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EDITORIAL



In the centenary year of Indian Council of Medical Research, we organized two very important meetings that brought together scientists working on HIV and AIDS from India and overseas. A meeting of policymakers, programme implementers, scientists, clinicians and academicians was held to discuss important gaps in the care and management of HIV infected persons. A consultative meeting with theme ‘Galvanizing Evidence for HIV Management’ was held at NARI from 27th to 29th January 2011. Three important areas were discussed threadbare; HIV and TB, sub-optimal response to ART and HIV and HPV. The lead article in this issue reports the deliberations of the meeting. The other important meeting was focused on HIV Basic biology and Translational research. The meeting was held in Goa from 12th to 14th January 2011. The meeting was attended by the leading researchers from India and abroad. A brief report of this meeting is presented in the issue.

Dr. Jayanta Bhattacharya has described in his articles the status of the knowledge on neutralizing antibody response. He goes on to describe the progress made so far and what needs to be done. Mr. Chaturbhuj and Ms. Gautam have reviewed two interesting articles on application of dried blood sample for drug resistance genotyping and an important molecule in innate immune mechanisms, tetharin respectively.

Besides this we have Robert (Bob) Grant, a Senior Investigator at the Gladstone Institute of Virology and Immunology and an Associate Professor of Medicine at the University of California San Francisco, as our researcher in focus by virtue of his significant contribution to Pre-exposure prophylaxis study that showed efficacy in protecting research participants from HIV infection.

Other activities at our Institute have been reported besides highlighting one of our Gems, Co-receptor biology research group. This quarter was crowded with activities that I have tried to summarise.

The Bulletin is now a year and half old and the young Editorial Board is ever striving to improve the quality. We look forward to your suggestions and comments.


Dr. R.S. Paranjape
 Director, NARI

INDEX

Editorial	
Galvanizing Evidence for HIV Management: A Consultative Meet	1
Researcher in Focus	8
Challenges of immunogen design that can elicit broadly neutralizing antibodies to HIV-1	9
Commentary	11
Happenings at NARI	12
Publications	16
Highlighting our Gems: The "Co-receptor usage by HIV"	17

Cover Photo : "AIDS can be prevented with a helping hand. We are there to help create a better future..... Spread Awareness. Stop HIV!".
 Artwork by Ms. Nisha Jariwala, Laboratory Technician, Division of Microbiology.

Photo on Last Page : "End of the day is not the end of life. Setting sun says, "I am going to bring a new morning".
 Photograph of a beautiful sunset at Vagator Beach, Goa by Mr. Sudhanshu Shekhar Pandey, CSIR-SRF.

Galvanizing Evidence for HIV Management: A Consultative Meet (NARI, Pune. 27-29 Jan 2011)

National AIDS Research Institute has focused its research efforts towards care and treatment of HIV infection in addition to focus on epidemiology and prevention. The Institute has partnered with various National as well as International agencies such as National AIDS Control Organization, World Health Organization and National Institutes of Health, USA. Indian Council of Medical Research, the parent organization of NARI has entered its centenary year. As a part of the celebrations a consultative meet was organized by NARI to discuss available evidence in the context of some of the important issues in care and management of HIV infected persons.

Nearly 25 years since the detection of HIV in India, the HIV epidemic seems to be stabilizing and valuable knowledge and experience has been accumulated as a result of extensive interventions for control of the epidemic. In 2009 it was estimated that 2.4 million people were living with HIV in India. According to NACO, just over half of the adults estimated to be in need of Anti Retroviral Treatment (ART) in India were receiving it. The increase in CD4 counts from baseline level to that at one year and survival rates up to three years after initiating ART in Indian ART rollout are similar to those reported from the developed countries. Thus, access to ART has increased the quality of life and survival of persons who have advanced HIV disease. However, a small proportion of persons who receive ART do not show a sizable increase in CD4 counts and continue to reveal a sub-optimal level of immunological response in presence of virological suppression. This observation poses numerous clinical challenges in clinical settings that range from understanding its determinants, management and differentiation from treatment failure.

Tuberculosis (TB) continues to be the most common opportunistic infection (OI) in India. Both Tuberculosis and HIV infection have a synergy that can affect diagnosis, prevention and control strategies adversely. Use of ART in the early stages of HIV has been shown to reduce the incidence of TB. Despite evidence from many trials, the fear of emergence of drug resistance precluded adoption of chemoprophylaxis for tuberculosis as a public health policy in most endemic countries. Emerging evidence on INH Preventive Therapy (IPT) and the use of daily regimen suggests that these methods are superior to tri-weekly Directly Observed Therapy short course DOTS which merits its risk benefit evaluation afresh in India.

Human Papilloma Virus (HPV), which is associated with development of cancer cervix and other malignancies, is a common infection in India. Vaccines to prevent high risk HPV infection are now available in India. HPV infection is common among sex workers and men having sex with men. In the presence of concurrent HIV infection, it can increase the risk of HPV related malignancies due to non clearance of HPV (which normally happens in HIV uninfected population) and ART induced longevity that allows time for cancer development.

Recognizing the newly emerging global and local evidences, it was considered that a review of these in the backdrop of existent policies in India was necessary with a view of strategic revision in policies. The National AIDS Research Institute (NARI), under the aegis of Indian Council of Medical Research (ICMR) with support from Division of AIDS, National Institute of Health, USA, and World Health Organization convened a three day consultative

meeting entitled '*Galvanizing Evidence for HIV Management.*'

The meeting was attended by representatives, stakeholders - policy makers and programme managers, National AIDS Control Organization (NACO), Revised National Tuberculosis Control Programme (RNTCP), researchers and academics.

The meeting was inaugurated on 27th January, 2011 by Shri. Sayan Chatterjee, Secretary, Department of AIDS Control & Director

General of NACO. Dr R. S. Paranjape, Director, NARI, in his welcome address, spoke about the centenary year of ICMR which is being celebrated from 15th Nov 2010 to 14th Nov 2011. In his inaugural address, Dr. Chatterjee pointed out that new HIV infections in India had declined by 50% in the last 10 years as a result of HIV prevention programmes. He detailed the facilities available for HIV care in India: 292 Anti Retroviral Therapy Centres catering to more than 11 lakh registered HIV infected persons, of which, more than 3.7 lakh were availing free ART. 2nd line and alternative 1st line ART are also being dispensed by eight Centres of Excellence. He outlined the NACO's future plan of making 2nd line ART available to all People Living with HIVs (PLHIVs) who require it and provision of 'smart card' for them. He encouraged the experts to review research findings which could be taken up by the Technical Resource Group (TRG) for consideration and implementation. He lauded the initiative taken by the National AIDS Research Institute in this direction. Following the inaugural address, Dr Dan Kuritzkes, Chair of Adult AIDS Clinical Trial Group (ACTG) Network, in his theme address



Inauguration by Dr. Sayan Chatterjee



Lamp Lighting by Dr. Sriram Tripathy

'Evidences that Transformed Health Policy' elaborated on factors that drove US HIV treatment guidelines and the evidences from research that led to policy changes in the US.

Thereafter, the proceedings of the three days meeting focused on the three identified tracks in HIV management namely, 'Response to First-line Antiretroviral Therapy', 'HIV & TB' and 'HIV & HPV'.

Response to First-line Antiretroviral Therapy

As on January 2011, 3, 93, 632 adult patients are taking ART from NACO ART centers in India. The initiation of ART leads to a rapid reduction in plasma HIV-1 RNA levels and an increase in CD4+ lymphocytes. The success of ART depends upon various factors such as potency of the regimen, drug adherence, baseline plasma viral load, nadir CD4+ cell count and rapid reduction of viremia in response to treatment (i.e. $>1 \log_{10}$ in 1-4 months). According to DHHS 2011 guidelines, an adequate CD4 response for most patients on therapy is defined as an increase in CD4+ cell count in the range of 50-150 cells/mm³ per year. However, a significant proportion of patients show virological suppression without immunological improvement and continue to have low CD4 count, while a small proportion of patients exhibit an increase in CD4+ cell count in presence of detectable viral load. Based on the nature of discordance between plasma viral load and CD4 count these patients are classified as immunological non-responders [fully suppressed viral load but no increase in CD4 count] and virologic non-responders [rise in CD4 despite detectable viral load]. Virological non response might be explained by the selection of mutant virus with decreased fitness compared with wild-type virus and it has also been suggested that protease inhibitors (PI) seem to inhibit lymphocyte apoptosis independently of their antiviral effect.

In developed countries, discordant responses, termed as sub-optimal responses (SOR), have been reported to occur in 20-30% of patients within first 6 months to 2 years of initiation of ART. But Indian data on discordant response are limited. Available data from Chennai confirm that 21% of patients with immunological

failure on first line ART showed discordant response. Various studies in industrialized countries have demonstrated that the probability of not achieving optimum rise in CD4 count increases with age, progressive decrease in thymus size, low CD4 counts at baseline, injection drug use, and use of AZT+ 3TC. Persistent HIV-1 replication in lymphoid tissues, low adherence to ART and previous treatment interruptions have also been associated with immunologic discord. Moreover, patients showing such discordance are more likely to develop chronic morbidities and increased risk of disease progression in terms of AIDS/non AIDS defining events and death while on first line ART medication. In addition, it is very important to understand the nature of discordance as it has implications on treatment modifications and management.

Despite the recognition of discordant response, there is no universally acceptable definition of immunologic non response in terms of CD4+ cell count threshold. A rise in CD4 T cells, more than 50/ μ l after 6 months or more than 100/ μ l during follow-up, are some of the definitions that are being used by researchers. Similarly, less than 50 copies/ml, less than 500 copies/ml or less than 1,000 copies/ml are some of the threshold values that are being employed to define virological response. Presently response to treatment is being monitored by CD4 cell count in NACO ART roll-out programme. Plasma viral load testing is available only at the Centres of Excellence for patients suspected to have clinical or immunological treatment failure. A recent World Health Organization study showed that basing treatment decisions on CD4 count alone, without viral load monitoring, led to missed cases of virological failure that could have occurred before immunological failure. More over it led to unnecessary treatment switches when drugs used were in reality, effective in suppressing viral load.

Evidence based data from India on prevalence, predictors and outcome of suboptimal response that could advise ART programme are limited. Recognizing the programmatic and research implications, immunologic non-response was considered as an important issue for in depth deliberations in the consultative meeting.

The plenary session included four talks which focused on prevalence of sub-optimal response to first line ART, immunologic basis of CD4/virological disconnect phenomena, determinants of sub-optimal response, its application and management. Subsequent to the presentations, discussion concentrated on definition of optimal and sub-optimal response to ART, its determinants and its implications in disease management. The following points emerged out of the deliberations:

Definition of sub-optimal response- In general, patients who do not show an increase of at least 100 CD4 lymphocytes within a year of ART initiation in presence of suppressed viral load should be considered as having sub-optimal response and less than 400 copies/ml could be considered as the threshold for viral load suppression. Threshold for early detection of SOR at 6 months

should also be defined if ART is initiated at higher counts [i.e. 350 cells per cu mm].

Interpreting data- Data collected at State AIDS Clinical Expert Panel (SACEP) should be analyzed to understand the prevalence, determinants and outcome of suspected immunological and virological failure in patients in India on first line ART.

Determinants for Sub-Optimal Response- Confounders for sub-optimal response in India include malnutrition, AZT based first-line therapy, concomitant drugs, and low adherence to ART. Tests to detect co-infection of HIV 1 and 2 should be made available at ART centres so that these patients will not be initiated on NNRTI-based ART and present as immunologic non- responders.

Monitoring Patients on ART- Immunologic failure should be distinguished by monitoring viral suppression since virological failure precedes immunological and clinical failure. Under national programme guidelines, targeted viral load can be done when immune response is inadequate at 12 months. However, when SOR is detected in the first six months, adherence counseling & clinical assessment to detect any underlying Opportunistic Infections (OI) or malignancy could be prioritized. There was a consensus that the plasma viral load should at least be offered at one year of ART initiation to distinguish between early treatment failure and sub-optimal response among those who do not show immunologic reconstitution.

Managing patients with sub optimal response- Data from various studies show that switching or intensification of regimen does not demonstrate any additional benefit over continuing primary regimen. The use of immune-modulator interleukin-2, a T cell growth factor which increases naive T-cells and CD4+CD28+ T-cells, has not shown any promising results in management of immunological non-response.

Chemoprophylaxis- TMP/SMX chemoprophylaxis and azithromycin wherever indicated should be continued if CD4 count is less than 200 cells cu mm or less than 100 cells/cu mm respectively.

Researchable area- It was felt that a well designed prospective research study to investigate immunological non-response, its determinants and clinical significance was urgently needed, with NARI taking the lead in developing this multicentric protocol. To summarize, it emerged out of the deliberations that Sub Optimal Response (Immunological non response) to ART needs to be defined and that viral load monitoring should be offered in the National ART programme to identify early failures. It was also clear that data on Sub Optimal Response in Indian context were scarce and the need for generating such data to guide the programme was strong.

Further reading:

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HIV and TB Co-infection

Tuberculosis is the most common treatable OI in HIV-infected patients as well as the leading cause of their death. The global impact of the converging epidemics of TB and HIV is one of the major public health challenges of our time. Globally an estimated 1.8 million people died of TB in 2008, of which about 0.5 million were patients with TB/ HIV co-infection.

India has one of the world's highest burdens of both TB (1.96 million cases annually) and HIV infection (2.31 million prevalent cases). With about 50% lifetime risk of developing TB disease in HIV infected people, it is projected that 50-60 percent of the HIV-infected persons in India will develop TB disease during their lifetime. Comparable data from various Indian studies conducted between 1994 and 2006 also report prevalence of HIV among TB patients to be in the range of 3 to 28%.

This HIV/TB dual epidemic has posed many challenges. It is crucial to screen for and diagnose TB infection in the HIV infected to reduce the morbidity and mortality. However, sensitive and effective diagnostic techniques such as the automated Nucleic Acid Amplification Test (NAAT), Liquid Medium Culture (MGIT) are out of reach for developing countries where the disease burden is very high. More over in advanced HIV infection



Dignitaries in audience



From left; Dr. B. C. Das, Dr. Nomita Chandhiok and Dr. S. M. Mehendale

with low CD4 count, extra pulmonary involvement, disseminated TB, smear negative TB with normal chest radiography are manifested more commonly, making the diagnosis difficult.

Prevention of re-activation of latent TB with chemoprophylaxis through rigorous screening using clinical parameters including Tuberculin Skin Test (TST) is being prescribed by countries where the TB epidemic is being driven by HIV epidemic. But currently this is not being implemented in most countries where TB is endemic due to fear of development of resistance.

In addition, this co-infection complicates the treatment of both diseases with possible complications from interactions between the drugs used to treat both HIV and TB. In India the Revised National Tuberculosis Control Program (RNTCP) regimen under DOTS strategy is being followed for the management of TB in HIV infected as well as HIV uninfected subjects.

However, newer evidences do suggest that

1. Active co-infection with TB needs to be diagnosed early and managed more aggressively with daily anti-TB regimen in intensive phase and continued with intermittent dosage regimen for longer period.
2. Early initiation of ART has also been advocated to reduce resistance, improve response and avert re-infection.

Recognizing the importance of diagnosing TB early, instituting optimized Anti-TB treatment in terms of dosage, frequency and duration, initiating ART at the most favorable time after the initiation of anti-TB treatment and to examine the necessity and feasibility of treatment of latent TB in HIV infected in India, this topic was chosen for critical review and deliberations in the consultative meeting.

Definition of TB problem and diagnosis in HIV infected individuals

Plenary sessions included presentations of Indian data which gave insights into program perspectives of HIV/TB co-infection in India, its policies, practices and results of Intensified TB case

finding (ICF) in Integrated Counseling and Testing Centres (ICTCs) and ART centres. The number of HIV detection and treatment centers is significantly fewer than the TB centers in India. As a result, there is a service-delivery gap between identification and treatment of HIV in TB infected population. The audience was also briefed with a synopsis of the state of the art research on HIV/TB co-infection in India. Global epidemiology and evidence on HIV/TB co-infection from Africa, approaches to screening and diagnosis of TB as well as information related to TB infection control in South Africa was also presented. Data from Africa showed that the newer diagnostic method using automated Nucleic Acid Amplification Test (NAAT) which requires less expertise but provides faster results, helped early initiation of treatment. Identification of active tuberculosis through symptom screening using report of any one of the four symptoms, cough, fever, weight loss or night sweats in the last 24 hours performed very well in the clinical as well as community set up with negative predictive value of over 95%.

The plenary session was followed by lively discussions and debate, which focused on the use and need for Ultra Sonography (USG) in diagnosing Extra Pulmonary TB (EPTB), use of Microscopic-Observation Drug-Susceptibility (MODS) assay in early diagnosis of TB, settings and frequency of screening for TB. Following points emerged out of the discussions:

Settings for and frequency of screening for TB- TB screening should be introduced at all 'points of care' accessed by the PLHIV and they should preferably be screened at baseline and at every visit. Symptom based screening should be supported with follow up evaluation for confirmation of TB among the TB suspects. Every client attending any HIV care setting should be screened for TB symptoms at every visit. However, ICF based on symptoms may not be effective in sub-clinical TB in HIV infected with low CD4 count.

Diagnostic tools- USG and other higher investigations necessary to diagnose EPTB should be made available where ever feasible. Need for introduction of newer rapid diagnostics into the programme for cheap and fast diagnosis of TB where ever feasible was also expressed.

To summarize, it emerged out of deliberations that HIV and TB control programme should have an integrated approach for TB case finding and management in HIV infected. A felt need for better diagnostic facilities in national programme was also evident.

Prevention of TB in HIV Infected individuals

There were four presentations in this plenary session which included, INH Prophylaxis Treatment (IPT) for PLHIV, programme perspectives on Prevention of TB among PLHIV, ART among HIV-TB patients in India and issues complicating TB research. It emerged that case definitions, ascertainment of end-points which listed lack of clinical case definition, definition of cure, lack of surrogate markers in TB, inability to differentiate between reactivation and reinfection, etc were some of the main issues complicating TB research. Experiences from the field based

studies on TB management at the field level were also shared. Issues relevant to IPT were discussed by the group. These include mechanism of action of IPT, necessity and efficacy of IPT, duration of IPT, IPT for contact of smear positive PLHIV and bio markers. Following points emerged out of the discussions:

Need for IPT- Since Indian TB epidemic was not driven by HIV epidemic unlike in South Africa, India should generate its own data which would instruct whether IPT is needed, especially with the background of expected revision of CD4+ count threshold from 250 to 350 for initiating ART by NACO.

Mechanism of action of IPT- In India most of PLHIV get TB infection as a reinfection and not reactivation. The actual mechanism of IPT was not known though it was believed to work on rapidly multiplying bacilli. More information is required to understand this.

Tuberculin Skin Test (TST)- Since IPT is being advocated for those testing positive (more than 5mm) for TST, an algorithm for TST reactivity interpretation in the immunologically compromised HIV infected needs to be developed.

IPT for TB contacts- There was a consensus that all HIV positive contacts of sputum smear positive individuals should receive IPT

Biomarker: It was recommended that identification of a biomarker to predict reactivation of TB and disease activity must be taken up on a priority basis.

To summarize, the deliberations on this topic underlined the need for Indian data on efficacy of IPT at various CD4+ cell count levels to take evidence based decision on introduction of IPT for HIV infected in India. Need for biomarkers to predict reactivation of TB was also evident.

Treatment of TB in HIV Infected

There were four presentations in the plenary session which highlighted various aspects of TB management in HIV infected patients. Findings of meta-analysis of data from studies on length and duration of treatment of TB in HIV infected, outcome among patients in Chennai treated with short course intermittent anti-TB treatment were discussed. Findings from a study of efficacy and safety of thrice weekly DOTs in TB patients with and without HIV and programme perspectives of treatment of TB among HIV-TB patients in India were also presented.

The discussion centered around the issues of improving diagnosis of HIV in TB patients, the time of starting ART in HIV-TB patient and the duration & frequency of treatment of TB in PLHIV.

Early initiation of ART- It was felt that ART should be initiated early in HIV TB patients to reduce mortality. The 'point of care' testing for HIV at the TB clinics could facilitate rapid initiation of ART in HIV infected TB patients (without the patient being lost to follow up during the referral to ICTC). ART should be initiated at the earliest, preferably after two weeks of anti-TB treatment, especially among smear positive TB Patients.

Frequency of Anti Tuberculosis Treatment: The primary

evidence from the meta-analysis which formed the basis of WHO recommendation (of daily anti-TB treatment during Intensive phase among PLHIV) was examined in detail. In view of the fact that most evidence was from pre-ART or early ART era and the contrasting evidence of equivalent success in ART era presented by TRC, Chennai, the group agreed unequivocally that the evidence was too weak to warrant a change in RNTCP regimen. However, the group recommended for expediting generation of evidence to resolve basic questions of frequency, dosage and duration of TB treatment among PLHIV.

Treatment of MDR and XDR- Since the disease severity is higher among PLHIV with DRTB; RNTCP should prioritise PLHIV for diagnosis of DRTB and make second line ATT available more widely to HIV/TB patients who have MDR-TB

Research need- It was recommended that more information on the prevalence of MDR and XDR in different regions of India in HIV/TB patients should be generated.

To summarize, the deliberations on HIV TB treatment resulted in consensus that ART should be started early (2-4 weeks after ATT initiation) in HIV TB co-infected patients. In addition, the need for stronger evidences for change to daily anti-TB regimen of longer duration, i.e. 9 months was reiterated.

Further reading

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HIV and HPV

It has been established that HIV-infected men and women are at a higher risk of HPV associated ano-genital and oral malignancies than the general population. It has been estimated that 80-90% of women in general, get infected with HPV in their life time but most infections clear over a period of few months. However in HIV infected women, infection with high risk types of HPV persists in the milieu of compromised immunity with gradual accumulation of altered cellular genetic material. This leads to development of grades 1, 2, 3 Cervical Intraepithelial Neoplasia (CIN) and eventually invasive cervical cancer. HIV HPV co-infected individuals tend to have multiple types of HPV which are associated with a greater risk of HPV-related disease, and are more likely to progress rapidly to intraepithelial neoplasia notably at lower CD4 counts.

HPV based screening for CINs has been shown to be more effective in preventing cervical cancer incidence and deaths, but the technique is expensive and availability is confined to few laboratories. Low cost and sensitive tests for HPV that could give quick results are expected to be available in the near future.

Although progression from intraepithelial neoplasia to malignancy typically takes many years, it is likely that many co-infected persons would face increased risk of malignancy due to prolonged life expectancy in the era of Highly Active Anti Retro Viral Therapy, HAART. This heightened risk of HPV related neoplastic disease and malignancy potentially adds additional burden in India where 366.58 million women are at risk of cervical cancer. However, there is a window of opportunity to identify and treat pre-cancerous lesions and thus avert progression to



Lecture by Dr. Joel Palefsky

malignancy by strengthening screening programs, although systematic surveillance and detection of early lesions could be challenging in India. While, HPV vaccines are being recently marketed in India, studies indicate that HPV 16- and 18-based vaccines may not be adequate to induce protective immunity in this population.

Acknowledging the risk of HPV related malignancies in HIV infected women in India where a large number of women are living with HIV, and recognizing the need for early diagnosis and management of cervical intraepithelial neoplasia in resource limited settings, efficacious inexpensive HPV tests and prevention through effective vaccines, this topic was selected for deliberations in the consultative meeting.

The plenary session included four presentations which focused on whether HPV was an important public health problem in HIV era, diagnostic challenges in HPV infection in resource limited settings, issues in early diagnosis & management of HPV related malignancies and prevention of HPV Infection in the Community.

Following the plenary session, the group focused on discussing following themes: screening for HPV and HPV related pre malignant and malignant conditions, place and frequency of screening and strategies to involve community in HPV vaccination programme. The points emerged out of the deliberations were as follows:

Screening for HPV and its related pre malignant conditions among HIV infected- The See and Treat strategy using Visual Inspection with Acetic acid (VIA) should be refined as a feasible option in India and to avoid overtreatment. HPV test in VIA positive cases would be more effective since HPV screening may not be feasible in general population. Screening for HPV could be done in the care and support settings of National AIDS Control Programme as well as in private health care settings which have resources, in order to maximize benefits.

Frequency of screening- Screening methods for cervical epithelial abnormalities HPV testing using DNA PCR and rapid HPV (adapted Digene HC2 assay), VIA /colposcopy & see-and-treat-strategy and Cytological screening (both conventional and liquid based cytology) were discussed. Screening for HPV in general population may not be cost effective, but in the HIV infected population it would be highly cost effective as the investment in screening for pre malignant condition in HIV infected women could translate into the prevention of 47 deaths for every 100000 screened women as cancer cervix mortality was high in HIV positive women. Furthermore, it was felt that the frequency of HPV testing in PLHIVs and screening at risk population for HIV also needs to be addressed.

HPV screening for men- HIV infected men should be screened for HPV and anal cytology as HPV prevalence in men was high and they could also transmit HPV infection to women.

HPV vaccination- Since the vaccination programme had to be stopped due to controversies; intensive programmes, advocacy,

media involvement and community mobilization should be taken up to create awareness about advantages of HPV vaccination which would lead to demand generation.

Research Needs- Need for generating data on HPV types, incidence of various HPV associated cancers in Indian women, feasibility study of HPV Triage for HIV positive and VIA positive women emerged from deliberations. Screening and long term follow up of the HPV vaccinated girls to assess occurrence of HPV infections in later life could give valuable data on the efficacy of the vaccine.

The deliberations on HIV HPV infection identified gaps in data on HPV HIV co-infection and HPV related malignancies in the Indian context. It emerged that HIV infected women need to be rigorously screened for CIN and VIA was a feasible low cost option. Testing for High risk HPV types in this population could be explored which if diagnosed and managed early would significantly reduce incidence of cervical cancer. It was also evident that community mobilization and demand generation for HPV vaccination through involvement of various stakeholders needs to be undertaken.

Further reading

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4. *HPV Screening for Cervical Cancer in Rural India. Rengaswamy Sankaranarayanan, M.D., et al N Engl J Med 2009; 360:1385-1394 April 2, 2009*

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Speakers of various sessions

Response to First-line Antiretroviral Therapy

Co-Chairs: *Dr Aradhana Johri, Dr. Suniti Solomon*
 Lead Discussant: *Dr R. R. Gangakhedkar*
 Rapporteurs: *Dr. Manisha Ghate, Dr. S.V. Godbole*

Dr. Rewari, NACO, New Delhi
Dr. O. C. Abraham, CMC, Vellore
Dr. R. S. Paranjape, Director, NARI
Dr. Atul Patel, Ahmedabad

HIV and HPV

Co-Chairs: *Dr B.C Das, Dr. Nomita Chandio*
 Lead Discussant: *Dr S. M. Mehendale*
 Rapporteurs: *Dr Mallika Alexander, Dr Sampada Dayarkar*

Speakers

Dr Sten Vermund, Director, Vanderbilt University Institute for Global Health
Dr Shastri, Preventive Oncology, Tata Memorial Hospital, Mumbai
Dr Sheela Godbole, Scientist D, NARI
Dr Neerja Bhatla, Prof Obst and Gyne, AIIMS, New Delhi

HIV-TB Co-Infection

Definition of the problem and diagnosis

Co-Chairs: *Dr. Po-Lin Chan and Dr. Sriram Tripathy*
 Lead Discussant: *Dr A. R. Risbud*
 Rapporteurs: *Dr Vijay Nema and Dr. Srikanth P. Tripathy*

Prevention of TB in HIV Infected

Co-Chairs: *Dr. Alka Deshpande & Dr. R.S. Paranjape*
 Lead Discussant: *Dr. Padma Priyadarshani*
 Rapporteurs: *Dr. Vidya, Dr. Annapurna Kaul*

Treatment of TB in HIV Infected

Co-Chairs: *Dr Rajasekaran and Dr. K.C. Mohanty*
 Lead Discussant: *Dr Srikanth. P. Tripathy*
 Rapporteurs: *Dr. Puneet Dewan and Dr. Sheela Godbole*

Speakers

Dr. Ian Sanne, Protocol Team Member for ACTG studies *Dr H Getahun, Stop-TB Dept., TB-HIV team leader* *Dr. Puneet Dewan, WHO, India*
Dr. Devesh Gupta, Chief Medical Officer, Central TB Division *Dr B Rewari, NACO Delhi* *Dr. Soumya Swaminathan, WHO*
Dr. Srikanth Tripathy, Scientist F, NARI *Dr. Raman Gangakhedkar, Scientist E, NARI* *Dr. S.K. Sharma*
Dr H Getahun, Stop-TB Department, TB-HIV team leader *Dr. Abhay Kudale, MAAS, Pune* *Dr. V.M. Ajay Kumar, WHO National Consultant*

In conclusion, this consultative meeting, which examined the available evidences on response to first line ART and Sub optimal response, implications of co-infections with TB or HPV in HIV infected has led to identification of many concrete approaches that could be implemented by the national programme. Also several areas where more data in Indian context are required have been identified which need further research to inform the national

programme. The process of translating some of the recommendations into action has been initiated by NARI.

Report Compiled by

Dr Sampada Dhayarkar, Dr. Sumitra Krishnan, Dr. Mallika Alexander, Dr. M. V. Ghate, Dr. S.V.Godbole, Dr. R. R. Gangakhedkar, Dr. S.P. Tripathy



**Galvanizing Evidence for HIV Management
A Consultative Meet at National AIDS Research Institute, Pune 27 to 29 Jan 2011**

Researcher in Focus



Robert M. Grant MS, MD, MPH

Robert (Bob) Grant is a Senior Investigator at the Gladstone Institute of Virology and Immunology and an Associate Professor of Medicine at the University of California San Francisco. He has over 26 years of experience with AIDS clinical care and research, which includes 4 years in leading roles in epidemiological studies in San Francisco and Uganda, followed by a fellowship in Molecular Medicine, after which he started the Gladstone/UCSF Laboratory of Clinical Virology in 1997 and the Gladstone Laboratory of Molecular Evolution in 2000.

Dr. Grant is currently the protocol chair for the Pre-Exposure Prophylaxis (or PrEP) Initiative study, which involves use of antiviral agents to block transmission of HIV-1 to highly exposed persons, in addition to standard prevention care. This global clinical trial has sites in Peru, Ecuador, Brazil, United States, South Africa, and Thailand - settings where HIV-1 continues to spread. Dr. Grant's laboratory is also leading research to understand the biological and social implications of PrEP, which may include immune responses due to viral antigen exposure during PrEP, low

level drug resistance, and alterations in HIV testing and disclosure in social networks. Dr. Grant's laboratory is also interested in HIV-1 superinfection, where persons already infected with HIV-1 might acquire additional strains of the virus. Understanding when superinfection occurs, and when it does not, could provide clues to protective immunity that would guide HIV vaccine development. Dr. Grant's team has also investigated the consequences of molecular evolution, including (1) the fitness of drug-resistant HIV-1 for replication, virulence, and transmission; (2) mechanisms of mutagenesis that underlie viral evolution; and (3) nonpathogenic simian immunodeficiency virus (SIV) infection in natural host species. Their long term goal is to understand the viral and social characteristics that underlie patterns of epidemic spread of HIV-1 in human communities.

Dr. Grant is a Betty Jean and Hiro Ogawa Endowed Investigator. He has served as a member of the US FDA Antiviral Advisory Committee. He is an author of over 95 publications.

Challenges of immunogen design that can elicit broadly neutralizing antibodies to HIV-1

Dr. Jayanta Bhattacharya and Mr. Rajesh Ringe
Division of Molecular Virology



Making an antigen capable of eliciting potent and broadly cross neutralizing antibodies (NAbs) against human immunodeficiency virus (HIV) is a daunting task. The approaches based on experimental findings and experiences have significantly failed and the results of two VaxGen efficacy trials of monomeric gp120 have disappointed HIV vaccinologists. Years of research on the HIV-1 envelope (Env) glycoproteins have shown that HIV-1 has many effective protective mechanisms with little vulnerability. HIV-1 is an enveloped virus and for fusion with its target cells, it uses a trimeric Env complex containing gp120 and gp41 subunits. The fusion potential of the Env complex is triggered by engagement of the CD4 receptor and a coreceptor, usually CCR5 or CXCR4. Neutralizing antibodies (NAbs) seem to work by binding to the mature trimer on the virion surface and preventing initial receptor engagement events, by binding after virion attachment and inhibiting the fusion process, and first binding to trimer complex and triggering gp120 subunit dissociate from gp41 transmembrane subunit thereby rendering the virion sterile to infection. HIV-1 has evolved multiple mechanisms to protect it from antibody binding. Env is heavily glycosylated, and the poorly or nonimmunogenic glycans prevents antibody access to the underlying epitopes. Trimerization of the gp120-gp41 structure can shield vulnerable epitopes that are better exposed on the individual monomeric subunits, something still not fully understood at the molecular level but that seems to involve conformational rearrangements. Kinetic and spatial constraints impede antibodies from accessing potentially vulnerable sites during receptor binding and membrane fusion processes. Finally, the variable loops of gp120 are a prime target for NAbs, but such NAbs usually, although not always, have a very narrow breadth of reactivity and variable loops are conformationally so flexible and recessed in the core structure that they are inaccessible on the primary viruses.

Env do have vulnerable sites to target

Despite so much of mutability, primary isolates of HIV-1 from different genetic subtypes can be neutralized by some broadly reactive human monoclonal antibodies (mAbs) such as b12, 2G12, 2F5, 4E10, VRC01, PG9/PG16. The very existence of these broadly neutralizing antibodies provides some clue and hope that a vaccine inducing NAbs can indeed be created, by showing vulnerabilities in the viral defenses that could be exploited. An immunogen that could efficiently elicit NAbs with these same specificities would be an effective remedy against HIV which today seems to be a distant hope. Therefore, it is important to understand how the mAbs described above interact with Env. The

first broadly neutralizing mAb identified was b12, an antibody that occludes the CD4 binding site on gp120 and prevents CD4 attachment. Env has a deep pocket in which CD4 inserts its residues (Phe43) and bind with avidity. Without this interaction, gp120 binds too weakly to CD4 for fusion to proceed efficiently. The b12 crystal structure shows that it has an unusually long protruding CDR3 loop that can plunge into this cavity in gp120 and prevent CD4 binding. Many other mAbs with similar specificity have been identified over the years that block CD4 binding, but none has the potential shown by b12 and lack the ability to neutralize primary HIV-1 isolates. It is important to know why b12 is unique and what can be done to induce b12 like NAbs. A second broadly neutralizing mAb, 2G12, recognizes a cluster of oligomannose residues on gp120 through a unique antibody structure. The unusual antigen binding site of this antibody is well suited to recognize the cluster of oligomannose residues formed as part of the protective sugar coat of gp120. The antibody has, in effect, been raised against the very defenses that the virus uses to ward off antibodies in the first place. The relative conservation of a particular glycan arrangement allows 2G12 to recognize many HIV-1 strains especially clade B viruses but not clade C which is a predominant subtype in India, China and South Africa. The problem with the elicitation of such antibodies is that the glycans moieties are poorly immunogenic and ability of glycans-peptide conjugates to elicit the antibodies should be worked out. The mAbs 2F5 and 4E10 recognize nearby but distinct epitopes on the membrane-proximal region of the gp41 ectodomain. These NAbs act by inhibiting the fusion process. The epitope for 2F5 is simple and linear yet all attempts to induce 2F5-like NAbs by vaccination with peptides or simple linear epitopes have failed; the resulting antibodies recognize the immunogen but not the virus itself. The mAb 4E10 in particular has a breadth of cross reactivity that needs to be understood and exploited. The 2F5 and 4E10 antibodies have specificity with lipids and recognize their epitopes better in the context of membrane. Therefore, in vivo antibodies like these are rarely produced. Additional studies are needed of both 2F5 and 4E10, preferably as complexes with more native Env structures, to show what immunization strategy might elicit NAbs with the same specificities. The four mAbs discussed above are the most broadly neutralizing antibodies identified and studied in detail. Recently few more antibodies have been discovered namely, VRC01, PG9 and PG16. These antibodies are specific to surface protein gp120. The epitopes of the PG9 and PG16 are made by V1V2 and

V3 loops (variable regions in gp120) and found to have breadth and potency of neutralization against diverse HIV-1 primary isolates.

The V3 loop in gp120 surface protein as a NAb target

The third variable loop V3 on the gp120 has an important role to play in binding with coreceptor post CD4 engagement. It is very long element divided into three parts as tip, stem and base and interacts with the core gp120 structure and stabilizes the association of trimer. The V3 loop of gp120 was once considered to be the principal neutralizing determinant but this term applies only to viruses that are adapted in cell culture. The V3 loop is of more limited importance for primary virus neutralization, but some V3 mAbs are active against a range of primary viruses. One such mAb is 447-52D, which recognizes the GPGR motif at the tip of the V3 loop and main-chain atoms along one side of the loop and neutralizes about 47% of clade B isolates. This antibody does not seem to recognize HIV-1 with a GPGQ motif at the tip of the V3 loop that is common to non-clade B isolates. The V3 specific antibodies neutralize cell line adapted viruses than primary isolates meaning that V3 is more accessible in them than primary envelopes. As V3 is more immunogenic, sera usually contain antibodies against it and so the viruses employ different mechanisms to hide V3 from immune surveillance. Another antibody, 58.2, also recognizes an epitope overlapping the tip of the loop and neutralizes about 33% of clade B isolates. There are some more anti-V3 antibodies recently discovered namely 2191, 2219, 2557, 2858, 3074 and 3869 showing limited breadth and potency and are being studied in more details.

The coreceptor site as a NAb target

The coreceptor binding site is not exposed on the Env protein in preCD4 state. CD4 binding induces structural changes in Env and reveals coreceptor binding site. The epitopes mostly conformational, present in this site are called as CD4 induced (CD4i) epitopes and can be targeted by NAb typified by mAb 17b. Antibodies to the CD4-induced epitope cluster have very limited activity against primary isolates. The possible explanation for this is that CD4i epitopes are presented only after virus binds to cellular receptor and there seems to be a space constraint to access CD4i epitope for a whole IgG. Unexpectedly, however, Fab fragments and single-chain Fv fragments of mAbs to CD4-induced epitopes can neutralize primary isolates very well, in contrast to the normal pattern in which IgG molecules are more potent than Fabs. Recently discovered Fab m36 has shown great potential to neutralize primary isolates. Unfortunately, the fact is that immune system does not produce Fabs but whole antibodies which lack neutralizing activity in this case. Studies with the mAbs induced against CD4i epitopes reveal the virus's ploy and battle of the immune system against it. Studies show that even if high affinity antibodies are somehow produced against coreceptor binding site they will fall victim to kinetic and spatial constraints imposed on antibodies by the geometry of interactions between

virus and the cell surface.

Transmembrane gp41: conserved but secured

The gp41 subunit is far more conserved than is gp120. Kinetic and steric constraints also probably protect potentially vulnerable regions of gp41 from NAb attack. MAbs namely 2F5, 4E10 and Z13 have been isolated from HIV positive patient and show considerable potency, 4E10 being the most broadly neutralizing of all. These MAbs bind to the intermediate conformation of gp41 during fusion process. The native gp41 seems to be inaccessible to antibodies and reveals epitopes during the fusion process. Therefore, either limited antibodies are produced against these epitopes as their exposure is too short to be recognized by B cell receptors to elicit antibodies against them or the antibody has a little window of opportunity to bind to short lived epitopes that are exposed only during fusion. A peptide mimetic of a gp41 subdomain, enfuvirtide (formerly called T-20), is an effective antiviral drug. It binds to the gp41 during fusion and impedes necessary conformational changes. The regions of gp41 that correspond to enfuvirtide and the enfuvirtide binding site are highly immunogenic, yet antibodies to these sites do not neutralize primary isolates probably because of the limited space available for large antibody molecules to gain access to most gp41 epitopes when they transiently become exposed during the fusion process.

Expectation and basis of immunogen design

How can one design immunogens that elicit broadly neutralizing antibodies? Based on the studies and what we have learned from the NAb described above an immunogen should elicit the antibodies that target multiple sites on the envelope. Polyclonal antibodies having different but distinct specificities would compromise the virus in multiple aspects during virus life cycle. The immunogen must expose short lived epitopes (CD4i and gp41 epitopes) for relatively longer time and better present Nab epitopes than wild type virus (Env). An approach that aims to target multiple epitopes would probably compromise the Envelope to mutate in response to antibody selective pressure and may cost the fundamental function of the env protein

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Commentaries

Dried Blood Spots (DBS) for Minority and Archived HIV drug resistance mutation

Devidas N. Chaturbhuj, Senior Research Assistant, Drug Resistance Lab, Molecular Virology.

HIV-1 Drug Resistance (HIVDR) testing is required for continued surveillance and/or individual HIVDR monitoring, which helps the clinical management of patients. Dried blood spots (DBSs) represent an attractive alternative to the conventional collection of whole blood in a tube. Due to HIVDR discrepancies observed between DBS and plasma questions have been raised regarding its potential use. To answer these questions, a study has been carried out to assess the utility and potential gain in genotypic information with sensitive testing of DBS compared to conventional bulk plasma genotyping, and to examine the correlation of majority and minority-level resistance mutations in DBS with treatment history.

The study includes 33 DBS and 17 corresponding plasma samples. To examine the contribution of RNA to minority resistance mutations for 16 samples for which corresponding plasma was not available, nucleic acid extracted from DBS was digested with DNase to ensure DNA was removed thoroughly. The sensitive drug resistance mutation testing was carried out by using RT-PCR with and without reverse transcriptase to selectively amplify DNA followed by majority and minority-level resistance, using real-time PCR for M41L, K65R, K70R, K103N, Y181C, M184V, and T215Y/F mutations. The bulk sequencing of DBS and plasma samples was performed using the ViroSeq HIV-1 Genotyping System.

The results were analyzed for detection of resistance mutations from DBS versus bulk plasma. The 98% majority level mutation from the original plasma bulk was also detected in the DBS using sensitive PCR assays. There were 13 additional mutations which

were not detected in plasma bulk sequences but were detected in DBS leading to 24% increase over what was identified by bulk sequencing of plasma, with substantial increases for K65R (4-fold) and K103N (3-fold). Of the 11 individuals with newly identified minority mutations in DBS, 6 individuals showed corresponding minority mutations in plasma sequences and remaining 5 individuals had no detectable minority resistance mutations in their plasma. This suggests that these mutations were exclusive to the DNA compartment. The results have also been analyzed for correlation of minority drug resistance mutations with treatment history. The presence of all 11 minority mutations was due to present or past ART treatment taken by individuals up to six years prior to the blood collection.

In summary, the findings of the study provided evidence with the use of sensitive resistance testing that DBS on Whatman 903 paper is a specimen type suitable for long-term storage. Expanded HIV-1 genotype analysis and identification of minority-level drug resistance mutations not detected by bulk genotyping are among the other advantages demonstrating robust stability of nucleic acids. The study reflected the presence of minority mutations with present or past drug regimens including K65R, which is known to be a short-lived, high fitness-cost mutation. Therefore it was demonstrated that DBS, if dried and stored properly, maintains high nucleic acid integrity for drug resistance testing. The data, in addition to the available literature, further support the global utility of DBS for drug resistance surveillance and clinical monitoring.

Further reading:

Wei X, Youngpairoj AS, Garrido C, Zahonero N, et al. Minority HIV mutation detection in dried blood spots indicates high specimen integrity and reveals hidden archived drug resistance. *J Clin Virol*. 2011 Feb;50(2):148-52.

Tetherin: Last barrier that limits virus release from cell.

Archana Gautam, Senior Research Fellow, Molecular Virology

Tetherin/*BST2* was identified in 2008 as the cellular restriction factor responsible for restricting HIV-1 replication at a very late stage in the lifecycle. Tetherin acts to retain virion particles on the plasma membrane after budding has been completed and limits the ability of HIV to escape from cells. Infected cells that express large amounts of tetherin display large strings of HIV virions that remain attached to the plasma membrane. Tetherin is an unusual Type II transmembrane protein that contains a GPI anchor at its C-terminus and is found in lipid rafts. HIV-1 in turn has evolved to express an accessory protein, Vpu that specifically counteracts the restriction to virus release contributed by tetherin. However, evidence that tetherin functions as a physical tether has thus far been indirect.

By using electron microscopy cryosectioning and immunogold labeling, Hammonds and colleagues have shown that tetherin is present as a physical link between virions and the plasma membrane and sometimes between virus particles in large clusters or strings. Their work addressed a simple question: is tetherin the physical connection that links retained virions to the plasma membrane and to each other? They demonstrated that tetherin is

present in concentrated fashion at focal sites of particle budding. The work in this paper has been performed in infected T cell-lines. They performed partial protease stripping experiments utilizing both over-expressed and endogenous tetherin sources and demonstrated the incorporation of tetherin into budding HIV-1 virions. They have shown that in the absence of Vpu, tetherin was enriched on filamentous structures connecting virions to the plasma membrane, and was present between chains of tethered viral particles present in focal accumulations in infected cells.

This is a classical study that has given direct evidence on how cellular host factor limits disease pathogenesis by preventing virus release. However, the study has been done in cell-lines, it would be tempting to speculate this phenomenon in primary cells like T cells and macrophages that represents natural targets of HIV in vivo. Studying interaction of tetherin with Vpu and other viral proteins will not only enhance our understanding but also provide key information about interaction of host factors with viral proteins.

Reference:

Hammonds J, Wang J-J, Yi H, Spearman P. [Immunoelectron Microscopic Evidence for Tetherin/BST2 as the Physical Bridge between HIV-1 Virions and the Plasma Membrane \(2010\). PLoS Pathog 6\(2\): e1000749.](#)

Happenings at NARI**Translational Research in HIV/AIDS in India (TRAJ)***Inauguration of TRAJ*

The Indian Council of Medical Research is currently celebrating its centenary. As a part of the centenary celebrations, a conference on **“Translational Research in HIV/AIDS in India” (TRAJ)** was jointly organized by National AIDS Research Institute (NARI), University of South Florida (USF), Tampa, USA and Community Resource Foundation, Goa, India, during 12-14 January 2011 in Goa. The conference was attended by around 100 Indian and international delegates including resource persons

working in the field of basic and applied HIV biology.

The meeting was inaugurated by Prof. Dileep Deobagkar, the Vice Chancellor, Goa University. The focus of the conference was HIV Basic Biology and Translational Research as the conference was primarily aimed at updating global and Indian research on the biology of HIV and taking the basic science discoveries to patient care through translational research. Basic science is very critical for new developments in the field of diagnostics, drugs and preventive strategies such as vaccines and microbicides. The meeting was expected to bring together some of the leading US scientists, AIDS International Training and Research Program (AITRP) investigators and Indian scientists working in the field of HIV and AIDS to discuss in depth research programmes in India and contextualize them with global programmes. The conference was also conducted with the objectives of deciding priorities in basic science research in India, establishing network of basic scientists and foster new collaborations and encouraging young scientists and graduate students.

To achieve these objectives nine different sessions pertaining to the high research priority areas in HIV/AIDS having translational

potential were selected. These included 'Immunity in HIV pathogenesis', 'Host and viral factors in HIV pathogenesis', 'Antiviral host factors and innate immunity', 'vaccine development and adaptive immunity', 'dynamics of HIV TB co-infection', 'microbicide development', 'nanotechnology application and HIV therapy and diagnostics', 'HIV diagnostics and resistance' and 'Bioinformatics'. A total of 38 renowned speakers from India and USA presented their work during these sessions with very vibrant interactions. The sessions were chaired by eminent senior scientists, who helped in provoking directed and constructive interactions during the discussions.

More than 35 posters were also displayed in the conference in addition to the oral presentations by different scientist including young scientists and graduate students. The special feature of the conference was presence of Dr. V. M. Katoch, Secretary, Department of Health Research and DG, ICMR. His plenary lecture was followed by interaction with different scientists from India and abroad. The conference was very successful in its objectives of bringing together Indian and USA scientists working on basic biology of HIV for discussing the priority areas and proposed collaboration. For making this platform available for such discussions frequently, it has been decided that similar event will be planned on yearly basis.

The in depth discussions during the conference highlighted the following points.

- There is a need for establishing cohorts that can provide base for good clinical and biological studies. It was also suggested that



Organizing team of TRA9

National agencies can support establishment of such cohorts.

- It was felt that since HIV-1 subtype C is predominant in India we need to develop the database on Indian HIV-1 subtype C. There is also need for carrying out meta-analysis on the data in public domain.
- It was felt that there is need for networking of the scientists. There is specific need for greater involvement of clinician scientists and interaction between basic scientists and clinical researchers.
- It was felt that there is need for studies on areas such as bioavailability of drugs, drug delivery, pharmacogenomics and adverse drug event monitoring.
- Need for translational research in the areas of vaccines, microbicides, anti-HIV drugs and diagnostics was expressed.

A workshop on “Information and Analytical Tools for the Medical Science Researchers”

NARI hosted a workshop on “*Information and Analytical tools for the Medical Science Researchers*” on the 15th Feb 2011. The workshop was jointly organized by the *Indian Council of Medical Research (ICMR)* and the *Elsevier*, the publishing company. The aim of the workshop was to raise awareness among ICMR scientists and researchers regarding the benefits of using online resources in their research activities. It was attended by about 75 researchers from National AIDS Research Institute, Pune; National Institute of Virology, Pune; and National Institute for Research in Reproductive Health (NIRRH), Mumbai.

In his inaugural speech, Dr R S Paranjape, Director, NARI, emphasized the role of publishing media and library in helping scientists doing research. Later, Dr K Satyanarayana, Scientist 'G' & Head of Publication & Information Division, ICMR, spoke about science indicators, such as Journal Impact Factor and Citation Indexes, which help scientists improve their research

performance and output. In the end, Mrs Padma Muralidharan and Mrs Priyanka Neogi, both officials of Elsevier, demonstrated the use of their products, such as Scopus, SciVerse, etc. for the benefits of scientific research.



Mrs. Priyanka Neogi from Elsevier

कुटिल वचन सबसे बुरा, जारी करे तन छार । साधु वचन जल रूप है, बरसे अमृत धार ।।

1. Invited lecture for NSD 1st March 2011

On the second day of National Science Day Celebration, an eminent and well known scientist of India, Dr G D Yadav Director and Vice Chancellor, Institute of Chemical Technology, Mumbai was specially invited. He gave a talk on '**Confluence of chemical and biological sciences for exciting future**'. Dr Yadav described the importance of chemistry in the life of the pre-historic man and how it has revolutionized the life of the modern man. This was an apt subject as 2011 has been declared by UN General Assembly as **the International Year of Chemistry**. This inspiring and thought provoking lecture was attended by all NARI staff and delegates at NARI who were attending the National Reference Laboratory Meeting at NARI. They included distinguished scientists and professors from the All India Institute of Medical Sciences, National Centre of Disease Control, National Institute of Enteric and Communicable Diseases etc. His session about the development and applications of chemistry and its confluence with biological sciences, nano technology and molecular biology in the imminent future inspired the audience. This was evident by the large number of questions asked by the learned audience to the Eminent Scientist, Dr G D Yadav. Director, Dr R S Paranjape thanked the distinguished guest for his wonderful talk and felicitated him with a NARI memento to commemorate the ICMR centenary-year celebration. Discussions continued over tea and this showed the impact of his very interesting talk.



Dr. G. D. Yadav with Dr. R. S. Paranjape

2. Open House at NARI on 28th Feb.2011.

2.1 Laboratory visit: Students from five colleges in and around Pune visited NARI which included Ramakrishna More College, Akurdi, M V College of DMLT, Nandurbar and JJ hospital Nursing College, Mumbai. The students had a tour of the NARI laboratories: Virology, Sequencing, Serology, Microbiology, Immunology and Immunogenicity; Liquid nitrogen storage and Cold room. The research being carried out in these laboratories was explained with the help of charts, presentations and exhibits. The varied queries of students were answered by experts in different laboratories. Following the visit to various laboratories, the students were taken to the auditorium for a video film and 'Panel' discussion.

2.2 Panel Discussion: A video film describing replication of virus and mode of action of different classes of antiretroviral drugs was shown to the students which was explained by Dr Dipak Bangar, Clinician at NARI. Subsequently an interactive session was held with three expert Panelists, in field of clinical science, social and behavioral sciences and laboratory. The three panelists: Dr Mallika Alexander, Medical Manager, Clinical Trials Unit, Mrs. Rajani Bagul, Technical Officer, Social and Behavioral Research Division and Dr. Vijay Nema, Scientist C, Department of Microbiology answered the queries raised by the students. Students from different colleges participated in the discussion with enthusiasm. Varied questions were asked as HIV infection is a part of the science college curriculum. The questions ranged from HIV vaccine to different classes of antiretroviral drugs, mother to child transmission, HIV PCR, symptoms of HIV in early and late stage of disease and also specific questions like the role of nurses and their job opportunities.



Young brains in discussion

5th Science Expo at Nehru Science Centre, Worli, Mumbai

NARI's exhibit at Nehru Science Centre

Nehru Science Centre, among the four National level Science Museums in NCSM, is working as the Western Zone Headquarters with five science centers in Nagpur, Calicut, Bhopal, Dharampur and Goa under its umbrella and caters to the people in the Western part of India. As a part of its activities, the Centre organizes regular extensive science education programmes, activities and competitions for the benefit of the lay people and students in particular. Science Expo is one of the popular educational programmes that is being organized since 2004, to give the general visitors and school students an opportunity to have live interaction with practicing scientists and to learn about various research institutes and their activities. Fifth Science Expo was organized between 20 and 24 Jan 2011. National AIDS Research Institute, Bhosari, Pune was invited to participate for the 5th Science Expo celebration at NSC. The team consisting of two clinicians (Dr. S. Gaikwad, Dr H. Nalgirkar); two counsellors (Mr. I. Mashal and Mr. A. Shinde) and two scientists (Dr. Nema & Dr. S. Dhayarkar) represented NARI at the expo.

The Expo was inaugurated by the renowned scientist and Director, National Environmental Engineering Research Institute, Nagpur, Dr. Tapan Chakraborty on 20 Jan 2011. The Expo included an exhibition with stalls from eminent institutes and other activities like quiz, elocution competition, essay writing competition and many more. Students from different schools and colleges from Mumbai visited the stalls and participated enthusiastically in these activities. Around 13000 people visited the exhibition on five days.

Participation by NARI consisted of Popular Science Lecture by Dr. Vijay Nema Scientist C from NARI, on “HIV, Science and

Adolescents”. Attendees were the students of class IX, X and XI with their science teachers. The vulnerability of adolescents and some measures to empower and prepare them to face the challenge and to contribute with their academic potential in future science was also discussed.

A posters exhibition focused mainly on important milestones of Indian HIV epidemic, modes of transmission, structure of HIV and replication, types of antiretroviral medicines, adherence to ART, and prevention methodologies and development stages of HIV vaccine. The team members explained this information to the visitors in simple language which helped general visitors and students understanding the various aspects related to HIV. NARI team displayed a working model demonstrating HIV life cycle. This model was self explanatory. An animated video of 'HIV replication and sites where of different antiretroviral drugs' act in the particular body cell to control replication of HIV was displayed for the viewers.

Dr. Sampada Dhayarkar, Scientist B, NARI, was invited to judge one elocution competition on “Challenging HIV through social networking” in which around 24 students from different schools participated.

INVITING LITERARY CONTRIBUTIONS

We encourage
original research articles
or review articles in the field
of HIV/AIDS for publication
in forthcoming issues
of NARI Bulletin

★ **Transitioning from HIV sentinel surveillance to programme based surveillance.**

Sanjay Mehendale.

Indian J Med Res 132, 2010: 245-247

Although HSS has remained the most reliable and readily available HIV data source in India, there have always been concerns about the survey methodology, robustness of sample size and generalizability of the findings. An emerging alternative source is HIV testing data at the PPTCT centers operated by the National AIDS Control Program. The PPTCT centers are documenting growing coverage of pregnant women in all the states of India. There is a suggestion to shift to program based surveillance and either reduce the frequency or scale of the ongoing HIV sentinel surveillance [HSS]. The PPTCT based HIV testing approach has an implicit advantage of access to counseling and treatment if necessary. But the significant limitation is inefficiency in timely management of data rendering difficulties in periodic report generation. Special efforts need to be taken at PPTCT centers to minimize women's drop out rates. Issues also exist related to differences in HIV testing protocols that need to be addressed. All these efforts will go a long way to enable us to eventually accept data on HIV prevalence in pregnant women generated all around the year at the PPTCT centers for strategic planning and policy decisions.

★ **Evidence of HIV Type 1 Clade C env Clones Containing Low V3 Loop Charge Obtained from an AIDS Patient in India That Uses CXCR6 and CCR8 for Entry in Addition to CCR5.**

Gharu L, Ringe R, Satyakumar A, Patil A, Bhattacharya J.

AIDS Res Hum Retroviruses 2011; 27 (2): 211-19.

HIV-1 clade C is the major subtype circulating in India and preferentially uses CCR5 during the entire disease course. Here, in this manuscript we amplified envelope clones from a terminal patient with AIDS NARI-VB52, infected with clade C HIV-1, and studied both coreceptor use and the corresponding envelope sequences. Earlier we found envelope clones from a late stage patient NARI-VB105 with the capacity to exploit several coreceptors, including CCR5 and CXCR4, and that contained an unusually high net positive charge of +8 in the V3 loop (Gharu et al., 2009) which is responsible for its expanded coreceptor tropism. However, unlike NARI-VB105 envelopes, NARI-VB52 envelope clones which we reported in this manuscript were found to contain a low net positive charge +3 and a typical GPGQ motif in the V3 loop despite using alternate coreceptors, CXCR6 and CCR8, indicating that regions outside the V3 loop in envelope possibly have influenced alternate coreceptor use.

★ **Predictors of Retention among Men Attending STI Clinics in HIV Prevention Programs and Research: A Case Control Study in Pune, India.**

Seema Sahay, Nikhil Gupte, Radhika G. Brahme, Amit Nirmalkar, Shilpa Bembalkar, Robert C. Bollinger, Sanjay Mehendale.

PLoS One 2011; 6 (3): e17448.

This paper focuses on retention of individuals in HIV prevention programs and research in India. An HIV prevention program can be successfully implemented through optimal primary prevention, valid incidence estimates and effective intervention/s evaluated through clinical trials respectively. Optimization in HIV prevention is highly dependent on minimizing loss to follow up [LTFU] all of these three varied components of prevention program. However achieving high retention and preventing drop outs is a challenge. Thus, our goal was to identify the predictors of retention in HIV prevention programs and research. We have used case control analysis to identify predictors of retention in predefined scenarios of retention in primary prevention, cohort studies and clinical trials. Predictors identified in this study can help in developing retention counseling checklist that might help a counselor to identify potential drop out in prospective studies.

Highlighting our Gems : The "Co-receptor usage by HIV"



*Group working on "Co-receptor usage by HIV"
(From left: Ms. Lavina Gharu, Dr. Jayanta Bhattacharya, Mr. Rajesh Ringe and Mr. Ajit Patil)*

AIDS is an immunodeficiency caused by HIV infection. The primary viral determinant which interacts with cellular receptors is the envelope glycoprotein. HIV-1 infection requires two sequential and specific binding steps by the envelope glycoprotein: first is the attachment to cell surface receptor CD4 present in CD4+ T cells, monocytes / macrophages and other cells; and second to a member of the chemokine receptor subfamily, within the G- protein coupled, seven transmembrane domain family of receptors, mainly CCR5 and/or CXCR4 (Peters et al., 2008; Clapham et al., 2002; Rossi et al., 2008). Hence, it is not only the **viral determinants** such as the envelope glycoprotein but also the **host determinants** such as different co-receptors as well as their expression levels that can have an impact on transmission mechanisms. Thus blocking the entry step can play a very crucial role in preventing the virus infection.

HIV-1 co-receptor tropism and biological aspects which modulates viral entry in the cells has been one of the focus areas of the research in the division of Molecular Virology. The Human Immunodeficiency Virus Type 1 (HIV-1) clade-C is the dominant genetic subtype circulating in India, Sub-Saharan Africa and China (Moore, 2001; Osmanov, 2002; Peeters, 2000; Shankarappa, 2001). This has been reported by many previous studies as well as our recent publications (Ringe et al., 2010; Gharu et al., 2009). In clade-C infections, CXCR4 using variants or a switch to any alternate co-receptor have rarely been detected in individuals in the late stages of disease; however the studies conducted in our lab (Gharu et al., 2009 and Gharu et al., 2010) indicated that viruses using alternate co-receptors do exist with respect to subtype C and that too in Indian scenario. Our group found envelope clones generated from two late stage Indian patients were found to use many different co-receptors in addition to CCR5 and also we were able to locate specific genetic determinants which modulates this phenomenon (not present in other strictly CCR5 using envelopes). This led us to further characterize such envelopes to understand their fitness in transmission mechanism as compared to R5 variants. By correlating the differential usage of the receptors with the sensitivities to different entry inhibitors, our findings indicated that in advanced disease stages, envelopes that are strictly CCR5 using with low CD4 dependence might enhance the ability of variants to exploit different cell types; thereby facilitate enhanced replication and subsequent disease progression by increasing the local viral load and/or transmission to T-lymphocytes.

Co-receptor inhibitors block HIV at the level of entry into target cells, and as such, they make up part of a larger group of drugs known as HIV entry inhibitors. Our lab has made a significant progress towards understanding of how co-receptor choice might impact HIV pathology and how co-receptor blockade may affect disease progression. Thus, greater focus on some of the key areas that are emerging now should be the main priority of co-receptor research and at a time when a CCR5 inhibitor, Maraviroc has been approved in the United States as the first in a new class of anti-HIV therapeutic drugs.



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