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Whereas federal expenditure in the United States on the development of an HIV/AIDS vaccine is approximately \$250 million, only \$25 million is spent on research and development for a malaria vaccine. It is not malaria but tuberculosis (TB) that is the poor relation. Government funds for a vaccine to fight this disease are in single figure millions. Stefan Kaufmann of the Max Planck Institute for Infection Biology examines obstacles in addition to funding that hinder the development of a new TB vaccine.

Is the development of a new tuberculosis vaccine possible?

“Since the time people have realised tuberculosis is preventable and since they have learned how to avoid infection, mortality rates caused by tuberculosis have declined in industrialised countries and signs are starting to appear that it can be eliminated. This is the right time to combat tuberculosis.” These words were spoken a century ago by the discoverer of the tubercle bacillus, Robert Koch. “I have a very distinguished group of leaders here ... who are profoundly interested in joining forces to fight against diseases that kill people and progress in the world’s poorest countries. Diseases like AIDS, tuberculosis and malaria...” With these words, United States President, Bill Clinton, opened a vaccine research meeting at the White House on 2 March this year.

Sadly, we are no closer to eliminating or even controlling tuberculosis (TB) today than we were when Koch first identified the causative agent, *Mycobacterium tuberculosis*. Every minute, more than 10 individuals develop TB, amounting to 8 million new cases annually (Fig. 1). Two to 2.5 million of these TB sufferers will die of the disease. These appalling figures put TB in the unfavourable list of the top major killers, together with AIDS and malaria¹. The situation is worsened by the increasing incidence of multidrug resistant (MDR) strains, and the deathly combination of TB with AIDS. Co-infection with HIV and *M. tuberculosis* increases the risk of developing TB 30-fold.

STEFAN H. E. KAUFMANN

The biggest burden of TB is in Southeast Asia where there are 3 million TB cases per year. Sub-Saharan Africa has nearly 1.6 million cases annually. But what we are seeing is not the re-emergence of a disease that had been controlled well in the past; rather, it is the resurgence in awareness in western countries of a problem that had always existed globally, but which had seemed to be in retreat in the industrialized world. For example, the incidence of TB increased in several parts of the United States in the early 1990s, incidence has risen sharply in several formerly socialist states², and the number of cases of TB in the UK has risen by 80% in the last 10 years.

Do we really need a new vaccine?

The current vaccine against TB, bacille Calmette-Guérin (BCG), was developed by the French scientists Calmette and Guérin in the first decade of the last century. BCG has been delivered for more than 70 years and has been given to more people than any other vaccine (more than 3 billion individuals and around 100 million newborns annually). Its side effects are tolerable, and it can prevent miliary and meningeal TB in young children to an appreciable degree. In striking contrast, BCG fails to protect against the most prevalent disease form, pulmonary TB in adults. In fact, data concerning the protective efficacy of BCG in adults range from 0% in South India to 80% in the UK (ref. 6). Although a meta-

TUBERCULOSIS**A global threat:**

- 2 billion infected
- 8 million new cases annually
- 2 million deaths annually

AIDS & TB, a dangerous liaison:

- >10 million coinfectd
- >1/2 million additional deaths annually

CONTROL MEASURES**Chemotherapy:**

- Works, but poor compliance
- Increasing incidences of MDR TB
- > 3% of all TB cases in several countries
- 50 million infected with MDR organisms
- 100-fold increase in cost

Vaccination:

- Prevents severe forms of childhood TB
- Ineffective against adult pulmonary TB

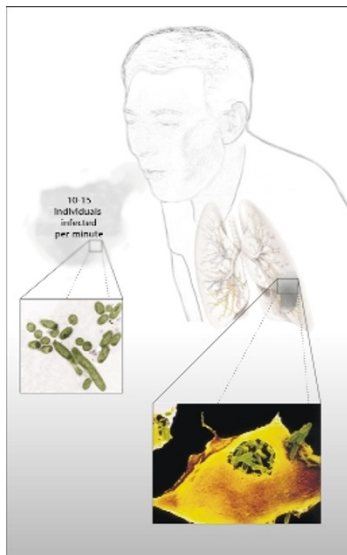


Fig. 1 TB: the continuous scourge of humankind.

analysis of all vaccination data available has yielded a theoretical efficacy rate of 50%, it has been estimated that only 5% of all vaccine-preventable deaths caused by TB could have been prevented by BCG. Thus, BCG is not a satisfactory vaccine.

But do we really need a new vaccine given that TB can be treated by chemotherapy? In this regard, two obstacles exist: first, TB drugs (isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol are typical of the drugs included in the directly observed treatment, short-course strategy (DOTS) program; see box) (ref. 2) must be taken for long periods of time (usually 6 months or longer); second, a combination of three specific drugs is required to avoid the development of drug resistance. Both obstacles severely affect compliance and with more than 1 billion bacteria in the lung of a patient suffering from active TB, poor compliance can easily lead to the development of resistant strains. Accordingly, TB therapy is expensive, costing from \$10 to

\$1000 and with the incidence of MDR-TB strains on the rise—currently 50 million people are infected with MDR strains of *M. tuberculosis*²—the disease can become virtually impossible to cure in poor countries, by reason of its prohibitive costs that can reach \$250,000. Thus, there is an urgent need to develop an effective and cheap medical intervention against this disease, namely an efficacious vaccine.

Is a TB vaccine possible at all?

Imagine the following scenario: one of the heroes in the TB field proclaims he has developed a therapeutic vaccine for TB based on his success in curing the disease in experimental animals. Because of the reputation of the scientist, and to proceed as fast as possible, controlled clinical trials comprising almost 2000 TB patients are immediately initiated by a governmental agency and the results already provided after 6 months. Due to great economic interest, the product is licensed to a pharmaceutical company, making the investigator a millionaire. The story is true. It occurred between August 1890 when Robert Koch, the highly respected discoverer of the TB bacillus, proclaimed at the 10th International Congress of Medicine that he had found a remedy for TB, and February 1891 when the official report on the clinical trials was published. Unfortunately, the report was crushing, with only 2% cured. We cannot risk such a fiasco again and it is therefore appropriate to ask whether the task of developing a TB vaccine is too formidable, even today.

Discussion on the feasibility of a TB vaccine normally receives support from the fact that less than 10% of the 2 billion individuals infected with *M. tuberculosis* develop active TB. It is generally assumed that active TB occurs because of a weakening of the immune system, which keeps *M. tuberculosis* in check as long as it is fully competent. A lot of truth lies in this assumption, which is best supported by the highly increased incidence of TB in AIDS patients. But is it also correct to assume that *M. tuberculosis* induces the maximum protection that can be achieved? Almost by definition, it is not the optimum protection because the pathogen is not eradicated. Thus, it is generally believed that individuals with endogenous *M. tuberculosis* infection do not become re-infected be-

Table 1 TB vaccine candidates

Vaccine Candidate	Potential Advantage	Potential Disadvantage
1. Subunit Vaccine: Antigen in adjuvant	Protective antigens, low side effects	Restricted number of T-cell clones, primarily CD4+, low immunogenicity, short-lived
Naked DNA	Protective antigens	Restricted number of T-cell clones, primarily CD8+, persistence, safety concerns
Recombinant carrier expressing antigen	CD4 ⁺ and/or CD8 ⁺ T cells, protective antigens	Restricted number of T-cell clones, safety concerns
2. Whole Bacterial Vaccine: <i>M. tuberculosis</i> deletion mutant	CD4 ⁺ CD8 ⁺ T cells, unconventional T cells	Safety concerns
rBCG expressing cytolysin	CD4 ⁺ CD8 ⁺ T cells, unconventional T cells	Devoid of TB-specific antigens, safety concerns
rBCG expressing cytokine	Improved immunogenicity, unconventional T cells	Primarily CD4 ⁺ T cells, devoid of TB-specific antigens, safety concerns
rBCG overexpressing antigen	Protective antigens, unconventional T cells	Primarily CD4 ⁺ T cells, safety concerns
3. Combination Vaccine: rBCG coexpressing immunomodulator + antigen	Improved immunogenicity, protective antigens	Safety concerns
r <i>M. tuberculosis</i> deletion mutant expressing immunomodulator	Improved immunogenicity	Safety concerns
Prime-boost	Improved immunogenicity	Safety concerns



Fig. 2 Looking back: Koch's greatest success and failure. Upper: Robert Koch, the discoverer of the TB bacillus, in his laboratory. Lower: Treatment of a TB patient with Koch's remedy, a therapeutic vaccine that turned out to be non-efficacious and even harmful.

tive immune response against TB, CD8⁺ T cells are needed as well. In addition to these two major T-cell populations, unconventional T cells, namely $\gamma\delta$ T cells and CD1-restricted $\alpha\beta$ T cells, apparently participate in optimum protection. In contrast to *M. tuberculosis*, BCG fails to adequately stimulate CD8⁺ T cells, probably because it has lost essential cytolysins. On the other hand, the ligands for $\gamma\delta$ T cells and CD1-controlled T cells are present in BCG.

How do these T cells function in protection? First, there is general agreement that activation of antimicrobial activities in macrophages by T cell cytokines is involved. Accordingly, interferon (IFN)- γ , which is a major macrophage-activating cytokine, and other Th 1 cytokines are critical. Second, direct killing of mycobacteria by T cells has been demonstrated. Third, mycobacteria reactive T cells lyse infected macrophages. Macrophage lysis appears to be a prerequisite for killing by T cells of microbes residing inside macrophages. Moreover, lysis of infected macrophages could promote release of mycobacteria from incapacitated macrophages to more proficient monocytes.

Although CD4⁺ T cells are considered as the major source of IFN- γ , other T cell populations have also been shown to produce this and other Th1 cytokines. Similarly, CD8⁺ T cells are mainly responsible for the killing activities although additional T cell sets, in particular CD1-restricted T cells, can perform these functions. This redundancy should not be misinterpreted as meaning that a single T cell population would suffice for protection. These T cell populations differ in other capacities, including antigen specificity, genetic restriction and activation requirements. Hence, it appears unlikely that any one T cell population could fully compensate for another. Applying this scenario to vaccine development, one can easily envisage two major strategies: The subunit vaccine approach would be satisfied with a few antigens recognized by CD4⁺ T cells and perhaps CD8⁺ T cells, whereas a whole bacterial vaccine approach would consider stimulation of as many T cell populations as possible to be more appropriate. Due to the ambiguous complexity of the immune response to TB, a distinct correlate of protection in man has not been determined. Probably a combination of quantitative correlates rather than a single qualitative read-out will be required. This will be essential before any human vaccine trial can be initiated.

Which animal model?

As for many infectious diseases, there is no ideal experimental animal model for TB. Although the mouse provides the deepest insights into the immune mechanisms underlying vaccination, it is more resistant to *M. tuberculosis* than man and does not fully mirror human pathology, for example, as regards the granulomatous lesion. To further complicate the situation, mouse strains with different susceptibility to TB exist and different strains of *M. tuberculosis* express different virulence. The guinea pig is extremely susceptible to *M. tuberculosis* and will rapidly die from only one or two virulent organisms, and the pathology in guinea pigs strongly resembles

cause of the efficacious immune response induced by the primary infection. With the availability of potent drug treatment and DNA fingerprinting for strain-specific diagnosis, the issue of immunity to re-infection has been re-examined³. It has now been shown that exogenous re-infection does occur, suggesting that natural immunity is insufficient. Consistent with this, *M. tuberculosis* infection in mice followed by chemotherapeutic eradication of the pathogen does not provide full protection against secondary *M. tuberculosis* challenge infection.

A conclusion from these findings would be that the immune response fails to control the pathogen in the long run, without necessarily being weakened by exogenous insult, thus allowing reactivation. In this scenario, the host's genetic predisposition and disturbance of the immune response by *M. tuberculosis* would explain disease in a substantial proportion of the 10% of infected individuals who develop TB. So vaccinologists are faced with a difficult hurdle—to design a vaccine that is superior to the pathogen with regard to the immune response evoked.

The immune response

M. tuberculosis misuses the phagosomal compartment of macrophages as its preferred habitat (Fig. 3) (ref. 7). Accordingly, its antigens have ready access to the antigen processing machinery encoded by the major histocompatibility complex (MHC) class II. This results in the activation of *M. tuberculosis*-specific CD4⁺ T cells of T-helper 1 type (Th1). Mouse experiments have also provided evidence for an additional participation in protective immunity of CD8⁺ T cells that are restricted by MHC class I. Thus, although CD4⁺ T cells play a major role in the build-up of an optimal protec-

that in man. Although non-human primates are susceptible to TB, these species have only recently been exploited in more depth for TB research, with the cynomolgus monkey, *Macaca fascicularis* being the most promising model⁸.

Thus, the mouse and guinea pig provide important and often complementary answers to TB vaccine questions. Because of the wealth of information on its immune mechanisms, the availability of a huge array of gene knockout strains, and because of its relatively low cost, the mouse appears to be very important for rational vaccine development. Before proceeding to human studies, however, protection assays in the guinea pig should show success. A more empirically oriented research approach would of course allow the reciprocal sequence, that is, screening for highly efficacious vaccine candidates in the guinea pig first and subsequent analysis of the underlying immune mechanisms in the mouse. Only the most promising vaccine candidates should then be considered for non-human primate experiments.

In summary, as for many infectious diseases, we don't have an ideal experimental animal model for TB. This is even truer if we think of models for latency and reactivation of TB and, as a corollary, of post-infection or immunotherapeutic vaccines rather than pre-infection vaccination of naïve animals. On a positive note, the TB system benefits from having a gold standard in BCG, which provides a significant degree of protection in all experimental animal models.

The new vaccine candidates

Given that T cells are central to protection against TB, future vaccine design should focus on T-lymphocyte populations. Unfortunately, there is no precedent for this because all successful vaccines in use today work through antibodies rather than T cells. Moreover, most vaccines do not prevent infection but instead disease, that is, they allow establishment of the pathogen in the host but prevent its harmful effects. Finally, vaccines are generally given pre-infection as opposed to post-infection—vaccination is generally preventive rather than therapeutic.

For TB, these issues deserve some reconsideration. With one third of the world population already infected with *M. tuberculosis*, and thus living with a time bomb, there is a sufficiently large group to be considered as targets for a post-infection vaccine. Moreover, with the increasing risk of MDR-TB and co-infection with HIV and TB, therapeutic vaccines may warrant specific consideration. A recent publica-

tion reported successful therapy of TB in mice by vaccination with a DNA construct encoding hsp60 (ref. 9). Obviously, a vaccine that could prevent infection would be ideal because the host initially encounters minute numbers of *M. tuberculosis* organisms. Here, a theoretical niche for antibodies exists. If a vaccine could induce antibodies that eliminate *M. tuberculosis* in the alveolar space before they invade macrophages, this would clearly solve all problems. Unfortunately, this scenario remains a dream with a low possibility of being fulfilled. A more likely prospect is vaccine-induced immunity that attacks the pathogen after it has established itself inside macrophages—a task that is the exclusive realm of T lymphocytes.

Table 1 shows current vaccine candidates. The subunit approach relies on the assumption that one or only a few antigens are sufficient to induce a protective immune response. With the recent availability of the *M. tuberculosis* genome and increasing information about gene expression from transcriptome and proteome analyses, the race for protective antigens is wide open^{4,10,11}. Whole genome DNA microarray techniques have revealed 129 *M. tuberculosis*-specific open reading frames that are absent in the genome of the BCG vaccine strains¹⁰. Obviously, the *M. tuberculosis* genes absent in BCG represent candidates not only for virulence factors, but also for protective antigens. Screening of the *M. tuberculosis* genome has thus initiated a hunt for genes of interest for vaccine development, either as targets for gene knockout or as candidates for protective antigens.

Despite the wealth of information from genomics and proteomics, clear parameters that define protective antigens are not available. Are there *M. tuberculosis*-specific antigens that are absent in BCG? Are they secreted or cell-bound antigens? Or are they antigens with unique functions? Perhaps they could be the most abundantly expressed proteins? Or they could be proteins expressed exclusively *in vivo* and absent in *in vitro* cultures? Current data do not provide a clear plan of action and we are left with the daunting task of screening each antigen as a vaccine candidate. This is probably best achieved with naked DNA constructs, which are easily made and which have a proven degree of vaccine efficacy. Protective antigens identified thus far include the members of the antigen 85 family and hsp60 (refs. 12,13) and both have afforded a degree of protection in mouse experiments although not exceeding that provided by BCG.

Malaria vaccine researchers have gone a step further and

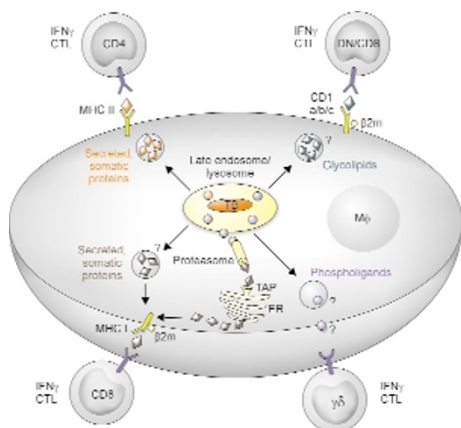


Fig. 3 The T-cell populations and antigens in protective immunity against TB. Compelling evidence suggests that the different T-cell populations shown participate in protective immunity against TB. *M. tuberculosis* resides in phagosomes at the late endosome/lysosome stage. From there, its antigens are transported by MHC class II molecules to the cell surface and stimulate CD4⁺ T cells. Some protein antigens are presented by MHC class I molecules, stimulating CD8⁺ T cells. Glycolipid antigens are presented by group 1 CD1 molecules (CD1a, b or c) to double negative (DN) or CD8⁺ T cells. Finally, small metabolites, which contain phosphate (phospholipids), are presented to $\gamma\delta$ T cells in the absence of known presentation molecules. All T cell populations have been shown to secrete IFN- γ and to express cytolytic T lymphocyte (CTL) activity to varying degrees.

DOTS strategy

To reduce the burden of TB, the WHO has actively promoted the DOTS strategy². One-hundred-and-nineteen of the 212 member states of WHO had adopted DOTS by the end of 1998, although coverage varied significantly. Where fully implemented, DOTS has proven its efficacy. WHO is aiming to detect 70% of existing cases of active TB, and to cure 85% of detected cases; yet by the end of 1998, only 21% of all TB cases were under DOTS treatment. DOTS requires daily delivery of drugs by health workers for 6 months and is reliant on a well-organized national TB control program. There are numerous difficulties in implementing DOTS, ranging from technical problems, such as detection difficulties due to antiquated tools and the lack of a single pill combining the drugs, to insufficient motivation and training of health workers, not to mention the difficulties in ensuring patient compliance. Hence, the impact of DOTS on global TB incidences probably will not fulfil expectations completely.

tackled the question of protective antigens with a vaccinomics approach, that is, an expression library immunization independent of the biological features of the candidate antigens¹⁴. Once protective antigens have been identified, synthetic polypeptides combining promiscuous protective epitopes from several antigens can be constructed. The success of any protein vaccine will depend not only on the identification of a protective antigen but also on a potent adjuvant that best improves its immunogenicity. Although naked DNA vaccines have appreciable immunogenicity, further improvements would be highly desirable. Recombinant carriers include attenuated salmonella and the vaccinia virus. The former has the advantage that it can be given orally and thus stimulates a mucosal immune response, the latter of being a potent stimulator of CD8⁺ T cells. Both have proven their feasibility, mostly in other systems.

The whole bacterial vaccine approach follows the notion that several antigens—proteinaceous and non-proteinaceous in nature—act together to achieve maximum protection (Table 1). In addition, whole bacterial vaccines profit from built-in adjuvanticity. The methodologies for establishing deletion mutants in *M. tuberculosis* have been refined and the proof of principle has been established, that is, deletion of single genes can cause sufficient attenuation¹⁵. Given that *M. tuberculosis* impairs the immune response and hence induces insufficient protection, targets for deletion would include not only classical virulence factors but also immunosuppressive components. These include inhibitors of macrophage activation because the best T cell fails if macrophages can't be fully activated. In a similar line, attempts have been made to improve the immunogenicity of BCG either by enhancing its CD8⁺ T cell stimulating capacity or by endowing it with Th1 cell-inducing cytokines¹⁶. BCG can also be engineered to over-express distinct antigens, either because it lacks the encoding gene or because it produces the antigen in insufficient quantity.

The combination-vaccine strategy aims to combine the best of the two approaches. Both BCG and *M. tuberculosis* might be improved combining two of the options mentioned above. Alternatively, prime-boost strategies, which have al-

ready demonstrated their efficacy in the experimental malaria model, are being applied to TB vaccination¹⁷.

All these approaches are at the stage of animal experiments, mostly in the mouse, and none has come up with a spectacular result, that is, markedly exceeding the efficacy of BCG. So, where should we set the threshold of satisfaction for a vaccine? Can we be satisfied with a candidate that affords a degree of protection equal to that of BCG if the new vaccine candidate was made because BCG is considered insufficient? Or should we wait for a candidate that achieves sterilizing immunity both pre- and post-infection, worrying that this goal will never be achieved. It can be argued that a subunit vaccine as efficacious as BCG would have certain advantages such as higher safety, in particular in immunocompromised hosts, as well as providing the possibility to distinguish between vaccinated and infected individuals. In contrast, a whole bacterial vaccine clearly needs to be significantly better than BCG if it is to be exploited further. Whatever the answers to these questions, any new vaccine seriously considered for human trials should prove its superiority to BCG in experimental animal models.

Is there a solution?

From what has been said, one can easily see why development of a vaccine for TB does not presently appeal to industry. First, proof of principle is still missing, namely the scientific rationale that something better than BCG can be designed. A second disincentive concerns clinical trials—they would easily comprise tens to hundreds of thousands of subjects, and involve protracted periods of time, given that the disease develops years or decades after primary infection. Clearly, this will be a long-term endeavor counted in decades rather than years, starting with experimental research, animal testing and phase I and II human trials, before proceeding to a large-scale long-term phase III field trial¹⁸. By the beginning of phase III trials at the latest, there needs to be accord about immunological correlates of protection, and perhaps pathogenesis, in humans. Even in populations with high TB incidences, pre-infection trials can easily exceed a decade. A post-infection vaccine trial in a high-risk population could be shorter, but would still last some years.

Fortunately, several incentives have recently been stipulated by President Clinton's Millennium Vaccine Initiative, such as tax credits for vaccines, ensured future markets in developing countries, low interest loans by the World Bank for health services, and significant increases in funding vaccine-targeted research to attract the private sector. Also the European Commission, the National Institutes of Health, and private foundations (notably the Bill and Melinda Gates Foundation), have instigated several mechanisms to support TB-oriented research and clinical trials. Hopefully, the sudden political interest in vaccine development will provide a unique opportunity for the TB research community.

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The discovery of coregulators and other recent advances in our understanding of the molecular biology of nuclear receptor action have generated expectations that these exciting basic advances will be translated into new diagnostic and therapeutic approaches for endocrine diseases such as breast cancer.

An issue of tissues: divining the split personalities of selective estrogen receptor modulators

Tamoxifen is the most popular prototype for a new arsenal of drugs, termed selective estrogen receptor (ER) modulators (SERMs), which elicit a complex array of tissue-specific effects. The ER belongs to the nuclear receptor (NR) superfamily—the largest group of metazoan transcription factors—whose members mediate the developmental, metabolic and physiological effects of steroid, thyroid, retinoid and vitamin D₃ hormones. The intense scrutiny to which ER α —and more recently, ER β —have been subject in breast cancer research attests to the historical association between estrogens and development of the disease. The fruits of this research have been realized in a 25% decline in breast cancer mortality rates in the UK and US over the last decade¹, a decrease attributed largely to the effects of ablative therapy using tamoxifen. Despite this success, a large number of breast tumors evade tamoxifen therapy and proceed to hormone-independent growth, a perennial problem in breast cancer treatment. Furthermore, as was discussed at great length in the recent 2nd Department of Defense Era of Hope Breast Cancer Research Program Meeting², the elaborate pharmacology of the SERMs presents obstacles to their broader clinical applications. By evaluating selected current models of ER pharmacology in breast cancer etiology, we will seek in this commentary to sketch the clinical implications of recent developments in NR signaling.

Estrogens bring their potent mitogenic stimuli to bear in the G₁ phase of the cell cycle. Recent evidence indicates that key factors in two pathways, namely c-Myc and cyclin D1, are central to these effects. A multistep pathway, involving phosphorylation of pRB by cyclin-dependent kinases and release of E2F transcription factors required for DNA synthesis, culminates in progression from the G₁ to the S phase of the cell cycle. ER ear-

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marks a primary breast tumor for an initially positive prognosis and the probability of a good response to hormone ablation therapy. Indeed, a striking pathogenetic symmetry exists between the role of the ERs in breast cancer and that of another NR superfamily member, the androgen receptor, in prostate cancer. Of the ER-positive breast tumors selected for tamoxifen treatment, roughly 70% will respond initially. As treatment progresses however, a considerable proportion of tumors will acquire hormone resistance and fail to respond to tamoxifen. To compound matters, tamoxifen is a two-edged sword. Although it opposes estrogen activity in the breast, it is an estrogen mimetic in the uterus, an important consideration in its prophylactic use against breast cancer. Conversely, raloxifene, an FDA-approved second-generation SERM, although estrogenic in its reduction of the severity of postmenopausal osteoporosis, is, broadly speaking, antiestrogenic in the breast and uterus.

Three central issues are raised by these clinical observations. First, what mechanisms in the breast tumor uncouple cell cycle progression from estrogen regulation? Second, how can the opposing effects of SERMs in different tissues in the body be reconciled, and how can these characteristics be manipulated to improve prospects for their future therapeutic applications? Third, can we accurately predict the tissue-specific effects of new NR ligands? Although full answers to these questions are beyond the scope of this commentary, we will provide clues that are now emerging from a rapidly developing chapter in NR action: co-regulators. Their characterization is fleshing out the linear depiction of NR function, which existed until the middle of the last decade, and is establishing a model in which the perplexing pharmacology of SERMs might finally be resolved.

The tripartite structure of NRs is defined by a central se-