Chapter 12
Anticancer Drug Repositioning Against Tropical Diseases: The Example of Methotrexate in the Treatment of Malaria

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12.1 Introduction

Chemotherapy remains one of the most important tools for the management and control of malaria. However, the rapid selection of malaria parasite resistant to antimalarial drugs can hamper this strategy. To slow the pace of selection of resistance, the World Health Organization has recommended the use of artemisinin combination therapies (ACTs) as first-line treatments of uncomplicated malaria [1]. In Africa, the combinations of lumefantrine/artemether (Coartem®) and amodiaquine/artesunate are currently the two main ACTs used to treat uncomplicated malaria [1–4]. However, recent reports indicate that resistance to artemisinin is emerging in South East Asia, and thus there is concern that this resistance could spread to Africa [5], a scenario which will not only render the current ACTs ineffective, but will also compromise the efficacy of pyronaridine/artesunate and piperaquine/dihydroartemisinin, the two next ACTs that are now in phase III/IV clinical development [6]. Intravenous artesunate has recently become the drug of choice in the treatment of severe malaria [7]. Unfortunately, the spread of artemisinin resistance will also affect the management of severe malaria. Thus, to counterbalance this burgeoning drug resistance problem, new drugs are urgently needed.

One of the strategies to discover new drugs is to reposition, repurpose or find new uses for drugs that have already been used for other indications. This approach, which has the advantage of reducing the cost and shortening the time of drug development, has become an important area of research by the pharmaceutical industry [8, 9]. For instance, in 2004, almost 40% of drugs registered by the Food and Drug Administration (FDA) found new uses in the treatment of various conditions in humans [8]. It is interesting to note, among the few available

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antimalarials, that some of them have been repositioned. For instance, quinine is used to treat muscle cramps [10], and chloroquine (CQ) (preferably hydroxylchloroquine) is used for the management of rheumatoid arthritis and systemic lupus erythematosus [11]. Artemisinin is being investigated for the treatment of schistosomiasis and cancer [12–14].

12.2 In Vitro Antimalarial Activities of MTX

Several reports indicate that MTX is potent against *Plasmodium falciparum*, including those resistant to the antifolate pyrimethamine (PM), which carry the Ileu-164-Leu *dhfr* codon (dihydrofolate reductase), the mutation associated with higher levels of antifolate resistance, with MTX $IC_{50} < 80$ nM (inhibitory concentration that kills 50% of parasitaemia) [15–18], and $IC_{90/99}$ (inhibitory concentration that kills 90–99% of parasitaemia) falling between 150 and 350 nM. Thus, if such concentration can be achieved in vivo with an acceptable toxicity profile, MTX could potentially become a useful antimalarial drug [18]. However, anticancers are perceived to be toxic, thus not suitable for the malaria treatment. Yet the literature is replete with examples of new uses of anticancers in the treatment of non-neoplastic diseases.

12.3 Anticancer May Be Safe at Low Dose

Most anticancers are used at high dose to block tumour cells, leading to the inhibition of the growth of normal cells. Affected most specifically are cells that multiply actively such as bone marrow cells, e.g. leukocytes; cells of the gastrointestinal mucosa; and hair follicle cells, explaining why bone marrow suppression, mucositis and alopecia (hair loss) are among the most salient side effects of anticancers. However, according to Paracelsus' Law, it is well known that for any drug (including anticancers), there is always a dose range at which the drug is safe.

Paracelsus' Law states "*Sola dosis facit venenum* (only dose makes the poison)," meaning that all substances are poisons, and there are none which are not. The right dose differentiates a poison from a remedy; this principle is also known as the "dose–response effect" [19, 20]. For any drug, there is always a dose range (concentration) that is without any effect, one with a pharmacological effect but with minimal toxicity (or acceptable safety profile) and another with pharmacological and toxic effects. Thus, a molecule becomes a drug if the dose required to treat a complication falls in the second range: pharmacologically active with minimal toxicity. Most drugs used in the treatment of human diseases fall in this group [21].

In this regard, the example of the antimalarial drug chloroquine (CQ) is noteworthy. CQ is used at 10 mg base/kg on days 1 and 2, and 5 mg/kg on day 3 [22]. At this dose, CQ has an acceptable safety profile. However, a dose of 20 mg/kg is

considered toxic [22], and fatal cases have been reported from doses as low as 30 mg/kg, only three times higher than the normal dose [23, 24]. This indicates that a slight dose increase shifts CQ's effect from the second range (acceptable safety profile with a pharmacological effect) to the third (life-threatening toxicity). Thus, CQ has a very low safety margin, and yet it has been used widely and is considered to be one of the safest antimalarials.

12.4 Safety of Low Dose of MTX in Human

MTX is one interesting example that vindicates Paracelsus' Law. MTX is used at high dose, up to 5,000–12,000 mg/m^2 per week (130–300 mg/kg) for several weeks for the treatment of cancer, and this dose can yield serum concentrations $>$1,000 μM, the concentration range that is associated with MTX life-threatening toxicity [25–27]. On the other hand, a 1,000-fold lower dose of MTX (LD-MTX) [0.1–0.35 mg/kg (7.5–25 mg per adult)] is used once weekly in the treatment of rheumatoid arthritis (RA), and sometimes, for many years. At this dose, MTX is safe and a mainstay in the treatment of RA in the Western world [28]. MTX is also the drug of choice for the treatment of juvenile idiopathic arthritis in children (including infants of less than 1 year old), a common rheumatic disease in the Western world [29].

Worldwide, it is estimated that 0.5–1 million adults and 50,000–100,000 children receive LD-MTX weekly for the treatment of rheumatoid and juvenile idiopathic arthritis, respectively. The drug is now being used in the African population as well, and its safety profile is similar to what has been reported in the Western world [30, 31]. LD-MTX is now considered to be one of the safest drugs used in the treatment of RA, and its safety profile has led to its new repositioning in the treatment of various conditions including multiple sclerosis [32], inflammatory bowel disease [33], urticaria [34], chronic cholestatic disorder [35], Wegener's granulomatosis [36], primary biliary cirrhosis [36] and systemic lupus erythematosus [37], among others.

12.5 Proof of Concept of MTX as an Antimalarial in Humans

The antimalarial potential of MTX has been established for almost 40 years now. Two relatively small clinical trials, involving seven patients, have demonstrated that doses as low as 2.5 mg/per day for 3–5 days are effective to treat malaria infection in humans (*Plasmodium falciparum* and/or *P. vivax*) [38, 39]. However, MTX has not come into widespread use because of concerns over toxicity [40, 41]. At the time of the aforementioned clinical trials (which were in the 1970s), no information was available on the safety of LD-MTX. Indeed, LD-MTX only started to be used for the treatment of arthritis from the 1980s, and before then, it was only used at high doses in cancer treatment, doses associated with toxicity. Thus, its use

as an antimalarial was abandoned. At present, we have 30 years' experience on the safety of LD-MTX in adults and children; thus, this drug could now be reconsidered and evaluated as a potential antimalarial. Unlike its use in immune diseases, in malaria, MTX will not be used on a chronic basis. It will be a "one-off treatment"; as a result, the risk of toxicity should be even lower.

This information on the in vitro activity of MTX, the safety and possible in vivo efficacy of LD-MTX has led us to re-evaluate the potential of MTX as an antimalarial.

12.6 Re-evaluation of the Antimalarial Potential of MTX in Humans

A phase I evaluation of MTX was conducted to assess the safety and pharmacokinetic profile of this drug in healthy adult male Kenyan volunteers [42]. Twenty five adult volunteers were recruited, admitted and received a 5 mg dose of MTX/day/5 days. Pharmacokinetics (PK) blood sampling was carried out at 2, 4, 6, 12 and 24 h following each dose, and patients were followed up to 42 days. The most important known side effects associated with the chronic use of LD-MTX were solicited. These included nausea, vomiting and oral ulcers, among others. The result clearly indicated the absence of drug-associated side effect during the 42-day follow-up [42].

Based on in vitro data, MTX $>$ 350 nM in blood will be required to clear malaria infection in humans [18]. However, PK analysis of the 25 volunteers showed that the achieved maximum concentration (C_{max}) fell between 160 and 200 nM only, and after 6 h, effective concentration (C_{eff}) was $<$ 150 nM. Thus, at a dose of 5 mg/day/5 days, the achieved MTX blood levels would not be high enough to clear malaria infection. This implies that further studies should be carried out to evaluate the dose range that will yield adequate plasma concentrations and still be well tolerated. One of the approaches would be to reduce the days of treatment from 5 to 3 only and increase the dose to 7.5, 10 or 12.5 mg per day. The total dose for each treatment course should, however, remain around 22.5–37 mg, which is close to the range of doses administered weekly in the treatment of inflammatory diseases (7.5–35 mg per adult). Thus, further studies are warranted to explore the antimalarial potential of MTX.

12.7 Other Anticancers with Antimalarial Potency

Our group has also shown that the anticancer antifolates aminopterin, trimetrexate and pemetrexed are potent against *P. falciparum*, with activity in the nanomolar range (IC_{50} $<$ 50 nM) ([18] and unpublished work). Since all these anticancers are inhibitors of DHFR enzymes, we hypothesise that other anticancer inhibitors of DHFR, such as edatrexate, talotrexin, pralatrexate and piritrexim [43], would also

be active against *P. falciparum*. In addition, several other anticancers but non-antifolates have proved potent against *P. falciparum*, and among these are the inhibitors of the microtubulin assembly tubulozole, vinblastine, docetaxel, paclitaxel and dolastatin [44–48]; the DNA cross-linking agent cisplatin [49]; and the proteasome inhibitor Bortezomib [50, 51]. If these drugs were active in vivo at low and safe doses, they could potentially become antimalarials.

12.8 Anticancer Drugs in the Treatment of Non-neoplastic Diseases

The use of MTX in the treatment of non-neoplastic diseases would not be unusual. Indeed, there are many anticancers that are used in the treatment of various complications other than cancer. For instance, cyclophosphamide is used in Behcet's syndrome, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura (ITP), nephritic syndrome, systemic lupus erythematosus (SLE), multiple sclerosis and Wegener's granulomatosis, among others [52, 53]; 6-mercapto-purine is used in Crohn's disease and ulcerative colitis [54]; thalidomide is becoming an important drug in Behcet's syndrome, Crohn's disease and SLE [52, 55]; and vincristine is used in ITP and thrombotic thrombocytopenic purpura [52, 56]. In relation to tropical diseases, the two anticancer agents miltefosine and eflornithine have been repositioned as drugs of choice in the treatment of leishmaniasis and trypanosomiasis, respectively [57, 58]. The common point in all these repositioned drugs is that they are used at doses lower that those used in the treatment of neoplasma; thus, they have better toxicity profiles. Therefore, the use of anticancers could be extended in the treatment of malarial diseases.

12.9 Conclusion

The pharmaceutical industry have now acknowledged the central importance of drug repositioning in the discovery of new active agents, even against diseases for which there are relatively sizeable human and financial resources such as cancer [59]. The rationale is that the large numbers of available drugs should permit, within a shorter time frame and a smaller financial cost, the discovery and development of new uses for drugs. This rationale is in fact more relevant with poverty related diseases such as malaria.

Among drugs that could be repositioned for malaria treatment are anticancer agents. For instance, many anticancers, including MTX, were shown to be potent against malaria parasite several decades ago; however, their perceived toxicity has prevented their development as antimalarials. Yet, as reported by Paracelsus more than 400 years ago, it is "the dose that makes a drug"; this principle has been exploited in the use of several anticancers (including MTX) at low doses in the

treatment of various non-neoplastic diseases. This concept could be extended to anticancer bearing antimalarial activity, such as MTX, as discussed in this chapter. Thus, anticancer pharmacopoeia could provide a good platform for the discovery of new antimalarials.

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References

1. Nosten F, White NJ (2007) Artemisinin-based combination treatment of falciparum malaria. Am J Trop Med Hyg 77:181–192
2. Adjei GO, Goka BQ, Binka F et al (2009) Artemether-lumefantrine: an oral antimalarial for uncomplicated malaria in children. Expert Rev Anti Infect Ther 7:669–681
3. Kokwaro G, Mwai L, Nzila A (2007) Artemether/lumefantrine in the treatment of uncomplicated falciparum malaria. Expert Opin Pharmacother 8:75–94
4. Sirima SB, Gansane A (2007) Artesunate-amodiaquine for the treatment of uncomplicated malaria. Expert Opin Investig Drugs 16:1079–1085
5. Dondorp AM, Yeung S, White L et al (2010) Artemisinin resistance: current status and scenarios for containment. Nat Rev Microbiol 8:272–280
6. Anonymous. http://www.mmv.org/sites/default/files/uploads/docs/essential_info_for_-scientists/3Q_Global_Malaria_Portfolio_Slide_by_therapeutic_type.ppt, entry of 15 Oct 2010
7. Sinclair D, Donegan S, Lalloo DG (2011) Artesunate versus quinine for treating severe malaria. Cochrane Database Syst Rev 3:CD005967
8. Ashburn TT, Thor KB (2004) Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov 3:673–683
9. Campas C (2009) Drug repositioning summit: finding new routes to success. Drug News Perspect 22:126–128
10. Miller TM, Layzer RB (2005) Muscle cramps. Muscle Nerve 32:431–442
11. Sibilia J, Pasquali JL (2008) Systemic lupus erythematosus: news and therapeutic perspectives. Presse Med 37:444–459
12. Efferth T (2007) Willmar Schwabe Award 2006: antiplasmodial and antitumor activity of artemisinin–from bench to bedside. Planta Med 73:299–309
13. Krishna S, Bustamante L, Haynes RK et al (2008) Artemisinins: their growing importance in medicine. Trends Pharmacol Sci 29:520–527
14. Utzinger J, Xiao SH, Tanner M et al (2007) Artemisinins for schistosomiasis and beyond. Curr Opin Investig Drugs 8:105–116
15. Dar O, Khan MS, Adagu I (2008) The potential use of methotrexate in the treatment of falciparum malaria: in vitro assays against sensitive and multidrug-resistant falciparum strains. Jpn J Infect Dis 61:210–211
16. Fidock DA, Nomura T, Wellems TE (1998) Cycloguanil and its parent compound proguanil demonstrate distinct activities against *Plasmodium falciparum* malaria parasites transformed with human dihydrofolate reductase. Mol Pharmacol 54:1140–1147

17. Kiara MS, Okombo J, Masseno V et al (2009) In vitro activity of antifolate and polymorphism in dihydrofolate reductase of *Plasmodium falciparum* isolates from Kenyan coast: emergence of parasites with Ile-164-Leu mutation. Antimicrob Agents Chemother 53:3793–3798
18. Nduati E, Diriye A, Ommeh S et al (2008) Effect of folate derivatives on the activity of antifolate drugs used against malaria and cancer. Parasitol Res 102:1227–1234
19. Langman LJ, Kapur BM (2006) Toxicology: then and now. Clin Biochem 39:498–510
20. Rozman KK, Doull J (2001) Paracelsus, Haber and Arndt. Toxicology 160:191–196
21. Nzila A, Okombo J, Becker RP et al (2010) Anticancer agents against malaria: time to revisit? Trends Parasitol 26:125–129
22. Taylor WR, White NJ (2004) Antimalarial drug toxicity: a review. Drug Saf 27:25–61
23. Cann HM, Verhulst HL (1961) Fatal acute chloroquine poisoning in children. Pediatrics 27:95–102
24. Riou B, Barriot P, Rimailho A et al (1988) Treatment of severe chloroquine poisoning. N Engl J Med 318:1–6
25. Fahey JB, DiMaggio C (2007) High-dose methotrexate and primary central nervous system lymphoma. J Neurosci Nurs 39:83–88
26. Fong CM, Lee AC (2006) High-dose methotrexate-associated acute renal failure may be an avoidable complication. Pediatr Hematol Oncol 23:51–57
27. Mantadakis E, Cole PD, Kamen BA (2005) High-dose methotrexate in acute lymphoblastic leukemia: where is the evidence for its continued use? Pharmacotherapy 25:748–755
28. Swierkot J, Szechinski J (2006) Methotrexate in rheumatoid arthritis. Pharmacol Rep 58:473–492
29. Niehues T, Lankisch P (2006) Recommendations for the use of methotrexate in juvenile idiopathic arthritis. Paediatr Drugs 8:347–356
30. Diouf ML, Diallo S, Mbengue M et al (2001) Methotrexate, liver and rheumatoid arthritis in tropical areas. Sante 11:195–200
31. Tahiri L, Allali F, Jroundi I et al (2006) Therapeutic maintenance level of methotrexate in rheumatoid arthritis. Sante 16:167–172
32. Gray OM, McDonnell GV, Forbes RB (2006) A systematic review of oral methotrexate for multiple sclerosis. Multiple Scler 12:507–510
33. Domenech E, Manosa M, Navarro M et al (2008) Long-term methotrexate for Crohn's disease: safety and efficacy in clinical practice. J Clin Gastroenterol 42:395–399
34. Montero Mora P, Gonzalez Perez Mdel C, Almeida Arvizu V et al (2004) Autoimmune urticaria. Treatment with methotrexate. Rev Alerg Mex 51:167–172
35. Novak K, Swain MG (2008) Role of methotrexate in the treatment of chronic cholestatic disorders. Clin Liver Dis 12:81–96, viii
36. Specks U (2005) Methotrexate for Wegener's granulomatosis: what is the evidence? Arthr Rheum 52:2237–2242
37. Wong JM, Esdaile JM (2005) Methotrexate in systemic lupus erythematosus. Lupus 14:101–105
38. Sheehy TW, Dempsey H (1970) Methotrexate therapy for *Plasmodium vivax* malaria. JAMA 214:109–114
39. Wildbolz A (1973) Methotrexate in the therapy of malaria. Ther Umsch 30:218–222
40. Ferone R (1971) Methotrexate therapy for *P. vivax* malaria. JAMA 215:117
41. Laing AB (1972) Methotrexate in malaria. Trans R Soc Trop Med Hyg 66:518–519
42. Chilengi R, Juma R, Abdallah AM et al (2011) A phase I trial to evaluate the safety and pharmacokinetics of low-dose methotrexate as an anti-malarial drug in Kenyan adult healthy volunteers. Malar J 10:63
43. Hagner N, Joerger M (2010) Cancer chemotherapy: targeting folic acid synthesis. Cancer Manag Res 2:293–301
44. Fennell BJ, Carolan S, Pettit GR et al (2003) Effects of the antimitotic natural product dolastatin 10, and related peptides, on the human malarial parasite *Plasmodium falciparum*. J Antimicrob Chemother 51:833–841

45. Koka S, Bobbala D, Lang C et al (2009) Influence of paclitaxel on parasitemia and survival of *Plasmodium berghei* infected mice. Cell Physiol Biochem 23:191–198
46. Schrevel J, Sinou V, Grellier P et al (1994) Interactions between docetaxel (Taxotere) and *Plasmodium falciparum*-infected erythrocytes. Proc Natl Acad Sci USA 91:8472–8476
47. Sinou V, Grellier P, Schrevel J (1996) *In vitro* and *in vivo* inhibition of erythrocytic development of malarial parasites by docetaxel. Antimicrob Agents Chemother 40:358–361
48. Usanga EA, O'Brien E, Luzzato L (1986) Mitotic inhibitors arrest the growth of *Plasmodium falciparum*. FEBS Lett 209:23–27
49. Nair L, Bhasin VK (1994) Cure with cisplatin (II) or murine malaria infection and *in vitro* inhibition of a chloroquine-resistant *Plasmodium falciparum* isolate. Jpn J Med Sci Biol 47:241–252
50. Kreidenweiss A, Kremsner PG, Mordmuller B (2008) Comprehensive study of proteasome inhibitors against *Plasmodium falciparum* laboratory strains and field isolates from Gabon. Malar J 7:187
51. Reynolds JM, El Bissati K, Brandenburg J et al (2007) Antimalarial activity of the anticancer and proteasome inhibitor bortezomib and its analog ZL3B. BMC Clin Pharmacol 7:13
52. Chabner B, Amrein P, Drucker B et al (2006) Chemotherapy of neoplastic diseases. In: Brunton L (ed) The pharmacological basis of therapeutics. McGraw-Hill, Washington, USA, pp 1345–1403
53. Nannini C, West CP, Erwin PJ et al (2008) Effects of cyclophosphamide on pulmonary function in patients with scleroderma and interstitial lung disease: a systematic review and meta-analysis of randomized controlled trials and observational prospective cohort studies. Arthritis Res Ther 10:R124
54. Prefontaine E, Sutherland LR, Macdonald JK et al (2009) Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. Cochrane Database Syst Rev CD000067
55. Wu JJ, Huang DB, Pang KR et al (2005) Thalidomide: dermatological indications, mechanisms of action and side-effects. Br J Dermatol 153:254–273
56. Mateos J, Perez-Simon JA, Caballero D et al (2006) Vincristine is an effective therapeutic approach for transplantation-associated thrombotic microangiopathy. Bone Marrow Transplant 37:337–338
57. Berman JJ (2008) Treatment of leishmaniasis with miltefosine: 2008 status. Expert Opin Drug Metab Toxicol 4:1209–1216
58. Burri C (2010) Chemotherapy against human African trypanosomiasis: is there a road to success? Parasitology 137:1987–1994
59. Duenas-Gonzalez A, Garcia-Lopez P, Herrera LA et al (2008) The prince and the pauper. A tale of anticancer targeted agents. Mol Cancer 7:82