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Short communication

Combination therapy for HIV-1 infection-overview: preclinical and clinical analysis of antiretroviral combinations

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Prolonged single-agent therapy for HIV-1 infection has been associated with development of drug-related toxicities, evidence of clinical drug failure, and emergence of drug resistance Johnson, 1994; Johnson et al., 1990; Johnson et al., 1991; Richman et al., 1991; Schinazi, 1991; Johnson et al., 1992; Richman, 1993; Richman et al., 1994; Caliendo and Hirsch, 1994; Belen'kii and Schinazi, 1994; Prichard et al., 1993. Based on clinical trials with available drugs to date, single-agent regimens will not likely be enough to provide long-term virus suppression in HIV-1-infected individuals. There are multiple factors contributing to drug failure in the clinic, including the dynamic nature of chronic viral infection (with tremendous viral burden and ultimate immunologic decline), emergence of drug-resistant HIV-1, detection of more cytopathic (syncytium-inducing) variants of HIV-1, and potential host cell drug resistance or insensitivity (due to altered drug metabolism and/or failure to achieve drug delivery into protected viral reservoirs/virus-infected cells) Johnson, 1994; Ho et al., 1995; Wei et al., 1995. As a result, there is much interest in the role of combination therapy for HIV-1 infection to limit viral spread. This approach has been successful in the treatment of a variety of bacterial and fungal infections, as well as in cancer chemotherapy. The goals for combination anti-HIV-1 therapy are: (1) To achieve more complete virus suppression in HIV-1-infected individuals, leading to longer disease-free intervals; (2) To limit the emergence of drug-resistant viruses; (3) To treat established drug resistance, based on observations that combined regimens including zidovudine may still be effective against zidovudine-resistant HIV-1 because virus populations exist as mixtures of zidovudine-sensitive and zidovudine-resistant HIV-1 Johnson et al., 1991; Richman et al., 1991.

What information can in vitro combination testing provide relevant to the design of clinical trials? The benefits of combination therapy testing include efforts to distinguish potential antiviral additive or synergistic combined drug effects, although in vitro efficacy should not be equated with in vivo benefit. The primary goal of combination therapy testing in vitro is to rule out antagonistic drug interactions (e.g. ribavirin's interruption of intracellular activation of zi-

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dovudine to its active triphosphate form) Caliendo and Hirsch, 1994. Ideally, these combination regimens should affect viral replication in a broad range of cell types, provide activity against heterogeneous viral populations, and should not display additional toxicity in combination or share cross-resistance. A number of methodologies for combined drug testing and mathematical analyses of combined in vitro drug interactions are now available. The ideal combined therapy in vitro assay should employ standardized methodology, should include testing of physiologic host cells using primary HIV-1 isolates, and should evaluate combined drug effects on both viral replication and potential drug-induced cytotoxicity. It should also be reproducible, quality-assured and amenable to mathematical analysis. Essential features of in vitro drug combination studies are that: (1) More than one concentration of each agent should be tested alone and in combination; (2) Uninfected drug-treated toxicity controls should be included to evaluate cellular proliferation and viability (tested as single agents and in combined regimens); (3) Multiple timepoints in culture should reproduce the phenomena, not just one harvest timepoint; (4) The terminology and the method of data analysis should be defined. Fortunately, most investigators agree with these consensus guidelines for combination drug testing in vitro.

There are several limitations to be considered regarding combination therapy testing in vitro. Evidence of superiority of one drug combination regimen over another may be a function of dose selection of each component agent. Evidence of in vitro 'cure' may be a function of experimental design (e.g. a short duration in culture, a low challenge of viral inoculum at the start of the experiment, removal of all infected cells during passage, etc.). Although it is difficult to assess dose-effect relationships in complex biological systems based on visual inspection of the data alone, it is also important to recognize that all methods of analyses are limited by the inherent difficulty in applying a mathematical model for analysis of complex biologic assays. For example, results obtained after breakthrough of viral replication has occurred during single and two-drug therapy in vitro may be determined by a mathematical model to be due to 'antagonism' by some methods of data analysis, rather than simply due to the expected lack of persistent drug effect following multiple rounds of viral replication. Thus, an understanding of the biologic phenomena that affect the results of in vitro studies is required to correctly interpret mathematical models that are applied to these biologic systems. Finally, it must be recognized that the nature of drug combination testing in vitro is 'crude' relative to drug effects in vivo, which are limited by complex pharmacologic factors and tremendous viral reservoirs that may overwhelm available agents at achievable drug concentrations.

There is now a general consensus that alternating therapy is inferior to combined simultaneous therapy for control of HIV-1 replication. Alternating drug regimens may limit drug toxicity, but this essentially represents a 'single agent' approach (i.e. one drug therapy is given at one time) that is not likely to maximally suppress viral replication. In contrast, simultaneous combination regimens may optimize antiviral efficacy by combining each agent at its maximally tolerated dose. In one in vitro study by T. Mazzulli et al., the efficacies of two-, three- and four-drug combinations given continuously or in alternating regimens were compared Mazzulli et al., 1994. Not surprisingly, there was greater suppression of HIV-1 infection as the number of drugs in the regimen was increased from one to four simultaneous drugs. Although alternating drug regimens were effective, they were not better than continuous administration of either single drugs or combinations of agents and were less effective than giving all drugs of an alternating regimen simultaneously Mazzulli et al., 1994. In one small study by de Jong et al., alternating nevirapine and zidovudine treatment did not prolong nevirapine activity in 10 HIV-1-infected patients with positive HIV-1 p24 antigen levels and no prior antiretroviral activity de Jong et al., 1994. These subjects were treated for 9–13 weeks with an alternating regimen of 1 week of nevirapine (200 mg/day) and 3 weeks of zidovudine (600 mg/day) to test the hypothesis that alternating courses with two RT inhibitors could potentially delay the

emergence of drug resistance by either limiting the time of drug exposure or by presenting different selection pressures on HIV-1 RT or both. Although serum p24 antigen levels declined during the first week of nevirapine treatment (median, 59%), subsequent courses of nevirapine were characterized by rising p24 antigen levels, whereas antigen levels remained stable or declined during zidovudine treatment. HIV-1 isolates obtained from two patients revealed 40- and 1000-fold reductions in nevirapine susceptibility after 8 weeks. This study concluded that alternating treatment with zidovudine and nevirapine does not prolong the effectiveness of nevirapine and does not prevent the development of nevirapine resistance de Jong et al., 1994. Therefore, it is unlikely that alternating single-drug strategies will be employed as a means to optimally impact on viral burden and viral dynamics. With the availability of more potent antiretroviral agents for two- and three-drug regimens, it may be possible to delay the emergence of drug resistance using a combination therapeutic approach.

Several RT inhibitors are associated with development of drug resistance-conferring mutations that 'suppress', 'reverse' or 'compensate' other RT inhibitor-associated mutations when these particular RT inhibitors are combined, based on in vitro molecular studies of drug interactions St. Clair et al., 1991; Larder, 1992; Tisdale et al., 1993; Larder et al., 1995. For example, the dual-presence of zidovudine-resistance and didanosine-resistance-conferring RT mutations may result in restored zidovudine susceptibility St. Clair et al., 1991. Similarly, the dual-presence of zidovudine-resistance and nonnucleoside-resistance-conferring RT mutations may reverse zidovudine resistance Larder, 1992. Moreover, when molecular clones are constructed with both zidovudine resistance-conferring mutations and a lamivudine (3TC) resistance-conferring mutation at RT codon 184 (Met → Val), these viruses have restored susceptibility to zidovudine in phenotypic drug assays when compared to molecular clones containing only zidovudine-resistant RT codon mutations Tisdale et al., 1993; Larder et al., 1995. These apparent phenotypic interactions that occur among co-expressed RT mutations suggest significant structural and functional flexibility of the RT enzyme. By exploiting these observations, combination therapy with selected two RT inhibitors may theoretically lead to sustained antiviral efficacy, even when zidovudine-resistant genotypes are present. There may ultimately be certain constraints on the ability of the HIV-1 RT to mutate, which represents the concept of 'convergent combination chemotherapy' Chow et al., 1993. These in vitro findings are currently being extended to clinical evaluation of multi-drug regimens that include RT inhibitors that are associated with 'suppressor' or 'compensatory' mutations, in the hope that more complete suppression will be achieved. However, drug testing of multiply RT-mutated HIV-1 molecular clones in vitro demonstrates that not all mutations are 'additive' or necessarily 'compensatory'. For example, molecular clones containing didanosine-conferring and nevirapine-conferring RT mutations are co-resistant Larder, 1992. Finally, some some RT mutations may attenuate viral virulence. For example, despite the rapid emergence of phenotypic and genotypic lamivudine resistance associated with development of a RT codon 184 mutation (M → V), sustained viral suppression is seen during lamivudine therapy in vivo, which is possibly due to attenuated viral virulence Larder et al., 1995; Wakefield et al., 1992. Clinical evaluation of combined RT inhibitor regimens that include lamivudine is currently underway based on these observations.

What strategies should be used to prioritize those combination regimens tested in vitro for future use in the clinical arena? To choose the most optimal drug therapies for larger-scale clinical evaluation in HIV-1-infected individuals, future antiretroviral trial designs will involve screening of combined therapy regimens in vivo for those strategies with maximal effects on virologic markers (i.e. reduction in viral burden, delay in detection of viral resistance, and delay in emergence of syncytium-inducing viruses) Johnson, 1994. This intensive virologic approach may then help identify promising combinations of active non-toxic agents for larger-scale efficacy trials. Recent data from ACTG protocol 155, ACTG protocol 175, and the European Delta Study sup-

port the need to intervene at earlier stages of HIV-1 infection with combined strategies to yield optimal effects on viral burden and to hopefully limit emergence of viral resistance. As drug resistance with current agents is probably not avoidable, we need combination therapy to achieve more complete virus suppression. Ultimately, we still need more potent non-toxic drugs, as well as a better understanding of the critical pharmacologic factors that influence sustained antiretroviral response. Promising strategies undergoing clinical evaluation currently include two-, three-, and four-drug regimens of RT inhibitors combined with other RT inhibitors and/or HIV-1 protease inhibitors, which combine agents that act at both early and late steps in the HIV-1 replicative cycle intracellularly. Ideally, antiviral intervention will be instituted at earlier stages of infection, including immediately following seroconversion. Efforts to halt immunologic deterioration, including combined strategies involving antiretrovirals with either immunomodulators, biologic response modifiers, or cytotoxic T lymphocytes, will be pursued to enhance host factors that limit viral spread and drug resistance. Further research efforts to identify better virologic markers will also continue to define when to best initiate combined therapeutic interventions in HIV-1-infected individuals.

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