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Invited Article

Chemotherapeutics challenges in developing effective treatments for the endemic malarias

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ABSTRACT

The endemic malarias threaten the several billion people residing where transmission occurs. Chemotherapeutic strategy pitted against these threats hinges upon species- and stage-specific treatments guided by diagnosis and screening against sometime dangerous contraindications. This approach suits malaria as it occurs among travelers in the developed, non-endemic world. However, limiting treatment to that which diagnosis affirms may not be rational in endemic zones. Most of the endemic malarias remain out of diagnostic reach, either by inaccessibility of the parasite stage, insensitivity of the technology, or unavailability of diagnostic services. The partial and fragmented chemotherapeutic attack of malaria guided by confirmed diagnostics leaves most of the endemic malarias unchallenged. Development of elimination therapy, a single course of treatment aimed at all species and stages, would significantly advance progress against the major killers known collectively as malaria.

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1. Introduction

Malariology may be rightfully considered one of the oldest and most developed technical disciplines among those composing the broad field called tropical medicine. At least 2 Nobel prizes (1902; 1908) have been awarded in malariology, and a third (1927) involved the use of malaria for therapy of tertiary syphilis. Another (1948) was discovery of the potent insecticide DDT that proved hugely effective against endemic malaria. The elimination of endemic malaria from much of North America and Western Europe during the early 20th century, and a global war in which malaria posed a serious threat, spawned armies of malariologists and great strides in the field. Soon after the war, malariologists marshaled their new tools and marched out an effort to eradicate malaria (Harrison, 1978). That campaign had tremendous impact but failed in its ultimate objective. The effort was formally abandoned in 1969 and malaria resurged powerfully between 1970 and 2000 (Baird, 2000).

Malaria today shows signs of retreat in many endemic zones (O'Meara et al., 2008; Behrens et al., 2008; Schmid et al., 2009; van Rijckevorsel et al., 2010; Prudhomme et al., 2010). The determinants of this trend may be poorly understood, but the emergence of strong economic, political, and social commitment against malaria since around 1995 certainly played a role (Feachem et al., 2010; Snow et al., 2010; Katz et al., 2011). Those commitments mobilized three new tools in malaria control: insecticide treated bed nets (ITN), rapid diagnostic tests (RDT), and the class of antimalarial drugs called the artemisinin-combined therapies (ACT). The fourth key malaria control tool, indoor residual spraying of insecticide (IRS), was nearly abandoned several decades ago but today is again emphasized as important (WHO, 2007). These tools, though they have almost certainly diminished morbidity and mortality due to malaria, may be inadequate to the far more complex and difficult task of malaria elimination – the sustained cessation of malaria transmission within a given geographic area.

In 2008 a sea change in global malaria strategy occurred when WHO endorsed a call for elimination as a strategic goal versus the sustainable control of the past four decades. Elimination strategy aims at, "…progressive elimination from the endemic margins, to shrink the malaria map." (Feachem, 2009). This strategy hinges largely upon successful diagnosis and treatment, along with preventive therapeutics and other interventions aimed at minimizing human contact with anopheline mosquitoes.

The principle strategic weakness in malaria elimination strategy may be the view of malaria as a single chemotherapeutic problem (Baird, 2010). Malaria manifests as many diseases, while ACT aims only at the acute attack, and principally that of a single species, *Plasmodium falciparum*. Many other malarias dominate in most endemic zones, and the single chemotherapeutic tool against most of them is primaquine, a 60-year-old drug. That 8-aminoquinoline, like others in the class, causes a mild to severe hemolytic anemia

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in patients having an inborn deficiency of glucose-6-phosphate dehydrogenase (G6PDd) (Beutler and Duparc, 2007). While routine screening of patients for this abnormality prior to therapy occurs in developed nations, the vast majority of patients who could benefit from this therapy, those resident in endemic zones, rarely have such access. The real risk of harm caused by primaquine, and the lack of capacities that could protect patients, explains its poor effectiveness in endemic zones (Baird, 2008).

Elimination of malaria will require chemotherapeutics that effectively deal with all of the malarias in endemic zones. That diverse set of infections includes many silent forms: asymptomatic and sub-patent infections of blood by asexual and sexual stages, and dormant stages in the liver called hypnozoites. The prevalence of these silent malarias appears far higher than has been appreciated, especially in zones of relatively very low transmission. Most of the silent malarias occur beyond the reach of standard and even state-of-the-art diagnostic technologies. Effective chemotherapeutic attack of them may require abandoning species- and stage-specific treatment guided by diagnosis as the keystone of malaria chemotherapeutic strategy and practice.

More than a century of great strides in modern malariology has not yet provided the technical foundations upon which the endemic malarias may be rationally and effectively attacked. Inadequate appreciation of the character of endemic malaria has driven the neglect of a broad array of essential areas of endeavor in chemotherapeutics. The developers of chemotherapeutic strategies and tools now have a key role to play in addressing this strategic weakness. This commentary aims to provide a conceptual framework for the formulation of chemotherapeutics research and development agendas aimed at malaria elimination.

2. Global problem

Authoritative agencies and experts, with the following of popular media, perceive global malaria as an overwhelmingly African problem dominated by *P. falciparum* (Finkel, 2007; United Nations, 2005; US CDC, 2011; Kitua et al., 2011). Recent studies, however, highlight the importance of unrecognized and generally unacknowledged burdens of morbidity and mortality associated with malaria outside of Africa (Hay et al., 2004, 2010; Snow et al., 2010; Dhingra et al., 2010). Estimates of the mortality burden caused by malaria as whole, much less any given species, range across orders of magnitude for mostendemic nations (Dhingra et al., 2010; Elyazar et al., 2011). The numbers of people at risk of any malaria in densely populated South and East Asia greatly exceed those at risk in endemic Africa (Hay et al., 2010; Guerra et al., 2011). If small children and pregnant women in Africa bear the brunt of mortality risk associated with malaria, then numbers at real risk of mortality must exclude a majority of the African population at risk of infection. In contrast, while much of Asia may be accurately characterized as relatively very low intensity transmission compared to Africa, risk of death with malaria occurs across demographic groups (Dondorp et al., 2008; Dhingra et al., 2010; Phu et al., 2010; Sahu et al., 2010; Abu Sayeed et al., 2011).

Recent studies challenging the view of *Plasmodium vivax* as benign and rarely fatal (Kochar et al., 2005, 2010; Barcus et al., 2007; Tjitra et al., 2008; Genton et al., 2008; Sharma and Khanduri, 2009; Valecha et al., 2009; Andrade et al., 2010) emphasize the importance of considering Asian and American malarias as perhaps substantial contributors to the global burden of morbidity and mortality associated with malaria. The global malaria problem weighs across endemic zones with all of the species and stages of the parasites responsible for the malarias. Tools that address them all advance the aim of elimination.

3. The toolbox for malaria

The tools available to national malaria control programs (NMCP) to strategize for control and elimination impose important limitations. Table 1 summarizes these. In each avenue of implementation, more research is required to maximize the extent to which NMCPs may approach interruption of transmission and its permanent elimination as a realistic goal. Despite hundreds of millions of RDTs being used annually, most malaria in most endemic zones is not diagnosed, not reported, and very often inadequately treated (Dhingra et al., 2010; Nsagha et al., 2011; Littrell et al., 2011; Ndo et al., 2011; Nyandigisi et al., 2011; Yeung et al., 2011; Elyazar et al., 2011; Mazumdar, 2011; Shaikh and Haran, 2011). Resistance to the pyrethroid insecticides threatens the effectiveness of ITNs (Chanda et al., 2011; Himeidan et al., 2011). Moreover, ITNs have relatively little impact in low transmission settings (Takken, 2002; Sochantha et al., 2006), especially where local mosquito vectors feed predominantly outdoors or in the early evening. The effectiveness of IRS may be limited by either a reluctance to use inexpensive and long-lasting DDT, or mosquito resistance to it. NMCPs typically depend upon relatively noxious, toxic, expensive, and short-lasting pyrethroids, carbamates or organophosphate insecticides. Those products – developed for and widely used in the agricultural sector – are also under threat of mosquito resistance. Despite the likely inadequacy of these tools to the task of elimination, diagnostics and interventions against mosquito vectors accounted for less than 5% of global malaria research expenditures between 2004 and 2009 (PATH, 2011).

Spending on vaccines, drugs and basic research dominated global malaria research & development at 87% of spending in 2009 (PATH, 2011). Development of vaccines for malaria suffers a lack of understanding of acquired immunity to the infection adequate to rational winnowing of candidate molecules (and their formulation and dosing) prior to phase III clinical trials (PATH, 2011; Baird, in press). Those trials must be large enough to evaluate protective efficacy against severe illness and death; the only end points of importance in the absence of achievable sterilizing immunity. One protein sub-unit vaccine, RTS, S, in development since 1987 reached phase III trials in 2010/2011. A distinct approach to vaccination involving live parasite challenge followed by suppressive chemoprophylaxis, achieves sterilizing protection and may thus be relatively quickly and inexpensively evaluated (Roestenberg et al., 2011).

Rolling cycles of parasite susceptibility yielding to resistance drive the continuous and chronically urgent search for new drugs (White, 2004). The life of any given antimalarial typically ranges from several years up to several decades, depending on myriad probable determinants. The one drug that seems to defy this rule, ancient quinine, may be the most impractical and potentially dangerous of antimalarial drugs (Townsend et al., 2004; Shine and Coates, 2005; Huston and Levinson, 2006; White, 2007). It is certainly the most unpleasant from the perspective of the patient, with serious adherence problems (Achan et al., 2009). Chloroquine, mefloquine, and the antifolate antimalarials have each in turn fallen as front-line therapies against acute attacks of falciparum malaria. ACTs seem poised to follow (Dondorp et al., 2009).

The generous funding provided to chemotherapeutics thus stands out as offering greatest promise for delivery of useful tools in the near future. Diagnostics, drugs, and interventions against the vector succeeded in eliminating endemic malaria from North America, most of the Caribbean, Europe, Japan, and numerous other islands in the Asia Pacific region during the 20th century. That precedent points to strategy emphasizing improvements to these tools for the tougher task of accomplishing the same feat across endemic Oceania, Asia, Africa, and the Americas.

Table 1
The malaria control and elimination toolbox.

	RDT	ACT	ITN	IRS
Name	Rapid diagnostic test	Artemisinin-combined therapies	Insecticide-treated nets	Indoor residual spraying
Sector	Diagnosis	Treatment	Prevention	Prevention
Application	Point-of-care kit suited to paramedics	Several days dosing post-diagnosis	Nightly in household	Quarterly to semi-annually in households
Primary advantage	Suited to endemic zones	Good efficacy against acute *P. falciparum*	Suited to endemic zones	Passive compliance
Primary disadvantage	Unable to detect most asymptomatic parasitemias	Ineffective against mature sexual blood stages and dormant liver stages	Low efficacy without high coverage rates or in low transmission settings	Requires substantial technical know-how and organizational infrastructure
Long term outlook	Less practical and relevant as transmission declines	Less effective as resistance rises	Donor exhaustion may threaten sustainability	Recipient and implementer exhaustion

4. Chemotherapeutics imperatives

No area of endeavor in malaria research and development supporting control and elimination is in greater need of a corrective course in strategy than chemotherapeutics. This arena has been dominated by a single imperative over the past six decades: treatment of the acute attack of *P. falciparum*. The view of malaria as a global problem almost entirely focused on that parasite and a single continent (where the other major cause of malaria, *P. vivax*, is relatively rare) surely helps explain this emphasis. But other key considerations also come into play: chief among those may be the ability to continuously maintain discreet lines of the disease-causing stages of *P. falciparum* in state-of-the-art laboratories in North America and Europe. In contrast, the same stages of *P. vivax*, and its important dormant liver stage, cannot be thus maintained. Unless *P. vivax* becomes similarly tamed to the laboratory, much of the work committed to this parasite will have to be done within convenient access to patients in endemic zones. There are compelling reasons to develop such access for chemotherapeutics research and development, and these are not limited to vivax malaria.

4.1. Malaria of travelers versus residents

Species- and stage-specific treatment of diagnosis-affirmed parasite targets in patients underpins malaria chemotherapeutics strategy and practice. This paradigm, while perfectly suitable for coping with the relatively rare imported malarias in North America and Europe, suffers serious drawbacks with respect to chemotherapeutic management of the many malarias in many millions of patients in endemic zones. Examination of the fundamental differences between malaria as it occurs in travelers versus residents draws attention to critically important issues that are directly relevant to chemotherapeutics strategy.

In a typical year almost all of the several tens of thousands of reported malaria cases occurring outside of endemic zones have a diagnosis of a single species of *Plasmodium*. The majority of these patients are non-immunes suffering acute malaria at relatively very low parasite densities in the blood (Mali et al., 2011). Relatively sensitive molecular diagnostics, however, detect those low parasitemias and are often applied in travelers with suspected malaria. Although missing that diagnosis sometimes ends tragically (Alunni-Perret et al., 2010), usually >99% of patients receive appropriate treatment and enjoy complete recovery. Among the approximately one-half diagnosed with *P. vivax*, most (87% (Muhlenberger et al., 2004)) receive the primaquine required to prevent relapses. Successful adherence to the 14-day regimen may require emphatic instruction or supervision. Virtually all patients prescribed primaquine would have been screened for both pregnancy and G6PDd, as not doing so invites exposure to risk of real harm. In short, travelers almost always receive chemotherapy informed by necessary laboratory evidence available to their providers.

In sharp contrast, most acute malaria in endemic zones is either self-treated or managed by a neighbor with some specialized training (see Section 3). An RDT at this ground level of care delivery may be available and permit treatment with ACT. If not, according to the national malaria treatment guidelines of many endemic countries, the patient will be administered chloroquine or sulfadoxine/pyramethamine, despite the frequent dominance of parasites resistant to these drugs. Further, primaquine is rarely prescribed because screening for G6PDd requires specialized equipment, laboratory skills, and a cold chain rarely available in endemic zones. Also, primaquine should not be given to pregnant women because it threatens the fetus of unknown G6PD status. The poor practicality of primaquine causes endemic gametocytes and hypnozoites to very often go unchallenged by any medical intervention.

In the context of control and especially elimination, silent malarias – those causing no illness and occurring beyond the reach of diagnostics – represent steep challenges. Infection by hypnozoites cannot be diagnosed by any standard means and their prevalence may be very high, even in areas of relatively low transmission. Douglas et al. (2011) evaluated 10,549 patients in Thailand treated with experimental therapies for acute *P. falciparum* malaria: among those given rapidly excreted drugs, 51% experienced attacks by *P. vivax* within 8 weeks. In addition to gametocytes and hypnozoites, low-density and asymptomatic asexual parasitemias also pose a significant threat to public health in endemic zones. A blood survey of over nine thousand people in the Solomon Islands documented 2.7% prevalence by expert microscopy, but 9% by PCR diagnostics (Harris et al., 2010). Only about 5% of those with parasitemia were febrile at examination, and most occurred well beyond the diagnostic reach of RDTs.

Treatment strictly guided by diagnosis fails to challenge most of the malarias that apparently dominate endemic zones. The majority occurs beyond diagnostic reach, and the most important tool for attacking many of them, primaquine, cannot be responsibly applied for want of a diagnostic device that safely excludes those at risk harm caused by that therapy. Chemotherapies tailored to malaria in travelers, informed by diagnostics and thereby partial and fragmented across species and stages, is not adequate to controlling malaria in endemic zones, much less eliminating it.

4.2. Appropriate therapies

Chemotherapy of malaria in endemic settings must take into account inadequate access to and sensitivities of diagnostics, the dominance of silent malarias, and the lack of clinical supervision. An unsupervised and safely administered single course of treatment effective against all of the malarias represents the strategic aim. Development of new drugs should be guided by that aim, but survey of already available drugs also yields possibilities in the nearer-term.

An assembled therapy suited to malaria elimination would include blood schizontocide(s), hypnozoitocide(s), and gametocytocide(s). Although many candidate blood schizontocides are available, primaquine (or tafenoquine, an 8-aminoquinoline in phase III trials in 2011) stands as the only option for the latter two therapeutic compartments. This brings focus to the primary problem: what regimen of primaquine may be safely and effectively administered with a blood schizontocide(s) without regard to offending species and stages of parasite(s) and G6PDd status? Hypnozoitocidal doses of primaquine providing incidental gametocytocidal coverage simplifies the problem to discovery of dosing regimens aimed at hypnozoites within the range of safe tolerability among the most sensitive variants of G6PD. Alternatively, discovery of a point-of-care device for safely excluding those at risk of harm may suffice. Both avenues merit immediate attention.

5. Chemotherapeutics research rationale

Overcoming the toxicity of primaquine or tafenoquine among G6PDd patients represents the primary technical objective in working towards elimination therapies. Avenues of doing so remain largely unexplored and at least several now command attention. Pursuit of these offers bright prospects of important discoveries and large strides in the ability to attack endemic malaria.

5.1. G6PDd RDT

Safe exclusion of G6PDd patients from exposure to primaquine with a point-of-care diagnostic that proves robust in endemic settings has not been pursued until very recently. Such a kit would certainly permit far broader application of primaquine as currently prescribed (0.25 or 0.5 mg/kg daily for 14 days), and could open the possibility of higher doses of shorter duration (e.g., 30 mg 3 times daily for 3 days) for the G6PD-normal majority. Prolonged dosing certainly curtails effectiveness, especially against a backdrop of NMCPs that may be reluctant to engage in advocacy for prescribing and adhering to a potentially harmful therapy against a silent infection.

RDTs for malaria have proven enormously useful in coping with acutely ill patients in endemic zones. These simple, durable, and inexpensive instruments work effectively at the ground level of healthcare delivery – the relatively impoverished endemic village. A similar kit for diagnosis of G6PDd would permit the unleashing of primaquine with greatly mitigated risk of serious harm. The technical task of fielding such a kit is complex and challenging due to the currently poor state of the knowledge base that must inform such work.

Primaquine sensitivity phenotypes among the hugely diverse family of clinically significant G6PD mutants remain very poorly characterized. The phenotype ranges from inconsequential to life threatening with primaquine challenge. The supposed inverse relationship between residual enzyme activity and sensitivity to primaquine derives from the presumption of cause-and-effect (the mechanism of primaquine-induced hemolysis is unknown) and the primaquine sensitivity phenotypes of just three variants. The African A-, Mahidol, and Mediterranean B- variants represent mild, moderate and severe hemolytic sensitivity to primaquine. These also happen to represent relatively high, moderate, and low levels of residual enzyme activity, respectively. Whether this correlation holds across variants has not been systematically evaluated, and at least some case reports suggest that important exceptions occur (Ziai et al., 1967). Development of a robust RDT for primaquine sensitivity based on G6PD activity will require confirmation of

the inverse correlation between that phenotype and residual enzyme activity.

Such an RDT will also require coping with other important variables. G6PDd is X-linked and thus heterozygous among females. Lyonization of the trait among them creates G6PDd phenotypes ranging from null to full expression. The kit must also deal with variable states of acute malaria and anemia and reliably exclude those at risk of harm, especially if higher doses over shorter duration are employed to improve primaquine effectiveness. In a strategic sense, the kit should not aim at diagnosis of G6PDd per se, but at primaquine "go/no go" decision-making by providers at the extreme periphery of care delivery. A G6PDd RDT in development was recently evaluated in Cambodia with promising results (Kim et al., 2011).

5.2. Less threatening dosing

Development of less threatening doses of primaquine, though a complex and nuanced issue, offers promise of substantial and relatively easily gained improvements to safety. Such development requires consideration of two important and unusual chemotherapeutic principles with primaquine: the total dose effect, and the synergistic effects of chloroquine and quinine on hypnozoitocidal activity.

5.2.1. Total dose effect and dosing strategy

The efficacy of primaquine against relapse hinges on the total dose delivered with almost no regard to schedule of dosing. Although evident in some human clinical trials, Schmidt et al. (1977) demonstrated this principle most convincingly in the *Plasmodium cynomolgi* model of relapse in rhesus macaques: the same total dose of primaquine whether administered as a single dose, or as smaller doses spread over many weeks showed equal efficacy. Incremental therapeutic activity against dormant hypnozoites appears to be irreversible and cumulative to complete efficacy over at least 8 weeks.

This effect offers great flexibility in dosing, and it was exploited by the developers of primaquine to field regimens of treatment with primaquine without the necessity of screening for G6PDd. The 14-day regimen delivered relatively less threatening doses and permitted early cessation of dosing. They also developed a regimen intended specifically for G6PDd patients: the single weekly dose of 45 mg primaquine for 8 weeks (Alving et al., 1960). These strategies were considered safe for most of the US Army recipients the developers had in mind, i.e., African-American soldiers with the African A- variant of G6PDd(safety of that regimen among other variants of G6PDd has not been evaluated). The total dose effect of primaquine provides great latitude in adjusting dosing to within safe tolerability of the most sensitive G6PDd variants. Defining those limits thus emerges as a vital evidence base for development of elimination therapies.

5.2.2. Synergism and dose optimizing

The synergism of primaquine activity against hypnozoites by drugs that otherwise have no known effect on that stage has not been understood or widely acknowledged. The developers of primaquine recognized the phenomenon at work with pamaquine (the 8-aminoquinoline predecessor of primaquine) and quinine, and later conclusively demonstrated it in a clinical trial designed and executed for that specific purpose (Alving et al., 1955). They randomized 57 prisoner volunteers to three treatment groups all experimentally challenged with sporozoites of Chesson strain *P. vivax* and receiving identical treatments with primaquine (15 mg daily for 14 days). Two groups of subjects also received identical daily doses of quinine known to have complete efficacy against the blood stages of Chesson *P. vivax* (by therapy of the same using

volunteers challenged with blood stages), but one group receiving quinine and primaquine concurrently, and the other group consecutively (quinine followed by primaquine after a two day pause). Relapse occurred in 1 of 19 volunteers receiving concurrent, and in 15 of 19 receiving consecutive therapies. Four subjects receiving chloroquine (concurrent with primaquine) relapsed. The experiment demonstrates not only a conspicuously important effect (concurrent versus consecutive quinine), but also what may be chemical class-specific synergistic activity (quinine versus chloroquine). There may be other compounds with greater synergistic effects that could be leveraged to deliver less threatening doses of primaquine. Recent work with tafenoquine showed a 10-fold reduction in the minimal effective dose against relapse of *P. cynomolgi* in rhesus macaques when administered with blood schizontocide (Dow et al., 2011).

5.3. Strategy

The total dose effect and synergy of primaquine activity provide means to explore regimens of therapy that do not threaten the most vulnerable among the G6PDd. Is the lowest possible effective total dose of primaquine or tafenoquine divisible over a practical period for safe and efficacious delivery to unscreened G6PDd patients? Answering this question would directly inform a key element of malaria control and elimination strategy. An affirmative answer would open the possibility of therapies safe and effective for all of the malarias of endemic zones without regard to diagnostic reach or G6PDd status.

6. Conclusions

Malaria chemotherapeutics research and development over the past six decades has not taken aim at the endemic malarias. Treatment guided by diagnosis and screening against contraindications has proven impractical in endemic zones, especially in the case of primaquine. Despite the wide availability of ACTs, their use is restricted to the diagnosed acute attack. Moreover, these therapies have not been systematically evaluated for use with primaquine to achieve the arrest of transmission or the prevention of relapses. The long neglect of primaquine and the G6PDd problem inhibited development of elimination chemotherapeutics strategies, but now offers bright prospects for discovery of practical and effective applications of primaquine (or tafenoquine) in elimination therapies. The extraordinary total dose and synergistic effects of this drug may be shrewdly exploited to develop treatments that may be within range of safe tolerability among patients without diagnosis and screening. In short, the chemotherapeutics potential of this 60-year-old drug for endemic zones may be considered effectively unexplored. Malaria chemotherapists developing evidence leading to elimination therapies will effectively arm NMCPs with an instrument that radically improves the likelihood of successful elimination of malaria transmission.

Nonetheless, even quick delivery of a safe and effective elimination therapy using primaquine or tafenoquine will leave important gaps. Safety concerns driven by a lack of compelling data will likely lead to the exclusion of pregnant women and very small children from its application. If a G6PDd RDT is applied in lieu of universally safe (and perhaps impractically prolonged) dosing, the G6PDd patients also become excluded from treatment. In the longer term, development of therapies that kill hypnozoites and gametocytes without threatening G6PDd patients should be the highest chemotherapeutic imperative for research. The 8-aminoquinolines, it should be recalled, were discovered and developed before the existence of the hypnozoite was known: the chemical universe of hypnozoitocidal possibility may be considered virtually unexplored.

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