Synopses of Research Articles

Casting Doubt on the Role of Mitochondria in Tumorigenesis

DOI: 10.1371/journal.pmed.0020373

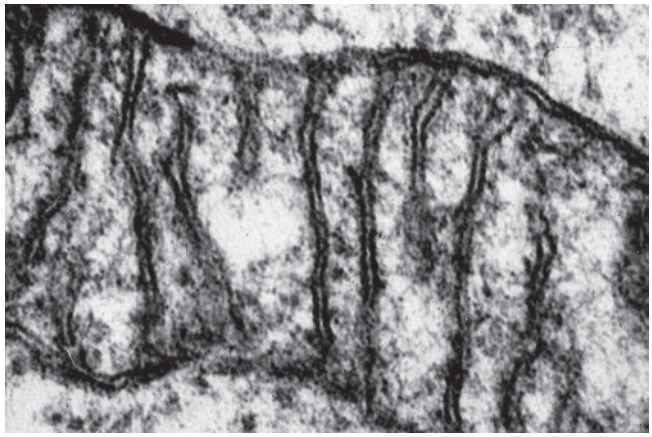
Mitochondrial DNA (mtDNA) has been intensively studied over the past two decades, and point mutations, more commonly known as deletions, of this DNA are known to be involved in several syndromes. Unlike nuclear DNA, with 46 chromosomes, half from each parent, mtDNA is just one piece of genome of which there are many copies, but all copies come only from the mother. Mitochondrial disease syndromes, such as MELAS, have a range of different clinical manifestations depending on how many copies of the abnormal mtDNA are present in affected cells.

There are several international resources of mtDNA sequences. From these sequences, it has been possible to show that different population groups have different patterns of substitutions in the mtDNA—so-called haplogroups; this information has been used, for example, in the investigation of the origin and migration patterns of human populations, and some investigators have even suggested that it could be used to trace back to earliest human history the founding mothers of humanity.

More recently, however, attention has turned to the question of whether mtDNA is involved in tumor formation. However, deciding whether mutations are harmful or innocuous has been difficult. One concern is that isolation of mtDNA from any tissue is not simple, and may be particularly difficult from tumor samples, which are often contaminated with exogenous DNA.

In a hard-hitting paper, Antonio Salas and colleagues cast more doubt over a causal role for mtDNA alterations in tumors; they have now reassessed many of the studies that have examined the role of mtDNA in tumorigenesis, and concluded that much of the data are at best questionable. The group used a phylogenetic approach to analyze the reported work, which compared the sequences under consideration with the current database of complete sequences of mtDNA. The authors believe that such an approach is essential to look at the overall picture of the mtDNA rather than assessing each substitution in the mtDNA independently.

Salas and colleagues conclude that more than 80% of published mtDNA sequencing studies contain obvious errors, and that many of the published results that implicate mutations in tumorigenesis are in fact part of normal population variability, and their presence must be due to contamination of the tumor sample. Salas does not hold back in his criticisms. He states that the result of such sequencing "disasters" is that flawed results are not filtered out from the clinical literature, which makes the task of interpreting the role of mtDNA in the tumor process very difficult.



DOI: 10.1371/journal.pmed.0020373.g001

The Role of Mitochondria in Tumorigenesis is not yet clear

The researchers draw analogies to the time when ancient DNA sequencing was beginning, and many contaminated samples were amplified and claimed to yield prehistoric DNA. They urge clinical geneticists to use checks similar to those proposed to assess authenticity for ancient DNA studies: special care should be taken in sequencing and documentation, raw sequence data should be made fully accessible to referees and readers, and the complete record of data from the population genetics field should be used to put the results in context.

The group's findings will undoubtedly cause much debate in this research community. With their conclusion that "there is no precedent that we know of in the genetics literature for such a high number of flawed papers (most of them published in high-rank journals), which affect a whole subfield of clinical research," they urge reconsidering the role of mtDNA in tumorigenesis.

Salas A, Yao YG, Macaulay V, Vega A, Carracedo Á, et al. (2005) A critical reassessment of the role of mitochondria in tumorigenesis. DOI: 10.1371/journal.pmed.0020296

First Trial of Male Circumcision against HIV

DOI: 10.1371/journal.pmed.0020391

Over 3 million people died of AIDS last year and about 5 million others became infected with HIV, bringing the total number of people living with the infection to nearly 40 million. The continuing rise in the number of new cases makes it a priority to investigate all possible measures that might reduce the risk of infection, particularly—but by no means only—in Africa, which has 10% of the world's population but two-thirds of the world's people with HIV.

In many African tribal groups, men are circumcised, usually in late childhood or as teenagers, and this is an important part of their cultural identity. In other African ethnic groups, men are not circumcised. From observational studies dating back to the 1980s, it has become clear that HIV infection rates are greater in those groups where men were not circumcised. It has, however, remained a matter of speculation as to whether it is circumcision itself or some other difference in behavior that has a protective effect. What has been needed to settle this question is a randomized controlled trial (RCT) of the use of circumcision as a preventive intervention.

Auvert et al. have completed the first such trial in the Orange Farm area, a semiurban region close to Johannesburg, South Africa. They offered young, heterosexual uncircumcised men the chance to have the operation, explaining that half of those who came forward

would be circumcised straightaway and the others (the control group) 21 months later. Some 3,000 men joined the study. It was planned that all the men would visit the research clinic four times during this 21-month period, and that they would be tested for HIV each time. They were instructed not to have sex for six weeks after the operation, and asked at each clinic visit to provide detailed information about their sexual activity. However, after 17 months, the number of new infections in the control group (49) was so much greater than the number in the treatment group (20) that it was considered unethical to continue the study, which was thus terminated early. Since infections were 60% lower in the treatment group, we now have strong evidence favoring the use of circumcision as part of prevention efforts.

Preliminary results from the Orange Farm study were presented in July at the 3rd International AIDS Society Conference in Rio de Janeiro, where there was considerable interest in the findings, although some cautionary notes were also sounded. Some observers have expressed concerns that the researchers did not tell participants of their HIV status, although they gave them every encouragement to attend a counseling and testing clinic in order to find this out. These and other issues are discussed in our accompanying Editorial (DOI: 10.1371/journal.pmed.0020293) and in two Perspectives, one by Peter Cleaton-Jones (DOI: 10.1371/journal.pmed.0020287), chair of the ethics committee that approved the study, and the second by Nandi Siegfried (DOI: 10.1371/journal.pmed.0020393), lead author of a Cochrane systematic review of male circumcision for prevention of heterosexual acquisition of HIV in men.

The authors have called for the promotion of male circumcision as part of AIDS prevention efforts in Orange Farm and in other parts of Africa. However, others will take a more cautious view, believing that the positive results of this one study must be confirmed by further studies. Such trials are currently under way in Kenya and Uganda. We must also remember that adult circumcision carries risks, especially if performed by medical personnel or traditional healers without proper training. A further concern is that circumcised men, considering themselves to be "protected," might be more likely to engage in unsafe sex. Research is also needed to find out whether male circumcision has a preventive effect only on female-to-male transmission, or whether it may also reduce male-to-female transmission or male-to-male transmission. In addition, it will be important to determine the mechanism by which circumcision exerts its apparent protective effect.

Many questions do therefore remain, but in the words of one of the study's peer reviewers, this first RCT may come to be regarded as "a landmark paper" in HIV prevention.

Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, et al. (2005) Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: The ANRS 1265 trial. DOI: 10.1371/journal.pmed.0020298

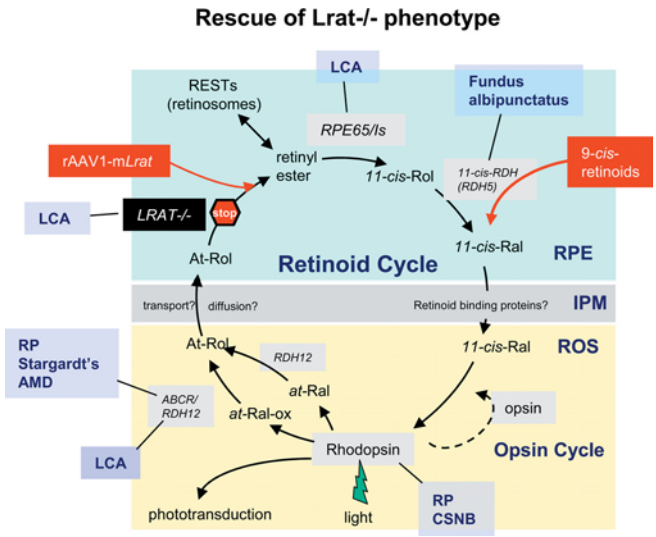
Tackling Inherited Blindness

DOI: 10.1371/journal.pmed.0020402

Imagine the eye is like a camera. The shutter, like the iris of the eye, opens and closes to let in the right amount of light. The lens helps focus light on the film. And the film is like the retina. Regardless of the quality of the camera, if the film is faulty, the developed pictures may be distorted or blurred. In this way, untreatable degenerative diseases of the retina, which affect millions of people worldwide, lead to varying degrees of irreversible blindness. These degenerative eye disorders include retinitis pigmentosa, which affects 1.5 million people, and age-related macular degeneration, which is a leading cause of blindness in North America. The list of inherited retinal dystrophies (degenerations) is long and includes Best disease, choroideremia, cone–rod dystrophy, congenital stationary night blindness, and Leber congenital amaurosis (LCA).

LCA is a collection of diseases all characterized by severe loss of vision at birth from retinal dysfunction. It is a leading cause of congenital blindness. Currently, there is no treatment for LCA; however, it is known that LCA can be caused by mutations in the gene encoding RPE65, a key protein involved in the production and recycling of the chromophore 11-*cis*-retinal (11-*cis*-RAL) in the eye. 11-*cis*-RAL is an integral part of rhodopsin and cone visual pigments, pigments essential for our vision. About 15% of patients with LCA have mutations in *RPE65*. Humans with this form of LCA and Rpe65-deficient mice models both have severely impaired rod and cone function.

Armed with this knowledge, scientists are honing in on various therapeutic strategies for genetic eye diseases. These strategies include somatic gene therapy, infusion of protective proteins, and embryonic cell transplantation. The hope is that such interventions will converge and lead to treatments that slow down or prevent the blindness characteristic of many degenerative eye diseases.



DOI: 10.1371/journal.pmed.0020402.g001

Pharmacological and rAAV Gene Therapy Rescue of the Retinoid Cycle

There have been several attempts to restore vision in patients with LCA using interventions such as calcium channel blockers and intraocular injection of neurotrophic factors. In most cases, the effects of these treatments lasted less than a month; hence, repeated administrations were required. Another approach is to bypass the biochemical block in mice without functional Rpe65 using synthetic *cis*-retinoids administered orally; such treatments have induced dramatic improvement in photoreceptor physiology.

Also, somatic gene therapy has been very successful in many animal models of retinal degeneration. In this issue of *PLoS Medicine*, Krzysztof Palczewski and colleagues attempted to combine two approaches to restore visual function with intraocular gene therapy and oral pharmacologic treatment with novel retinoid compounds in lecithin retinol acyl transferase (LRAT)–deficient mice. LRAT is a key enzyme involved in storage of vitamin A in the form of retinyl esters in structures known as retinosomes. In mice without LRAT, no 11-*cis*-RAL chromophore is produced, and visual function is severely impaired. *Lrat* mutations have been detected in a subset of patients with LCA.

The team found that gene therapy using intraocular injection of recombinant adeno-associated virus carrying the *Lrat* gene successfully restored electroretinographic and pupillary light responses in $Lrat^{-/-}$ mice. Production of 11-*cis*-RAL was also restored. Pharmacological intervention with orally administered pro-drugs 9-*cis*-retinyl acetate and 9-*cis*-retinyl succinate also caused long-lasting restoration of retinal function in *Lrat*-deficient mice. Combining interventions produced markedly increased levels of visual pigment, and 1,000-fold improvements in pupillary light response and electroretinogram sensitivity. Direct comparison of each treatment was difficult, but both therapies provide efficient recovery of higher order visual responses. One advantage of oral retinoid treatment was its ease of administration compared with the subretinal injections required for viral vectors. Another factor was that the orally administered compounds were not stored in the liver for long, and were quickly oxidized and secreted. Pharmacological treatment could also be given multiple times; several low-dose treatments show cumulative effects. The main disadvantage of oral treatment was the potential for long-term systemic toxicity compared with vector targeting of LRAT to the RPE, which needs to be examined in future studies.

Interestingly, the researchers observed that chromophore supplementation and somatic gene therapy were optimally effective in combination, particularly when chromophore supplementation was continued at low doses for longer periods of time. The authors suggest that the combined approach might be more suitable for treating a wider age range of patients. Although much more preclinical testing is required, it is likely that pharmacologic and somatic gene therapeutic approaches could be used together if such testing proves safe and successful in human trials. The authors speculate that treatment of patients with oral retinoids could begin in infancy to avoid amblyopia while also avoiding the difficulties associated with surgery in very young patients. For older patients, a long-lasting drug-free treatment might be achieved by surgical introduction of viral vectors.

Batten ML, Imanishi Y, Tu DC, Doan T, Zhu L, et al (2005) Pharmacological and rAAV gene therapy rescue of visual functions in a blind mouse model of Leber congenital amaurosis. DOI: 10.1371/journal.pmed.0020333

Malaria Vaccine Yields Parasite-Killing Antibodies in Human Volunteers

DOI: 10.1371/journal.pmed.0020408

Many adults living in malaria-endemic areas have acquired clinical immunity against the disease: even though they are constantly reinfected and frequently carry parasites, they are able to control the level of parasitemia and don't develop clinical symptoms. The passive transfer of serum IgG from such individuals can confer a similar level of protection to individuals without acquired immunity. Searching for the targets of those protective antibodies, Pierre Druilhe and colleagues had previously identified *Plasmodium falciparum* merozoite surface protein 3 (MSP3).

Subsequent work from Druilhe's group and others provided additional support that MSP3 might be a good candidate for a malaria vaccine: (1) naturally occurring or artificially raised antibodies against MSP3 can, in collaboration with monocytes, inhibit parasite growth in vitro, (2) an MSP3-based vaccine can confer immunity in primates (and protection correlates with anti-MSP3 antibody titers), and (3) anti-MSP3 antibody transfer can protect a malaria-susceptible humanized mouse model.

Druilhe and colleagues have now completed the first human trial of an MSP3-based vaccine. Some 30 healthy volunteers received three subcutaneous injections, each over several months of different doses of MSP3 peptide together with either aluminum hydroxide or Montanide ISA720. (Some 36 individuals were originally enrolled, but five of the recipients of MSP3 plus Montanide developed local erythema after the second injection and, per protocol specifications, received no third injection, and a sixth dropped out during the follow-up.) Details of the trial and the safety outcome will be published elsewhere (Infect Immun 73. DOI: 10.1128/IAI.73.12).

Both vaccine formulations induced cellular and humoral immune responses in the majority of the volunteers, even at the lowest peptide doses. Specific T-cell responses were seen in 29 of 30 participants, vaccine-specific antibodies developed in 23 of 30, and 18 of 30 reacted with the parasite native protein. Like those of the naturally immune individuals from endemic areas, the vaccine-induced antibodies were primarily of the IgG1 and IgG3 subclasses. Antibodies elicited by MSP3 plus alum were generally of higher affinity (compared with the Montanide formulation), and similar to those from individuals with acquired immunity. Some of the MSP3 plus alum responses lasted for up to 12 months.

To test for functional immunity, Druilhe and colleagues showed that serum antibodies from those volunteers who had MSP3-specific antibodies were able to inhibit parasite growth in collaboration with monocytes. They also tested the antibody in vivo in a humanized mouse model and found that they enabled parasite clearance from the blood.

"A particular strength of the MSP3 trial is that functional assays were utilized to assess the quality of the antibody response produced," say Brendan Crabb and James Beeson in an accompanying Perspective. However, they also point out that important questions remain, some of which will only be answered in efficacy-based trials. Encouraged by the results of the initial trial, Druilhe and colleagues have started a phase II efficacy field trial of MSP3 plus alum in malaria-exposed individuals. The results are eagerly awaited.

Druilhe P, Spertini F, Soesoe D, Corradin G, Mejia P, et al. (2005) A malaria vaccine that elicits in humans antibodies able to kill *Plasmodium falciparum*. DOI: 10.1371/journal.pmed.0020344

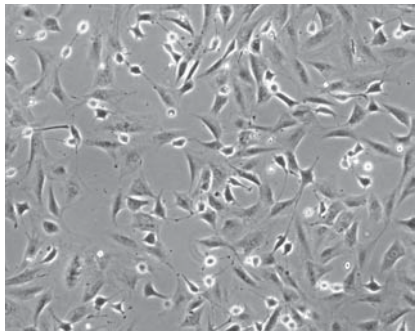
EGFR Mutations and Lung Cancer

DOI: 10.1371/journal.pmed.0020374

Tyrosine kinases of the epidermal growth factor receptor (EGFR) family are frequently mutated in human cancers. Mutations in the tyrosine kinase domain of EGFR (encoded by exons 18–24) have mostly been found in lung cancers. Some, but not all, lung cancers carrying such mutations are responsive to treatment with small-molecule EGFR inhibitors, including the two FDA-approved drugs erlotinib and gefitinib.

Matthew Meyerson and colleagues undertook a systematic study of the different classes of EGFR mutations found in lung cancers in order to understand their roles in tumorigenesis on one hand, and their relation to drug sensitivity on the other. They introduced different altered EGFR versions into fibroblast and lung epithelial cells, and found that all of the mutant proteins transformed both cell types in an EGF-independent manner. Transformation was associated with constitutive kinase activity and with the activation of known downstream signaling pathways.

While the various mutant receptors had similar transforming capabilities,



DOI: 10.1371/journal.pmed.0020374.g001

Morphology of fibroblasts expressing mutant EGFR

cells expressing them differed in their response to EGFR inhibitors. Transformation of cells expressing mutations in exons 18, 19, and 21 was inhibited by 100 nM erlotinib or gefitinib, whereas no significant inhibitory effect on cells expressing an exon 20 insertion mutation was seen even at much higher concentrations of either drug. This result is consistent with the lack of clinical responses to erlotinib or gefitinib in three lung cancer patients with exon 20 mutations. In contrast, when the researchers tested another experimental EGFR inhibitor called CL-387,785, they found cells expressing the exon 20 insertion mutation to be sensitive, consistent with previous studies that had found similar patterns with other EGFR exon 20 mutations.

These results highlight the problems and the possibilities of individualized cancer therapy. One drug is unlikely to fit all tumors, not even all tumors with mutations in a specific oncogene. On the other hand, having a collection of drugs against a particular target increases chances that one of them will prove effective, and that alternatives exist when tumors develop resistance. Developing such a collection and selecting the right drug for the right patient is a challenge not only scientifically but also economically.

Greulich H, Chen TH, Feng W, Jänne PA, Alvarez JV, et al. (2005) Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. DOI: 10.1371/journal.pmed.0020313

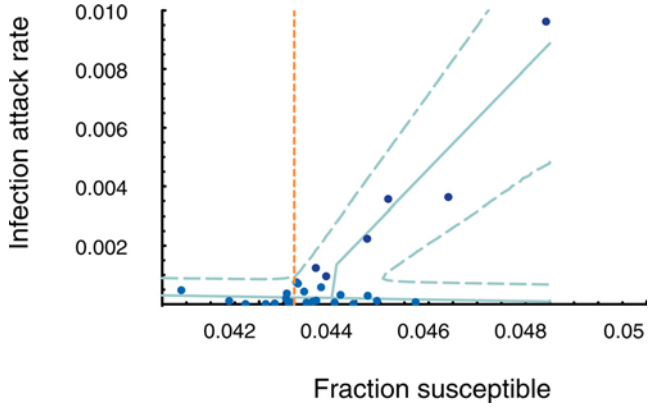
Modeling Measles Epidemics

DOI: 10.1371/journal.pmed.0020384

Measles is still a leading cause of death among young children, despite the availability of an effective vaccine for the past 40 years. Although it is rare in many developed countries, it remains a common illness in many developing countries, and more than half a million people, mostly children, died from measles in 2003. There is a global effort to reduce measles mortality, which aims to achieve routine vaccination coverage of at least 90% of people. In countries with high vaccination coverage, the epidemic pattern has changed from a roughly biennial cycle to an irregular sequence of outbreaks—mainly from imported cases and people refusing vaccination.

A proper understanding of the size and timing of these outbreaks is crucial for monitoring a vaccination program and assessing the risk of future measles outbreaks. Previous studies have shown that the size of an outbreak depends on the fraction of susceptible individuals in a population but also on chance events in the transmission process. These previous studies presupposed the existence of a critical threshold level for the fraction of susceptible individuals, below which introduction of infection will lead to only minor outbreaks. The probability that the outbreak will be major increases with the fraction of susceptible individuals in excess of the threshold.

In a *PLoS Medicine* paper, Jacco Wallinga and colleagues stress the need for direct observations that show the relationship between fraction of susceptible individuals and infection attack rate during irregular measles outbreaks in highly vaccinated



DOI: 10.1371/journal.pmed.0020384.g001

Relation between fraction of susceptible individuals and attack rate

populations. Such observations could shed light on how health authorities should interpret infection attack rates and assess the risk of future measles outbreaks, they say.

To overcome this lack of information, the team investigated measles outbreaks over 28 years in a Dutch population. They estimated the fraction of susceptible individuals and infection attack rates for each epidemic year during the study period. The average vaccine coverage during this period was 93%, and there was no sustained measles transmission. Several

measles outbreaks occurred in communities with low vaccine coverage, but these ended without intervention. The team found a clear threshold value for the fraction of susceptible individuals below which only minor outbreaks occurred, and above which both minor and major outbreaks occurred. They suggest that a precise quantitative relationship exists between the fraction of susceptible individuals in excess of this threshold and the infection attack rate during major outbreaks: one additional unvaccinated person is associated with more than one—almost two—infected persons in subsequent major outbreaks.

These findings update previous thinking on measles vaccination strategies. Previous approaches relied on a statistical description of outbreak sizes based on the theory of solid herd immunity in a homogeneous population. However, the authors' observations are suggestive of occasional, localized lapses in herd immunity, which would allow for major outbreaks within communities with low vaccine coverage. The authors suggest that major outbreaks can be averted only if additional vaccination programs, first tested in mathematical models, target communities at risk, or if measles cases are prevented from entering those communities.

The authors warn that great care should be taken in interpreting a time series of measles case notifications without a corresponding time series of the fraction of susceptible individuals. A few measles cases over several years might not necessarily signal that solid herd immunity has been achieved. It might in fact indicate an increasing risk of a future major measles outbreak. Therefore, these findings could also be used to improve the design of disease-surveillance systems by including monitoring of the changes in fraction of susceptible individuals through time. And whenever the transmission chain is interrupted, the estimated change in fraction of susceptible individuals could be used to assess the risk of future major measles outbreaks.

Wallinga J, Heijne JCM, Kretzschmar M (2005) A measles epidemic threshold in a highly vaccinated population. DOI: 10.1371/journal.pmed.0020316

Where Do People Get Their Information and Contraceptives From?

DOI: 10.1371/journal.pmed.0020382

There are 123 million women around the world, mostly in developing countries, who are not using contraception in spite of having expressed a desire to space or limit the numbers of their births. About 38% of all pregnancies worldwide every year are unintended, and around six out of ten such unplanned pregnancies result in an abortion. By helping women to exercise their reproductive rights, family planning programs can improve the social and economic circumstances of women and their families.

Often contraception is available only in family planning clinics and hospitals, where it is given by trained providers; however, worldwide supplies of contraceptives are available from diverse places. Studies have suggested that conventional family planning clinics might discourage some groups, such as younger people, from using their facilities, an observation backed by findings that report high awareness of contraception in some communities but low usage. But other barriers to contraceptive use include cultural issues, religion, cost, husband/partner's refusal, availability, accessibility, and fear of side effects.

In *PLoS Medicine*, Boniface A. Oye-Adeniran and colleagues say that a better understanding of families' preferences for certain distribution centers would enable providers to deliver better service. And getting contraceptives, especially condoms, closer to people in acceptable, culturally sensitive, and friendly environments could not be more urgent than now, with the need to contain the HIV/AIDS pandemic.

The team did a community-based study to examine the sources of contraceptives for users in Nigeria to identify whether there was a preference for some distribution centers. Of the 2001 respondents aged 15–49 years, 1,647 (82.3%) were sexually active, out of which only 244 (14.8%) were using contraception at the time of the study. The team found some striking trends. Most respondents got their contraceptives from chemist/patent medicine shops (19.7%), whereas only 0.8% went to family planning clinics. Married respondents preferred hospitals, health centers, and clinics—perhaps indicating their need for long-term contraception, that is, intrauterine devices and injectables. However, young unmarried respondents, who preferred condoms and pills, went to pharmacies and over-the-counter services.

The small number of respondents who got their contraceptives from family planning clinics was worrying, but showed a similar trend to Ghana, where there was also a shift from public to private sources. One factor for this development might be general staff attitude in clinics, said the authors, and the lack of youth-friendly services.

There were religious differences in usage: Catholics and Muslims preferred to use chemists/patent medicine shops for contraceptives rather than hospitals and clinics, which may have had something to do with their religions' disapproval of contraceptive use. The age of the respondent was also very important in how individuals obtained contraceptives. Most adolescents preferred chemist/patent medicine shops, but at 25 years of age and older, more people went to general or private hospitals. This behavior perhaps reflected a cultural disapproval of sex when unmarried, the group to which most of these young persons belong. The authors noted that the most popular contraceptives for younger age groups (condoms and pills) were also available over the counter. The observations in this age group are particularly relevant, said the authors, since 15–24 year olds have the greatest incidence of unwanted pregnancy and unsafe abortion.

Altogether, the study highlighted the inadequate information and counseling on the various types of contraceptive methods in Nigeria. The authors suggest that when planning contraceptive services, planners must take into account the sources of contraception identified here, and work on improving culturally relevant delivery methods, such as youth-friendly clinics.

Oye-Adeniran A, Adewole IF, Umoh AV, Oladokun A, Gbadegesin A, et al. (2005) Sources of contraceptive commodity for users in Nigeria. DOI: 10.1371/journal.pmed.0020306

KwaZulu–Natal's Successful Fight against Malaria

DOI: 10.1371/journal.pmed.0020371

The resurgence of malaria remains a major global concern. Artemisinin-based drugs are increasingly seen as one of the best hopes for, at last, making progress in the battle against malaria. Trials of artemisinin-based combination therapy (ACT) in control programs in Southeast Asia have been very encouraging. However, we need to know whether similar levels of effectiveness are achievable in Africa, where the majority of the world's cases of malaria are found.

One part of Africa that has seen increases both in the number of malaria cases and in drug resistance is South Africa's KwaZulu–Natal province. The rise in malaria in this area has been dramatic, with a 15–fold increase in cases taking place during the 1990s. Control efforts during this period involved mosquito control with pyrethroid insecticides (which had replaced DDT) and sulfadoxine-pyrimethamine (SP) as a first-line treatment. (SP was introduced in 1988 in response to high levels of chloroquine resistance.)

In the year 2000, new measures were put in place to address KwaZulu–Natal's malaria crisis. The key elements of this new strategy were the introduction of an ACT drug, artemether-lumefantrine (AL), and an intensification of mosquito control efforts. While pyrethroids were retained for indoor residual spraying of western-style structures, DDT was also reintroduced for spraying traditional homesteads. Karen Barnes and colleagues now present the first comprehensive description and evaluation of the program.

The researchers reviewed four years of malaria morbidity and mortality data at four representative health-care facilities within KwaZulu–Natal's malaria-endemic area. They found that, in the year following improved vector control and implementation of AL treatment, malaria-related admissions and deaths declined by 89%, and outpatient visits decreased by 85%. By 2003, malaria-related outpatient cases and admissions had fallen by 99%, and malaria-related deaths had decreased by 97%. There was a marked and sustained decline in malaria throughout the province. AL cured 99% of those study patients who were followed up for 42 days. Consistent with the findings of focus group discussions, a household survey found that self-reported adherence to the six-dose AL regimen was 96%. Two surveys in subsets of patients receiving AL revealed no serious adverse events resulting from the treatment.

These are impressive results, but they are not solely due to the introduction of ACT. As the authors say, "the ready access to treatment in a relatively well-developed rural primary health-care infrastructure, coupled with an effective vector control programme [are] important factors for deriving the greatest benefit from ACT implementation. Equally important are the strong community perceptions that malaria diagnosis and treatment should be sought urgently at public health-care facilities and treatment then completed."

DOI: 10.1371/journal.pmed.0020371.g001

Artemisinin is extracted from *Artemisia annua*, the annual wormwood (Photo: Scott Bauer)

In an accompanying Perspective (DOI: 10.1371/journal.pmed.0020368), Patrick Duffy and Theonest Mutabingwa highlight lessons learned from the "notable success" in KwaZulu–Natal "amid the dire statistics showing a deadly resurgence of malaria." They also discuss how economic and noneconomic conditions in other parts of sub-Saharan Africa differ from KwaZulu–Natal in ways that are likely to affect the influence of ACT.

Barnes KI, Durrheim DN, Little F, Jackson A, Mehta U, et al. (2005) Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu–Natal, South Africa. DOI: 10.1371/journal.pmed.0020330