200mg, 600mg, 1000mg, and 1200mg was recently completed. Final results are expected by early fall 2008. If funding allows, TB Alliance intends to study PA-824 in both drug-susceptible and drug-resistant disease.

Two other new compounds are currently in early phase clinical trials: SQ109 by Sequella and Lupin's Sudoterb/LL3858. In phase I studies SQ109 has shown synergistic effect with RIF and INH, increasing the activity of the drugs. Early PK data indicates that SQ109 has no interactions with other TB drugs. Sequella will be initiating a phase Ib study comparing multiple doses of SQ109 to placebo. Because SQ109 has a long half-life similar to TMC207, it might be dosed a few times a week after a daily loading dose, and therefore an extra cohort has been added to evaluate an intermittent therapy regimen. Additional phase I and II trials are planned for later this year.

Lupin has been quite tight-lipped about its new drug, the pyrrole Sudoterb/LL3858, but it appears that it is equally potent against drug susceptible and resistant organisms. A series of phase I dose escalation studies found Sudoterb/LL3858 to be well tolerated.

TB/HIV Coinfection Treatment Strategies

In addition to the studies listed above, a number of trials are evaluating different treatment strategies in TB/HIV coinfecting persons. The majority of these trials are trying to determine when to begin ARV treatment, and assess the interaction between ARVs and TB treatment. Despite these efforts to better understand how to treat coinfecting persons, there is a dearth of research dedicated to better understanding how to treat extrapulmonary TB, and patients with smear-negative TB are often excluded from participating in these drug trials.

Because people with HIV are more likely to have extrapulmonary and smear-negative TB, this gap in evidence leaves them at even greater risk. Similarly, there is also very little evidence on how best to treat children (who are also more likely to have smear-negative and extrapulmonary TB) and pregnant women. In fact, pregnancy was a reason for exclusion from every clinical trial. Children did not fare much better. Of the trials mentioned in this review, only the TBTC Study 26 is currently enrolling children. And of the institutions pursuing novel drug candidates, the TB Alliance is the only one to mention including children in its long-term drug development plan. This despite mounting evidence that TB disease progression is more aggressive in children and pregnant women.

In more promising news, Sanofi Aventis, the maker of rifapentine, has recently announced that after years of neglect it has decided to reinvest in developing the drug. Currently the CDC/ATS guidelines recommend RPT be used in the continuation phase for HIV-negative patients, and it is not registered for use in the European Union. The goal of the redevelopment plan is to get RPT approved for use for people with HIV, inclusion in the WHO recommended list of TB drugs, and inclusion as part of a four-month first-line treatment regimen. The company has stated it would like to fast-track this process but first needs to rebuild its internal capacity to study TB.

After the 40-plus-year lull in TB drug development, it is not difficult to feel optimistic about the number of studies underway and the many players joining the fight against TB. However, the first new drug is not expected to hit the market until 2012. And because a single new drug will not constitute a new regimen, the search for ways to maximize the current drugs must continue to 2012 and beyond. It is important to stay vigilant and push for better, shorter, more adaptable, and easier-to-tolerate treatment that is effective against LTBI, drug-susceptible, and drug-resistant TB in all populations.

Tuberculosis Diagnostics

BY JAVID SYED

Introduction

Obtaining an accurate tuberculosis (TB) diagnosis is the Achilles' heel of TB control. The most commonly used TB diagnostic test, sputum smear microscopy, is 126 years old; it involves visually identifying rod-shaped *Mycobacterium tuberculosis* (MTB) bacteria (bacilli) through a microscope. If the bacteria are detected, then the source patient is diagnosed with *smear-positive* pulmonary tuberculosis. Failure to see the bacilli does not rule out the possibility of *smear-negative* pulmonary TB disease, which can be diagnosed by TB culture. Smear microscopy is time-consuming for both the laboratory technician and the patient, often requiring repeat visits to gather the two or three sputum samples. Moreover, sputum smear microscopy is not a sensitive way to detect TB, since it routinely misses over half of active TB disease cases. Even a person with active TB replicating in the lung may have low numbers of bacilli in their sputum (paucibacillary TB) and people with disease outside the lungs (extrapulmonary TB) will have none. Children, and people with HIV—two groups with an increased risk of death from TB—are more likely to have paucibacillary or extrapulmonary disease; thus, sputum smear microscopy is even less reliable for diagnosing TB in these populations.

Besides direct microscopic detection of the TB bacilli in samples taken from patients, the organism can be grown (cultured) on a solid or liquid culture medium under laboratory conditions. Samples containing the organism can be taken from sputum, from blood, from other body fluids (e.g., pleural effusions from the sac lining the lung), or from a biopsy. TB culture in solid media dates from the 1880s. TB culture is a sensitive way to diagnose disease because it allows a very small number of bacilli to grow until they are easy to detect. However, growing TB in both solid and liquid media culture is unacceptably slow. The organism can take at least two weeks to grow in liquid culture and up to eight weeks on solid media. Detection of TB after it has been cultured provides a diagnosis of *culture-positive* TB disease, although a *culture-negative* patient may still be infected and have symptoms. The major disadvantages of culture are the time it takes for a result, sample contamination, and the danger of laboratory workers becoming infected from culture samples. The technique requires relatively sophisticated laboratory infrastructure and equipment, reagents, trained staff, clean water, and access to uninterrupted electricity—resources often unavailable in less-developed countries. Culturing TB organisms in the presence of anti-TB drugs can identify an organism's drug susceptibility pattern. If the bacilli grow despite the presence of an anti-TB drug, then it is a *drug-resistant* strain.

Besides direct detection of TB organisms, a person's immune response to MTB can be measured with skin or blood tests. These tests are used to determine exposure and/or infection with MTB but do not reliably distinguish between latent TB infection (LTBI) and active TB disease. One of these tests, the Tuberculin Skin Test (TST), also known as the Purified Protein Derivative (PPD) or Mantoux test, elicits this immune response (so-called delayed type hypersensitivity reaction) by injecting a combination of MTB proteins under the skin and measuring the local reaction. A more recent blood test measures the release of gamma interferon after exposing the blood specimen to MTB antigens. Interferon gamma is an immune system messenger molecule released by immune cells responding to MTB antigens, and its detection is an indication of TB infection. Interferon gamma release assays (IGRAs) are discussed below. A positive TST or PPD test can indicate latent TB infection (LTBI) or might be caused due to prior immunization with the BCG (Bacille Calmette-Guérin) vaccine, which is derived from a live TB organism. Similarly, a positive IGRA test cannot distinguish between active and latent disease. The presence of these immune responses in the absence of any clinical symptoms of TB disease, such as fever, weight loss, a cough lasting longer than three weeks, or blood in the sputum, indicates the likelihood of latent TB infection. Since people with LTBI have not yet progressed to active TB disease, the PPD test identifies persons who would benefit from preventive TB therapy with isoniazid (INH, an anti-TB drug) to reduce the chance of developing disease. People with HIV or with compromised immune systems can go undetected by most immune response tests despite having TB because their immune systems do not mount adequate responses to the MTB antigens.

Sensitivity and specificity are two important measures of a diagnostic tool's performance. A TB test with high sensitivity correctly identifies a very high proportion of the people tested who have TB infection and gives very few false positive results. A test with high specificity correctly discriminates between those who have TB infection and those who do not and gives very few false negative results. The best tests will be both highly specific and highly sensitive.

How useful a test will be in a given health care setting is limited by the resources required to perform the test, such as laboratory equipment, biosafety measures, clean water, electricity, and trained technicians. High-tech referral laboratories at the national or supranational levels have the infrastructure to perform very sophisticated tests, yet they serve only a fraction of the people in need of TB services. Peripheral laboratories, often associated with TB clinics and district hospitals, can perform basic laboratory services. Peripheral laboratories may be available to about 60% of people in need of services but this access can vary widely depending on the country context. For instance, even relatively simple smear microscopy is available in only 18% of

public health facilities in Kenya. Community health posts are locations with no laboratory infrastructure, yet they may provide the only accessible health care for a great proportion of the people in a country. A simple yet accurate TB test that could be used in rural community health posts would be a revolutionary addition to the diagnostic tool kit.

An ideal TB diagnostic tool—one that is accurate, fast, cheap, easy to use at the health post level, causes no hardship for the person seeking diagnosis or health care provider, and is safe for both—does not yet exist. Instead, several different diagnostic tools are often used in succession to arrive at a correct diagnosis. The path a health provider takes to make a diagnosis and begin treatment is called a *diagnostic algorithm*, and each successive diagnostic tool used contributes to greater certainty about the diagnosis. For instance, a health care provider will often use the results from a clinical symptom assessment, chest X-ray, HIV test, and sputum smear test to determine whether it is likely that a person has TB. If the chest X-ray and clinical symptoms show a high likelihood of TB, but the smear test results are negative, the clinician may ask for a culture test, if available, to further investigate the potential for TB disease. Although only the microscopy and culture tests can confirm the presence of TB, the symptom screen and chest X-ray provide important additional information to identify persons at risk of active TB.

Since TB is primarily a disease of the poor, there has been little profit incentive to develop new and more effective or more accessible diagnostics. The Foundation for Innovative and New Diagnostics (FIND), a product development partnership; a few academic laboratories; the World Health Organization–based Special Programme for Research and Training in Tropical Diseases (TDR); and a few diagnostic companies are attempting to develop better TB diagnostic tests. The WHO monitors emerging diagnostic tools and strategies and recommends those that would be appropriate for use in high-burden settings. Its approval is seen as a vital step in validating new methods and tools for the global effort to stop TB. Development of TB diagnostics is coordinated in part by the Stop TB Partnership’s New Diagnostics Working Group, which like the rest of the Partnership is a public-and-private collaboration consisting of donors, National TB programs, technical agencies, researchers, civil society organizations, and activists. However, with TB research and development overall getting about \$430 million in funding in 2006, and diagnostics getting only \$31 million, it is no surprise that the ideal tool that will save the most lives has not appeared in the TB diagnostic research pipeline. Unfortunately, we are not anticipating a revolution in TB diagnostics any time soon unless a major unexpected breakthrough occurs—or until a major investment of time, resources, and expertise is committed to the challenge.

TB diagnostics researchers are working to maximize the utility of the currently available tools while working in parallel on improved tests and strategies, some of which have recently been recommended by the WHO for use in high-TB-burden settings. Although no new test on its own provides a solution for the current TB diagnostics crises, some may offer incremental improvements. This TB diagnostics pipeline report thus provides an overview of some of the new tools and methods that are being developed and organizes them according to the level of the health care system level where they are likely to be used. Where available, sensitivity and specificity data is provided. Many of the processes or products do not yet have extensive peer-reviewed data available. Others are still being optimized to increase their utility. The future of certain tools or processes being developed by academic laboratories also seems uncertain as they will need partners to take them through the development and validation process if they are ever to be approved by the WHO and regulators for global use.

Table 1: TB Diagnostic Tests or Processes in the Pipeline

Name of Test or Process	Sponsor/ Developer	Technology	Application	Anticipated Time to Adoption in High-TB-Burden Settings
Health Post				
MPT-64 skin patch	Sequella	Antibody production to MPT-64 exposure	Detection of TB infection and disease	2009
Antigen detection test*	FIND	Detects antigen	Detection of TB infection and disease	2011
Peripheral Level				
One positive-smear case definition*	TDR	Sputum smear test through microscopy	TB detection	Available
Front-loaded smear microscopy*	TDR	Conduct same day sputum smears	TB detection	2009
LED adaptor	TDR	Fluorescent microscopy	TB detection	2009
Primo Star iLED microscopy	Carl Zeiss Inc. and FIND	Fluorescent microscopy	TB detection	2009
Sputum digestion process by sodium hypochlorite (bleach)*	TDR	Laboratory processing to improve microscopy yield	Sputum processing with bleach for microscopy	2009
Filter concentration*	Academic laboratories	Laboratory processing to improve microscopy yield	Sputum concentration for microscopy	2009
Fluorescent vital dye staining*	Academic laboratories	Stains only live TB bacteria	Detection of live TB	2010
Eiken	FIND and EIKEN Chemical Co. Ltd	Nucleic acid amplification	TB Detection	2010
LAM antigen test	Inverness-Chemogen	Detects the LAM antigen from urine	Detection of TB infection and disease	2010

Name of Test or Process	Sponsor/ Developer	Technology	Application	Anticipated Time to Adoption in High-TB-Burden Settings
Reference Laboratory Level				
MGIT	Becton Dickinson & Co.	Automated liquid culture	TB detection /DST	Available
MODS*	Academic laboratories, PATH, Tulip Diagnostics	Inverted light microscopy that detects growing TB	TB detection/DST	2009
Thin layer agar (TLA)*	Academic laboratories, FIND	Solid media culture and light microscopy to detect growth	TB detection/ DST for rifampicin and isoniazid	2009
Nitrate reductase assay (NRA)*	Academic laboratories	Solid media; TB growth causes color change	DST	2009
Colorimetric DST*	Academic laboratories	Solid or liquid culture; TB growth causes color change	DST	2009
FASTPlaque	BIOTEC Laboratories, Ltd.	Phage based DST for rifampicin on solid culture	DST	2010
Capilia test	Tauns and FIND	Lateral flow technology uses antibodies to detect presence of MTB	Speciation	2009
INNO-Lipa	Innogenetics	Line probe assay	DST for rifampicin	Available
HAIN test MDR TB Plus	HainLifescience GmbH and FIND	Line probe assay	DST for rifampicin and isoniazid	Available
GeneXpert	Cepheid and FIND	Nucleic acid amplification of TB DNA	TB detection/ DST for rifampicin	2010
Urinary nucleic acid amplification*	University College of London, Spaxen, and FIND	Nucleic acid amplification	TB detection	2011
QuantIFERON-TB Gold test	Cellestis, Inc	Interferon-gamma release assay	Detection of latent TB infection	2009
T-SPOT.TB ELISpot	Oxford Immunotec Ltd.	Interferon-gamma release assay	Detection of 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.

What Is in the TB Diagnostic Pipeline?

Health Post Level

The real revolution in TB diagnostics will occur when a rapid, accurate, and inexpensive diagnostic test is developed that can be used in settings with little to no infrastructure—specifically health posts that serve the majority of people in need of access to health services. Unfortunately, the TB diagnostic pipeline has few tests appropriate for this health service level.

The MPT-64 skin patch test is being developed by Sequella to identify active TB by detecting an immune response to MPT-64, a TB protein. Initial studies in healthy persons with BCG or with a TST positive test who did not have TB disease showed that exposure to the MPT-64 patch test did not produce a false positive response. In persons with active TB disease who were on treatment, the immune response decreased with the duration of therapy. This test might be able to distinguish subclinical active TB disease from latent TB infection and be useful in identifying active TB disease. A patch containing MPT-64 is applied to a person's skin; if the person's immune system recognizes the protein, a reddish reaction will occur at the site within 72 hours. There is little data published on this test and it needs to be validated in people with HIV, children, and in programmatic settings. There is also some concern that the reaction is difficult to read on dark skin. Some BCG does have the MPT-64 protein, leaving a potential for false positives. The test is being developed and plans for a larger trial are underway.

Finally, there is ongoing research to identify appropriate antigens or antibodies to be used to develop a **dipstick TB detection test**. If appropriate antigens or antibodies were discovered soon, the test could quickly come into wide-scale field testing, but efforts to date are weak, underfunded, uncoordinated, and inadequate to meet the dire need for a cheap, accessible, accurate, sensitive, and specific diagnostic test for active TB disease.

The Peripheral Laboratory Level

The pipeline includes a number of technological improvements and standardization measures targeted for use at peripheral laboratories, such as may be found in district hospitals. These low-cost incremental improvements are needed to make the most of the currently available tools, improve clinical outcomes immediately, and reduce the burden on persons accessing services as well as the health care system.

Revised Case Definition of a Smear-Positive Case

In 2007, the WHO changed the case definition of active TB from requiring at least two sputum smear-positive results to one smear-positive result and also reduced the number of bacilli required to be detected in each smear to be classified as a case in

countries that have laboratories with a well-functioning external quality assurance system. This relatively simple change in case definition can allow for cases to be detected in one day, increase case finding, and decrease the number of patients that drop out during the diagnostic process. The reduction in sputum tests also reduces the costs for the health system and the burden on the laboratory technicians. The recommendation has been made and now needs to be implemented on the country level, and its impact on clinical outcome for the patients needs to be documented.

Front-Loaded Smear Microscopy

Though the revised case definition for smear-positive cases creates potential for same-day diagnoses of smear-positive cases, in reality most patients are asked to come back the next day to provide a morning specimen. Front-loaded smear microscopy proposes that a second smear be done one hour after the first one if the initial smear is negative. Since 85% of cases are detected by the first smear, and an additional 10% are detected by the second smear, this will increase the chance of a person getting an accurate same-day diagnosis, will reduce the burden of testing, and prevent attrition during the diagnostic process. Data from studies that look at the impact of front-loaded microscopy need to be considered by WHO to make same-day diagnoses possible.

Optimizing Microscopy

One improvement in the pipeline focuses on optimizing microscopy by upgrading a conventional light microscope to a fluorescent microscope. TB fluoresces when stained with an acid-fast fluorochrome dye and lit with an intense light source (usually a high-pressure mercury vapor lamp). Fluorescence microscopy provides a 10% increase in sensitivity and reduces the time a laboratory technician needs to examine each slide. However, there are various problems associated with fluorescent microscopy, including the need for a dark room, the cost of the mercury vapor lamp, and the seemingly unfounded fear of becoming impotent from exposure to the bright light. The WHO-based Special Programme for Research and Training in Tropical Diseases (TDR) is evaluating an **LED adaptor** that can convert most light microscopes to perform fluorescence microscopy. FIND, along with its commercial partner Carl Zeiss Inc., is also working to develop an LED microscope (**Primo Star iLED** fluorescent microscope) that will provide increased access to fluorescence microscopy at a cost similar to a conventional microscope and can be battery powered. This microscope, which is capable of white light LED and UV light fluorescent microscopy and has potential utility for the diagnosis of malaria, as well, is currently in demonstration projects in India and will become available in November 2008. The LED adaptor manufactured by LW Scientific is already commercially available. Bright light fluorescent microscopy still needs to go through WHO approval.

In order to improve the chance of detecting TB in sputum, laboratories process sputum with various procedures before examining it. Attempts at improving microscopy yield are being made by standardizing two of these procedures, the **sputum digestion process by sodium hypochlorite** (bleach) and the concentration of bacilli through the use of a filter. Both the use of bleach and filter concentration are low-cost improvements that have been shown to increase the yield of smear microscopy. There is some data on the bleach and sedimentation method's contribution to improved yield of smear microscopy in high-HIV-burden settings. This impact of bleach and that of fluorescent microscopy on improving the accuracy of smear microscopy for HIV-positive individuals needs to be further confirmed.

Besides confirming diagnosis, the smear test also is used to monitor treatment success. However, dead bacilli can retain conventional stains and be visible under the microscope. Academic laboratories have developed a test to distinguish between viable and dead bacilli through use of a fluorescent dye that will only stain live bacilli. This **fluorescent vital dye staining** can therefore identify persons who are failing treatment and might have resistant strains of TB. The test is under early evaluation.

The LAM urine antigen detection test detects the mycobacterial protein lipoarabinomannin (LAM) in urine and is being developed by Inverness-Chemogen. Preliminary data showed that it had 79% sensitivity in detecting mycobacterial LAM among smear- and culture-positive people with HIV. For all others the test showed low sensitivity (42%) in HIV-negative, smear-positive patients and overall 28% sensitivity for smear-negative, culture-positive patients. Unless the sensitivity is improved for smear-negative cases, the utility of the test is uncertain. FIND dropped the LAM test from its project pipeline. The current version is an ELISA test. Inverness-Chemogen continues to try to optimize and improve this test.

Molecular Technology

The Eiken LAMP nucleic acid amplification test from Eiken and FIND uses a LAMP (loop mediated method of nucleic acid amplification) process that can carry out the amplification at one temperature (isothermally) at 65 degrees Celsius and doesn't require heating and cooling cycles. There is limited data available in the peer-reviewed literature about the test, and one study of this easier-to-perform test has 97% sensitivity for smear-positive cases, but only 49% sensitivity in smear-negative, culture-positive cases. The test is able to give results within an hour, provide easily readable results (the presence of TB can be detected by the naked eye under UV light), can be used on sputum sample or culture, requires little retraining of the laboratory workers, has potential as a cross-disease platform, and is a vast improvement over current tools. Attempts are being made to improve its accuracy for smear-negative, culture-positive cases.

The Reference Laboratory Level

There are 13 diagnostic test platforms currently in the pipeline appropriate for use in the reference laboratory. Two of them are liquid culture and drug susceptibility testing (DST) systems—the **BACTEC MGIT** and **MODS**, both of which have a sensitivity of nearly 100% and are relatively fast (7–11 days). The BACTEC MGIT (Mycobacteria Growth Indicator Tube) from Becton Dickinson and Co. (BD) is a high-throughput automated liquid-culture system with a fluorescing oxygen sensor at the bottom of the tube that glows when the growing TB bacilli consume oxygen. BACTEC MGIT is expensive and comes in fully automated and manual versions (FIND’s negotiated price is \$5 per culture test tube and \$35,000 for an automated MGIT machine). The automated system reads the fluorescence automatically and can process more than 900 tests at the same time. The cheaper manual system is read by a laboratory worker under an ultraviolet lamp. In 2007 the WHO recommended the use of liquid culture in high-burden settings, thereby endorsing the use of a number of commercial systems, of which only BACTEC MGIT has been validated in a high burden settings and is being championed by FIND for use for case detection and DST. Through a partnership with CREATE (a Johns Hopkins University-based TB/HIV research consortium), FIND conducted a 60,000-person test in South Africa, Zambia, and Brazil to evaluate MGIT’s utility and cost effectiveness. Data from studies in Kenya, Nepal, Russia, the Philippines, and Uzbekistan are currently being analyzed to assess MGIT’s utility for TB case detection and drug susceptibility testing (DST).

The BACTEC MGIT can provide positive culture results in 11 days on average, and DST results in an additional 11 days. Negative culture results can take up to 42 days to establish. This makes it difficult to schedule a return appointment for patients. In early validation studies MGIT had a high level of contamination (15%). According to FIND, contamination levels have been reduced to about 10% through the implementation of stricter operating procedures. Liquid culture also requires a high level of laboratory safety to protect lab workers from infection as they handle live cultures. Furthermore, BACTEC MGIT, like many other techniques, also requires sputum processing and often identifies non-TB mycobacteria (NTM), which requires speciation testing to identify. FIND is attempting to resolve the NTM issue by using the Capilia speciation test (described below), which distinguishes between MTB and other mycobacteria, though it still requires a higher-level biosafety lab, which is not easy to come by in most high burden settings.

MODS, or microscopically observed drug susceptibility, is a non-copyright-restricted, open-source technology system for TB detection and DST. It is cheaper than MGIT and available for anyone to work with, though it does use a proprietary liquid medium produced by BD. MODS has been used successfully in some field

settings and is being implemented by the Peruvian TB Control Program. It requires special reagents, equipment, and careful processing procedures. PATH, along with Tulip Diagnostics, is developing a MODS kit, which will need validation trials. MODS is much cheaper than MGIT, depends on direct observation of mycobacterial growth, and gives results in 7–21 days with the detection of cordlike mycobacterial growth through an inverted light microscope.

Two rapid, solid-culture and drug susceptibility systems being developed for reference laboratories are the **Thin Layer Agar (TLA) test** and the **Nitrate Reductase Assay (NRA)**. The TLA system has sensitivity of 92% for detecting TB, low rates of contamination and detection of NTM, and does not require a special microscope. TLA systems do require a carbon dioxide incubator. Demonstration trials are needed to confirm the utility of TLA in high-burden settings, and FIND is involved in this effort. NRA, on the other hand, also known as the Griess method, is routinely used in Peru for drug susceptibility testing and is well validated on clinical isolates. For detecting rifampicin and isoniazid drug resistance it has a sensitivity and specificity of greater than 94% and 92%, respectively. However, it still needs to be validated for direct use with smear-positive sputum samples in order to reduce time to diagnosis. Both TLA and NRA are slightly slower and a little less sensitive than MODS and MGIT.

New methods of conducting drug sensitivity testing (DST) are being developed and are expected to lead to a product that may be available for use in reference laboratories by 2010. These methods are described below.

Colorimetric DST systems developed by academic laboratories can detect multidrug-resistant (MDR) TB that is resistant to two of the most powerful first-line anti-TB drugs, rifampicin and isoniazid. The tests use MTT (a tetrazonium salt) or resazurin (a dye) to indicate TB growth by changing color. They require culture, but have sensitivity and specificity between 89% and 100% in detecting rifampicin and isoniazid resistant growth. The tests are cheap and can provide overnight results. The use of these tests on direct specimen samples needs to be demonstrated.

A phage-based test commercially known as **FASTPlaque** identifies rifampicin resistance in two days. It is being developed by Biotec Laboratories and can be used on smear-positive samples. Rifampicin resistance in many settings indicates resistance to isoniazid as well. Cell cultures containing MTB are infected with a TB-specific mycobacteriophage, and then all the cells other than the MTB are killed. The MTB is then grown in a rifampicin-containing media. The mycobacteriophages inside the MTB will replicate if the bacteria are able to survive in the rifampicin-rich media and they will appear as spots on the culture plate that indicates resistance. However, about 10–20% of the tests have an indeterminate result, and the very high

level of contamination shown in one study (close to 40%) carried out in Zambia highlights challenges for its utility in high-burden settings unless this problem can be addressed. Of the tests that could be read, FASTPlaque has a sensitivity and specificity of greater than 95%.

Once a mycobacterium culture is grown, there is a need for speciation to ensure that the mycobacterium is indeed MTB and not one of a number of non-TB mycobacteria. Some of these can also cause disease (such as those that cause MAC infections) and require specific treatment, while others may be nonpathogenic environmental mycobacteria. The **Capilia test** can do just this, and will potentially replace more complex and slow speciation tools. The Capilia test, manufactured by Tauns and being developed in partnership with FIND, is a lateral flow assay that uses a TB antibody to distinguish between MTB and other non-TB mycobacteria from a culture sample in 15 minutes with sensitivity of greater than 92% and specificity of 100% and is currently being evaluated under routine program conditions.

Nucleic Acid Amplification Tests (NAAT) that can identify MTB hold the potential to revolutionize TB diagnosis in the referral laboratories similar to the way polymerase chain reaction (PCR) made possible RNA testing for HIV plasma levels and genotypic testing for HIV drug resistance. The three NAAT tests in the current pipeline are fast and accurate.

The **Hain MDR TB Plus** and **INNO-Lipa** are two tests based on the line probe technology endorsed by the WHO for widespread use for rapid drug resistance testing. INNO-Lipa has been developed by Innogenetics for detection of rifampicin resistance and requires culture samples. Developed by Hain Lifescience GmbH and FIND, the Hain test is a PCR-based test that can accurately identify isoniazid and rifampicin resistant isolates and has the relative advantage of doing so from sputum specimens, dried sputum, and culture in a couple of hours. The test's sensitivity for detecting rifampicin resistance alone was 99%, and about 90% for both rifampicin and isoniazid resistance. The sensitivity for isoniazid resistance is greater than 90%. The test requires manual processing to extract and amplify the DNA and then uses a strip with gene probes to identify MTB mutations associated with isoniazid and rifampicin resistance patterns. Demonstration projects are currently being conducted in South Africa, Uganda, Turkey, Vietnam, Thailand, China, India, the Philippines, and Indonesia to further validate this test.

The fully automated PCR-based **GeneXpert system** developed by Cepheid (being used in the U.S. postal system to detect anthrax) is being adapted for TB detection in collaboration with FIND. This test is an automated closed system that is being developed to detect TB in sputum. As it requires only one simple step of adding a

reagent to the sputum, it reduces the need for training and the burden on laboratory technicians. Initial data shows the test's sensitivity and specificity to be 89% and 99%, respectively, in detecting rifampicin-resistant gene mutations within a few hours. The test could potentially be used as a multidisease diagnostic platform, making it more broadly useful. The Cepheid test is still being developed and will need to be tested in field settings. It is likely to be relatively expensive.

Urine from TB patients contains MTB DNA fragments and is an easier specimen to collect than sputum or blood. University College London, Spaxen, and FIND are developing a test that exploits this fact and will apply PCR-based technology to amplify and identify **MTB DNA from urine**. A small study was able to amplify and identify TB DNA from urine in 79% of TB patients. Initial studies used different PCR methods and had varied sensitivity of between 40% and 100% and consistently high specificity of greater than 98%. The sensitivity is highest among people with HIV and pulmonary TB. If the sensitivity can be improved for smear-negative and extrapulmonary cases, the test might offer an improvement over current diagnostics, especially for people with HIV and pediatric TB cases.

Immune-Based Tests

There are two tools based on detecting an immune response on exposure to TB as a way to determine the presence of latent TB infection. They both need relatively sophisticated laboratory equipment and trained staff.

The **QuantIFERON Gold Test (QFT Gold)** from Cellestis exposes a blood sample to TB antigens that are not part of the BCG vaccine. If the person has TB infection, the blood sample will react by producing interferon gamma (IFN-gamma). The test is a type of IGRA. As the TB antigens in the test are not present in BCG, unlike PPD, this test will not show a false positive result due to previous BCG vaccination. There is a lot of uncertainty about whether IGRA levels reflect active disease or resolution of disease as opposed to latent disease. Discrepancies between TST and QFT Gold results are not yet fully understood. The test needs to be conducted within 12 hours of collecting the blood sample and can provide results in 24 hours. The test is currently being rolled out in some developed countries but its utility, especially in high-burden settings, in people with HIV, and in children still needs to be clarified.

The ELISpot **T-SPOT.TB** test is another IGRA, this one made by Oxford Immunotec. In a small study in people with HIV the test was able to detect interferon gamma production despite low CD4 levels, and in patients with extrapulmonary TB, it showed greater sensitivity and specificity (95% and 88%, respectively) than TST (47% and 86%, respectively). The interferon gamma-producing immune T cell is detected within 24 hours. The test still requires a blood

sample that needs to be processed within eight hours of collection, is complicated to implement, and needs much larger studies to be validated in high-burden settings, in children, and in people with HIV.

Conclusion

Currently the goal of a TB diagnostic test that can be used at point of care or at the health post level remains out of reach. With the shamefully small amount of resources directed to TB diagnostics (\$31 million in 2006), current efforts are unlikely to yield dramatic progress in the near future. Basic science research for TB in general needs to be better funded if the current diagnostic challenges in TB are to be met. The National Institutes of Health (NIH), the leading funder of TB research in the world, has not seen a funding increase since 2004. Among other challenges are the lack of large and well-characterized specimen banks that could be used to examine leads, and the lack of a clear scientific blueprint for developing a point-of-care test for active TB disease. As the pipeline demonstrates, many of the products need more peer-reviewed data to back them, and nearly all need to be further optimized or validated for use in high-burden settings. In addition, many of the products currently being developed in academic laboratories need to have their methods systematized so that they can be replicated with accuracy. Many of these methods discovered by academic laboratories need a development plan and resources to validate them and bring them to market. Some of the resource and research challenges, particularly for applied and late-stage product development, are being addressed with support from the Gates Foundation, the New Diagnostics Working Group, TDR, and FIND. The increased involvement of Becton Dickinson, Eiken, Inverness-Chemogen, and other private companies in the diagnostics field is encouraging as well. In the meantime, the TB community is doing what it does best: making do with limited and inadequate resources and focusing on optimizing smear microscopy, expanding laboratory capacity, and accelerating research on improved diagnostic tests for use mostly at the referral center level. These efforts need to happen concurrently with a massive push to achieve progress in TB research and development overall—and for TB diagnostics in particular.

Tuberculosis Vaccines

BY CLAIRE WINGFIELD

The first and only successful tuberculosis (TB) vaccine was introduced 87 years ago, in 1921. Today, the Bacille Calmette-Guérin (BCG) vaccine is the most widely administered vaccine in the world. However there are still many questions about its efficacy and safety. It appears that BCG loses most, if not all, of its effectiveness by adolescence and additional doses do not extend the period of protection. Therefore, once an individual reaches adolescence she lacks effective induced immunity to TB.

BCG's effectiveness is considered variable even against the two forms of extrapulmonary TB it appears to protect against—miliary (disseminated throughout the body) and meningal (TB of the lining of the brain). The World Health Organization (WHO) estimates that BCG saves the lives of over 40,000 children per year; it is therefore included as part of standard infant immunization in most of the world, particularly where TB is endemic.

Recent evidence suggests that the BCG vaccine may be harmful to HIV-positive infants, causing a potentially fatal focal or disseminated disease (BCGitis or BCGosis). The reaction is so severe that even when considering that TB is the most common cause of death among people with HIV, the WHO issued a recommendation in May 2007 warning against vaccinating infants with a confirmed HIV diagnosis. However, since most infants are not diagnosed with HIV due to the lack of HIV RNA testing in many settings, it is often not practical to get a confirmed HIV diagnosis within the vaccination timeline; many infants are thus put at risk for this serious adverse reaction. BCG also appears to offer scant immune benefit in this population as it induces very few memory cells. Additionally, it is suspected that BCG may potentially aggravate HIV disease progression in HIV-positive infants and children by causing immune activation.

The limitations and uncertainties surrounding BCG underscore the need for a finer and more nuanced understanding of how the immune system responds to TB. There is still much that needs to be understood about how TB-specific T- and B-cell immunity are induced, how they evolve, and how they reflect different stages of infection (primary, latent, subclinical, and active in adults and children, and among those with intact and damaged immune systems). Why is it that 90% of those infected with TB never develop active disease? How can a long-lasting and potent cell-mediated immune response to TB be induced in *all* populations?

The current list of TB vaccine candidates includes constructs designed to confer protection prior to exposure or augment immunity after infection with

Mycobacterium tuberculosis (MTB). Preventive vaccines are intended for persons who have not been infected with TB bacilli. Postinfection vaccinations aim to boost immunity so that latent infection does not progress to active disease. The new generation of TB vaccine candidates deploy several strategies to induce a cell-mediated immune response. They are categorized as *priming*, *boosting*, and *immunotherapeutic*, and refer to the timing and the role that the vaccine construct plays in this process. Some candidate TB vaccines in development may play multiple roles.

A priming vaccine induces an initial immune response. This prime response may be “boosted” by another vaccine months or years later. This concept is not new to the vaccine world but has not been proved effective with BCG alone. A priming vaccine teaches the immune system how to respond to an organism by stimulating the creation of memory immune cells specific to whatever is in the vaccine, such as attenuated (weakened) live TB organisms (as with BCG) or TB proteins. A *booster* vaccine may be given months or years later to strengthen the induced immune response by increasing the number or broadening the activity of these specific immune cells. Immunotherapeutic vaccines strengthen the immune system postinfection, thereby preventing disease progression or improving the impact of treatment.

Eight TB vaccine candidates have entered early-stage clinical trials. To show efficacy, phase III vaccine trials require large numbers of participants and a long follow-up period and will be expensive. According to the Aeras Global TB Vaccine Foundation, a product development partnership (PDP) with six novel TB vaccine candidates entering clinical trials this year, there is not yet enough funding for phase III vaccine studies. However, when considering the lives saved and the long-term benefits of a more effective and universally accessible vaccine, the research costs will be a bargain.

Table 1: TB Vaccine Candidates

Agent	Type	Description	Sponsor	Status
MVA85A	Prime boost	MVA vector	Oxford University	Phase II
GSK M72	Prime boost	Recombinant protein	GlaxoSmithKline	Phase II
<i>Mycobacterium vaccae</i>	Prime boost	Heat killed NTM	SR Pharma	Phase II

Three of eight vaccine candidates in clinical trials have moved into phase II studies: The University of Oxford’s MVA85A, GlaxoSmithKline’s (GSK) M72, and an inactivated *Mycobacterium vaccae* from SR Pharma. Both the University of Oxford and GSK have partnered with Aeras, which received substantial funding from the Bill and Melinda Gates Foundation as well as support from the governments of Denmark, the Netherlands, Norway, and the United States, to bring these constructs to market. SR Pharma has received financial and technical support from the Elizabeth

Glaser Pediatric AIDS Foundation, the Sigrid Juselius Foundation, the Hitchcock Foundation, Muhimbili University, Dartmouth University, and the U.S. National Institutes of Health to move its vaccine through the pipeline. All three of these new vaccines are being investigated in phase II studies as part of a BCG-prime boost strategy—each candidate will be given to persons previously immunized with BCG.

MVA85A, recombinant modified vaccinia virus Ankara expressing antigen 85A, uses an attenuated version of the vaccinia virus (cowpox) that cannot cause disease combined with MTB antigen 85A. The antigen 85A stimulates a strong TB-specific immune cell response, thereby boosting the immune recognition of TB initiated by the BCG prime. A series of phase I studies have showed that MVA85A is safe and well tolerated in healthy volunteers and stimulates a good cell-mediated response, particularly among those who had previously been vaccinated with BCG. Oxford in collaboration with the University of Cape Town is currently recruiting for a phase II study of MVA85A in 108 healthy infants and children, ages 6 months to 11 years, in South Africa. This is a nonrandomized, open-label trial comparing safety and immune response of three dosages of MVA85A compared with placebo. All participants were BCG vaccinated within the first four weeks of life. Expected study completion is December 2009. The past and ongoing clinical trials of MVA85A have been sponsored by the University of Oxford with funding from the Wellcome Trust and the European Commission. The university has partnered with Aeras to conduct a Phase IIb proof of concept trial that is planned for later this year. This trial will take place in South Africa and plans to randomize 2,800 infants to receive boosted MVA85A or placebo followed by two years of monitoring.

GSK Biologicals, a subsidiary of GlaxoSmithKline, has developed GSK M72. This candidate vaccine is a protein subunit vaccine that induces an immune response to TB protein M72, and was designed to provide protection against pulmonary disease in infants and adolescents in disease endemic countries as well as at-risk adults with latent TB infection. The vaccine is being developed to boost preexisting immunity induced by BCG and/or MTB. GSK Biologicals in partnership with Aeras are conducting a phase II trial to measure the safety and immunogenicity in PPD-positive adults who have been exposed to TB either through BCG vaccination or by infection in Africa and the Philippines. In another phase II trial currently ongoing in partnership with Aeras and TBVAC, the safety and immunogenicity of the GSK M72 is being evaluated in HIV-positive individuals. Information on the initial safety and immunogenicity profile of the vaccine in these populations should be available by early 2009. Proof of concept studies to evaluate the efficacy of the vaccine are planned to start in early 2010 and if successful, and funding is secured, will lead to phase III licensure trials starting in 2012.

Mycobacterium vaccae (*M. vaccae*) is a heat-killed non-TB mycobacterium (NTM) being considered for both preexposure boosting and postexposure immunotherapy. It is farthest along in the pipeline as part of a BCG boosting regimen. However, *M. vaccae* has been plagued by controversy. Results from studies evaluating this construct as part of immunotherapy for those who are already infected with TB have been underwhelming. Evidence suggests that *M. vaccae* has absolutely no impact on infection, disease progression, or death. Questions have been raised about the study design and whether its results will be relevant, as participants had been given only one dose of *M. vaccae*. Surprisingly, it is still being considered for immunotherapy in multiple doses, and has in fact been approved by the Chinese regulatory authorities as part of treatment for TB.

Mycobacterium vaccae is now being studied as a preventive vaccine for disseminated TB among people with HIV. The hypothesis is that immunization with *M. vaccae* early in HIV infection will significantly reduce the risk of developing HIV-associated disseminated TB as well as pulmonary TB disease. The DARDAR Health Study, a partnership between Muhimbili University in Tanzania and Dartmouth Medical School in the United States with funding from NIAID is conducting a phase II/III randomized, double-blind study comparing five doses of *M. vaccae* to placebo among BCG vaccinated people with HIV. Participants will be followed for three to five years to assess any incident cases of pulmonary or disseminated TB, as well as risk factors for disseminated TB. Silence Therapeutics, a subsidiary of SR Pharma, is planning to initiate phase I clinical trials of *M. vaccae* as a therapeutic vaccine for persons with TB in 2009.

Recent evidence from an HIV vaccine trial has raised some concerns about another TB vaccine construct that has entered into human trials. AERAS-402/Crucell Ad35 from Crucell NV and Aeras is a heat-inactivated adenovirus (Ad35) that serves as a vector for DNA expressing TB antigens 85A, 85B, and 10.4. Adenoviruses have been used as candidate vaccine vectors for a number of diseases, including HIV, herpes, and rabies, because they can be modified to include genetic material from an organism to trigger an immune response, and are potent inducers of CD8 cell responses. The concern over adenoviral vector vaccines is based on evidence from the Merck/HIV Vaccine Trials Network STEP Study that was evaluating an Adenovirus 5 (Ad5) based HIV vaccine candidate. The trial and its companion trial in South Africa were stopped last fall when preliminary data suggested that those immunized with the Ad5 vector had a higher rate of HIV infection, especially when they had high preexisting immunity to adenoviruses. This development is not completely understood and intensive follow-up investigation is ongoing, but it has raised questions about the safety of using adenoviruses as vaccine vectors. Though Ad5 is different from Ad35, and there is no evidence from the ongoing phase I trial of AERAS-402/Crucell Ad35 to suggest that the Ad35 construct will increase the risk

of acquiring HIV infection, Aeras has instituted additional safety precautions in its trials of this candidate.

The fact that the vaccine candidates that are farthest along in the pipeline are part of a BCG prime-boost regimen begs the question, What are the options for HIV-positive infants and children? Because BCG vaccination is not recommended for these groups it would seem that none of these constructs will be useful in this population. What alternate strategies can be used to help protect them against TB infection? There is some research being considered evaluating using early antiretroviral therapy (ART), IPT, and/or a combination of BCG while on ART among HIV-positive children to bolster their immune function. But at this point there are no easy answers.

The most optimistic scenario has the first new TB vaccine coming to market in 2015. While there are some promising vaccine candidates in the pipeline there are not enough resources to properly evaluate them. There are also many questions about the candidates' relevance and safety for all populations that need to be addressed in clinical studies, particularly among HIV-positive infants and children who are most at risk for being left out. However, there is no doubt about the need for a vaccine that is safe, tolerable, easy to administer, and able to provide lifetime protection against all forms of TB infection and disease in all populations and age groups.

Immune-Based Therapies and Preventive Technologies Pipeline

BY RICHARD JEFFERYS

In 2007, the landscape for immune-based HIV therapies and preventive technologies (vaccines, microbicides, and preexposure prophylaxis, or PrEP) was shaken by a minor earthquake: the premature cessation of immunizations in two phase IIb trials (called the STEP/HVTN 502 trial and the Phambili/HVTN 503 trial) of Merck's HIV vaccine candidate due to lack of efficacy and concern that the vaccine may have increased susceptibility to HIV infection among a subset of participants. Results from these trials were not anticipated until 2009 at the earliest, and while few believed there was any chance that the vaccine would protect against acquisition of infection, there was considerable optimism that it might improve postinfection control of HIV replication to some significant degree. The fallout from the vaccine's failure was unpleasant and confused: several major media articles declared that no progress had been made in vaccine research and that the trial showed that all animal models were useless; neither claim is supported by the data, and in fact the evidence suggests that the simian immunodeficiency virus (SIV)/macaque model predicted the outcome quite accurately. The trial has, however, cast a cloud over the use of weakened adenoviruses as vaccine vectors. This platform had become popular because adenovirus serotype 5 (Ad5) induces CD8 T-cell responses in around 70% of participants—a vastly superior response rate compared to previously studied vectors. But the results from the Merck trial show that receipt of Ad5 was associated with significantly increased susceptibility to HIV infection among the subset of trial participants with antibodies against Ad5, particularly uncircumcised gay men. The uncertainties now attending to the safety of Ad5 have shone a harsh spotlight on the dearth of vectors able to induce CD8 T-cell responses in a similar proportion of recipients, and finding alternate options is now a vital priority for the preventive vaccine field.

Microbicides also experienced failure in 2008 with the announcement that the product Carraguard had failed to show any efficacy in a large clinical trial. Given the travails of the biomedical prevention field over the past few years, it was perhaps some relief to researchers that there was no evidence of any adverse effects of Carraguard use, although the data suggested that adhering to regular microbicide application is a daunting challenge that needs to be addressed by better delivery methods. In the area of PrEP, the long-planned efficacy trial among gay men in Peru and Ecuador is underway (with sites now being added in the United States) and another trial comparing oral PrEP to a microbicide is in the advanced planning stages.

Immune-based therapies remain neglected in 2008. This is, in part, a testament to the efficacy of antiretroviral drugs and the availability of newer compounds with fewer side effects, such as Truvada and Isentress. However, there are still voids in the therapeutic armamentarium that effective IBTs could conceivably fill.

Table 1: Preventive Vaccines Pipeline 2008

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 Phase I (in infants)
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 Phase I (in infants)
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 containing <i>gag/pol</i> genes from HIV-1 clade B and <i>env</i> genes from clades A, B, and C	NIH Vaccine Research Center, GenVec, Vical	Phase IIb (PAVE 100)
pGAZ/JS7 DNA MVA/HIV62	DNA prime and MVA booster vaccines encoding <i>gag</i> , <i>pol</i> and <i>env</i> from HIV-1 clade B	NIAD, Geovax	Phase IIA
ISS P-001	Recombinant Tat protein from HIV-1 clade B	ISS, Excell	Phase IIA
LIPO-5	5 lipopeptides containing CTL epitopes (from Gag, Pol and Nef proteins)	ANRS	Phase II
HIVIS 03 DNA-MVA prime-boost HIV-1 vaccine candidate	Prime: HIVIS DNA encoding <i>env</i> (A,B,C), <i>gag</i> (A,B), <i>RT</i> (B), <i>rev</i> (B) Boost: MVA-CMDR encoding <i>env</i> (E), <i>gag</i> (A), <i>pol</i> (E)	Karolinska Institute, SMI, Vecura, USMHRP	Phase I/II
GTU-Multi-HIV	DNA vaccine containing <i>nef</i> , <i>rev</i> , <i>tat</i> , <i>gag</i> , <i>pol</i> , <i>env</i> , and CTL epitopes	FIT Biotech	Phase I/II
DNA-C + NYVAC-C	Prime: DNA vaccine encoding clade C <i>env</i> , <i>gag</i> , <i>pol</i> , <i>nef</i> Boost: NYVAC-C attenuated vaccinia vector encoding clade C <i>env</i> , <i>gag</i> , <i>pol</i> , <i>nef</i>	EuroVacc Foundation, GENEART	Phase I/II

Vaccines

In last year's report, TAG described the narrative arc of HIV vaccine research, leading to the current focus on the clinical evaluation of T cell–based approaches that might reduce viral load, lessen the chance of onward transmission, and prevent or slow disease progression in immunized individuals. This focus has been driven by compelling evidence from animal and human studies that T-cell responses play a critical role in viral load control, and also by evidence that the challenge of inducing effective T-cell responses, particularly CD8 T-cell responses, is solvable in the relatively near term. In parallel, laboratory work has continued to try and tackle the more daunting problem of developing effective neutralizing antibody-based approaches that might eventually lead to vaccines capable of offering complete protection against HIV infection.

In 2008, it is fair to say that this narrative has been complicated by the plot twist of the Merck vaccine efficacy trial results. Any hopes that simply inducing a CD8 T-cell response that is detectable with currently used tests would be sufficient to control HIV replication have been dashed. But because the vaccine did at least induce detectable CD8 T-cell responses in the majority of recipients, the trial has made it possible for researchers to begin to address questions about how the magnitude, functionality and specificity (which parts of HIV were being targeted) of these CD8 T cells may have impacted the outcome.

One exploratory analysis of STEP that has received considerable attention revealed a possible inverse correlation between the magnitude of vaccine-induced Gag-specific CD8 T-cell responses (measured by interferon gamma ELISpot) and post-infection viral load set point, but only in participants with no detectable anti-Ad5 antibodies. While this analysis may offer some solace in the face of the vaccine's failure, it's important to stress that the data are derived from only 12 people and the statistical significance is extremely fragile. Another post-hoc analysis of the STEP results suggests that the vaccine may have improved control of viral load in individuals with favorable immune response genes (class I HLA genes such as HLA B*57 and B*27, which govern the targeting of CD8 T-cell responses), a phenomenon that has been well described in the SIV macaque model. In the SIV challenge study conducted with Merck vaccine, there was no significant effect on viral load overall but immunized animals possessing the known favorable class I Mamu (the macaque HLA) gene A*01 did show significantly enhanced immune control of SIV replication compared to equivalent placebo recipients. If the Merck vaccine had a similar effect in humans, it would strongly suggest that the SIV/macaque model is a valid means of evaluating the potential efficacy of T cell–based vaccines. Therefore, if a next-generation T-cell vaccine can have a significant impact on viral load in this animal model, it would provide a compelling justification for advancing the candidate into an efficacy trial.

In the meantime, plans for what was scheduled to be the next HIV vaccine efficacy trial have had to be completely overhauled because the candidate also includes an Ad5 vector (as a booster immunization, after three shots of a DNA vaccine). This prime-boost approach was developed by researchers from the Vaccine Research Center (VRC) at the National Institutes of Health (NIH) and was originally slated to undergo an efficacy evaluation in an 8,500-person trial dubbed PAVE100. However, the association between Ad5 immunization and enhanced susceptibility that was seen in the STEP trial means that the VRC's vaccine cannot be given to anyone with preexisting antibody responses to Ad5. An alternate trial design, called PAVE100A, is now being considered. The new trial would enroll 2,400 gay men and limit the population to circumcised individuals with no detectable antibodies against Ad5 to ensure safety. It is currently unclear if PAVE100A will take place and a number of scientists and AIDS activist groups—including TAG—have suggested that the trial should not go forward.

TAG also stated in last year's report that if both the Merck and VRC candidates failed, then "the prospects for all T cell-based approaches will dim and the field will need to turn back to neutralizing antibodies." The unpleasant surprise regarding the enhancement of susceptibility by the Ad5 vector has muddied this picture. The prospects for T cell-based approaches have been dimmed by the Merck failure, but the data has also shone light on some areas for improvement such that the hope for an effective T-cell vaccine is not extinguished. In particular, the STEP data identifies several shortcomings of the immune responses induced by Merck's vaccine that future candidates can potentially address:

- CD4 T-cell help: The Merck vaccine induced HIV-specific CD4 T-cell responses in less than half the recipients. This indicates that, surprisingly, the much-derided ALVAC vector is a better CD4 T-cell immunogen than Merck's vaccine.
- T-cell breadth: 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).
- T-cell function: Recent studies indicate that Ad5 vectors may not induce an ideal CD8 T-cell response due the persistence of the vector. Specifically, the ability of CD8 T cells to proliferate (copy themselves) appears a key component of protection in animal models, but CD8 T cells induced by Ad5 vectors proliferate poorly.

A review of the current preventive HIV vaccine pipeline suggests that there are approaches that may offer better CD4 T-cell responses and perhaps improved CD8 T-cell functionality. However, none appear likely to do better when it comes to the

key issue of breadth. Also, at the current time, no approach in the pipeline other than the VRC's has come close to inducing CD8 T-cell responses in as high a proportion of recipients as the Merck Ad5 construct. It is therefore extremely unlikely that any of the current candidates in the preventive HIV vaccine pipeline will advance to efficacy testing without significant alterations, at the very least.

In recognition of the challenges facing the HIV vaccine field, the National Institute of Allergy and Infectious Diseases (NIAID) sponsored a summit on March 25, 2008, to discuss how to encourage innovative research and contributions from young investigators. A webcast of the summit is archived online at: http://www.macrovolt.com/live/dgi_032508/

NIAID has also sought input on priorities in vaccine research and on a possible program to foster innovative ideas in the realm of biomedical prevention of HIV infection.

Ideal Elements of Vaccines

The ideal vaccine would be safe, affordable, and easy to administer (e.g., a single injection). It would deliver long-lasting immunity, with efficacy against multiple HIV subtypes and complete protection against HIV infection in as many recipients as possible. It also would be easy to manufacture on a large scale, and to ship and distribute globally.

Adenovirus-Based Vaccines. As already discussed, the STEP results revealed an unsuspected interaction between Ad5 vectors and preexisting antibody responses to Ad5, which appears to have enhanced susceptibility to HIV infection. Because most people have antibodies against Ad5, these findings mean that Ad5 vectors likely cannot be used as a platform for future HIV vaccines. To try and address this problem, several alternate adenovirus vectors are being developed by the companies Crucell and GenVec. These include adenoviruses from less prevalent serotypes (Ad35 and Ad26), a chimeric vector comprised of Ad5 with the highly variable region replaced with components from the rare Ad48 serotype, and a chimpanzee adenovirus. The chimpanzee adenovirus vector is being developed by IAVI in partnership with GlaxoSmithKline.

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% to 20% of recipients, leading to considerable skepticism about its potential efficacy. A version of ALVAC is undergoing an efficacy evaluation in a 16,000-person Thai trial initiated by researchers affiliated with the U.S. Military HIV Research Program. The trial is fully enrolled and a Data Safety Monitoring Board (DSMB) review in July 2007 determined that no safety issues had emerged and that the study could progress to completion. Results are anticipated around 2009–2010.

Modified Vaccinia Virus Ankara strain (MVA) is an attenuated, nonpathogenic derivative of the cowpox virus. Data suggests 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 also entering a phase IIA immunogenicity trial under the aegis of the HIV Vaccine Trials Network. Two other MVA-based HIV vaccine candidates are also in human studies, one 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 NIH Division of AIDS.

DNA Vaccines. In the early 1990s, vaccine researchers were surprised to discover that simply injecting DNA sequences encoding protein antigens could induce substantial immune responses in mice. For a time there was much excitement about the potential of these “naked DNA” vaccines, particularly because they are extremely cheap and easy to produce. However, as studies escalated into those of larger animals and humans, it quickly became apparent that the immunogenicity of the vaccines declined dramatically in these settings. Several candidates have abjectly failed to induce detectable immune responses in humans.

Nevertheless, researchers have continued seeking to improve DNA vaccine immunogenicity, and the questions about the safety of viral vectors raised by the STEP trial have provided additional impetus for these efforts (unlike viral vectors, DNA vaccines have little in the way of extraneous components). A number of companies and researchers are exploring the use of cytokines such as IL-2 and IL-15 as DNA vaccine adjuvants, but results to date with these approaches have been profoundly disappointing. A more promising approach may be electroporation, which delivers a brief electrical charge to muscle cells into which the DNA vaccine has been injected. The electricity opens transient pores in local cell membranes, allowing the DNA vaccine 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. Wyeth has recently published promising animal immunogenicity data using this approach, and the International AIDS Vaccine Initiative (IAVI) has signed an agreement with the company Inovio to advance electroporated DNA vaccines for HIV into human testing. The laboratory of dendritic cell (DC) doyen Ralph Steinman has also recently published encouraging preclinical data with an approach that targets DNA vaccines to DCs, but this strategy has yet to be studied in humans.

Evaluating T-Cell Immunogenicity

ELISpot (Enzyme-Linked ImmunoSpot). This test 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,” or SFC). 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, and this is increasingly viewed as inadequate for capturing the full magnitude and functionality of a vaccine-induced T-cell response.

Intracellular Cytokine Staining (ICS). This test 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 year 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). These “polyfunctional” T cells produce a much greater quantity of cytokines on a per-cell basis than other T cells. Recent data suggests that these assays provide important additional information compared to interferon gamma ELISpot, but additional work is needed to standardize how these tests are performed in different laboratories.

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 a lab dish.

Table 2: PrEP and Microbicides Pipeline 2008

Product	Type	Manufacturer/Sponsor	Status
Tenofovir (Viread, TDF)	Nucleotide reverse transcriptase inhibitor	Gilead Sciences, Inc.	Phase III
Truvada (FTC, TDF)	Combined nucleoside and nucleotide reverse transcriptase inhibitors	Gilead Sciences, Inc.	Phase III
PRO 2000/5 Gel	Adsorption inhibitor	Indevus Pharmaceutical, Inc.	Phase III, Phase II/Ib (with BufferGel)
BufferGel™	Acid-buffering agent	Reprotect, LLC	Phase II/Ib (with PRO2000)
Tenofovir/PMPA Gel	Reverse transcriptase inhibitor	Gilead Sciences, Inc.	Phase IIb
Invisible condom	Entry/fusion inhibitor	Laval University (Division of Microbiology)	Phase I/II
Dapivirine (TMC120)	Reverse transcriptase inhibitor	International Partnership for Microbicides (IPM)	Phase I/II
VivaGel (SPL7013 gel)	Entry/fusion inhibitor	Starpharma Ltd.	Phase I/II
UC-781	Reverse transcriptase inhibitor	Biosyn, Inc.	Phase I
PC 815 (Carraguard + MIV-150)	Combination adsorption inhibitor/reverse transcriptase inhibitor	Population Council	Phase I
vaginal tablet	Uncharacterized mechanism	Talwar Research Foundation	Phase II
Terameprocol	Antiviral (inhibits Spt-1-regulated proteins)	University of Maryland/Erismos Pharmaceuticals	Phase I
Combination monoclonal antibodies (C2F5, C2G12 and C4E10)	Neutralizing antibodies	Polymun, EMPRO (European Microbicides Project)	Phase I

PreExposure Prophylaxis (PrEP)

PrEP is the prophylactic use of antiretroviral drugs to prevent HIV infection. Currently two drugs are being evaluated in phase II 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 CDC is sponsoring two ongoing efficacy trials of PrEP. 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 safety and acceptability study in 400 U.S. gay men will be completed next year. An NIH-sponsored efficacy trial of Truvada as PrEP in high-risk gay men in Peru and Ecuador—which underwent a long period of community consultation, planning, and

preparation—is now enrolling, with additional U.S. sites being added. The trial was initially slated to enroll 1,400 participants but will likely be expanded to 3,000 in order to bolster the statistical power and ensure that the efficacy results can be clearly interpreted. Three additional efficacy trials are also in the late stages of planning. The Microbicide Trials Network's VOICE study intends to compare three strategies in 4,200 African women: oral PrEP using tenofovir or Truvada versus a tenofovir-containing vaginal microbicide gel. Family Health International will study Truvada as PrEP in 3,900 African women and the University of Washington is launching a trial of tenofovir versus Truvada in 3,900 serodiscordant couples.

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. One major advantage to such interventions, if they are to work as intended, is that they could potentially be used by women who may not be able to control whether or not their partners use condoms. However, paralleling some of the debates that have occurred in the HIV vaccine field, there has been controversy regarding the potential effectiveness of the current lead microbicide compounds and the process by which candidates are selected for efficacy trials. Microbicide research has suffered a number of setbacks in the last few years, with two compounds, Savvy and Carraguard, failing to show efficacy and one, Ushercell, showing evidence of enhancement of HIV risk in some recipients. Questions have also arisen from the Carraguard trial regarding the inconvenience and difficulty of adhering to regular microbicide application, highlighting the importance of developing novel sustained-release delivery methods.

On a more positive note, the reorganization of the NIH clinical trials apparatus led to the formation of a specific microbicides trials network (MTN) under the leadership of Sharon Hillier. The first MTN efficacy trial will be the PrEP/microbicide comparison mentioned above.

The Ideal Elements of a Microbicide

The four guiding principles of microbicide design are that it be cheap, safe, effective and acceptable. Furthermore, it would be highly advantageous if someone could use a microbicide without detection by the sexual partner. A rectal product is also desirable, and studies in humans of these approaches are now getting underway.

Adsorption Inhibitors. These block the binding of HIV to target cells. After the failure of the adsorption inhibitor carageenan (Carraguard), announced early in 2008, PRO 2000 remains the only such product undergoing efficacy evaluation. Results from these trials are anticipated in 2009.

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, is being studied in a phase IIb efficacy trial with PRO 2000.

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 evaluated in an ongoing phase IIb efficacy trial in South Africa called CAPRISA 004. The reverse transcriptase inhibitor UC-781, originally developed by Uniroyal, is in a phase I trial sponsored by BioSyn. The International Partnership for Microbicides (IPM) is developing a nonnucleoside reverse transcriptase inhibitor, dapirivine gel (licensed from Tibotec and formerly known as TMC120); the organization hopes to move it forward into a phase III trial within the next few years. Following on the heels of these compounds are preclinical candidates that target attachment and entry of HIV; IPM has licensed the CCR5 inhibitor CMPD 167 from Merck and the attachment inhibitor BMS-378806 from Bristol-Myers Squibb.

An outlier among antiretroviral compounds is terameprocol, which is reported to have broad activity against HIV, HSV, and HPV due to blocking interactions with a group of host proteins (called Sp1) that are required for viral replication. Terameprocol is being developed by researchers at the University of Maryland. Another novel antiviral approach is being studied by the European Microbicides Project (EMPRO). The approach involves combining three monoclonal antibodies known to have broad neutralizing activity against HIV (2F5, 2G12 and 4E10) in a microbicide gel. A phase I dose comparison trial was launched in 2007.

Immune-Based Therapies (IBT) Pipeline 2008

Immune-based therapies (IBTs) are a broad category of treatments that aim to exert therapeutic effects by acting on the human 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-2 and 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.

The development of IBTs is hampered by the lack of a clear pathway toward approval. Given the success of combination antiretroviral therapies (ART), the logical focus for IBTs is addressing the limitations of ART. For example, studies show 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 studies consistently estimate to be ~5% of individuals on ART).

There is also a rationale for studying approaches, such as therapeutic vaccination, that might reduce dependence on ART by allowing safe interruptions of therapy. It was once thought that intermittent ART may be a viable treatment strategy on its own; this was evaluated in the 5,742-person Strategies for the Management of Antiretroviral Therapy (SMART) trial, which had to be stopped early because interrupting ART was associated with an increased risk clinical disease and death, and also an increased risk of cardiac, kidney and liver problems that heretofore were widely assumed to represent drug toxicities. Analyses of the SMART data indicates that the persistent immune activation that has long been known to be caused by HIV infection is driving these events by increasing levels of inflammatory cytokines (IL-6, for example). IBTs designed with the goal of allowing ART interruption will therefore need to reduce both viral load and immune activation in order to be worthy of further development.

Ideal Elements of Immune-Based Therapies

In addition to improving immune reconstitution and clinical health of people who remain immune suppressed despite ART, there are a number of other conceivable goals for IBTs:

- elimination of the need for ART by replacing ART or inducing post-ART remission/cure
- delaying the initiation of ART
- allowing safe intermittent use of ART
- supplementing the anti-HIV effects of ART (allowing ART to work for longer and/or enhancing the anti-HIV effects of ART)
- maintaining immune function in people for whom ART is failing
- targeting drug-resistant HIV in people with multidrug resistance

Each scenario presents its own challenges in terms of designing efficacy trials that could lead to licensure. As the IBT pipeline currently stands, however, few products are close to this stage of development.

Beyond the potential uses listed above, the desired characteristics of an IBT would be much the same as other therapies: broadly effective, safe, cheap, and convenient.

Table 3: Therapeutic Vaccines Pipeline 2008

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
AGS-004	Mature dendritic cells coelectroporated with autologous HIV-1 RNA and CD40L RNA	Argyros Therapeutics, ACTG	Phase I /II
VRC-HIVADV014-00-VP	Adenovirus serotype 5 vector containing <i>gag</i> , <i>pol</i> , and multisubtype (A, B, and C) <i>env</i> genes	VRC/NIAID	Phase I/II
LC002, a DermaVir Vaccine	DNA expressing all HIV proteins except integrase formulated to a mannosilated particle to target antigen-presenting cells	Research Institute for Genetic and Human Therapy (RIGHT), NIAID/ACTG	Phase I/II (adults) Phase I/II (children and young adults ages 6–23)
Autologous HIV-1 ApB DC Vaccine	Therapeutic immunization with autologous dendritic cells pulsed with autologous, inactivated HIV-1 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 cells pulsed w/ALVAC (vCP1452)	Canarypox vector encoding <i>env</i> , <i>gag</i> , the protease-encoding portion of the <i>pol</i> gene and CTL epitopes from the <i>nef</i> and <i>pol</i> gene products	ACTG/Aventis	Phase I
Autologous dendritic cell HIV vaccination w/ conserved HIV-derived peptides		University of Pittsburgh	Phase I
Multiepitope DNA	21 CTL epitopes and proprietary, non-HIV derived “universal” CD4 T-cell epitope	Pharmexa-Epimmune	Phase I
Tat vaccine	Recombinant protein	Sanofi Pasteur	Phase I
GTU- <i>nef</i> DNA vaccine	DNA encoding the subtype B <i>nef</i> gene	FIT-Biotech	Phase I
GSK protein HIV Vaccine	Recombinant Tat, Nef, and gp120 proteins in AS02A adjuvant	GlaxoSmithKline, Marcus Altfeld,	Phase I
VRC-HIVDNA016-00-VP	Six separate DNA plasmids containing <i>gag</i> , <i>pol</i> , and <i>nef</i> genes from HIV-1 subtype B, and <i>env</i> genes from subtypes A, B, and C	NIH Vaccine Research Center	Phase I
GW825780	DNA vaccine encoding a fusion protein incorporating epitopes from RT, Gag and Nef (delivered coated onto gold particles via gene gun)	GlaxoSmithKline	Phase I
GX-12	Multiantigen + IL-12 DNA vaccine	Genexine Co., Seoul National University Hospital	Phase I

Therapeutic Vaccines

Data generated by studies of long-term nonprogressors have played a key role in guiding the development of therapies aimed at bolstering the immune response to HIV. Recent studies have found that CD4 and CD8 T cells capable of performing multiple functions have advantages over those with more limited abilities 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.

A Farewell to Remune

The year 2007 saw the demise of the longest-running therapeutic vaccine candidate, Remune, a whole-killed, gp120-depleted immunogen developed by the late Jonas Salk in the 1980s. The manufacturer, the Immune Response Corporation (IRC), changed its name to Orchestra Therapeutics just in time to serenade the sinking ship of its therapeutic HIV vaccine program, which ended a few months later. Remune was widely derided by both scientists and community activists due the amount of hype that attended its initial development, hype that was not borne out when it failed to show any efficacy in a large phase III clinical end-point study that was carried out prior to the widespread availability of combination ART. However, immunogenicity studies indicate that it reliably induced HIV-specific CD4 responses, particularly Gag-specific responses, so it is ironic that it has become unavailable just as the importance of both CD4 responses and the targeting of Gag is receiving widespread acceptance.

The efforts of scientists at the Partners AIDS Research Center in Boston to study therapeutic vaccines provide a stark illustration of the frustrating illogic that has attended this area of HIV research. A small, placebo-controlled immunogenicity study of Remune led by Marcus Altfeld clearly demonstrated the induction of HIV-specific CD4 T-cell responses and, furthermore, showed that these responses are associated with a restoration of the proliferative capacity of HIV-specific CD8 T cells in study participants. HIV-specific CD8 T-cell proliferation has also emerged as a potentially key correlate of immune control over the past several years. However, the plans of Altfeld and colleagues to investigate whether these responses could enhance control of viral load in the context of ART interruption were stymied

shortly after the trial got underway, because Pfizer—who had optioned the vaccine from IRC—ended further development. Altfeld is now conducting a similar study with a protein-based vaccine developed by GlaxoSmithKline that does not contain HIV Gag, despite the fact that much of the data demonstrating the importance of targeting this antigen has come from researchers at Partners.

The Therapeutic Trial of Merck's Ad5 Vaccine

As described in the preventive vaccine section, Merck's Ad5-based HIV vaccine candidate failed to show efficacy and has now been discontinued. However, a prototype of the construct (encoding only HIV Gag) was also studied in a therapeutic trial conducted under the aegis of the AIDS Clinical Trials Group (ACTG). The trial design involved an analytical ART interruption in order to explore the potential of vaccine-induced T-cell responses to control HIV replication. Results were presented by Robert "Chip" Schooley at the Conference on Retroviruses and Opportunistic Infections (CROI) in 2008. The trial enrolled 114 individuals on ART with CD4 T-cell counts over 500 and viral loads that had been suppressed below 500 copies/ml for at least two years. Participation was limited to people with anti-Ad5 antibody titers of less than 1:200 units. The vaccine (or placebo) was administered on the same schedule used in the STEP study (months 0, 1, and 6) and ART was interrupted three months after the final immunization. Randomization was 2:1 vaccine versus placebo and 110 of the participants (73 vaccine, 37 placebo) continued into the ART interruption phase of the trial. ART was interrupted for 16 weeks but therapy was restarted if CD4 or viral load safety thresholds were crossed, or if a participant requested.

The coprimary end points of the trial were the area under the curve (AUC) of all viral load measurements during the 16-week treatment interruption—AUC is a method for calculating how much virus each individual was exposed to as a function of time—and viral load set point (the average of viral load levels at weeks 12 and 16 postinterruption). Schooley reported that favorable trends were observed for both endpoints ($p = 0.024$ and $p = 0.059$, respectively), but the prespecified level of significance (which required that both endpoints achieve p values <0.05) was not attained. Perhaps most interestingly, there was a very strong inverse correlation between preinterruption Gag-specific CD4 T-cell responses and postinterruption viral load ($p = 0.0001$). Since Merck's Ad5 is a poor CD4 T-cell immunogen (inducing responses in less than 50% of recipients in immunogenicity studies conducted in HIV-negative individuals), it appears possible that these results could be improved upon.

It is illuminating to contrast the response to these data with the discussion of the weak inverse correlations between preinfection HIV-specific CD8 T-cell responses and post-infection viral load that have emerged from the Ad5-seronegative

participants in the STEP trial. The preventive vaccine field is viewing these results as potentially very important, despite the fact the correlations are weak and based on 12 people. In contrast, the much more significant correlations reported in the therapeutic trial, which are derived from 110 people, appear to have generated no public discussion whatsoever.

A Narrowing Pipeline

In addition to the departure of Remune and Merck's Ad5 vaccine, therapeutic studies of the fowlpox and modified vaccinia Ankara strain (MVA) candidates being developed by Therion have fallen by the wayside because the company went out of business (the NIH's Division of AIDS has picked up the constructs for continued evaluation in the preventive context). But a number of new or modified candidates have emerged since TAG's 2007 report. A company called Bavarian Nordic has been developing an MVA candidate encoding the HIV Nef protein, and at CROI in 2008 reported that receipt of the vaccine was associated with significantly lower viral load subsequent to ART interruption compared to individuals that received MVA without any HIV antigens; however this was not a large study (there were a total of 37 participants that rolled over from an immunogenicity study into an ART interruption phase). Bavarian Nordic is now launching a phase I study of a modified version of this MVA construct encoding seven additional HIV antigens.

A novel idea that was proposed several years ago is that it might be possible to induce CD8 T-cell responses that specifically target drug-resistant strains of HIV. The National Cancer Institute recently initiated the first trial of such an approach, involving a peptide vaccine designed to stimulate responses against the M184V 3TC (Efavir) resistance mutation. Sharon Riddler from the University of Pittsburgh has been working for a long time on developing a dendritic cell (DC)-based therapeutic vaccine approach that involves pulsing DC with inactivated HIV-infected cells, and a phase I/II trial is now getting underway.

A Norwegian company, Bionor Immuno AS, is planning a phase II trial of a therapeutic vaccine containing peptides from the HIV-1 Gag p24 protein. The decision to move the candidate forward is based on results from earlier phase trials, which suggested that individuals mounting a strong response to the vaccine were able to undergo a prolonged interruption of ART (the magnitude of response was associated with a lack of clinical indication for reinitiating ART two years subsequent to interruption).

The only other new candidate to enter trials is a DNA vaccine developed by Genexine in Korea called GX-12. The vaccine encodes multiple HIV antigens and the cytokine IL-12, and a phase I trial is recruiting participants at the Seoul National University Hospital.

Table 4: Cytokine, Immunomodulator, and Gene Therapy Pipeline 2008

Agent	Type	Sponsor	Status
Valganciclovir	Antiviral	Roche Pharmaceuticals/ UCSF	Phase IV
Interleukin-2 (IL-2)	Cytokine	Novartis	Phase III
Chloroquine phosphate	Antimalarial	University of Minnesota	Phase II
Pyridostigmine	Acetylcholinesterase inhibitor	Dept. of Infectious Diseases, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán	Phase II
Atorvastatin	HMG-CoA reductase inhibitor	Pfizer/NIAID	Phase II
Pegasys (peginterferon alfa-2a)	Cytokine	Roche Pharmaceuticals	Phase Ib/II
PEHRG214 Passive Immunotherapy	HIV-specific goat antibodies	Virionyx Corporation	Phase I/II
Palifermin (recombinant human keratinocyte growth factor)	Fibroblast growth factor	Amgen	Phase I/II (ACTG 5212)
Interleukin-7 (IL-7)	Cytokine	Cytheris	
	Phase I		
HLA-B*57 cell transfer	Cell infusion	NIH Clinical Center	Phase I
OZ1 ribozyme gene therapy	Antiviral ribozyme targeted against the <i>tat</i> gene, introduced into CD4 T cells via stem cells	Johnson and ohnson	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

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. Since last year's report, a number of small, investigator-initiated trials have been launched. Drugs under study include pyridostigmine, a drug approved for treating muscle weakness in myasthenia gravis; atorvastatin, a lipid-lowering agent; and valganciclovir, an anti-CMV drug. The malaria drug chloroquine phosphate is also being studied for both direct anti-HIV and anti-inflammatory effects.

Cell infusion and gene therapies. Several phase I/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. Early phase studies have indicated that the anti-HIV genes being employed can persist in CD4 T cells, but the therapeutic impact remains unknown. Results from these trials should be forthcoming over the next several years.

The intramural at the National Institutes of Health has launched a novel phase I trial of a cell infusion approach. The study will take white blood cells from individuals possessing the class I HLA gene B*57 who are controlling their viral load to undetectable levels without treatment (elite controllers) and transfer them to individuals with the same HLA B*57 gene who show evidence of disease progression (CD4 count less than 400 and viral load >10,000 copies).

Palifermin (recombinant human keratinocyte growth factor). Palifermin, manufactured by Amgen, is a recombinant form of a naturally occurring human protein, keratinocyte growth factor (KGF). Palifermin is licensed by the FDA to reduce the incidence of severe mucositis in people receiving cancer chemotherapies. The ACTG is conducting an ongoing trial to evaluate whether Palifermin can help restore CD4 T-cell counts in individuals with a discordant response to HAART (controlled viral load but an inadequate rise in peripheral blood CD4 T-cells). Studies in macaques undergoing transplantation have reported that KGF significantly increases naive T-cell counts and improves the immune response to new immunizations.

IL-7. This 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 manufactured by Cytheris is now in phase I trials. Glycosylation improves the pharmacokinetics of IL-7 and should allow weekly dosing.

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