SUMMARY

The high incidence of malaria, coupled to the increasing occurrence of parasite resistance against commonly used anti-malaria drugs and the absence of an effective vaccine, makes the implementation of alternative strategies to combat this killer disease imperative. Antisense technology is one such alternative strategy, which makes use of complementary oligodeoxynucleotides designed to inhibit specific parasite mRNA sequences. These bind to their complementary mRNA target sequences and inhibit their translation. In this study α-I-tubulin was chosen as protein target due to its vital role in cell division.

Three antisense ODNs (ASL-1, ASL-2 and ASL-3) were designed, complementary to different regions of the parasite α-I-tubulin mRNA. ASL-1 was complementary to the region surrounding the translation initiation codon, with ASL-2 and ASL-3 being complementary to nucleotides 92-118 and 118-144, respectively. These regions were predicted to be accessible for ODN hybridization by RNA secondary structure determination and subsequent analysis. The above ODNs were modified to offer protection against nuclease degradation, by the inclusion of a 15 nucleotide, self-complementary 3' terminal loop. The stability of these modified ODNs was investigated in serum-containing culture medium, by means of reversed-phase HPLC. These studies showed that the ODN was not significantly degraded after 6 hours of incubation at 37°C in complete culture medium.

A sequence-specific inhibition of parasite proliferation of 20% was obtained after antisense ODN, ASL-1 was incubated with ring-phase parasitised cultures. The inclusion of cationic liposomal transfection reagent, DOSPER, abolished inhibition of parasite growth. Only minimal parasite inhibition was observed with ASL-2 and ASL-3. Studies on the effect of parasite phase on ODN uptake showed ODN uptake into trophozoite phase cultures to be 10x higher than in ring-phase cultures. No inhibition was obtained after ASL-1 ODN incubation with trophozoite-phase cultures. Higher uptake in the trophozoite-phase could be explained by modifications introduced into the erythrocyte membrane, by the maturing parasite (72% of trophozoite and only 14.6% of ring-phase parasitised cultures contained ODNs).

Retrospective analyses showed that alpha-I-tubulin mRNA was available for ASL-1 hybridization only 40%-50% of the time. The lack of inhibition of the trophozoite developmental stage, by any of the ODNs tested, suggests that the targeted site is no longer available for hybridization. Since ASL-1 was directed to the initiation site and the surrounding sequences it is likely that the ODN is unable to compete with the initiation complex during active translation. The low inhibition observed during the ring phase appears to be due to two factors, low uptake in this phase and availability of the target site prior to translation. Retrospective analysis further showed that the mismatch control ODNs, particularly MSL-1, are complementary to several parasite nucleotide sequences. Inhibition due to antisense ODN ASL-1 could therefore be increased to 33%, when sequence-specific MSL-1 inhibition was not deducted.

Inhibitory efficacy of ODNs targeted against the malaria parasite is therefore dependent on the choice of target protein, the local mRNA secondary structure of the target sequence and the transcription and translation stages of the target mRNA and protein, respectively, which include the parasite phases during which these take place.

REFERENCES

Addae-Kyereme, J., Croft, S.L., Kendrick, H. and Wright, C.W. (2001) Antiplasmodial activities of some Ghanaian plants traditionally used for fever/malaria treatment and of some alkaloids isolated from Pleiocarpa mutica; in vivo antimalarial activity of pleiocarpine. *J. Ethnopharmacol*. **76**, 99-103.

Agrawal, S. (1996) Antisense oligonucleotides: toward clinical trials. *Trends Biotechnol.* **14**, 376-387.

Agrawal, S., Tang, J.Y. and Brown, D.M. (1990) Analytical study of phosphorothioate analogues of oligodeoxynucleotides using high performance liquid chromatography. *J. Chromatogr*. **509**, 396-399.

Akhatar, S. (1998) Antisense technology: selection and delivery of optimally acting antisense oligonucleotides. *J. Drug Targeting* **5**, 225-234.

Allred, D.R. (2001) Molecular technology and antigenic variation among intraerythrocytic hemoparasites: do we see reality. *Vet. Parasitol.* **101**, 261-274.

Alonso, P.L., Sacarlal, J., Aponte, J.J., Leach, A., Macete, E., Milman, J., Mandomando, I., Spiessens, B., Guinovart, C., Espasa, M., Bassat, Q., Aide, P., Ofori-Anyinam, O., Navia, M.M., Corachan, S., Ceuppens, M., Dubois, M-C., Demoitié, M-A., Dubovsky, F., Menéndez, C., Tornieporth, N., Ripley Ballou, W., Thompson, R. and Cohen, J. (2004) Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomized controlled trial. *Lancet* **364**, 1411-1420.

Altman, K.H., Kesselring, R. and Pieles, U. (1996) 6'-carbon-substituted carbocyclic analogs of 2'-deoxynucleosides – synthesis and effect on DNA/RNA duplex stability. *Tetrahedron* **52**, 12699-12722.

Anderson, R.A., Knols, B.J.G. and Koella, J.C. (2000) *Plasmodium falciparum* sporozoites increase feeding-associated mortality of their mosquito hosts *Anopheles gambiae* s.1. *Parasitol*. **120**, 329-334.

Apffel, A., Chakel, J.A., Fischer, S., Lichtenwalter, K. and Hancock, W.S. (1997) New procedure for the use of high-performance liquid chromatography – electrospray ionization mass spectrometry for the analysis of nucleotides and oligonucleotides. *J. Chromatogr. A* **777**, 3-21.

Askari, F.K. and McDonnell W.M. (1996) Antisense-olgonucleotide therapy. *N. Engl. J. Med.* **334**, 316-318.

Auf, G., Carpentier, A.F., Chen, L., Le Clanche, C. and Delattre, J.Y. (2001) Implication of macrophages in tumor rejection induced by CpG-oligodeoxynucleotides without antigen. *Clin. Cancer. Res*. **7**, 3540-3543.

Baek, K.H., Ha, S.J. and Sung, Y.C. (2001) A novel function of phosphorothioate oligodeoxynucleotides as chemoattractants for primary macrophages. *J. Immunol*. **167**, 2847-2854.

Bannister, L.H.,. Hopkins J.M, Fowler, R. E., Krishna, S., Mitchell, G. H. (2000). A brief illustrated guide to the ultrastructure of Plasmodium falciparum asexual blood stages. *Parasitol. Today* **16**, 427-33.

Barker, R.H., Metelev, V., Coakley, A. and Zamecnik, P. (1998) *Palsmodium falciparum*: effect of chemical structure on efficacy and specificity of antisense oligonucleotides against malaria in vitro. *Exp. Parasitol.* **88**, 51-59.

Bansal, R., Chen, H.X., Marshall, J.L., Tan, J., Glazer, R.I. and Wainer, I.W. (2001) Detection of mixed-backbone oligonucleotide (GEM 231) in liver and tumor tissues by capillary electrophoresis. *J. Chromatogr B* **750**, 129-135.

Bell A. (1998) Micotubule inhibitors as potential antimalarial agents. *Parasitol. Today* **14**, 234-240.

Benimetskaya, L., Tonkinson, J. and Stein, C.A. (2000) Determination of cellular localization of fluoresceinated oligonucleotides. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp. 287-297.

Bennett, C.F., Chiang, M., Chan, H., Shoemaker, J.E. and Mirabelli, C.K. (1992) Cationic lipids enhance cellular uptake and activity of phosphorothioate antisense oligonucleotides. *Mol. Pharmacol*. **41**, 1023-1033.

Berendt, A. R., Ferguson, D. J. P., Gardner, J., Turner, G., Rowe, A., McCormick, C.; Roberts, D., Craig, A., Pinches, B., Elford, B. C. and Newbold, C. I. (1994) Molecular mechanisms of sequestration in malaria. *Parasitol.* **108**, S19-S28.

Bianco, A.E., Battye, F.L. and Brown, G.V. (1986) *Plasmodium falciparum*: Rapid quantification of parasitaemia in fixed malaria cultures by flow cytometry. *Exp.Parasitol*. **62**, 275-282.

Birkholtz, L-M, Joubert, F., Neitz, A.W.H. and Louw, A.I. (2003) Comparative properties of a three-dimensional model of *Plasmodium falciparum* ornithine decarboxylase. *Proteins: Structure, Function and Genetics* **50**, 464-473.

Bojang, K.A., Milligan, P.J., Pinder, M., Vigneron, L., Alloueche, A., Kester, K.E., Ballou, W.R., Conway, D.J., Reece, W.H., Gothard, P., Yamuah, L., Delchambre, M.,Voss, G., Greenwood, B.M., Hill, A., McAdam, K.P., Tornirporth, N., Cohen, J.D.

and Doherty, T. (2001) Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in the Gambia: a randomised trial. *Lancet* **358**, 1927-1934.

Borque, A.J. and Cohen, A.S. (1993) Quantatative analysis of phosphorothioate oligonucelotides in biological fluids using fast anion-exchange chromatography. *J. Chromatogr*. **617**, 43-49.

Borst, P., Bitter, W., McCulloch, R., Van Leeuwen, F. and Rudenko, G. (1995) Antigenic variation in malaria. *Cell* **82**, 1-4.

Bozdech, Z., Llinás, M., Pulliam, B.L., Wong, E.D., Zhu, J. and DeRisi, J. (2004) The transcriptome of the intraerythrocytic development cycle of *Plasmodium falciaprum*. *PLoS Biol*. **1**, 1-16.

Braasch, D.A. and Corey, D.R. (2002) Novel antisense and peptide nucleic acid strategies for controlling gene expression. *Biochemistry* **41**, 4504-4510.

Brossalina, E., Pascolo, E. and Toulme, J. (1993) The binding of an antisense oligonucleotide to a hairpin structure via triplex formation inhibits chemical and biological reactions. *Nucleic Acids Res*. **21**, 5616-5622.

Brown, P. (1994) Guarded welcome for malaria vaccine. *New Scientist* November, 14-15.

Bruice, T.W. and Lima, W.F. (1997) Control of complexity constraints on combinatorial screening for preferred oligonucleotide hybridization sites on structural RNA. *Biochemistry* **36**, 5004-5019.

Bruna-Romero, O., Gonzalez-Aseguinolaza, G., Hafalla, J.R., Tsuji, M. and Nussenzweig, R.S. (2001) Complete, long-lasting protection against malaria of mice

primed and boosted with two distinct viral vectors expressing the plasmodial antigen. *Proc. Natl. Acad. Sci. USA*. **98**, 11491-11496.

Carey J.V. (2001) Innovative malaria vaccine to undergo clinical development. *Trends in Parasitol*. **117**, 571-572.

Catteruccia, F., Nolan, T., Loukeris, T.G., Blass, C., Savakis, C., Kafatos, F.C. and Crisanti, A. (2000) Stable germline transformation of the malaria mosquito *Anopheles stephensi*. *Nature* **405**, 959-962.

Chen, Q., Fernandez,V., Sundstrom, A., Schlichtherle, M., Datta, S., Hagblom, P. and Wahlfren, M. (1998) Developmental selection of *var* gene expression in *Plasmodium falciparum*. *Nature* **394**, 392-395.

Chen, I.J., Neamati, N. and MacKerrel, A.D. jr. (2002) Structure-based inhibitor design targeting HIV-I integrase. *Curr. Drug. Targets. Infect. Disord*. **2**, 217-234.

Cirino, N.M., Li, G., Xiao, W., Torrence, P.F. and Silverman, R.H. (1997) Targeting RNA decay with 2',5' oligoadenylate-antisense in respiratory syncytial virus-infected cells. *Proc. Natl. Acad. Sci. USA*. **94**, 1937-1942.

Clark, D.L., Chrisey, L.A., Campbell, J.R. and Davidson, E.A. (1994) Non-sequence specific antimalarial activity of oligodeoxynucleotides. *Mol. Biochem. Parasitol*. **63**, 129-134.

Coates, C.J. (2000) A mosquito transformed. *Nature* **405**, 900-901.

Cody, V. (2002) Mechanisms of molecular recognition: crystal structure analysis of human and rat transthyretin inhibitor complexes. *Clin. Chem. Lab. Med*. **40**, 1237-1240.

Cohen, J.S. and Hogan, M.E. (1994) The new genetic medicines. *Scientific American* December, 50-55.

Cook, P.D. (1998) Antisense medicinal chemistry. Targeted Drug Delivery (Juliano, R.L. ed), Springer-Verlag, Berlin, pp 51-101.

Cooke, B. M., Wahlgren, M. and Coppel, R. L. (2000) *Falciparum* malaria: sticking up, standing out and out-standing *Parasitol. Today* **16,** 416-420.

Cooke, B.M., Nicoll, C.L., Baruch, D.I. and Coppel, R.L. (1998) A recombinant peptide based on PfEMP-1 blocks and reverses adhesion of malaria-infected red blood cells to CD36 under flow. *Mol. Microbiol*. **30**, 83-90.

Crooke, S.T. (2000) Progress in antisense technology: the end of the beginning. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp 3-45.

Dagle, J.M. and Weeks, D.L. (2000) Selective degradation of targeted mRNAs using partially modified oligonucleotides. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp 420-436.

Davies, D.R., Interthal, H., Champax, J.J. and Hol, W.G. (2002) Insights into substrate binding and catalytic mechanism of human tyrosyl-DNA phosphodiesterase (TdP1) from vavdate and tungstate-inhibited structures. *J. Mol. Biol*. **324**, 917-932.

De-Koning-Ward, T.F., Janse, C.J. and Waters, A.P. (2000) The development of genetic tools for dissecting the biology of malaria parasites. *Ann. Rev. Microbiol.* **54**, 157-185.

Delves, C.J., Alano, P., Ridley, R.G., Goman, M., Holloway, S.P., Hyde, J.E. and Scaife, J.G. (1990) Expression of α and β tubulin genes during the asexual and sexual blood stages of *Plasmodium falciparum*. *Mol. Biochem. Parasitol*. **43**, 271-278.

Desai, S.A. (1999) A nutrient-permeable channel on the intraerythrocytic malaria parasite. *Novartis Found. Symp*. **226**, 89-95.

Deshmukh, R.R., Cole, D.L. and Sanghvi, Y.S. (2000) Purification of antisense oligonucleotides. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp, 203-226.

Desjardins, J., Mata, J., Brown, T., Graham, D., Zon, G. and Iverson, P. (1995) Cholesteryl-conjugated phosphorothioate oligodeoxynucleotides modulate CYP2B1 expression in vivo. *J. Drug Targeting* **2**, 477.

Doktycz, M.J., Larimer, F.W., Pastrnak, M. and Stevens, A. (1998) Comparative analyses of the secondary structures of synthetic and intracellular yeast *MFA2* mRNAs. *Proc. Natl. Acad. Sic. USA*. **95**, 14614-14625.

Douglas, K.T. (1994) Rational drug design in parasitology. *Parasitol. Today* **10**, 389-392.

Douglas, R.G.Jr. (1994) How vaccines are developed. *Curr. Clin. Top. Infect. Dis*. **14**, 192-204.

Downing, K.H. and Nogales, E. (1998) Tubulin and microtubule structure. *Curr. Opinion in Cell Biol*. **10**, 16-22.

Eguchi, Y., Itoh, T. Tomizawa, J.-I. (1991) Antisense RNA. *Annu. Rev. Biochem*. **60**, 631-652.

Escalante, A., Grebert, H.M., Chaiyaoj, S.C., Riggione, F., Biswas, S., Nahlen, B.L. and Lal, A.A. (2002) Polymorphism in the gene encoding Pfs4845 antigen of *Plasmodium falciparum*. XI. Asembo Bay Cohort Project. *Mol. Biochem. Parasitol*. **119**, 17-22.

Felgner, J.H., Kumar, R., Sridhar, C.N., Wheeler, C.J., Tsai, Y.J., Border, R., Ramsey, P., Martin, M. and Felgner, P.L. (1994) Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. *J. Biol. Chem*. **269**, 2550-2561.

Fischer, P.R. and Bilek, R. (2002) Prevention of malaria in children. *Clin. Infect. Dis*. **34**, 493-498.

Foehler, B.C., Wadwani, S., Terhorst, T.J. and Gerrard, S.R. (1992) Oligodeoxynucleotides containing c-5 propyne analogs of 2'-deoxyuridine and 2'-deoxycytidine. *Tetrahedron Lett*. **33**, 5307-5310.

Foley, M. and Tilley, L. (1995) Home imrovments: Malaria and the red blood cell. *Parasitol. Today* **11**, 436-439.

Forsdyke, D.R. (2002) Selective pressures that decrease synonymous mutations in Plasmodium falciparum. *Trends in Parasitol*. **118**, 411-417.

Francois J., Thuong, N.T. and Helene, C. (1994) Recognition and cleavage of hairpin structures in nucleoc acids by oligodeoxynucleotides. *Nucleic Acids Res*. **22**, 3943-3950.

Francois, J-C., Lacoste, J., Lacroix, L. and Mergny, J-L. (2000) Design of antisense and triplex-forming oligonucleotides. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp74-95.

Frauendorf, A. and Engels, J.W. (1996) Interaction of linear and folded modified antisense oligonucleotides with sequences containing secondary structure elements. *Bioorg. Med. Chem. Lett*. **4**, 1019-1024.

Frederich, M., Dogneacute, J.M., Angenot, L. and Moluc P (2002) New trends in anti-malarial agents. *Curr. Med. Chem.* **9**, 1435-1456.

Furdon, P.J., Dominski, Z. and Kole, R. (1989) RNase H cleavage of RNA hybridized to oligonucleotides containing methylphosphonate, phosphorothioate and phosphodiester bonds. *Nucleic Acids Res*. **17**, 9193-9204.

Gamain, B., Gratepanche, S., Miller, L.H., Baruch, D.I. (2002) Molecular basis for the dichotomy in *Plasmodium falciparum* adhesion to CD36 and chondroitin sulfate A. *Proc. Natl. Acad Sci. USA*. **99**, 10020-10024.

Gardner, M.J., Tettelin, H., Caucci, D.J., Cummings, L.M., Smith, H.O., Fraser, C.M., Venter, J.C. and Hoffman, S.L. (1999) The *Plasmodium* genome sequencing project: complete sequence of *Plasmodium falciparum* chromosome 2. *Parasitologia* **41**, 69-75.

Gardner, M.J. (2001) A status report on the sequencing and annotation of the *P. falciparum* genome. *Mol. Biochem. Parasitol*. **118**, 133-138.

Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., Carlton, J.M., Pain, A., Nelson, K.E., Bowman, S., Paulsen, I.T., James, K., Eisen, J.A., Rutherford, K., Salzberg, S.L., Craig, A., Kyes, S., Chan, M., Nene, V., Shallom, S.J., Suh, B., Peterson, J., Angiuoli, S., Pertea M., Allen, J., Selengut, J., Haft, D., Mather, M.W., Vaidya, A.B., Martin, D.M.A., Fairlamb, A.H., Fraunholz, M.J., Roos, D., Ralph, S.A., McFadden, G.I., Cummings, L.M., Subramanian, G.M., Mungall, C., Venter, J. C., Carucci, D.J., Hoffman, S., Newbold, C., Davis, R.W., Fraser, C.M. and Barrell, B. (2002). Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **419**, 498-511.

Geary, R.S., Yu, R.Z. and Levin, A.A. (2001) Pharmacokinetics of phosphorothioate antisense oligodeoxynucelotides. *Curr. Opinion Inv. Drugs* **2**, 562-573.

Gilar, M., Belenky, A., Smisek, D.L., Borque, A. and Cohen, A.S. (1997) Kinetics of phosphorothioate oligonulceotide metabolism in biological fluids. *Nucleic Acids Res*. **25**, 3615-3620.

Giles, R.V. and Tidd. D.M. (1992) Increased specificity for antisense oligodeoxynucleotides targeting of RNA cleavage by RNase H using chimeric methylphosphonate/phosphodiester structures. *Nucleic Acids Res*. **20**, 763-770.

Giles, R.V., Spiller, D.G. and Tidd, D.M. (2000) Chimeric oligodeoxynucleotide analogs: chemical syntesis, purification and molecular and cellular biology protocols. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp 95-135.

Gilles, H.M (1993): The malaria parasites. In *Bruce-Chwatt's Essential Malariology.* Edited by Giles, H.M, Warnell, D.A, Edward Arnold: 12-34.

Ginsburg, H. and Stein W.D. (1987) New membrane permeation pathways induced by the malarila parasite in the membrane of its host erythrocyte: potential routes for targeting of drugs into infected cells. *Biosci. Rep*. **7**, 455-463.

Ginsburg, H. (1990) Some reflections concerning host erythrocyte-malarial parasite interrelationships. *Blood Cells* **16**, 225-235.

Ginsburg, H. (1994) Transport pathways in the malaria-infected erythrocyte. Their characterization and their use as potential targets for chemotherapy. *Biochem. Pharmacol*. **48**, 1847-1856.

Ginsburg, H. (1999) The permeability properties of the parasite cell membrane. *Novartis Found. Symp*. **226**, 99-108.

Goodyer, I.D., Pouvelle, B., Schneider, T.G., Trelka, D.P. and Taraschi, T.F. (1997) Characterization of macromolecular transport pathways in malaria-infected erythrocytes. *Mol. Biochem. Parasitol*. **87**, 13-28.

Goodwin, L; (1992) Protection postponed. *Nature* **356**, 300.

Graves, P and Gelband, H. (2000) Vaccines for preventing malaria. Cochrane Database Review (http://www.update-software.com/abstracts/mainindex.htm).

Gunasekera, A.M., Patankar, S., Schug, J., Eisen, G., Kissinger, J., Roos, D. and Wirth, D.F. (2004) Widespread distribution of antisense transcripts in the *Plasmodium falciparum* genome. *Mol. Biochem. Parasitol*. **136**, 35-42.

Hall, J.L. and Cowan, N.J. (1985) Structural and restricted expression of a human α-tubulin gene. *Nucleic Acids Res*. **13**, 207-223.

Hartl, D.L., Volkamn, S.K., Nielsen, K.M., Barry, A.E., Day, K.P., Wirth, D.F. and Winzler, E.A. (2002) The paradoxical population genetics of *Plasmodium falciparum*. *Trends in Parasitol*. **118**, 266-272.

Harwaldt, P., Rahlfs, B. and Becker, K. (2002) Glutathione S-transferase of the malarial parasite *Plasmodium falciparum*: characterization of a potential drug target. *Biol. Chem*. **383**, 821-830.

Hauel, N.H., Nar, H., Priepke, H., Ries, U., Stassen, J.M. and Wienen, W. (2002) Structures-based design of novel potent non-peptide thrombin inhibitors. *J. Med. Chem*. **45**, 1757-1766.

Hess, F.I., Kilian, A., Soellner, W., Nothdurft, H.D., Proell, S. and Loescher, T. (1995) *Plasmodium falciparum* and *Plasmodium berghei*: Effect of magnesium on the development of parasitaemia. *Exp. Parasitol*. **80**, 186-193.

Ho, S.P., Britton, D.H.O., Stone, B.A., Behrens, D.L., Leffet, L.M., Hobbs, F.W., Miller, J.A. and Trainor, G.L. (1996) Potent antisense oligonucleotides to the human multidrug resistance-1 mRNA are rationally selected by mapping RNA-accessible sites with oligonucleotide libraries. *Nucleic Acids Res*. **24**, 1901-1907.

Holloway, S., Sims, P., Delves, J. and Hyde, J. (1989) Isolation of alpha-tubulin from the human parasite, *Plasmodium falciparum*: sequence analysis of alpha-tubulin. *Mol. Microbiol*. **3**, 1501-1510.

Hopkins-Sibley, C., Hyde, J.E., Sims, P.F.G., Plowe, C.V., Kublin, J.G., Mberu, E.K., Cowman, A.F., Winstanley, P.A., Watkins, W.M. and Nzila, A.M. (2001) Pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: what next? *Trends Parasitol.* **17**, 582-588.

Hoppe, H.C. (1993) Identification and characterization of selected merozoite-stage antigens in South African isolates of *Plasmodium falciparum*. PhD. Dissertation, University of Pretoria.

Hoppe, H.C., van Schalkwyk, D.A., Wiehart, U.I.M., Meredith, S.A., Egan, J. and Weber, B.W. (2004) Antimalarial quinolines and artemesinin inhibit endocytosis in *Plasmodium falciparum*. *Antimicrob. Agents Chemother*. **48**, 2370-2378.

Howley, P.M., Israel, M.A., Law, M.F. and Martin, M.A. (1979) A rapid method for detecting and mapping homology between heterologous DNAs. Evaluation of polyomavirus genomes. *J. Biol. Chem*. **254**, 4876-4883.

Hughes, J., Astriab, A., Yoo, H., Alahari, S., Liang, E., Sergueev, D., Ramsey Shaw, B. and Juliano, R.L. (2000) *In vitro* transport and delivery of antisense oligonucleotides. *MethodsEnzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp. 342-358.

Hyde J.E. (2002) Mechanisms of resistance of *Plasmodium falciparum* to anti-malarial drugs. *Microbes and Infection* **4**, 165-174.

Hyrup, B. and Nielsen, P.E. (1996) Peptide nucleic acids (PNA): synthesis, properties and potential applications. *Bioorg. Med. Chem*. **4**, 5

Inouye, M. (1988) Antisense RNA: its functions and applications in gene regulation – a review. *Gene* **72**, 25-34.

Itin, C., Burki, Y., Certa, U and Doebli, H. (1993) Selective inhibition of Olasmodium falciparum aldolase by a tubulin derived peptide and identification of the binding site. *Mol. Biochem. Parasitol*. **58**, 135-144.

Ito, J., Ghosh, A., Moriera, L.A., Wimmer, E.A. and Jacobs-Lorena, M. (2002). Transgenic Anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature* **417**, 452-455.

Jaeger, J.A., Turner, D.H. and Zuker, M. (1989) Improved predicitions of secondary structures. *Proc. Natl. Acad. Sci. USA*. **86**, 7706-7710.

Jan, S., Mao, C., Vassilev, A.O., Navara, C.S. and Uckun, F.M. (2000) COBRA-1, a rationally designed epoxy-THF containing compound with potent tubulin depolymerization activity as a novel anticancer agent. *Bioorg. Med. Chem. Lett*. **10**, 1193-1197.

Janse, C.J. and Waters, A.P. (1995) *Plasmodium berghei*: The application of cultivation and purification thechniques to molecular studies of malaria parasites. *Parasitol. Today* **11**, 138-143.

Jones, T.R., Gramzinski, R.A., Aguiar, J.C., Sim, B.K., Narum, D.L., Fuhrmann, S.R., Kumar, S., Obaldia, N. and Hoffman, S.L. (2002) Absence of antigenic competition in Aotus monkeys immunized with *Plasmodium falciparum* DNA vaccines delivered as a mixture. *Vaccine* **20**, 1675.

Jordan, M.A. and Wilson, L. (2004) Microtubules as a target for anticancer drugs. *Nature Rev*. **4**, 253-265.

Joubert, F., Neitz, A.W.H. and Louw, A.I. (2001) Structure-based inhibitor screening: A family of sulfonated dye inhibitors for malaria parasite triosphosphate isomerase. *Proteins: Structure, Function, and Genetics* **45**, 136-143.

Kanagaratnam, R., Misiura, K., Rebowski, G. and Ramasamy, R. (1998) Malaria merozoite surface protein antisense oligodeoxynucleotides lack antisense activity but function as polyanions to inhibit cell invasion. *Int. J. Biochem. Cell Biol*. **30**, 979-985.

Kandimalla, E.R., Manning, A., Zhao, Q., Shaw, D.R., Byrn, R.A., Sasisekharan, V. and Agrawal, S. (1997) Mixed backbone antisense oligonucleotides: design, biochemical and biological properties of oligonuceotides containing 2'-5'-ribo- and 3'-5'-deoxyribonucleotide segments. *Nucleic Acids. Res*. **25**, 370-378.

Kasis, I., Lak, L., Adler, J., Choni, R., Shazberg, G., Fekede, T.D., Shoshani, E., Miller, D. and Heyman, S. (2001) Medical relief operation in rural northern Ethiopia: addressing an ongoing disaster. *Isr.l Med. Assoc. J.* **3**, 772.

Keillor, J.W., Lherbert, C., Castonguay, R., Lapierre, D., Martinez-Oyanedel, J., Fothergill-Gilmore, L.A. and Walkinshaw, M.D. (2003) Expression, purification, crystallization and preliminary crystallographic analysis of *Trypanosoma brucei* phosphofructokinase. *Acta Crystallogr. D. Biol. Crystallogr*. **59**, 532-534.

Kemp, G. (1998) Capillary electrophoresis: a versatile family of analytical techniques. *Biotechnol. Appl. Biochem*. **27**, 9-17.

Kester, K.E., McKinney, D.A., Tornieporth, N., Ockenhaus, C.F., Heppner, D.G., Hall, T., Krzych, U., Delchambre, M., Voss, G., Dowler, M.G., Palensky, J., Wittes, J., Cohen, J. and Ballou, W.R. (2001) Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. *J. Infect. Dis*. **183**, 640-647.

Kimelman, D. (1992) in Gene Regulation Biology of Antisense RNA and DNA (Erikson, R.P. and Izant, J.G., eds), Raven Press, New York, pp. 1-10.

Knapp, B., Hundt, E. and Kupper, H.A. (1990) *Plasmodium falciparum* aldolase: gene structure and localization. *Mol. Biochem. Parasitol*. **40**, 1-12.

Krettli, A.U. and Miller, L. (2001) Malaria: A sporozoite runs through it. *Current Biology* **11**, R409-R412.

Krieg, A.M. (1999) Direct immunologic activities of CpG DNA and implications for gene therapy. *J. Gene Med*. **1**, 56-63.

Krieg, A.M. and Stein, C.A. (1995) Phosphorothioate oligodeoxynucleotides – antisense or anti-protein? *Antisense Res. Dev*. **5**, 241.

Krishna, S., Woodrow, C,J., Burchmore, R.J.S., Saliba, K.J. and Kirk, K. (2000) Hexose transport in asexual stages of *Plasmodium falciparum* and kinetoplastidae. *Parasitol. Today* **16**, 516-521.

Krueger, S.K. and Williams, D.E. (1995) Quantitation of Digoxigenin-labeled DNA hybridized to DNA and RNA slot blots. *Anal. Biochem*. **229**, 162-169.

Kwiatkowski, M., Sandstrom, A., Balgobin, N. and Chattopadhyaya, J. (1984) Use of reverse phase ion pair chromatography to fractionate and purify DNA fragments and monomeric components of RNA. *Acta. Chem. Scand. B* **38**, 721-733.

Kyes, S.A., Rowe, J.A., Kriek, J.A. and Newbold, C.I. (1999) Riffins: a second family of clonally variant proteins expressed on the surface of red cells infected with *Plasmodium falciparum*. *Proc. Natl. Acad. Sci.USA*. **96**, 9333-9338.

Lambros, C. and Vanderberg, I.P. (1979) Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J. Parasitol*. **65**, 418-420.

Lappalainen, K., Urtti, A., Soderling, E., Jaaskelainen, I., Syrjanen, K. and Syrjanen, S. (1994) Cationic liposomes improve stability and intra-cellular delivery of antisense oligonucleotides into CaSki cells. *Biochim. Et. Biophys. Acta*. **1196**, 201-208.

Lavigne, C., Yelle, J., Sauve, G. and Thierry, A.R. (2002) Is antisense an appropriate nomenclature or design for oligodeoxynucleotides aimed at the inhibition of HIV-1 replication. *AAPS Pharm. Sci*. **4**, E9.

Leeds, J.M., Graham, M.J., Truong, L. and Cummins, L.L. (1996) Quantitation of phosphorothioate oligonucleotides in human plasma. *Anal. Biochem*. **235**, 36-43.

Leontis, N.B. and Santa-Lucia, J. (1997) Overview. Molecular Modeling of nucleic acids (Leontis, N.B; Santa-Lucia, J. eds), American Chemical Society, Washington, DC, pp. 1-16.

Lima, W.F. and Crooke, S.T. (1997) Binding affinity and specificity of *E. coli* RNase-H1: Impact on the kinetics of catalysis of antisense oligonucleotide-RNA hybrids. *Biochemistry* **36**, 390-398.

Lima, W.F., Brown-Driver, V., Fox, M., Hanecak, R. and Bruice, T.W. (1997) Combinatorial screening and rational optimization for hybridization to folded Hepatitis C virus RNA of oligonucleotides with biological antisense activity. *J. Biol. Chem* **272**, 626-638.

Lima, W.F., Monia, B.P., Ecker, D.J. Freier, S.M. (1992) Implication of RNA secondary structure on antisense oligonucleotide hybridization kinetics. *Biochemistry*, **31**, 12055-12061.

Lledo, P., Desmaisons, D., Carleton, A. and Vincent, J. (2000). Physiology and molecular biology brought to single-cell level. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp. 143-156.

Lobo, C.A., Dhar, R. and Kumar, N. (1999) Immunization of mice with DNA-based Pfs-25 elicits potent malaria transmission-blocking antibodies. *Infect. Immun*. **68**, 5530-5538.

Loke, S.L., Stein, C.A., Zhang, X.H., Mori, M., Nkanishi,C., Subasinghe, J.S., Cohen, J.S. and Neckers, L.M. (1989) Characterization of oligonucleotide transport into living cells. *Proc. Natl. Acad. Sci. USA*. **86**, 3474.

Lonnberg, H. and Vuorio, E. (1996) Towards genomic drug therapy with antisense oligonucleotides. *Ann. Med*. **28**, 511-512.

Makler, M.T., Lee, L.G. and Recktenwald, D. (1987) Thiazole orange: a new dye for *Plasmodium* species analysis. *Cytometry* **8**, 568-570.

Makler, M.T., Piper, R.C. and Milhous, W.K. (1998) Lactate dehydrogenase and the diagnosis of malaria. *Parasitol. Today* **14**, 376-377.

Malhotra, P., Dasaradhi, P.V., Kumar, A., Mohmmed, A., Agrawal, N., Bhatnaggar, R.K. and Chauhan, V.S. (2002) Double-stranded RNA-mediated gene silencing of cysteine proteases (falcipain-1 and –2) of *Plasmodium falciparum* . *Mol. Microbiol*. **45**, 1245-1254.

Matthews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters involves prediction of RNA secondary structure. *J. Mol. Biol*. **288**, 911-940.

McKenzie, F.E. (2000) Why model malaria. *Parasitol. Today* **16**, 511-516.

McRobert, L. and McConkey, G.A. (2002) RNA interference (RNAi) inhibits growth of *Plasmodium falciparum*. *Mol. Biochem. Parasitol*. **119**, 273-278.

Metelev, V. and Agrawal, S. (1992) Ion-exchange high-performance liquid chromatography analysis of oligodeoxynucleotide phosphorothioates. *Anal. Biochem*. **200**, 342-346.

Michel, F. and Westhof, E. (1990) Modelling of the three-dimensional architectural group I catlytic introns based on comparative sequence analysis. *J. Mol. Biol*. **216**, 585-610.

Miller, L. H., Good, M. F. and Milon, G. (1994) Malaria pathogenesis. *Science* **264,** 1878.

Miller, L.H., Hudson-Taylor, D., Gamain, B. and Saul, A.J. (2002) Definition of the minimal domain of CIDR1 of *Plasmodium falciparum* PfEMP1 for binding CD36. *Mol. Biochem. Parasitol*. **120**, 321-323.

Milner, N., Mir, K.U. and Southern, E.M. (1997) Selecting effective antisense reagents on combinatorial oligonucleotide arrays. *Nat. Biotechnol*. **15**, 537-541.

Moon, I., Lee, Y., Kwak, C., Choi, K., Schreiber, A.D. and Park, J. (2000) Target site search and effective inhibition of leukaemic cell growth by a covalently closed multiple anti-sense oligonucleotide to *c-myb*. *Biochem. J*. **346**, 295-303.

Munasinghe, A., Patankar, S., Cook, B.P., Madden, S.L., Martin, R.K., Kyle, D.E., Shoaibi, A., Cummings, L.M. and Wirth, D.F. (2001) Serial analysis of gene expression (SAGE) in *Plasmodium falciparum*: application of the technique to A-T rich genomes. *Mol. Biochem. Parasitol*. **113**, 23-34.

Murillo, L.A., Tenjo, F.A., Clavijo, O.P., Sampaio, S., Kalil, J.and Patarroyo, M.E. (1992) A specific T-cell receptor genotype preference in the immune response to a synthetic Plasmodium falciparum malaria vaccine. *Parasite Immunol.* **14**, 87-94.

Nacher, M. (2001) Malaria vaccine trials in a wormy world. *Trends Parasitol.* **17**, 563-565.

Nakai, D., Sewita, T., Iwasa, T., Aiwasa, S., Shoji, Y. and Mizushima, Y. (1996) Cellular uptake mechanism for oligonucleotides: involvement of endocytosis in the uptake of phosphodiester oligonucleotides by a human colorectal adenocarcinoma cell line HCT-15. *J. Pharamacol. Exp. Ther*. **278**, 1362-1372.

Newton, C.R. and Krishna, S. (1998) Severe *falciparum* malaria in children: current understanding of pathophysiology and supportive treatment. *Pharmacol. Ther*. **79**, 1-53.

Niederwieser, A. and Brenner, M. (1965) Polyzonal thin-layer chromatography. Chromatographic solvent separation and its use for the separation of mixtures. 1. Duozonal thin-layer chromatography. Theory and Practice. *Experentia* **21**, 50.

Nielsen, P.E., Egholm, M., Berg, R.H. and Buchardt, O. (1991) Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science* **254**, 1497-1500.

Nikiforov, T.T.and Connolly, B.A. (1991) The synthesis of oligodeoxynucleotides containing 4-thiothymidine residues. *Tetrahedron Lett*. **32**, 3851-3854.

Nosten, F and Brasseur, P. (2002) Combination therapy for malaria: the way forward? *Drugs* **62**, 1315-1329.

Ockenhaus C.F., Sun, P.F., Lanar, D.E., Wellde, B.T., Hall, B.T., Kester, K., Stoute, J.A., Magill, A., Krzych, U., Farley, L., Wirtz, R.A., Sadoff, J.C., Kaslow, D.C., Kumar, S.,

Church, L.W. Crutcher, J.M., Wizel, B., Hoffman, S., Lalvani, A., Hill, A.V., Tine, J.A., Guito, K.P., de Taisne, C., Anders, R., Ballou, W.R. et al. (1998) PahseI/Iia saftey, immunogenicity, and efficacy trial of NYVAC-Pf7 pox-vectored, multiantigen, multistage vaccine candidate for *Plasmodium falciparum* malaria. *J. Infect. Dis.* **177**, 1664-1673.

Osta, M.A., Christophides, G.K., Vlachou, D. and Kafatos, F.C. (2004) Innate immunity in the malaria vector *Anopheles gambiae*: comparative and functional genomics. *J. Exp. Biol*. **207**, 2551-2563.

Patarroyo, G., Franco, L., Amador, R., Murillo, L.A., Rocha, C.L., Rojas, M. and Patarroyo, M.E. (1992) Study of the safety and immunogenicity of the synthetic malaria SPf66 vaccine in children aged 1-14 years. *Vaccine* **10**, 175-178.

Phillips, M.I. and Zhang, Y.C. (2000) Basic principles of antisense oligonucleotides in vitro. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, 46-56.

Phillipson, J.D. and Wright, C.W. (1991) Antiprotozoal agents from plant sources. *Planta. Med*. **57**, S53-59.

Piper, K.P., Hayward, R.E., Cox., Day, K.P. (1999) Malaria transmission and naturally aquired immunity to PfEMP-1. *Infect. Immun*. **67**, 6369-6374.

Pouvelle, B., Spiegel, R., Hsiao, L., Howard, R.J., Morris, R.L., Thomas, A.P. and Taraschi, T.F. (1991) Direct access to serum macromolecules by intraerythrocytic malaria parasites. *Nature* **353**, 73-75.

Povoa, M.M., Adagu, I.S., Oliviera, S.G., Machado, R.L.D., Miles, M.A. and Warhurst, D.C. (1998) *Pfmdr1* $^{Asn}1042^{Asp}$ and $^{Asp}1246^{Tyr}$ polymorphisms , though to be associated

with chloroquine resistance, are present in chloroquine-resistant and sensitive Brazilian field isolates of *Plasmodium falciparum*. *Exp. Parasitol*. **88**, 64-68.

Price R.N., Nosten, F., Luxemborger, C., ter Kuile, F.O., Paiphun, L., Chongsuphajaisiddhi, T. and White, J.N. (1996) Effects of artemisinin derivatives on malaria transmissibility. *Lancet* **347**, 1654-1658.

Quartin, R.S., Brakel, C.L. and Wetmur, J.G. (1989) Number and distribution of methylphosphonate linkages in oligodeoxynucleotides affect exo-and endonuclease sensitivity and ability to form RNase H substrates. *Nucleic Acids Res*. **17**, 7253-7262.

Rathold, P.K. and Phillips, M.A. (2003) Prized malaria drug target nailed. *Nat. Struct. Biol*. **10**, 1-3.

Reber-Liske, R., Salako, L.A., Matile, H., Sowunni, A. and Sturchler, D. (1995) [NANP]19-5.1. A malaria vaccine field trial in Nigerian children. *Trop. Geogr. Med*. **47**, 61-63.

Riesner, D. (1997) Nucleic acid structures. Antisense Technology: A practical approach, (Lichtenstein, C. and Nellen, W. eds), Oxford University Press, Oxford, 1-24.

Rojas, M.O., De-Castro, J., Marino, G and Wasserman M. (1996) Detection of genomic polymorphism in *Plasmodium falciparum* using an arbitrarily primed PCR assay. *J. Eukaryot. Microbiol*. **41**, 323-326.

Roper, C., Pearce, R., Bredenkamp, B., Gumede, J., Drakeley, C., Mosha, F., Chandramohan, D. and Sharp, B. (2003) Antifolate antimalarial resistance in southeast Africa: a population-based analysis. *Lancet* **361**, 1174-1181.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1983) *Molecular cloning : A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 202-203.

Saul, A. and Battistutta, D. (1990) Analysis of the sequences flanking the translation start site of *Plasmodium falciparum*. *Mol. Biochem. Parasitol*. **42**, 55-62.

Schulze, D.L.C., Makgatho, E.M., Coetzer, T.L., Louw, A.I., van Rensburg, C.E.J. and , Visser, L. (1997) Development and application of a modified flow cytometric procedure for rapid *in vitro* quantification of malaria parasitaemia. *S.A. J. Sci*. **93**, 156-158.

Semple, S.C., Klimuk, S.K., Harasym, T.O. and Hope, M.J. (2000) Lipid-based formulations of antisense oligonucleotides for systemic delivery applications. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp 322-341.

Senior, J.H. (1987) Fate and behavior of liposomes in vivo: a review of controlling factors. *Crit. Rev. Ther. Drug. Carrier. Syst*. **3**, 123.

Shaw, M.K., Compton, H.L., Roos, D.S. and Tilney, L.G. (2000) Microtubules, but not actin filaments, drive daughter cell budding and cell division in *toxplasma gondii*. *J. Cell. Sci*. **113**, 1241-1254.

Simons, R.W. and Kleckner, N. (1983) Translational control of IS10 transposition. *Cell* **34**, 683-691.

Smith, L., Andersen, K.B., Hovgaard, L. and Jaroszewski, J.W. (2000) Rational selection of antisense oligonucleotide sequences. *Europ. J. Pharm. Sci*. **11**, 191-198.

Sohail, M. and Southern, E.M. (2000) Selecting optimal antisense reagents. *Adv. Drug. Del. Rev*. **44**, 23-34.

Southern, E.M., Case Green, S.C., Elder, J.K., Johnson, M., Mir, K.U., Wang, L. and Williams, J.C. (1994) Arrays of complimentary oligonucleotides for analysing the hybridization behaviour of nucleic acids. *Nucleic Acids Res*. **22**, 1368-1373.

Southern, E.M., Mir, K.and Shchepinov, M. (1999) Molecular interactions on microarrays. *Nat. Med. Suppl*. **21**, 5-9.

Spielman, A. (1994) Research priorities for managing the transmission of vector-borne disease. *Prev. Med*. **23**, 693-699.

Stanley, S.L. (1998) Malaria vaccines: are seven antigens better than one? *Lancet* **352**, 1163-1164.

Stein, C.A. and Cheng, Y.C. (1993) Antisense oligonucleotides as therapeutic agents – is the bullet really magical? *Science* **261**, 1004-1012.

Stowers A.W. and Miller, L.H. (2001) Are trials in New World Monkeys on the critical path of blood-stage malaria vaccine development? *Trends in Parasitol.* **17**, 415-419.

Stull, R.A., Taylor, L.A. and Szoka, F.Jr. (1992) Predicting antisense oligonucleotide inhibitory efficacy: a computational approach using histograms and thermodynamic indices. *Nucleic Acids Res.* **20**, 3501-3508.

Stull, R.A., Zon, G. and Szoka, F.C.Jr. (1996) An *in vitro* messenger RNA binding assay as a tool for identifying hybridization-competent antisense oligonucleotides. *Nucleic Acid Drug Dev*. **6**, 221-228.

Summerton, J. and Weller, D. (1997) Morpholino antisense oligomers: design, preparation and properties. *Antisense Nucleic Acid Drug Develop*. **7**, 187-195.

Tanner, M. and Evans, D. (1994) Vaccines or drugs: Complementarity is crucial. *Parasitol. Today* **10**, 406-407.

Taraschi, T.F. (1999) Macromolecular transport in malaria-infected erythrocytes. *Novartis Found. Symp*. **226**, 114-120.

Thayer, J.R., McCormick, R.M. and Avdalovic, N. (1996) High resolution nucleic acid separations by high-performance liquid chromatography. *Methods Enzymol.*, volume 313, Academic Press, San Diego, pp. 147-174.

Thierry, A.R., Rahman, A., Dritschilo, A. (1993) Overcoming multi-drug resistance in human tumor cells using free and liposome encapsulated antisense oligodeoxynucleotides. *Biochem. Biophys. Res. Commun*. **190**, 952-960.

Tinoco, I., Borer, P.N., Dengler, B., Levine, M.D., Uhlenbeck, O.C., Crothers, D.M. and Bralla, J. (1973) Improved estimation of secondary structure in ribonucleic acids. *Nature New Biol.* **246**, 40-41.

Thomas, B.E., Manocha, M., Haq, W., Adak, T., Pillai, C.R. and Rao, D.N. (2001) Modulation of the humoral response to repeat and non-repeat sequences of the circumsporozoite protein protein of *Plasmodium vivax* using novel adjuvant and delivery systems. *Ann. Trop. Med. Parasitol*. **95**, 451-472.

Thomas, S.L.Y and Lew, V.L. (2004) *Plasmodium falciparum* and the permeation pathway of the host red blood cell. *Trends in Parasitol*. **20**, 122-125.

Thompson, J.D. (2002) Applications of antisense and siRNAs during preclinical drug development. *Drug Discov. Today* **7**, 912-917.

Toulme, J.J., Cazenave, C. and Moreau, S. (1997) Selecting, preparing and handling antisense oligodeoxynucleotides. In Antisense Technology: A practical approach (Lichtenstein, C. and Nellen, W, eds), Oxford University Press, Oxford, pp. 39-74.

Toulme, J.J., Tinevez, R. and Brossalina, E. (1996) Targeting RNA structures by antisense oligonucleotides. *Biochemie* **78**, 663-673.

Trager, W. and Jensen, J.B. (1976) Human malaria parasites in continuous culture. *Science* **19**, 673-675.

Trager, W. (1995) Malaria vaccine. *Science* **267**, 1577.

Uhlmann, E. and Peyman, A. (1990) Antisense oligonucleotides: A new therapeutic principle. *Chem. Rev*. **90**, 544-584.

Uhlmann, E., Peyman, A., Ryte, A., Scmidt, A. and Buddecke, E. (2000) Use of minimally modified antisense oligonucleotides for specific inhibition of gene expression. *Methods Enzymol.*, volume 313 (Phillips, M.I. ed), Academic Press, San Diego, pp. 268-284.

Ulla, E., Tschudi, C. and Chakraborty, T. (2004) RNA interference in protozoan parasites. *Cell. Microbiol*. **6**, 509-519.

Upston, J.M. and Gero, A.M. (1995) Parasite-induced permeation of nucleosides in *Plasmodium falciparum* malaria. *Biochim. et. Biophys. Acta*. **1236**, 249-258.

Van Vianen, P.H., van Engen, A., Thiathong, S., van der Keur, M., Tanke, H.J., van der Kaay, H.J., Mons, B., Janse, C.J. (1993) Flow cytometric screening of blood samples for malaria parasites. *Cytometry* **14**, 276-280.

Vernes, A., Haynes, J.D., Tapchaisir, P., Williams, J.L., Dutoit, E. and Diggs, C.L. (1984) *Plasmodium falciparum* strain-specific antibody inhibits merozoite invasion of erythrocytes. *Am. J. Trop. Med. Hyg*. **33**, 197-203.

Vickers, T.A., Wyatt, J.R. and Freier, S.M. (2000) Effects of RNA secondary structure on cellular antisense activity. *Nucleic Acids Res*. **28**, 1340-1347.

Vollmer, J., Janosch, A., Laucht, M., Ballas, Z.K., Schetter, C. and Krieg, A.M. (2000) Highly immunostimulatory CpG-free oligodeoxynucleotides for activation of human leukocytes. *Anisense Nucleic Acid Drug Dev*. **12**, 165-175.

Wagner, R.W. (1994) Gene inhibition of hepatitis C virus expression by antisense oligodeoxynucleotides. *J. Biol. Chem*. **269**, 14205-14210.

Wanidworanum, C., Nagel, R.L. and Shear, H.L. (1999) Antisense oligonucleotides targeting malarial aldolase inhibit the asexual erythrocytic stages of *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* **102**, 91-101.

White, J.J. (1997) Assessment of the pharmacodynamic properties of anti-malarial drugs in vivo. *Antimicrob. Agents Chemother.* **41**, 1413-1422.

White, N. J. (1998) Malaria Pathophysiology In: *Malaria Parasite biology, pathogenesis and protection* (Sherman, I. W. ed), ASM Press, Washington, D. C.

White, N.J., Nosten, F., Looareesuwan, S., Watkins, W.M., Marsh, K., Snow, R.W., Kokwaro, G., Ouma, J., Hien, T.T., Molyneux, M.E., Taylor, T.E., Newbold, C.I., RuebushII, T.K. and Danis, B.M. (1999) Averting a malaria disaster. *Lancet* **353**, 1965-1967.

Wickham, M.E., Rug, M., Ralph, S.A., Klonis, N., McFadden, G.I., Tilley, L. and Cowman, A.F. (2001) Trafficking and assembly of the cytoadherence complex in *Plasmodium falciparum*-infected human erythrocytes. *EMBO Journal* **20**, 5636-5649.

Withers-Martinez, C., Carpenter, E.P., Hackett, F., Ely, B., Sajid, M., Grainger, M. and Blackamn, M.J. (1999) PCR-based gene synthesis as an efficient approach for expression of the A + T-rich malaria genome. *Protein Eng*. **12**, 1113-1120.

Wooster, M.M. (2001) DDT is a good chemical. *American Enterprise* January, 1.

Wu-Pong, T., Weiss, L. and Hunt, C.A. (1994) Calcium dependent cellular uptake of *c-myc* antisense oligonucleotides. *Cell. Mol. Biol*. **40**, 843

Yu, D., Kandimalla, E.R., Zhao, Q., Cong, Y. and Agrawal, S. (2001) Immunostimulatory activity of CpG oligonucleotides containing non-ionic methylphosphonate linkages. *Bioorg. Med. Chem*. **9**, 2803-2808.

Yuthavong, Y. (2002) Basis for antifolate action and resistance in malaria. *Microb. Infect*. **4**, 175-182.

Yuvaniyama, J., Chitnumsub, P., Kamchonwongpaisan, S., Vanichtanankul, J., Sirawaraporn, W., Taylor, P., Walkinshaw, M.D. and Yuthavong, Y. (2003) Insights into antifolate resistance from malaria DHFR-TS structures. *Nat. Struct. Biol*. **10**, 357-365.

Zamecnik, P. and Stephenson M. (1978) Inhibition of the Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. *Proc. Natl. Acad. Sci. USA*. **75**, 280-284.

Zhao, Q., Matson, S., Herrera, C.J., Fischer, E., Yu, H. and Krieg, A.M. (1993) Comparison of cellular binding and uptake of antisense phosphodiester, phosphorothioate

and mixed phosphorothioate and methylphosphonate oligonucleotides. *Antisense Res. Dev*. **3**, 53-66.

Zambrano-Villa, S., Rosales-Borjas, D., Carrero, J. and Ortiz-Ortiz, L. (2002) How protozoan parasites evade the immune response. *Trends in Parasitol*. **118**, 272-278.

Zuker, M. (1989) On finding all suboptimal foldings of an RNA molecule. *Science* **244**, 48-52.