CCR5 ANTAGONISTS IN HIV THERAPY

REPORT OF THE FORUM FOR COLLABORATIVE HIV RESEARCH
CHEMOKINE CORECEPTOR ANTAGONIST WORKING GROUP

ROUNDTABLE I: REGULATORY REQUIREMENTS AND TROPISM DIAGNOSIS

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FORUM FOR COLLABORATIVE HIV RESEARCH

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FOREWORD

This report summarizes the discussions at the CCR5 Antagonists for HIV Therapy Working Group Roundtable Discussion held on May 23, 2005, in Washington, D.C[*]. This was the first of a planned series of discussions engaging the key players in new drug development and clinical research -- the patient community, the pharmaceutical and diagnostic industry, regulatory agencies, and researchers. We envisioned that regular meetings involving all parties, held in a neutral setting, would contribute to the development of new HIV therapeutic agents by providing a platform for discussion of concerns and issues brought to the table by each of the constituencies. Such a process is especially needed in the setting of new drugs from new drug classes with which the HIV community has had very limited experience. In the current case, such a process would also facilitate new drug development by garnering "intersponsor" experience gained in clinical trials sponsored by various companies.

All new drug classes present many "unknowns" until such a time that the HIV community develops long-term experience regarding the efficacy, effectiveness, drug resistance patterns, tolerability and long term safety characteristics of the individual drugs and in combination with other antiretroviral therapies. For chemokine antagonists, however, the issues and concerns extend further, to concerns regarding the long term implications of targeting a host cellular receptor involved in immune responses, the efficacy/effectiveness implications of specific targeting of viruses with specified tropism, and the unknown long term effects of tropic-specific inhibition of viral replication. These concerns are compounded by the lack of experience with tropism detection assays and the clinical relevance of changes in viral tropism. While these concerns exist, there is general agreement within the HIV community that new drug development *must* continue in order to provide better treatment options for HIV-infected patients.

The first roundtable discussion of the series focused on the pressing issues of clinical trial design, expectations and requirements from U.S. and European regulatory agencies, and diagnosis of viral tropism. Several developments have taken place since the time of the first roundtable – namely unexpected adverse events[1] and in some situations, less than optimal antiviral activity[2]. These events illustrate the need for continued open dialogue involving all key players, as the HIV community gains further experience in the optimal use of this new drug class.

[*] This first roundtable focused primarily on CCR5 antagonist; subsequent roundtables have included CXCR4 and CCR5 antagonists, thus the name of the working group has been changed to "Chemokine Coreceptor Antagonist Working Group" as reflected in the title of this report

The second roundtable discussion focused on potential biologic and immunologic effects of chemokine antagonist treatment[3]. A third meeting, sponsored jointly by the FDA and the Forum for Collaborative HIV Research, will provide an opportunity for public input regarding the necessity of, and mechanisms for, long term follow up of patients participating in chemokine antagonist clinical trials.[4]

Subsequent discussions will be planned with the project's steering committee. The discussion series will continue for as long as there is a need to do so.

EXECUTIVE SUMMARY

The May 23, 2005 CCR5 Antagonists in HIV Therapy Working Group Roundtable was the first in a series of discussions planned to focus on issues specific to the development of coreceptor antagonists, a new class of drugs for the treatment of HIV infection.
The Forum for Collaborative HIV Working Group consists of representatives from the patient community, academia, regulatory agencies, and industry.

GOALS AND OBJECTIVES

The goals of this meeting were:

- To review and discuss regulatory requirements in the US and Europe for the development of CCR5 antagonists in treatment-naïve and treatment-experienced patients
- To identify unanswered questions and discuss approaches to filling the gaps in knowledge related to the safety and efficacy of CCR5 antagonists in the context of drug development and expanded access programs
- To discuss issues related to the diagnosis of viral tropism before and after treatment, before and after the development phase
- To discuss and identify mechanisms for long-term follow-up of patients enrolled in CCR5 antagonist clinical studies

REGULATORY CONCERNS

CCR5 antagonists target a host cell receptor rather than a viral enzyme, leading to concerns regarding the best use of this drug class, and the approach to patient follow-up. In addition to the "usual" safety issues associated with new drug classes, CCR5 antagonists face scrutiny regarding their potential effect on viral tropism and changes therein, as well as potential immunologic safety issues. At this time in development, there is some disagreement regarding the relative importance of these risks. Clinical studies in the early phase of development are expected to contribute information regarding safety and efficacy, helping to delineate the risk:benefit of this drug class. These data will guide the design of phase 3 studies. Safety is the most important consideration for clinical trial design, and the oversight role of the Data and Safety Monitoring Board will be critical.

Differences in the FDA and EMEA recommendations

The timing of studies in treatment-naive patients:

- The EMEA would prefer that studies in treatment-naive patients, especially those with low CD4+ cell counts, not be started until phase 3 studies
- The FDA would like data on treatment-naive patients from closely monitored phase 2b trials, if early phase 1 and 2 data, resistance data, and preclinical studies indicate minimal safety concerns; otherwise, studies in treatment-naive subjects should wait for phase 3

Phase 3 minimum follow-up:

- The EMEA requests 2 years of follow-up for all study participants.
- The FDA requests 5 years of follow-up on subjects with virologic failure.

Issues that need to be addressed include:

- Coreceptor specificity and occupancy by CCR5 antagonists
- Potential changes in viral coreceptor tropism associated with CCR5 antagonist therapy
- Long-term safety of blocking endogenous CCR5 receptors and of coreceptor tropism changes
- Length of follow-up needed to address safety issues, which, because of the host issues, needs to be longer than in previous drug trials
- The placement of CCR5 antagonist in the regimen strategy and sequence of antiretroviral regimens
- Identification of the class of antiretroviral drugs that CCR5 receptor antagonists will replace, if any
- The adequate frequency of testing for drug resistance and tropism changes during CCR5 antagonist therapy
- Treatment options following virologic failure due to resistance or tropism changes
- Identification of populations that will benefit most from CCR5 antagonist therapy
- Dose adjustment requirements, if any, for drug interactions and for different types of populations (e.g., treatment-naive versus heavily treatment-experienced patients, adults versus adolescents and children, CCR5 antagonist-naive vs. CCR5-antagonist-experienced)
- Phase 3 trial designs to answer these questions

Research on viral tropism and drug resistance has revealed the following:

- Viral resistance to entry inhibitors is dependent on the mechanism of action of the inhibitor
- Reductions in viral susceptibility to fusion inhibitors is best described by increases in IC_{50} due to competitive inhibition mechanisms, analogous to reverse transcriptase and protease inhibitors
- The ability to inhibit viral replication 100% at high drug concentrations is consistent with a competitive mechanism of inhibition and escape
- Reductions in viral susceptibility to some receptor and coreceptor antagonists is associated with an uninhibited fraction at high drug concentrations (plateau <100%)
- The inability to inhibit viral replication 100% at high drug concentrations is consistent with a noncompetitive mechanism of inhibition and escape
- The method of viral escape depends on the mechanism of action of the drug.
- In noncompetitive inhibition, escape variants may be using inhibitor-bound receptor or coreceptor complexes
- Infectivity is not a marker for fusibility, and vice versa

The following additional studies are needed to better understand the interaction of HIV, cell surface receptors, and receptor antagonists:

- Additional comparisons of coreceptor inhibitor-resistant variants using multiple-cycle and single-cycle assays to better understand what is being measured
- Evaluation of the influence of coreceptor copy number on phenotypic resistance profiles, because the number of coreceptors on the cloned cell lines is higher than on in vivo cells
- Phenotypic and genotypic evaluation of HIV-1 *env* genes derived from patients who fail coreceptor inhibitor-containing regimens to help develop clinical guidelines
- Establishment of resistance assay cutoffs based on clinical response
- Natural history studies of viral tropism changes from CCR5 to CXCR4 and from CXCR4 to CCR5 with or without coreceptor antagonist treatment and in treatment with drugs other than entry inhibitors

Issues that remain with the use of tropism testing are:

- Distinguishing between dual-tropic and mixed-tropic virus
- Identifying minor viral variants in the development of drug resistance
- Clarifying the role of viral tropism in pathogenesis
- Clarifying the role of viral compartmentalization in pathogenesis
- Elucidating novel mechanisms of drug resistance in evaluating changes in viral susceptibility to coreceptor antagonists

- Defining further the relative sensitivity of genotypic and phenotypic assays
- Clarifying the determinants of fusogenicity in CCR5 or CXCR4 viral pg120
- Developing validated guidelines for phenotypic and genotypic resistance testing to help physicians know when to change therapy and whether alternative choices exist within the same class of CCR5 antagonists

It is imperative in these early studies to learn:

- The real relationship between viral tropism and pathogenesis
- Viral changes occurring under the pressure of exposure to coreceptor antagonists
- The type and frequency of diagnostic and clinical tests required for best management of patients on CCR5 antagonists

LONG-TERM FOLLOW-UP

One requirement for approval of CCR5 antagonists is obtaining sufficient follow-up of clinical trial participants to assess the safety of this new class of drugs. The FDA requests 5 years of follow-up on patients who experience virologic failure in phase 2 and 3 studies. Assessments should occur 2-3 times a year for CD4+ cell counts, viral load, viral tropism, and occurrence of AIDS-defining illnesses and death. These approach focuses on the long-term effects of viral tropism changes, with the goal of increasing our understanding of the relationship between a change to X4 virus and disease progression.

Three issues concerning long-term follow-up that require regulatory clarification are:

- How to define the control group
- How to evaluate data after patients switch antiretroviral therapy one or more times after stopping CCR5 antagonist therapy, switch other antiretroviral agents in the investigational regimen or the control group, or are lost to follow-up
- How to harmonize data requirements among the FDA, the EMEA, and perhaps organizations from other countries, such as Health Canada

There are several other research questions that long-term follow-up might help answer, including:

- Will disease progression be different in patients who congenitally lack CCR5 receptors versus those who "lose" receptor function on treatment?
- Is the natural history of HIV the same when the outgrowth of X4 virus occurs naturally or when it is driven by CCR5 antagonist therapy?
- What changes will occur in the immune status of patients taking these drugs?
- How will receptor antagonists affect intravenous drug users?
- What are the potential immune system changes on CCR5 antagonists and their potential impact?

- What will be the effect on β-chemokines, CD8+ B lymphocytes, and response to intercurrent infections?
- What will be the effect on other inflammatory conditions?
- How will new drugs (both receptor antagonists and other antiretroviral agents in development) affect the collection and analysis of data over the long term?
- What statistical tests will be used to make meaningful comparisons between investigational and control groups?

The Adult AIDS Clinical Trials Group and various pharmaceutical companies have long-term follow-up protocols that might provide good models for long-term follow-up of patients from CCR5 antagonist studies.

NEXT STEPS

The following issues for further discussion were identified:

- More US and EMEA regulatory clarity:
  - in data collection requirements
  - in implementation of long-term follow-up
  - in the use of CCR5 antagonists in treatment-naive study populations
- The role of CCR5 antagonists for HIV prevention:
  - in microbicides
  - in oral chemoprophylaxis
  - in mother-to-child transmission
- The therapeutic use of CCR5 antagonists:
  - in multidrug combinations
  - with CXCR4 antagonists
  - in sequencing of antiretroviral regimens
- The cost-effectiveness of CCR5 use and laboratory follow-up
- Criteria for expanded access to CCR5 antagonists
- Clarification of need for and/or the timing of tropism testing (e.g., routine, optional, none and the role of resistance in virologic failure)
- CCR5 antagonists in pediatric antiretroviral therapy
- Clarification and education about viral tropism and coreceptor antagonist therapy to the community
- Mechanisms to obtain more data on the use of CCR5 antagonists in women and minorities
- Clarification of the natural history of coreceptors and definition of additional basic science required to better understand the use of coreceptor antagonists
  - Current studies are looking at coreceptor genotype, and the FDA has requested stored samples for further testing
- The systemic distribution of CCR5 antagonists and the effect of CCR5

antagonists on compartmentalization of HIV
- CCR5 and CXCR4 interactions and their long-term implications (perhaps in joint discussion with the NIH study section on host interactions)
- CCR5 drug-drug interactions and the development of drug resistance
- The value of genotyping and/or phenotyping in researching coreceptor antagonist therapy
- Clarification of immune markers and/or infection definitions for data collection
- Testing in IV drug users for potential differential effects of CCR5 antagonists in these subgroups

CONCLUSIONS AND SUGGESTIONS

- More data are needed on the distribution of R5- and X4-tropic viruses in compartments other than peripheral blood
- Long-term immune function effects of CCR5 treatment need to be defined, with special emphasis on their potential effect in pediatric populations
- Investigators from large long-term cohorts should be invited to the next meeting.
- Specialists in pharmacoeconomics would be helpful in answering some of the cost-benefit issues

INTRODUCTION

The Forum Chemokine Coreceptor Antagonist Development Working Group was established to address issues of concern to the HIV community, including patients, academicians, regulatory agencies and industry. The first roundtable discussion sponsored by this working group addressed clinical trial design and regulatory requirements, as well as the diagnosis of viral tropism, issues in the development of CCR5 antagonists.

CCR5 antagonists are members of a new class of drugs for the treatment of HIV infection. Drugs in this class block host cellular receptors – the chemokine coreceptors – rather than viral enzymes. Chemokine receptors normally transmit signals in response to chemokines (e.g., CCL3 [MIP-1α], CCL4 [MIP-1β], and CCL5 [RANTES]), with chemotactic, inflammatory and other functions.

HIV infection requires the binding of gp120 to CD4, triggering a conformational change within the viral envelope glycoprotein structure that enables it to bind to the chemokine coreceptor. This is followed by a conformational change within the viral gp41, which allows the initiation of fusion of viral and host cell membranes and access of the viral core to the cytoplasm. HIV uses two coreceptors: CCR5 and CXCR4, and viruses are classified as CCR5 (R5) tropic, CXCR4 (X4) tropic or R5X4 (dual) tropic. The latter category includes viruses which are truly dual-tropic (individual viral particles may use either coreceptor) or virus populations containing mixtures of R5- and X4-tropic viruses. For the purposes of this report, mixtures will be referred to as R5+X4 virus populations.

CCR5 receptors are found on T cells, B cells, macrophages, and monocytes in circulating cells, lymphoid tissues and other compartments, such as resident or trafficking cells in the central nervous system (CNS) [5].

R5-tropic viruses predominate early in infection and throughout most of the disease course. X4-tropic viruses may be found as predominant or as minority populations in advanced disease or in isolated patients homozygous for the Δ32 deletion in the CCR5 receptor. An estimated 90% of HIV-1 infections involve host cell entry by R5 virus[6]. Although originally R5-tropic HIV-1 entry into cells was thought to require both the CD4 receptor and the CCR5 coreceptor, genetic variants of HIV-1 have been found that gain cell entry in the absence of CD4 receptors[7, 8]. Viruses using the CXCR4 chemokine receptor (R4 viruses) arise later in the course of infection. The cause-effect relationship between the emergence of X4 viruses and deteriorating immune function has not been established.

The development of a new drug class targeting host receptors raises concerns in several areas. The CCR5 antagonists were first identified by their ability to block chemokine binding to CCR5. Thus, they block normal host inflammatory processes; the short-term and long-term effects of this blockade are unknown. Special issues may exist for the pediatric population. Secondly, there is concern that CCR5 receptor blockade will result in upregulation of CXCR4 receptors, favoring the emergence X4-tropic virus or R5X4 viruses. Given the uncertainty of whether X4 viruses are causally related to disease progression, drug developers, regulators, patients and the research community agree on the need for extensive and comprehensive studies early in the development of this drug class. Concern also exists regarding the efficacy and safety of CCR5 antagonists in relation to the genetic polymorphism of chemokine receptors in different populations, and in relation to the relative expression of CCR5 and CXCR4 in coinfections (e.g., tuberculosis, hepatitis C or other opportunistic infections). Finally, the relationship between CCR5 expression in brain and CNS tissue, the effect of CCR5 antagonists and their potential interaction with opioid addiction has not been elucidated.

The Chemokine Antagonist Working Group, consisting of government regulators and research sponsors, patient advocates, industry representatives, and clinical investigators, was formed to address issues in the development and regulatory approval of CCR5 antagonists. These issues include:

- Effects of CCR5 antagonist treatment on viral tropism, escape, and susceptibility
- Long-term safety issues in adults and children
- Effects of viral and/or host receptor heterogeneity on CCR5 antagonist treatment
- Role of CCR5 antagonists in treatment strategies
- Potential use of CCR5 antagonists in prevention of HIV transmission

The May 23, 2005 roundtable discussion was the first of several discussions that will focus on these issues over the coming months. A bibliography of literature relevant to the development of CCR5 antagonists and to this roundtable discussion is provided in Appendix A.

GOALS AND OBJECTIVES FOR ROUNDTABLE 1

The goals specific to the first Chemokine Antagonist Roundtable discussion were:

- To review and discuss regulatory requirements in the US and Europe for the development of CCR5 antagonists in treatment-naïve and treatment-experienced patients

- To identify unanswered questions and discuss approaches to filling the gaps in knowledge relating to the safety and efficacy of CCR5 antagonists in the context of drug development and expanded access programs
- To discuss issues related to the diagnosis of viral tropism before and after treatment, pre- and post development phase
- To discuss and identify mechanisms for long-term follow-up of patients enrolled in CCR5 antagonist clinical studies

REGULATORY PERSPECTIVES

Representatives from the US Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA) presented their agencies' concerns for the conduct of clinical trials seeking drug approval for CCR5 antagonists. These presentations sought to clarify the current thinking by these agencies and to solicit suggestions on future development of CCR5 clinical trials.

FDA PERSPECTIVE

From the perspective of the FDA's Division of Antiviral Drug Products (DAVDP), the development of CCR5 antagonists will occur in the general context of antiretroviral drug development, with specific attention to the issues presented by the development of this particular drug class, including the agency's questions regarding safety, efficacy, significance of coreceptor tropism changes, and development of drug resistance for CCR5 antagonists.

FDA approval for CCR5 antagonist class will essentially follow the principles applicable to all antiviral drugs. These general requirements include data on HIV-1 RNA levels and CD4+ cell counts over at least 48 weeks (for traditional approval) or through 24 weeks (for accelerated approval). The supporting data must be derived from two adequate and well-controlled clinical trials. When submitting a new drug approval application (NDA), sponsors are requested to interpret the data in relation to available information for both drug class and the specific drug. Review of these data may result in the agency's request for additional data or longer follow-up. The FDA requests that pediatric studies be developed at the time Phase II plans are presented to the agency.

Generally, an indication for treatment of HIV infection in antiretroviral-experienced patients requires a minimum of 24 weeks of treatment data. An indication in antiretroviral-naive patients requires a minimum of 48 weeks of treatment data. For CCR5 antagonists specifically however, follow-up of treatment-naive patients to 96 weeks is required to help define the risk-benefit ratio of potential coreceptor tropism changes within the viral population, which may be different in treatment-naive patients than in treatment-experienced patients.

After proof-of-concept, the timing of clinical studies will depend on safety data from preclinical and early clinical studies, including drug resistance studies. If warranted by safety concerns, the first studies will be conducted in treatment-experienced patients and studies in treatment-naive patients may follow if deemed appropriate based on all available data. In the absence of safety concerns, studies may start simultaneously in

both groups of patients. Prior to clinical trials, particularly in treatment-experienced patients, drug-drug interaction studies must be conducted to support the coadministration of multiple antiretroviral agents in the background regimens.

The DAVDP's CCR5 antagonist drug development working group, which includes the primary reviewers, aims to provide consistent advice on the amount and type of data required for drug approval while allowing flexibility in overall development plans so that new data may be obtained from successive trials. The specific evaluations that CCR5 antagonists must undergo include:

- Safety and activity in patients with R5-tropic virus
- Safety and activity in patients with mixed or dual-tropic (R5X4) virus
    - Assess whether CCR5 antagonists are causing harmful changes in viral tropism
    - Further evaluate the limitations of the current tropism assay in terms of assay sensitivity and distinguishing mixed populations from dual-tropic populations
- Adverse events in all patients, particularly
    - Stringent adjudication of new AIDS-defining events by an independent review committee, as is currently required for all antiretroviral drugs
    - Class-specific adverse events, such as changes to the immune system as a result of the antagonist's effect on CCR5 receptors (e.g., increased frequency of infection)
    - Tropism changes and the effect of such changes on disease progression

Monthly tropism, viral load, and CD4 data are currently sent to the FDA from ongoing clinical trials. In addition, baseline samples need to be stored for future analysis and made available additional analysis in the event of viral failure associated with a change in coreceptor tropism. In these cases, the DAVDP wants to determine:

- Whether the tropism change is due to selection of new viral variants
- Whether the increased viral load represents proliferation of a minor viral population undetected at screening
- Whether viral resistance (independent of tropism change) developed
- Whether resistance to the background antiretroviral drugs developed

The following are required to assess coreceptor tropism changes from R5 to either X4 or R5X4 populations:

- In vitro susceptibility (phenotype) to the CCR5 inhibitor
- Nucleotide sequence analysis (genotype) of the gp120 region to identify amino acid changes (verified by site-directed mutagenesis), and analysis of protease inhibitor and reverse transcriptase inhibitor mutations

- Clonal evaluations of pretreatment and on-treatment samples to establish the extent of minor variant outgrowth
    - Currently, clonal analyses at screening/baseline and at viral failure are required
    - Other time points would be appropriate for studies of viral evolution
- Phylogenetic analysis to assess the relationship of emerging X4 virus to the original R5 virus

The FDA also requests 5 years of follow-up for all patients who experience virologic failure to obtain long-term data on viral load, CD4+ cell count, tropism changes, AIDS-defining events, and death, collected two or three times per year (see section on Long-Term Follow-Up).

Sponsors and regulatory teams face numerous challenges as the first drugs in this class are developed for treatment. The planning of clinical trials and trial designs will need to take into consideration what the potential impact of coreceptor tropism changes on safety and on virologic response or failure is. It is unclear at the moment what amount and type of data, and what collection frequency will be necessary to provide a reasonable assurance of safety. The same may be said for the extent and quantity of drug resistance and coreceptor tropism data that will be required for drug approval. For example, will data from a subset of patients be sufficient? Furthermore, the feasibility and mechanism for the 5 year follow-up will need to be considered up front.

Another "unknown" is the place that the CCR5 antagonists will take within the treatment armamentarium. Which current drug class – if any – will they replace?

Recommendations on the use and timing of tropism assays for clinical practice by the time a CCR5 antagonist is approved will need to be developed. Whether these recommendations are developed via treatment guidelines or product drug label will depend on the data available at time of approval. For example, if the consensus is that tropism assays are appropriate for a subgroup of patients, the recommendation for their use will be in the drug label.

EMEA Perspective

The EMEA considerations for approval of CCR5 antagonists include four new concerns:

- Potential drug interaction with the host
- Association between disease progression and receptor tropism status (nature of the causal relationship, if any)

- Potential limitations of the PhenoSense™ Entry assay (Monogram Biosciences, South San Francisco, CA) used to track R5 and X4 viral activity, such as limits of sensitivity in identification of minority virus populations
- Potential role of the PhenoSense Entry assay post approval of CCR5 antagonists

The EMEA convened a workshop on 8 November 2004 to discuss the potential need for revisions of guidelines for HIV drug development to specifically address issues in receptor antagonist development, soliciting comments through the end of May 2005. A major focus of the draft changes was to allow more flexibility in clinical trials. Up to the time of the workshop, no companies had requested scientific advice on CCR5 antagonists from the EMEA

The EMEA will want to see data on CCR5 antagonist receptor specificity and occupancy. Phase 2 studies should relate CCR5 receptor occupancy with viral load decrease. At this time, it is uncertain which pharmacokinetic parameters will most correlate with efficacy, but receptor occupancy may be an important measure for determining the appropriate dose.

The EMEA is reviewing concerns raised by the European AIDS Treatment Group (EATG) regarding the suitability of treatment-naive patients with CD4+ cell counts less than 200/mm^3 for dose-finding studies. These concerns are based on the possibility that patients in some dosing arms will be under- or over-exposed and the potential risks associated with this, as well as the hypothesis that patients with more advanced disease are more likely to have dual- or mixed-tropic viral populations, and thus be more at risk for outgrowth of X4 virus. Whether such changes in viral population tropism will have long-term clinical impact is not known, nor is the extent to which the R5 virus may regain dominance at a later time. Therefore, the EATG recommends that these patients be given regimens of proven efficacy first.

The possibility that different doses will be needed for treatment-naive versus heavily pretreated patients is another issue to be considered; viral strain also might influence dose requirements. For CCR5 antagonists specifically, it would be of interest to determine whether different doses are needed for patients with CD4+ cell counts above or below 200/mm^3. Thus, the ethical and scientific considerations of using a diverse treatment-naive patient population in early studies need further discussion.

Determining the appropriate or ideal population for phase 3 studies will also require considerable discussion. Treatment-naive patients were considered ideal because heavily pre-treated patients may have more mixed- or dual-tropic virus populations than patients earlier in disease. For example, in the TORO 1 and TORO 2 studies[9], mean baseline viral loads were 5.2 and 5.1 $\log_{10}$ copies/mL, respectively and mean baseline CD4+ cell counts were 80 and 98 cells/μL. In both studies, the average number of antiretroviral drugs taken previously was 12 and the percentage of patients

with 5 or more primary mutations was 80% and 90%, respectively. Baseline tropism studies were available for 627 participants. In this group, 12 (2%) patients had non-B HIV clades; 311 (50%) had R5 virus, 304 (48%) had mixed/dual-tropic virus, and 12 (2%) had X4 virus. More studies on the tropism characteristics of viral populations in populations pre-treated to different extents will need to be conducted. Given the fact that the impact of CCR5 antagonist treatment in patients with dual or mixed viruses is unknown, careful analysis of population changes and clinical disease status are urgently needed.

The choice of inclusion and exclusion criteria and pretreatment stratification will be important to phase 3 study design. The simplest approach would be to base inclusion criteria on the combination of viral load and CD4+ cell count without relying on a tropism diagnosis. However, assessment of viral tropism at baseline will be important, for tracking tropism changes during treatment. Exclusion of patients with X4 or R5X4 virus will limit interpretation of the study results, so it might be best to explore results in all three tropism groups to facilitate extrapolation of results to clinical practice. It will be important to stratify by CD4+ cell count, especially if the CD4+ cell count is not specified as an inclusion criterion. Another important stratification variable will be prior exposure to other entry inhibitors (e.g., enfuvirtide, or T20). The percent of patients with undetectable viral load will be sufficient for a primary endpoint, since this has been established as an appropriate surrogate endpoint in antiretroviral studies.

Head-to-head comparison of drug regimens is the classical approach to phase 3 antiretroviral studies, for both treatment-experienced and treatment-naive patients. In treatment-experienced populations, superiority of the investigational drug is demonstrated against placebo when both are administered with an optimal background regimen. In treatment-naive populations, non-inferiority of the investigational drug is demonstrated against an active comparator drug when given with two other drugs. The choice of comparator drug is uncertain and may depend upon which class of currently approved antiretrovirals CCR5 antagonists are thought to replace.

Other issues specific to CCR5 inhibitor clinical trials include unique criteria for virologic failure, such as the rate of change in viral tropism on the CCR5 antagonist regimen compared to the comparator regimen. The concern that measurement of viral tropism may lead to unblinding will need to be addressed. Unblinding may occur if CCR5 antagonist treatment results in a greater incidence of tropism change compared to the comparator arm. To date, information on this potential problem is not available. Data from the ongoing and planned studies should help to establish to what extent tropism changes do occur and provide the mechanism of resistance for this class of drugs. The stop-treatment rules for these studies, such as defining acceptable versus unacceptable rates of viral tropism change, must be carefully thought out. The role of the data and safety monitoring boards (DSMBs) for these studies will be critical. They

will need to confirm the reversibility of changes in viral tropism and watch for indications of immune toxicity, such as an increased incidence of infection.

The EMEA currently recommends 48 weeks of patient follow-up in drug approval studies, with conditional approval possible in antiretroviral-experienced patients with more than 16 weeks of follow-up. However, longer follow-up is being planned for CCR5 antagonists to assess the potential risk for immunotoxicity, reversibility of a change to X4 virus and any associated clinical consequences.

Another concern for the EMEA is the ability to assess viral tropism. The PhenoSense assay has been used to characterize baseline tropism and identify an on-study change in viral tropism. However, current assay limitations may preclude the detection of low level minority viral populations and this may impact the selection of the study population. In fact, sensitive clonal analysis has allowed the detection of minor X4 variant populations in patients experiencing virologic failure. These mixed populations had not been detected in the baseline samples using population based tropism testing [10]. Should tropism diagnosis be a requirement for clinical use post-approval, steps will need to be taken to ensure the wide-spread availability of the assay.

In conclusion, the EMEA is most concerned with:

- Defining the potential risks:
    - A change in viral tropism may have a negative impact on disease progression
    - CCR5 inhibitors may inhibit proper immune function
- Developing flexibility in the guidelines for drug approval that will balance risks and benefits in the studies of CCR5 and CXCR4 receptor antagonists and will adapt to changes in risk assessment during the clinical research phase
- Soliciting comment at this meeting on the draft amendments to the European HIV guidelines proposed by the EMEA
    - Comment on the draft revisions closed on May 31st, but the draft guidelines are available at: http://www.emea.eu.int/pdfs/human/ewp/063302en.pdf

DISCUSSION

Study evaluations

The amount of data requested by the regulatory agencies will require a substantial investment in terms of cost and logistic strategies. Thus, the agencies are working with industry and external experts to define appropriate subpopulations for the more intense and/or long-term analyses. From this perspective, information gained through phase 2 studies will be critical for the design and execution of phase 3 studies. Phase 2 studies

may clarify to what extent viral tropism changes occur and help to identify predictors of such change.

Site-directed mutagenic assays to verify nucleotide sequence changes associated with tropism changes may be difficult to do, as the underlying mechanisms for tropism change are more complex than simple substitution of one or two nucleotides. Furthermore, such changes often affect viral replication. However, to the extent that a particular drug-specific mutation is identified, confirmation by site-directed mutagenesis should be attempted.

Risks

CCR5 antagonist therapy may pose additional risks than those already discussed (immunotoxicity and altered coreceptor tropism). For example, changes in HIV receptor affinity may accompany exposure to CCR5 antagonists, as demonstrated in R5 virus escaping suppression, a mechanism that is independent of changing receptor tropism[11-15]. Another concern was raised by a report of increased infectivity of macrophages -- via the CXCR4 receptor and associated with neurotropism -- in a CCR5Δ32 homozygous subject [16]. Additional risks may be identified as studies progress.

In early CCR5 antagonist studies, different doses showed overlapping effects (CD4+ cell counts and viral loads) and a clear separation of efficacious or non-efficacious doses was not possible. Thus the best dose for clinical use will depend heavily on the safety profile of the drugs; if a clear difference cannot be detected, the lowest doses tested will be the ones used in the later studies. However, 10-day studies indicate that dose affects the speed of receptor occupancy. Dose is expected to impact development of resistance, but early studies have not demonstrated an association between dose and increase in the X4-tropic virus during CCR5 antagonist treatment.

Treatment-experienced patients with multidrug resistant HIV clearly experience the greatest unmet medical need; however, therapeutic options for treatment naïve patients are far from ideal. Efavirenz has been classified as a class D (a teratogen) drug by the FDA, thus complicating its use in women of child-bearing potential. Thymidine analogues are associated with mitochondrial toxicity leading to lipodystrophy syndromes. Ritonavir-boosted protease inhibitors, while effective, have substantial toxicity and pharmacokinetic interaction issues. These issues need to be considered when deciding what population to use in the proof-of-concept studies.

As discussed above, there is a lack of information on the suspected and other potential risks associated with CCR5 antagonist therapy. Traditionally, phase 2 dose-finding studies are followed by phase 3 studies in large populations; this approach does not lend itself well to the problem at hand. For CCR5 antagonist development, it may be useful to define a subset of patients for a large phase 3 study based on a closely

monitored phase 2 lead-in study. The phase 3 segment of the study would enroll a larger population than the phase 2 segment, depending on the available information at the time of phase 3 initiation. An example of this is the Pfizer phase 2b-3 study, with uniform inclusion criteria and intense monitoring for both parts of the study. Strict program stopping rules based on predetermined failure rates have been established (and such stopping rules may be appropriate for all phase 2b studies). The critical elements are close monitoring of the patients and careful characterization of safety issues, with a significant role played by the DSMB. Additionally, the FDA receives ongoing reports from all the coreceptor studies; these, as well as the minutes from DSMB meetings, are reviewed by the agency and will help identify cross-study issues. Expert input from immunologists will be valuable for identifying baseline factors that may impact tropism changes and the role of CD4+ cell counts and treatment experience in CCR5 antagonist treatment outcome.

In the US, nearly one third of HIV+ patients are intravenous (IV) drug users. The safety profile of therapeutic drugs may be different for these patients, and in this context, better knowledge of the immunologic safety of coreceptor antagonists is needed. The inclusion of HIV+ IV drug users will help to elucidate the particular concerns for this population [17].

Selection of study populations – Healthy Volunteers

Healthy volunteers play a vital role in identifying drug-drug interactions, especially for drugs that are metabolized by the CYP3A4 pathway. These studies may uncover a need for dose adjustments; thus, HIV-positive patients are not an ideal study population since they may be exposed to risk of developing resistance, receiving inadequate doses of needed drugs, or of having greater side effects from increased plasma drug concentrations.

Treatment-naive versus treatment-experienced

Issues that arise from the EMEA guidelines for inclusion of treatment-naive patients include:

1. It will be easier to assess differences in outcome when comparing CCR5 antagonists to nonnucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitor-based regimen in treatment-naive or minimally pretreated patients.

2. EMEA draft guidelines state that studies that include treatment-naive patients should have entry criteria that meet current treatment guidelines. Current criteria recommend no treatment for asymptomatic treatment-naive patients unless the CD4+ cell count is less than 200/mm^3 (or less than 350/mm^3 with high viral load). If the use of treatment-naive patients were restricted to severely immunocompromised patients

only, it would delay learning whether patients with CD4+ cell counts greater than 200/mm^3 would benefit from CCR5 antagonist treatment.

3. The reticence to do early studies in treatment-naive populations is difficult to reconcile with the need to find a correct dose for these patients. Hypothetically, if a new NNRTI were developed that did not have the hypersensitivity, hepatic toxicity, and teratogenesis of current NNRTIs, it would fill an unmet need for a safer drug in a class that is considered, world-wide, as the treatment of choice for a first regimen. Because drugs in this class are metabolized by the cytochrome P450 pathway, it would be essential to have dose-finding studies in treatment-naive patients.

Many, but not all, questions can be answered in treatment-experienced patients. Patients with CD4+ cell counts over 200/mm^3 but under 450/mm^3 and a high viral load might also be suitable candidates for phase 2 studies. Pfizer has tested maraviroc in an international phase 2a study using treatment-naive patients with CD4+ cell counts greater than 250/mm^3 and viral loads greater than 5,000 copies/mL[18]. An efficacious dose in these patients would likely be effective in those with lower CD4+ cell count; the phase 2b/3 studies will provide those data.

The FDA supports phase 2 studies in treatment-naive patients, because (1) the studies follow a limited number of patients very closely for an extended period of time, and (2) the agency is interested in obtaining safety information for these patients before drug approval. The EMEA would prefer to see a step-wise progression in clinical trial development, beginning with treatment-experienced patients. Once safety is shown in treatment-experienced patients, dose-finding studies could be done in treatment-naive patients with CD4+ counts greater than 200/mm^3 to assess whether a lower dose could be appropriate. Phase 3 studies would then allow entry of treatment-naive patients with any CD4+ cell count, and the data on those with less than 200/mm^3 would confirm the findings in patients with higher CD4+ cell counts. Because the reversibility of a change in tropism is unknown, precautionary measures are critical during development of the CCR5 antagonists, and trials with treatment-naive patients should have stringent stopping rules.

The use of treatment-naive patients in dose-finding clinical trials has been discussed extensively within the HIV/AIDS community. Patient advocates in Europe have expressed clearly their view that the safety concerns favor exclusion of treatment-naive patients from dose-finding studies. The EMEA has not taken a final position on the inclusion of treatment-naive patients in dose-finding clinical trials; draft language is general and states that the inclusion criteria should be written to ensure the safety of the patients. The EMEA is considering more specific language that will ensure greater level of harmonization across Europe. Although the issue of testing new drugs in naïve patients was raised specifically for CCR5 antagonists, any changes in EMEA guidelines resulting in exclusion of treatment-naive patients from dose-finding studies

have repercussion for drugs in all classes. Any EMEA restriction of dose-finding studies to non-naïve patient populations would have an impact on studies in non-European countries, thus affecting drug development world-wide. A change in European guidelines will likely influence practice in other areas of the world. Study results from treatment-experienced patients, with their accompanying problems of drug interactions, are not easily extrapolated to treatment-naive patients. Additionally, drugs approved in the US will be widely available to all patients regardless of restrictions to specific patient populations (e.g. treatment experienced patients) in the drug label; therefore, the US advocates support obtaining dosing information in treatment-naive patients. Given that, potentially, these drugs may be more effective in treatment-naive populations but at different doses required for treatment-experienced patients, restriction on dose-finding studies to treatment experience patients raises concern. Thus, compatibility of approach between the European and North American programs would be of interest to all patients world wide.

Treatment guidelines and drug approval guidance documents are revised frequently, as new information and a better understanding of antiretroviral treatment, its effectiveness, and its long-term consequences, are gained. It will be important to glean as much information as possible from the early CCR5 antagonist studies to inform the future research and development agenda in order to place these drugs in their most advantageous treatment regimen and strategy positions.

Investigators on current CCR5 antagonist studies will be expected to modify studies as warranted by new emerging information, but mid-study amendments are confusing to both investigators and patients. Thus, recognizing, considering and discussing potential issues prior to initiating clinical trials programs would be the preferable option.

Additional clinical trial issues

Binding efficacy and binding specificity are two distinct issues that need to be considered in early clinical trials. The energy barrier involved in CCR5 interactions may differ for different strains of virus and may be related to ethnicity[19]. Changes in binding efficacy between acute and chronic HIV infection has been demonstrated[20]. Binding efficiency has important implications for transmission, dendritic cell uptake of HIV, and development of resistance. Binding efficiency will affect dose, and binding efficiency may change during HIV evolution.

SUMMARY OF REGULATORY ISSUES

CCR5 antagonists represent a new class of antiretroviral drugs that interact with the host rather than the virus. The fact that a host receptor is targeted raises concerns about

how best to test these drugs and follow patients. Safety and efficacy data are needed, and the risk-benefit ratio of these drugs is unknown.

The roundtable participants agreed that:

- Although the risks of treatment with CCR5 antagonists are becoming better known (or will be known as studies progress), disagreement as to the significance and hierarchy of various risks does exist
- More data on safety and efficacy are needed to guide the design of phase 3 studies
- Safety is the most important consideration for clinical trial design
- The role of the DSMB will be critical

Differences in the FDA and EMEA recommendations are:

- The timing of phase 2 studies in treatment-naive patients:
    - The EMEA would prefer that studies in treatment-naive patients, especially those with low CD4+ cell counts, be restricted to phase 3 studies
    - The FDA would like data on treatment-naive patients from closely monitored phase 2 trials, given early indication of minimal safety concerns from phase 1 and 2 data, resistance data, and preclinical studies; otherwise, studies in treatment-naive patients should wait for phase 3
- Phase 3 minimum follow-up:
    - The EMEA requests 2 years of follow-up
    - The FDA requests 5 years of follow-up for patients who experience virologic failure

Issues that need to be addressed include:

- Coreceptor specificity and occupancy by CCR5 coreceptor antagonists.
- Potential changes in viral tropism associated with CCR5 coreceptor antagonist therapy
- Long-term safety of blocking endogenous CCR5 receptors and of coreceptor tropism changes
- Length of follow-up needed to assess safety issues, which, because of the host issues, needs to be longer than in previous drug trials investigating drugs targeting viral enzymes
- The placement of CCR5 antagonists in the regimen strategy and sequence of antiretroviral regimens
- Identification of the class of antiretroviral drugs that CCR5 receptor antagonists will replace, if any
- Identification of populations that will benefit most from CCR5 antagonist therapy
- The adequate frequency of testing for drug resistance and tropism changes during CCR5 antagonist therapy

- Treatment options following virologic treatment failure due to drug resistance or tropism changes
- Dose adjustment requirements, if any, for drug interactions and for different types of populations (e.g., treatment-naive versus heavily treatment-experienced patients, adults versus adolescents and children, CCR5 antagonist-naive vs. CCR5-antagonist-experienced)
- Phase 3 trial designs that will provide a platform to answer the above questions (It appears that the phase 3 studies should follow classic designs, but they will need somewhat different monitoring and longer follow-up)

VIRAL TROPISM

Viral tropism has been studied for a long time, beginning with the identification of syncytium-inducing (SI) and nonsyncytium-inducing (NSI) viruses[21]. Tropism change is one potential escape pathway; viruses can also develop drug resistance independent of tropism change.

UNDERSTANDING TROPISM

The viral tropism issue is far more complex issue than originally realized, creating some challenging areas for research, including:

- The natural history of viral tropism in the absence of treatment
- The effects of treatment (including reverse transcriptase and protease inhibitors) on viral tropism
- The compartmentalization of viruses of differing tropism
- The differential pathogenesis of X4 vs R5 tropic virus[†]
- The correlation of HIV-1 fusogenicity with viral sequences[‡]
- The extent to which in vitro observations predict preferential coreceptor use by viruses in vivo i.e. the possibility that virus dual-tropic virus in vitro is in fact monotropic in vivo

An assumption of a causal relationship between tropism and clinical progression has dominated much of the thinking in this field, and underlies the concern for risk to patients who may undergo tropism change while on treatments. However, the data available so far does not allow a differentiation between two possibilities: that X4 viruses drive clinical progression, or that a higher degree of immunodeficiency favors the appearance of X4-tropic virus. In particular, the relevance of transient appearances of X4 viruses in predominantly R5 viral populations is not clear. Some of the relevant data will be discussed below.

In a study of 100 hemophiliac HIV-infected patients, viral tropism and replicative capacity were both associated with disease progression.[22] AIDS-free survival was >90% in patients with R5 virus and about 60% in patients with R5X4 virus.

In the HOMER study of 979 antiretroviral-naive adults who initiated therapy between 1996 and 1999 and whose viruses were phenotyped for viral tropism, approximately

[†] Nearly half of individuals who die of AIDS have R5 tropic virus, rather than the seemingly more pathogenic X4 variant.
[‡] It is not certain how gp120 sequences relate to syncytium formation since R5 viruses can be more fusogenic than X4 HIV-1, and not all X4 viruses are fusogenic.

82% of patients had R5-tropic virus, 18% had dual-tropic virus, and 0.1% (1 patient) had only X4-tropic virus[23]. The detectable presence of X4 virus was related to CD4+ cell count (9.2% incidence in patients with CD4+ cell counts >200/mm^3; 54.4% incidence in patients with CD4+ cell counts <25/mm^3). These data are consistent with what has been published other studies[16, 24].

Mullins and colleagues have demonstrated that X4 strains are transient, cycling in and out of the viral population over many years. In his small series of minimally treated patients X4 virus was more prevalent at the maximum divergence of the viral quasispecies[25].

Huang et al.[26] collected longitudinal tropism data in 20 of 28 patients with drug-resistant virus who underwent a structured treatment interruption. Prior to the treatment interruption, the dominant plasma virus was exclusively R5 tropic in 10 patients and either dual/mixed or X4 tropic in 10 patients. Concurrent with emergence of drug-susceptible "wild-type" HIV in the absence of drug selective pressure, the virus changed coreceptor use in 5 patients. No consistent trends in the direction of the change were observed: 2 changed from dual tropic to either R5 or X4; 2 from R5 to dual tropic; and one from X4 to dual tropic. After controlling for baseline CD4 and change in viral load, there was no evidence of difference in CD4 declines between those with pure R5 vs. those with dual-tropic (P=0.20) virus. Thus, the relative impact of R5 vs. X4 on CD4 cell counts was not predictable in this study; further elucidation of the nature of the association between viral tropism and CD4 cell decline will require careful observation in larger cohorts.

Tropism and CCR5 Genotype and CCR5 expression

Only about 10 CCR5Δ32 homozygote patients are reported, of which only 3 are fully characterized[27-29]. One patient with X4 virus experienced a rapid loss of CD4+ cells and early death. On the other hand, another patient with both R5- and X4-tropic virus, had a much slower decline in CD4+ cell count.

The predominance of R5- or X4-tropic viruses may also be influenced by the level of expression of the relevant receptors, as illustrated by Hoshino et al.[30] in their study of viral tropism and CXCR4 expression on alveolar macrophages.

The industry is working with several large cohort studies to assess the natural history of viral tropism, including the Multicenter AIDS Cohort Study (MACS); the Amsterdam Cohort Study (ACS); the Swiss HIV Study; the Women's HIV Interagency Study (WHIS); and a recently established Rwandan cohort.

Compartmentalization

Discordance between blood and other compartments needs to be kept in mind when thinking about the role of the tropism assay and follow-up of patients. In addition to the cerebrospinal fluid (CSF) study mentioned below[31], discordance has also been documented between circulating and genital tract isolates. In patients with moderately depressed CD4+ cell counts, the circulating virus was R5 tropic, whereas the genital tract isolates were X4 tropic[32]. Discordance in the presence of drug-resistant variants has also been observed. Some compartments are not easily tested, and cost becomes an issue. Little is known currently regarding the extent of drug penetration into the various compartments; more research on drug distribution and the potential effects of differing drug levels on viral tropism is needed.

Spudich et al.[31] investigated viral tropism in matching plasma and cerebrospinal fluid (CSF) samples from 46 patients. Approximately 90% of samples were concordant for R5 virus, but 10% (N=5) were discordant. Two of the five had R5X4 virus in CSF and R5 virus in plasma. The R5X4 tropism was associated with lower CD4+ cell counts. All of the patients (N=4) with AIDS dementia complex had R5 virus in the CSF.

Hosino et al. have demonstrated an increase in CXCR4 receptors on alveolar macrophages obtained by bronchoalveolar lavage from patients infected with Mycobacterium tuberculosis[30]. These changes were specific to this tissue, and not seen in the pleural space. The increase in X4 expression was associated with an increase of X4 viral entry into alveolar macrophages. An accompanying increase of CCL4 and CCL5 decreased cell entry by R5, with the net result of increased X4 viral production. This has implications for any conditions that increase inflammation and demonstrates the complexity of understanding apparent changes in tropism.

Assessing Changes in Viral Tropism & Clinical Implications

An interesting question is whether tropism is different at the CD4 nadir than after immunologic recovery in response to highly active antiretroviral therapy (HAART). These data may already be available, or may be gathered through cohort studies. A study has shown that as disease progresses, R5 viral variants develop resistance to CCL5 and increase their ability to use variant CCR5 receptors that are phenotypically CCR5 but behave more like CXCR4[33]. This could explain some instances of treatment failure.

The potential implications of tropism change were discussed at length in the previous section. It will be important to learn as much as possible from early studies regarding the real relationship between viral tropism and pathogenesis and the extent of and/or

durability of viral changes under the pressure of exposure to coreceptor antagonists. Early studies will also provide information on the type and extent of clinical monitoring that will be required.

TREATMENT IMPLICATIONS

The key to using CCR5 coreceptor agents is pairing them with agents that block X4 virus, whether this be a standard combination chemotherapy, a fusion inhibitor[34], or a yet-to-be developed entry inhibitor[35]. The possibility that the standard 3-drug regimen may not provide sufficient activity in mixed viral populations needs to be considered.

The proof-of-concept studies for CCR5 antagonists have been in patients infected with R5 virus. The regulatory agencies are interested in the treatment effects of these drugs in patients with R5X4 virus, yet CCR5 antagonists are not expected to suppress X4 virus. Treatment with AMD3100, a CXCR4 antagonist, had no effect on R5 virus but both X4 virus and R5X4 virus disappeared[36]. These minority viral populations reappeared when treatment stopped. Most likely, however, different dual-tropic virus populations will respond differently. Previous studies have shown that HAART preferentially suppresses X4 virus[37-39]. The suppression, however, is transient, indicating that the X4 virus remains sequestered in the body[37, 40].

Ultimately, very little is known about changes in tropism at the population level. Changes from predominantly R5 to X4 mixed populations occur in the absence of CCR5 antagonist treatment, and it has been suggested that changes from X4 to R5 populations may also occur[25]. In an STI study[26], R5 virus was associated with more negative effects on the immune system than X4 virus. The issue is whether changes in viral receptor affinity that are associated with significant clinical outcome can be detected. High viral load is a significant risk factor, regardless of viral tropism. The clinical trial control arms will also show coreceptor tropism changes, although it may be that tropism changes occur faster in regimens with CCR5 antagonists. These "unknowns" underscore the need for long-term follow-up, as requested by the regulatory agencies.

It may be difficult to confirm clinical efficacy of CCR5 antagonists in R5X4 populations because of the antiviral activity exerted by the background antiretroviral drugs. If the CCR5 antagonist is paired with two other antiretroviral drugs, resistance to the background drugs could develop relatively quickly in someone with X4 virus present in the viral populations, because the X4 virus would be exposed to only two effective drugs rather than the currently recommended three effective drugs. Safety is the major concern, thus the FDA requires that the initial studies in patients with R5X4

virus be small, including extensive and comprehensive analysis. If the drugs prove promising in patients with mixed tropism viral populations, the FDA may request the companies to perform extra registrational studies to characterize these issues for product labeling.

DRUG SUSCEPTIBILITY

HIV entry into the cell is a multistep process, each step of which is a potential target for entry inhibitors. Resistance to entry inhibitors may involve competitive inhibition (discrimination), noncompetitive inhibition (use of an inhibitor-receptor complex), outgrowth of X4 virus, and receptor escape (e.g. CD4-independent entry). Competitive inhibition is well understood, and outgrowth of X4 virus has been discussed above.

The experiments of Riley et al.[41] with Schering C (SCH-C) illustrate noncompetitive inhibition. Virus was passed through PM1 cells in the presence of increasing doses of SCH-C. Although the experimental conditions presented the opportunity for outgrowth of X4 virus, such outgrowth did not occur. However, the viral susceptibility to SCH-C was significantly reduced by week 14, regardless of drug concentration (a plateau effect). Susceptibility continued to fall with further passages and was nearly 0% by week 104. Genotypic analysis detected mutations in the V3 loop, and the accumulation of mutations correlated with increasing levels of drug resistance[42]. The isolated mutation pattern makes construction of mutated viruses for further study and confirmation of these results, difficult. Additionally, virus infectivity was reduced as resistance increased, as has been observed with protease inhibitors, and can be reasonably expected with other receptor antagonists.

Westby et al.[43] obtained similar results in experiments with maraviroc (UK-427,857). Early passage isolates displayed maximal inhibition of 75%, again illustrating the plateau effect. After multiple passages, maximal inhibition levels dropped below 30%. Clonal analysis showed mutations in the V3 region that were related to the drug resistance level. The authors are currently working to relate single-cycle results to multiple-cycle results by inserting multipassaged *env* into replication-competent virus. The complete inhibition seen with multiple cycle assays, which is not evident in single-cycle assays, may be related to (1) over-expression of CCR5 in engineered cell lines or (2) increased opportunities to inhibit the virus during multiple passages.

Tsamis et al.[44] have proposed that in the normal conformation of HIV, the CCR5 binding area of the virus is hidden by the V1/V2 and V3 loops. When the viral gp120 binds to the CD4 receptor, it causes a conformational change that opens the CCR5 binding site, the V3 region, to interact in two places with the CCR5 receptor. In the

presence of a CCR5 receptor inhibitor, one of the receptor's extracellular loops normally involved in binding the virus to the CCR5 receptor is altered such that it no longer interacts with V3, effectively blocking HIV entry. In this model, both HIV and the CCR5 antagonist are bound to the cell, but the HIV cannot complete the process for cell entry. Viral escape may be due to receptor binding to alternate non V-3 regions (this would need to be demonstrated with site-directed mutagenesis or loop 2 deletions studies) or – given the variability in *env* mutation a more likely explanation – due to conformational changes in the same binding region.

In clones of maraviroc-resistant virus assessed with the PhenoSense assay, the inhibition curve plateaus created reproducible markers of resistant virus. In dilution studies, although the signal decreased, the plateaus did not. This indicates that the inhibition plateaus are not affected by viral titer.

REGULATION OF ANTIRETROVIRAL DRUG RESISTANCE AND TROPISM ASSAYS

Commercially available drug resistance assays are classified as laboratory-developed or "home brew" assays. Home brew assays and the reagents used in these assays are developed and validated by the laboratory conducting the testing and are not assembled into kits intended for retail distribution. Home brew assays that are developed and performed in clinical reference laboratory settings within the US are regulated by the federal Clinical Laboratory Improvements Act (CLIA) under the jurisdiction of the Centers for Medicare and Medicaid Services (CMS). In addition, certifications by the College of American Pathologists (CAP) and state regulatory agencies are routinely obtained by clinical labs performing high complexity tests, such as HIV phenotypic and genotypic assays. Currently, the FDA does not regulate these home brew assays, except for any analyte specific reagents (ASR) that are required to perform the assay and are purchased from outside vendors. In Europe, home brew drug resistance assays are considered service based information and must be performed in compliance with EN45001 guidelines for diagnostic services. Reference laboratories that conduct drug resistance testing must be CLIA and EN45001 certified, and the test procedures and validation data must be reviewed and approved by the appropriate regulatory agencies to ensure high clinical laboratory standards. Both CLIA and EN45001 regulations are intended to ensure consistency and reliability by setting specifications for reference laboratory personnel (qualifications and training), documentation (procedures and test results), and test procedures (performance characteristics and validation). Quality control (QC) and quality assurance (QA) of phenotypic drug susceptibility assays are the responsibility of the clinical reference laboratory. Specific QC procedures vary among laboratories but typically involve the use of external proficiency panels, in-process controls and references, reagent qualification, routine instrument calibration, assay performance tracking, and daily data review. QA measures include the use of validated procedures and systems, routine training, internal proficiency testing, monitoring of quality indicators, report and sample retention, and regulatory compliance.

ENTRY INHIBITOR/TROPISM ASSAYS

Monogram Biosciences has developed phenotypic and genotypic assays to aid academic and pharmaceutical collaborators in the preclinical and clinical evaluation of entry inhibitors. These assays may also be useful for guiding therapy decisions as these drugs become commercially available for treatment of HIV-infected patients. The phenotypic assay provides a direct measure of the coreceptor tropism of HIV-1 clinical isolates. Coreceptor tropism data can be used both, to select patients for treatment with coreceptor antagonist drugs or to monitor patient on treatment for the emergence of X4-tropic variants.

Currently, this is the only assay used in the development programs. However, other groups, including VIR*alliance*[45] and the New York State Department of Health are developing assays, although information on the performance and utility of these assays is not yet available. To what extent the original SI-NSI assay[46] will be of use remains to be determined.

PhenoSense HIV Entry Assay

This assay is a modification of the PhenoSense HIV assay developed and commercialized by ViroLogic (now Monogram Biosciences) to measure susceptibility to protease and reverse transcriptase inhibitors. In the PhenoSense Entry Assay, an HIV genomic vector (pHIVlucΔU3) containing a luciferase reporter gene is co-transfected with a plasmid that expresses HIV envelope (pHIVenv). The HIV *env* sequences may be derived from a patient HIV sample or may represent control sequences from well-characterized laboratory strains of HIV (e.g., HXB2), primary virus isolates (e.g., JRCSF) or patient plasma viruses. The patient-derived *env* sequences are tested as pools or libraries of sequences that represent the diversity of the viral *env* sequences present in the viral quasi-species population for each patient. Viral stocks are prepared by co-transfecting HEK293 cell cultures with pHIVenv and pHIVlucΔU3 (Figure 1A). Recombinant pseudotyped virus particles are harvested from the transfected cell cultures and used to infect U87 cells expressing CD4 and CCR5 or CXCR4 coreceptors (U87-CD4-CCR5, U87-CD4-CXCR4, respectively). Production of luciferase in target cells is dependent on virus entry and the completion of one round of virus replication. Drug susceptibility is measured by adding serial concentrations of entry inhibitors to target cells at the time of infection (Figure 1B). Drugs that inhibit virus entry reduce luciferase activity in a dose-dependent manner, providing a quantitative measure of drug susceptibility.

Coreceptor tropism determinations

A procedure for the determination HIV-1 coreceptor tropism has been developed. Coreceptor tropism is determined by measuring the ability of recombinant viruses to infect U87 CD4+ cells expressing either CXCR4 or CCR5 coreceptors. Viruses that efficiently infect U87/CD4/CXCR4 cells but not U87/CD4/CCR5 cells are designated X4 tropic. Conversely, viruses that efficiently infect U87/CD4/CCR5 cells but not U87/CD4/CXCR4 cells are designated R5-tropic. Viruses that efficiently infect U87/CD4/CCR5 and U87/CD4/CXCR4 cells are designated dual tropic (X4/R5). Coreceptor designations are confirmed by measuring the ability of CXCR4- and CCR5-specific antagonists to block infection of U87/CD4/CXCR4 and U87/CD4/CCR5 cells, respectively.

Genotypic analysis

Sequencing of *env* is compounded by unique problems. Conventional sequencing methods work well with the gp41 region, which has a complexity similar to protease and reverse transcriptase sequences, and it has been possible to establish a population sequence as a reference against which to report amino acid substitutions for this region. Sequencing of gp41 has been successfully used in enfuvirtide studies. The relevant region within gp120 region is more difficult to sequence because of its high variability; usually, clonal analysis is required. Researchers are still working on conventional annotation for the gp120 region. Nevertheless, gp120 sequencing has proven useful for studying coreceptor tropism, entry inhibitor resistance, and, in vaccine studies, neutralizing antibody escape. As for the phenotype, sensitivity to minor populations is an issue for genotyping.

A separate issue for genotype assays is the accuracy of tropism predictions based on amino acid sequence. V3 loop-based algorithms are able to predict X4 use with good sensitivity, but poor specificity. Thus, it is possible to predict X4 use but it is difficult to distinguish X4 from X4/R5 dual-tropic viruses. Inclusion of genetic determinants outside of V3 will likely be required to improve genotype interpretation algorithms.

The *GeneSeq HIV Envelope Assay* is a modification of the *GeneSeq HIV Assay* developed by Monogram Biosciences to detect drug resistance mutations in HIV-1 protease and reverse transcriptase. The assay uses 16 HIV-1 envelope specific primers to determine the nucleotide sequences of patient virus envelope genes derived by amplification from patient plasma samples (pHIVenv). Primers are not located in variable regions, and fewer primers are added for the gp41 region. Envelope mutations associated with coreceptor tropism, entry inhibitor resistance, and antibody neutralization or escape, are identified by comparing the sequence of the patient virus to the sequence of one of several well-characterized drug susceptible reference viruses (e.g. JRCSF, HXB2). Methods to accommodate envelope sequence length heterogeneity (insertions/deletions) were developed and incorporated into data analysis.

Figure 1. Construction of test vectors and assay overview

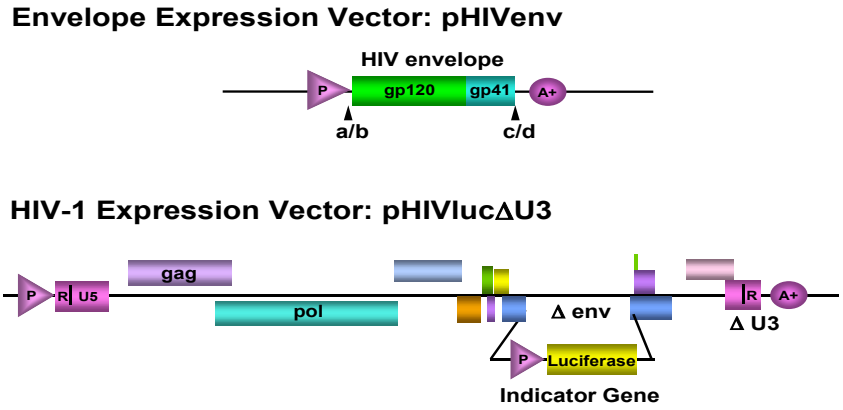

Figure 1A. The pHIVenv *env* expression vector was constructed by inserting amplified *env* gene segments from patient plasma samples into an expression vector, using *Pin*AI and *Mlu*I restriction sites (vertical arrows). The amplified fragment comprises the entire open reading frame of HIV-1 gp160. To monitor virus replication, a luciferase indicator gene cassette was inserted into a deleted region of the *env* gene of the NL4-3 strain of HIV-1, creating the pHIVlucΔU3 HIV-1 expression vector.

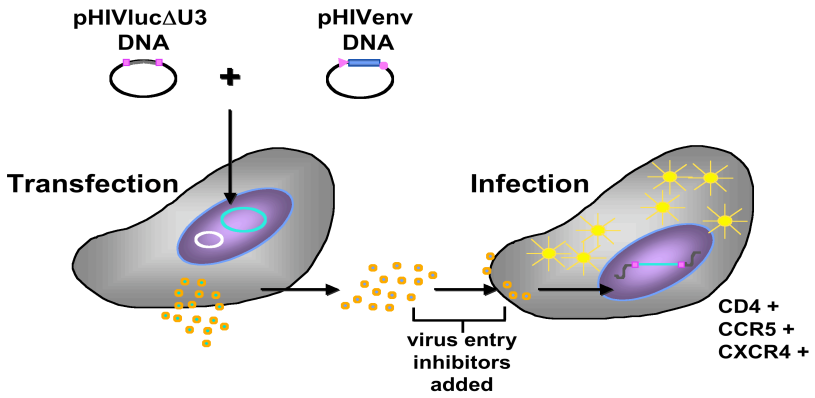

Figure 1B. Pseudotyped virus particles are produced by co-transfecting embryonic kidney 293 cells with pHIVenv and pHIVlucΔU3. Following transfection, the virus particles are harvested and used to infect fresh target cells (U87 cells expressing CD4 and one or both of the CCR5 and CXCR4 coreceptors). The ability of virus particles to complete a single round of replication in the presence or absence of viral entry inhibitors is assessed by measuring luciferase activity in target cells.

CLINICAL REFERENCE LABORATORY OPERATIONS

The development of assays for use in a clinical reference laboratory setting pose additional operational challenges beyond the technical challenges typically associated with assays intended for discovery research or high throughput screening. In a clinical laboratory setting, sample integrity is of the utmost importance and requires "mistake proof" process design that eliminates opportunities for cross-contamination or sample mix-ups. Since clinical laboratory test results are used to manage patient care, the process must be completed quickly and with reliable due date performance, and also provide convenient mechanisms for retesting. Routine procedures must also be sufficiently robust to accommodate variation in patient samples without the need for special handling. As part of today's medical care, clinical testing is also subject to significant price pressure, thus assay costs must be minimized without sacrificing quality. In the clinical laboratory environment, all new assays and processes must be validated and meet stringent standards for data quality. Implementation requires the development of detailed Standard Operating Procedures (SOPs), as well as procedures for personnel training and equipment maintenance. Process validations and implementation of new assays consume significant production capacity and development resources.

Many simple clinical laboratory tests are performed using a limited number of "reaction" and "recovery" steps and little attention to workflow is required to achieve acceptable due date performance (or Turn-Around-Time, TAT). In contrast, phenotypic and genotypic drug resistance assays require many successive reaction and recovery steps. Without careful attention to workflow, samples can quickly accumulate at constraints in the process, which adversely affects TAT and customer satisfaction. Monogram Biosciences continues to automate its test procedures to minimize such constraints. Although many laboratory equipment manufacturers have built instruments capable of performing similar operations, the integration and flexibility of such systems cater to research and high-throughput screening applications, and often are incompatible with the dedicated stepwise workflow requirements of a clinical laboratory process (small batch sizes and short cycle times) or with the specific process steps required for high complexity phenotypic or genotypic assays. Also, the many unnecessary features found in such commercial instrument systems add to their overall cost, including instrument "down time" and maintenance costs associated with their inherent complexity. Finally, little attention is given to "mistake-proofing" the design of commercially available instruments. In view of these limitations, Monogram Biosciences is pursuing a strategy of designing and developing process-specific, reliable "unit" operation equipment to meet the needs of its clinical laboratory operations.

Assay Sensitivity

When the sensitivity of the gp160 *env* amplification was tested, amplification was 93.8% successful at viral loads of 500-1000 copies/mL and 100% successful at viral loads of 5000 copies/mL or greater. At viral loads of 1000 copies/mL or greater, the successful amplification rate was greater than 94%. Thus, the recommended minimum viral load is 1000 copies/mL.

The assay sensitivity for detection of minority viral populations was also investigated with a mixture of X4-tropic and R5-tropic clones that expressed comparable amounts of luciferase. When minority variants were present at 5% of the total, they were detected in 83% of repeat assays; when present at 10%, they were detected 100% of the time.

Different types of cells will have different levels of receptors. Studies are underway or planned to assess the effect of tropism on infectivity in various cell types. These data should help clarify the extent to which the behavior of virus in the U87 cells

corresponds to viral behavior in vivo. These studies will also assess whether changes in the receptor levels of various cell types (e.g., monocytes) correlate to viral susceptibility in vitro, as, based on current assumptions, there are more CCR5 receptors on the engineered U87 cells than on most cells in vivo.

In general, fusibility is not a good marker for infectivity, nor is infectivity a good marker of fusibility. Cavrois et al. have studied dendritic cells in HIV fusion assays and found that fusion is 50% in dendritic cells compared to 5% in T cells[47]. This difference was attributed to the high number of CCR5 receptors on dendritic cells.

Another question is whether mutations develop in other portions of the genome that affect its pathogenicity post-entry[48, 49]. Although Monogram Biosciences has not experimented with mixed genotypes from clonal and primary virus, there is no question that viruses change characteristics during disease progression, as is seen in the differences in fusion of virus from chronic infection compared to early infection[50]. R5 *env* from late virus is as infectious as primary X4 *env*. Evidence for increased viral tropism for macrophages over time in cross-sectional [51] and longitudinal[52] studies exists.

Monogram Biosciences is giving a lot of thought to a second-generation tropism assay, and many of the issues were discussed at this meeting. Any one of these issues can be dealt with alone, but together they create a complex problem. These issues include cost, sensitivity to minor species, rate of successful amplifications, turn-around time, and access (portability).

Viral Subtypes

The tropism assay was validated with all the major HIV subtypes, and panels are being developed with additional subtypes for more detailed characterization. Efforts need to be made to also test more rare subtypes. AIDS Clinical Trials Group (ACTG) study A5211, a study of vicriviroc (SCH 417690, SCH-D), requires that all patients be genotyped for pol and be tested for viral tropism. A few of the patients have clade non-B virus, and the characterization of R5 to X4 virus in these clades will be assessed.

The R5 to X4 ratio in chronic versus early infection in different subtypes is unknown. In one study[53], results were different in clade B virus compared to clade C virus. It appears, however, that this may have been explained, at least partially, by differences in the length of time to transmission of the virus. Data have also been published on differences between clade A and clade B viruses[54].

Regulatory Issues

Both the FDA and EMEA recognize that the Monogram Biosciences tropism assay is the only one currently available and that this is the assay used in all CCR5 antagonist development programs. The regulatory agencies need more data to determine the utility and accuracy of the assay, especially for minority populations. To what extent is the detection of X4-tropic virus after CCR5 antagonist treatment in cases where no X4-tropic virus was detected at baseline an indication of outgrowth of an undetected minority population or of viral evolution? Results from the currently ongoing studies are expected to contribute to clarification of these issues. A better understanding of the clinical implications of tropism change will contribute to a better sense of how critical the ability to detect tropism change will be.

The current clinical studies are providing information regarding the performance of the assay in newly or chronically infected patients. The "success" of diagnosis of exclusively R5-tropic viruses has varied, depending on the study and the patient population. The presence of mixed- or dual-tropic viruses has been a frequent reason for ineligibility of screened patients.

Study sponsors have reported X4 virus emerging after treatment in a small proportion of patients, and in some of these cases, clonal analysis revealed X4 virus in baseline samples[10]. The fate of the X4 virus upon treatment discontinuation has varied – in some cases the viral population changed to pure R5 virus, in some patients, mixed populations persisted[55]. The importance of gaining as much information as possible from the early studies can not be overstated.

REIMBURSEMENT & COST-BENEFIT

The PhenoSense Entry tropism assay is CLIA approved. However, reimbursement may require other approvals in addition to CLIA. For instance, in New York State, laboratory evaluations are required to have Clinical Laboratory Evaluation Program (CLEP) approval for Medicaid reimbursement. Monogram Biosciences is preparing the appropriate submissions in order to obtain such approval, which is expected within the time frame needed to meet patient testing requirements.

The experience of use and reimbursement of drug resistance testing may be helpful in illustrating the potential problems that may be encountered should the tropism assay be required prior to initiating CCR5 antagonist therapy. In the US, about 13 states do not fully reimburse for resistance testing in their AIDS Drug Assistance Program (ADAP); for instance, in some states, reimbursement rates are well below the national Medicare

limitation amount and below assay costs, although Monogram Biosciences still accepts full assignment for samples from these states. Two states (Texas and Michigan) have yet to establish coverage policies for resistance testing. Approaches to laboratory testing are different in Europe. In Italy, physicians use genotypes and phenotypes for screening, although phenotypes are used less frequently. Europe also has barriers to reimbursement for phenotypic resistance tests, since many investigators in Europe do not believe the advantages of phenotyping over genotyping justify the added costs of phenotyping. Access, therefore, will relate to the perceived clinical value of the assay.

It is difficult to estimate the manner and extent to which the tropism assay will be used in advance of knowing whether it will be needed and what the cost will be. If it is required for coreceptor antagonist treatment, might the cost of the test be included in the cost of the drug? Included in a cost-benefit analysis is the assessment of the cost of treating someone who was not tested and did not respond to therapy. Many, but not all, US insurers (private and public) will follow what the treatment guidelines say.

Patient advocates are concerned about the performance of the tropism assay and its cost, should it be necessary to include this, or another tropism test, once these drugs reach the market. Concerns include the general assay "success" rate (amplification step) as well as the ability to detect the presence of minor X4 variants. Again, the experience with drug resistance tests may offer guidance in developing a useful strategy to maximize the "success" of the assay. For example, some clinical trial sites have experienced higher than the expected assay failure rate due to RT-PCR amplification failure. Experience has shown that training made available to these sites, along with increased familiarity with the assay requirements, has led to improved success rates of the test.

The price of the test is not yet established but will be comparable to other types of viral phenotype assays. The future availability of the test is under evaluation now. One question is whether the risk of developing the assay commercially can be shared, since it is unclear whether the assay will be needed or used once the coreceptor antagonists are approved. The company is developing commercial partners in Europe to facilitate distribution of the test. It is also assessing future competition and alternative technologies.

Some European investigators have made the point that, given the relative uncommonness of X4 virus — even in late-stage disease — a regimen that includes approved drugs that will treat the X4 virus may be sufficient to ameliorate the need for a tropism assay. In this scenario, a lack of response in 1-2 weeks (the time for a response to CCR5 antagonists in phase 1 studies) would lead to dropping the antagonist from the regimen. This approach, however, conflicts with the EMEA guidelines currently under consideration. As more data become available, algorithms

for use of the test will be needed, similar to those developed for resistance testing with protease inhibitors.

This early in clinical research, most patient advocates strongly support the use of the tropism assay for patients treated with chemokine coreceptor antagonists to better understand who benefits from the antagonists and how best to use them. Some of the investigators agree that more data are needed, especially in mixed-tropism, treatment-naive patients. This is similar to the need for resistance testing. Although a minority of new infections have NNRTI-resistant virus (about 2%)[56], resistance testing is important in treatment-naive patients because of the risk of also developing lamivudine resistance if response to the NNRTI is compromised.

In the future, it may be possible to combine CCR5 and CXCR4 antagonists in treatment regimens, thus reducing the need for tropism diagnosis in patients receiving CCR5 antagonists, but such a combination is a distant reality. Clinicians do not want to guess about how to use the CCR5 antagonists, since patients should not be exposed to the potential toxicities or to the risk of losing the benefit of the drugs in the background regimen for partial or no benefit. Logistical problems in requiring patients to return after 14 days to document the level of treatment response and to institute a change in the treatment regimen are also a problem. Thus, the clinicians and advocates are in general agreement that as much data as possible need to be collected now, with the possibility that the number of tropism tests can be reduced once more is known about the behavior of the virus in the presence of coreceptor antagonists.

Summary of Tropism and Resistance Testing Issues

Issues relating to tropism and resistance testing are:

- Distinguishing between dual-tropic and mixed-tropic virus
- Identifying minor viral variants in the development of drug resistance
- Clarifying the role of viral tropism in pathogenesis
- Clarifying the role of viral compartmentalization in pathogenesis
- Elucidating novel mechanisms of drug resistance in evaluating changes in viral susceptibility to coreceptor antagonists
- Further defining the relative sensitivity of genotypic and phenotypic assays;
- Clarifying the determinants of fusogenicity in R5 or X4 viral gp120
- Developing validated guidelines for phenotypic and genotypic resistance testing to help physicians know when to change therapy and whether alternative choices exist within the same class of CCR5 antagonists

- Viral resistance to entry inhibitors is dependent on the mechanism of action of the inhibitor
- Reductions in viral susceptibility to fusion inhibitors is best described by increases in IC_{50} (competitive inhibition), analogous to reverse transcriptase and protease inhibitors
- The ability to inhibit viral replication 100% at high drug concentrations is consistent with a competitive mechanism of inhibition and escape
- Reductions in viral susceptibility to some receptor and coreceptor antagonists is associated with an uninhibited fraction at high drug concentrations (plateau <100%)
- The inability to inhibit viral replication 100% at high drug concentrations is consistent with a noncompetitive mechanism of inhibition and escape
- In noncompetitive inhibition, escape variants may be using inhibitor-bound receptor or coreceptor complexes
- Infectivity is not a marker for fusibility, and vice versa

Future Studies

The following additional studies are needed to better understand the interaction of HIV, receptors, and receptor antagonists:

- Additional comparisons of coreceptor inhibitor-resistant variants using multiple-cycle and single-cycle assays to better understand what is being measured
- Evaluation of the influence of coreceptor copy number on phenotypic resistance profiles, because the number of coreceptors on the cloned cell lines is higher than on in vivo cells
- Phenotypic and genotypic evaluation of HIV-1 *env* genes derived from patients who experience virologic failure of coreceptor inhibitor-containing regimens to help develop clinical guidelines
- Establishment of resistance assay cutoffs based on clinical response
- Natural history studies of viral tropism changes from CCR5 to CXCR4 and from CXCR4 to CCR5 with or without coreceptor antagonist treatment and in treatment with drugs other than entry inhibitors

LONG-TERM FOLLOW-UP – FINDING A MECHANISM

The discussion above has outlined the rationale for the requirement of long-term follow-up of clinical trial participants for safety assessment of this new class of drugs.

The FDA requests 5 years of follow-up for patients who experience virologic failure on phase 2 and 3 studies, focusing on patients with tropism changes. The primary reason for this follow-up is to learn more about the nature of the relationship between tropism change (from R5 to X4) and disease progression. Assessments should occur 2-3 times a year for CD4+ cell counts, viral load, viral tropism, and occurrence of AIDS-defining illnesses and death. These data focus on the long-term effects of tropism changes.

Investigators and industry representatives agreed that this long-term follow-up will need to include patients with and without tropism changes, and from the investigational as well as the control study arms. Characterization of tropism change and drug resistance to other drugs in the regimen should be included in all virologic failure cases.

Several issues surrounding follow-up of the control group were identified. Patients on the control arm may have viral failure not accompanied with tropism changes. They may need to be followed for a prolonged period to learn the failure rate compared to patients on the investigational arm and to assess tropism change in the absence of coreceptor antagonist therapy. This will be more difficult to assess in treatment-experienced patients who, if they do experience viral failure, are usually offered crossover to the investigational regimen. After assessing the follow-up from the phase 2 treatment failures (and control arm failures), the FDA may need to adjust its request for follow-up data.

Another issue is the difficulty of following trial participants for a prolonged period, even when they go to other clinical trials. The ACTG has a protocol, the Adult AIDS Clinical Trials Group Longitudinal Linked Randomized Trials Protocol (ALLRT), specially designed for long-term follow-up of patients from ACTG randomized clinical trials. Patients are followed regardless of what they do for subsequent treatment. When patients complete their original study, they may go to other ACTG studies or to studies and physicians outside the ACTG. However, patients in ALLRT return to the AIDS Clinical Trial Unit (ACTU) three times per year to have viral load, CD4+ cell counts, new AIDS-defining illnesses, immune status, and neurologic status assessed. If patients have already undergone these tests outside the protocol at a CLIA-approved laboratory, copies of the laboratory reports suffice, thus avoiding repeat testing. It might be possible for the pharmaceutical companies to partner with other institutions to set up a similar mechanism for follow-up. Similar programs would need to be set up in advance, however, because of institutional review board (IRB) and Health Information Privacy and Portability Act (HIPPA) regulations.

Patient advocates pointed out several problems with long-term follow-up. Historically, long-term follow-up has been poor in the US, and not all trial participants will have access to ACTUs. The limitations of the tropism assay open the question of whether a virologic failure for which there is no indication of tropism change was not, in fact, due to an undetectable change in tropism. The discussion so far has not indicated a role for data on the length of treatment before virologic failure. Patients on the investigational regimen and the control regimen may not have equivalent care, especially in studies of pretreated patients who may be on anything from a second to a third, fourth, or later regimen. The usefulness of the data obtained under these conditions is unknown.

Merck & Co. and Abbott have long-term follow-up databases that might have application to this discussion. Also, the FDA has a Drug Safety and Risk Management Advisory Committee that might provide useful suggestions. ACTG investigators have found that once patients are in the system, they form bonds with the study staff and are amenable to a few visits a year for follow-up. Some companies, (e.g., Pfizer) are already committed to following all their trial participants and in general, industry should follow all patients, even if only twice a year.

The EMEA questions whether long-term follow-up beyond 2 years is possible. Historically, the rate of discontinuation is very high after 2 years. At 2 years, investigators should be able to determine whether side effect data indicate an increased rate of opportunistic infections. The EMEA would like to see roll-over studies that could assess the reversibility of any tropism changes once viral load is again controlled.

Another issue for long-term follow-up is attaining the consent and participation from patients in the control arm, as there may be little incentive for them. It is likely that a case-control study will be needed to adequately interpret the data obtained in follow-up. The regulatory agencies will need to provide more guidance on the required number of patients from control arms, and this is likely to change over time. Informed consent issues include the need to state the follow-up plans in the consent form of the original, investigational trial (perhaps added by amendment to existing studies). Incentives may be seen by some IRBs as coercive. Treatment experienced patients will likely provide the most useful data, and they are also the hardest to follow as they are likely to move on to expanded access and other clinical trials. The industry has not been able to find a workable solution yet and would welcome further talks with the regulatory agencies, patient community and clinical researchers on how to achieve the goal.

The complexity of a long-term follow-up dataset and its impact on future analysis needs to be considered early in the plans for a suitable design. There will be many confounding variables, especially changes in treatment. It is expected that initially the data will be descriptive and hypothesis-generating. Currently, it is unknown how many virologic failures there will be, so it is impossible to do power calculations. There are only 10 descriptions of patients with X4 virus in the literature, and these were identified before HAART became available. Thus, long-term follow up studies in the context of CCR5 antagonist development represent a

unique opportunity to gather data. The FDA would be interested in data from existing cohort studies, too.

It may be possible that an analysis of the slope of CD4+ cell count decline leading to virologic failure might answer some of the questions earlier. Patients who experience a CD4+ cell count decline will likely have treatment changed and develop different endpoints from those who do not experience a significant fall in CD4+ cell counts.

A consortium may provide the ideal mechanism for following patients on a long-term basis. The ACTG is not ubiquitous even in the US, and is even more limited outside the US. Many of the sites used by industry are not set up to accept federal funding, and this may become an issue should federal funding become available for chemokine coreceptor antagonist long-term follow-up studies. On the other hand, the requirements for federal funding of a follow-up study are less rigorous than for a treatment study. The possibility of setting up a substudy within the ACTG should be considered. As the treatment studies move from phase 2 to phase 3 to expanded access, there will be more patients and more opportunity to obtain data.

Three issues concerning long-term follow-up that require regulatory clarification are:

- How to define the control group
- How to evaluate data after patients switch antiretroviral therapy one or more times after stopping CCR5 antagonist therapy, switch other antiretroviral agents in the investigational regimen or the control group, or are lost to follow-up
- How to harmonize data requirements among the FDA, the EMEA, and perhaps organizations from other countries, such as Health Canada

Other Potential Long-Term Issues

There are several other research questions that long-term follow-up might help answer, including:

- Will disease progression be different in patients who congenitally lack CCR5 receptors versus those who "lose" receptor function as a result of treatment (see [5])?
- Is the natural history of HIV the same when outgrowth of X4 virus occurs naturally or when it is driven by CCR5 antagonist therapy?
- What changes will occur in the immune status of patients taking these drugs?
- How will receptor antagonists affect IV drug users, who are a diverse population often on multiple drugs that change over time, women, and other minority populations?
- What are the potential immune system changes on CCR5 antagonist therapy, and what is their potential impact?
- What will be the effect on β-chemokines, CD8+ B lymphocytes, and response to intercurrent infections?
- What will be the effect on other inflammatory conditions?

- How will new drugs (both receptor antagonists and other antiretroviral agents in development) affect the collection and analysis of data over the long term?
- What statistical tests will be used to make meaningful comparisons between investigational and control groups?

Several ACTG studies/substudies have been set up to investigate specific questions, including a substudy to assess the impact of CCR5 inhibition on peripheral neurologic complications from HIV in ACTG A5211, and the effect of CCR5 antagonists on immune expression, PBMCs, and the inflammatory and antibody response to hepatitis A vaccine in patients treated with CCR5 inhibitors. Another substudy, led by Dr. David Haas, is looking at host factors related to the effect of anti-HIV therapies.

The potential for immune toxicity is of concern; however, to date there is not consensus of what look for in terms of immunologic adverse events. Although any opportunistic infections seen in phase 3 studies may be seen as indicative of immune deficiency, it will be difficult to interpret any changes in immune function based on clinical progression alone. In treatment-experienced patients, immune function may be highly variable. With no standard marker, it will be very difficult to interpret what is happening immunologically, especially in patients with coinfections (e.g., HCV, HBV).

The FDA is requiring that all new AIDS-defining events be recorded, as is traditional in any antiretroviral study in HIV patients. In addition, Pfizer is collecting all viral and bacterial infections and their incidence is to be reviewed by the DMSB. Standardized criteria should be used for systematic collection of this type of information, as was done for granulocyte colony-stimulating factor (G-CSF) studies. The ACTG has standardized definitions of AIDS-defining infections and HIV-1-related illnesses (about 60 definitions in all) and use of the definitions allows standardized confirmation of diagnosis or assessment as “probable”. These definitions were reviewed by the FDA, and approved for the pivotal delavirdine trial in the early 1990’s. Pharmacia-Upjohn augmented them for their delavirdine and tipranavir studies. Thus, these definitions may be available and appropriate for standardized and systematic collection of opportunistic and other associated infections in the setting of current CCR5 antagonist trials.

The pharmaceutical company sponsored trials include a diverse population of patients on whom an extensive array of data is being collected. Whereas the collecting the information is important, the challenge will be in the interpretation of these datasets. Generally speaking, patients on HAART have shown variable response with regard to the immunologic markers investigated to date; no single available surrogate marker has clearly gained predominance. The issue of appropriate surrogate immune markers is, of course, not unique to antiretrovirals. The only way to start getting answers to these many questions is to do design and carry out the appropriate studies.

NEXT STEPS

The following issues for further discussion were identified by the attendees:

- More US and EMEA regulatory clarity:
    - in data collection requirements,
    - in implementation of long-term follow-up, and
    - in the use of CCR5 antagonists in treatment-naive study populations.
- The role of CCR5 antagonists for HIV prevention:
    - in microbicides,
    - in oral chemoprophylaxis, and
    - in mother-to-child transmission.
- The therapeutic use of CCR5 antagonists:
    - in multidrug combinations,
    - with CXCR4 antagonists, and
    - in sequencing of antiretroviral regimens.
- The cost-effectiveness of CCR5 use and laboratory follow-up
- Criteria for expanded access to CCR5 antagonists
- Clarification of need for and/or the timing of tropism testing (e.g., routine, optional, none and the role of resistance in virologic failure)
- CCR5 antagonists in pediatric antiretroviral therapy
- Clarification and education about viral tropism and coreceptor antagonist therapy to the community
- Mechanisms to obtain more data on the use of CCR5 antagonists in women and minorities
- Clarification of the natural history of coreceptors, their genetic polymorphisms, and the basic science regarding receptor-ligand and receptor-antagonist interaction required to better understand the use of coreceptor antagonists (Current studies are looking at coreceptor genotype, and the FDA has requested stored samples for further testing.)
- The systemic distribution of CCR5 antagonists and the effect of CCR5 antagonists on compartmentalization of HIV
- CCR5 and CXCR4 interactions and their long-term implications (perhaps in joint discussion with the NIH study section on host interactions)
- CCR5 drug-drug interactions and the development of drug resistance
- The value of genotyping and/or phenotyping in researching coreceptor antagonist therapy
- Clarification of immune markers and/or infection definitions for data collection
- Testing in women and minorities and IV drug users for potential differential effects of CCR5 antagonists in these subgroups

- More data are needed on the distribution of R5- and X4-tropic viruses in compartments other than peripheral blood
- Long-term immune function effects of CCR5 treatment need to be defined, with special emphasis on their potential effect in pediatric populations
- Investigators from large long-term cohorts should be invited to the next meeting
- Specialists in pharmacoeconomics would be helpful in answering some of the cost-benefit issues

The regulatory agencies welcome feedback from industry, investigators and community on the issues raised in this discussion. In particular, the EMEA would be interested in whether their step-wise approach and cautious stopping rules in phase 2 dose-finding studies need revision or are acceptable, both to advance the receptor antagonist development and to protect treatment-naive patients. The EMEA needs to solicit its member countries' positions, as well, and will bring those discussions to the next meeting.

The Forum is committed to continuing the working group meetings until the participants think the major issues have been resolved. The Steering Committee will review the issues from this meeting to help direct the focus of the next meeting. In addition, the Forum is working on guidelines for clinical trials in treatment-naive patients in a separate working group with overlapping interest to the CCR5 antagonist group. The Forum encourages feedback from all participants and from invitees who were unable to attend the session.

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APPENDIX B: ACRONYMS

ACTG	AIDS Clinical Trials Group
ACTU	AIDS Clinical Trial Unit
ALLRT	Adult AIDS Clinical Trials Group Longitudinal Linked Randomized Trials Protocol
CCL3	A chemokine ligand (formerly MIP-1α) that binds to CCR5
CCL4	A chemokine ligand (formerly MIP-1β) that binds to CCR5
CCL5	A chemokine ligand (formerly RANTES) that binds to CCR5
CCR5	A cell surface coreceptor used by HIV for cell entry
CCR5Δ32	A CCR5 gene missing 32 base pairs, which results in no cell surface expression
CXCR4	A cell surface coreceptor used by HIV for cell entry
CLIA	Clinical Laboratory Improvements Act
CNS	Central nervous system
CSF	Cerebrospinal fluid
CYP3A4	Cytochrome P450, subfamily IIIA, polypeptide 4, often part of drug metabolism
DAVDP	FDA Division of Antiviral Drug Products
DSMB	Data and Safety Monitoring Board
EATG	European AIDS Treatment Group
EMEA	European Agency for the Evaluation of Medicinal Products
FDA	Food and Drug Administration
gp	glycoprotein (as in gp120)
HAART	Highly active antiretroviral therapy
HIPPA	Health Information Privacy and Portability Act
IC_{50}	50% inhibitory concentration
IRB	Institutional Review Board
IV	Intravenous
MIP-1α	Macrophage inflammatory protein 1 alpha (CCL3)
MIP-1β	Macrophage inflammatory protein 1 beta (CCL4)
NIH	National Institutes of Health
NNRTI	Nonnucleoside reverese transcriptase inhibitor
NSI	Nonsyncytium-inducing
pHIVlucΔU3	Plasma HIV genome vector containing luciferase
R5	CCR5 [tropic virus]
R5+X4	CCR5 and CXCR4 mixed [tropic virus]
R5X4	CCR5 and CXCR4 dual [tropic virus]
RANTES	Regulated on activation, normal T expressed and secreted (CCL5)
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase-polymerase chain reaction
SI	Syncytium-inducing
V1, V2, V3	Variable loop regions of the HIV RNA
X4	CXCR4 [tropic virus]

APPENDIX C: AGENDA

9:00—9:15	**Welcome and Introductions**	Veronica Miller
9:15—9:30	**Goals and Objectives**	Veronica Miller
9:30—10:45	**Session I: Regulatory Perspectives** Expectations for drug approval, concerns, gaps in knowledge	Moderators: Roy Gulick, Veronica Miller
9:30—9:40	US FDA	Kimberly Struble
9:40—9:50	EMEA	Nathalie Morgensztejn
9:50—10:45	Discussion	All
10:45—11:00	*Break*	
11:00—12:30	**Session II: Diagnostic & Resistance Issues**	Moderators: Dan Kuritzkes, Veronica Miller
11:00—11:45	Cell Entry Assay	Chris Petropoulos
11:45—12:30	Discussion	All
12:30—1:30	*Lunch*	
1:30—2:30	**Session III: Long-Term Follow-up: Finding a Mechanism**	Moderator: Veronica Miller
1:30—2:30	Discussion	All
2:30—3:00	**Session IV: Summing Up**	Moderator: Veronica Miller
2:30—3:00	**Next Steps**	All

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