ST. JOHN'S MEDICAL COLLEGE HOSPITAL

PROFORMA FOR APPROVAL OF RESEARCH PROJECT BY IERB

<u>Outline of previous work</u> in the field with relevant references : (Introduction, review of literature, justification for study, highlighting the need for the study, potential risks and benefits and outcome measures).

In clinical trials – drug detail in brief including information on the rationale for using the drug & safety profile. Information from earlier Phase 1-4 studies, if any. (*Not more than 2-3 pages*)

Pediatric HIV has benefited significantly since the advent of anti-retroviral therapy (ART). It is estimated that in India, of the 2.6 million people living with HIV/AIDS, around 5% are children <5 years of age. Non-nucleoside reverse transcriptase inhibitors (NNRTIs), notably nevirapine, are highly effective and commonly used to treat pediatric HIV-1 infections, particularly in resource-constrained settings. Nevirapine is initiated at a lower dose and increased in a stepwise fashion because it induces hepatic cytochrome P450 including 3A (CYP3A) and 2B6; auto-induction of metabolism occurs in 2 to 4 weeks, with a 1.5- to 2-fold increase in clearance[1]. Thus nevirapine is initiated with a single daily dose during the first 2 weeks to allow for auto-induction of the liver enzymes. At 14 days of therapy, nevirapine dose is escalated to the age-appropriate full dose administered twice daily. However, younger children (aged ≤8 years) have higher apparent oral clearance than older children and require a higher dosage to achieve equivalent drug exposure compared with children aged >8 years[2,3].

Studies, largely in adult cohorts, indicated the potential for greater drug toxicity when this dose escalation strategy was not used[4] although a consistent relationship between toxicity and nevirapine pharmacokinetic parameters has not been observed[5]. A randomized controlled trial in Zambia comparing full-dose nevirapine versus a dose escalation strategy among children, did not reveal higher degree of toxicity among those children who received full dose nevirapine at initiation[6]. Thus virological failure due to underexposure and potentially lower-than optimal dosing is of greater concern than toxicity due to overdosing in young children[7]. Maintaining optimal plasma levels of nevirapine is critical, because a single point mutation at specific positions on the *pol* gene on the HIV genome may confer NNRTI resistance. The single-dose nevirapine strategy used for prevention of mother-to-child transmission has been associated with drug resistance development in both the mother and the offspring, likely due to lingering suboptimal levels of nevirapine[8,9]. Additionally, widespread use of first-line NNRTI-based therapy (initiating nevirapine with dose escalation), has been shown to negatively impact the effectiveness of therapy and further reduce treatment options[10,11]. Direct sequencing is currently used for routine HIV-1 genotyping, but it only detects mutated viruses if they account for more than 10 to 20% of the virus population[12,13]. The recent development of next-generation sequencing allows the opportunity to look into the minor viral quasispecies[14]. There is growing evidence that the presence of minor mutated viruses at baseline has a deleterious impact on the subsequent response to ART[15].

We hypothesize that suboptimal NNRTI dosing exposure during the dose escalation period may inadvertently promote development of minor viral quasispecies with NNRTI-associated mutations that could potentially compromise treatment outcome among HIV-infected children.

Using next-generation sequencing to identify minor viral quasispecies, we aim to identify NNRTI-associated mutations following initiation of the nevirapine-based therapy among children. We also aim to assess plasma nevirapine levels among these children, and correlate with evolution of drug resistance to NNRTI.

If our findings indicate that nevirapine initiation at current dosing recommendations promotes development of minor populations of drug resistant virus, there will be compelling evidence to review and revise nevirapine-based ART dosing strategies globally. Thus our study will contribute towards optimizing pediatric HIV treatment in resource-constrained settings and can potentially lead to decreasing the burden of drug resistance among children and promoting better treatment outcomes.

References

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1. Aims and Objective(s) of the Study:

The overall aim of the current study is to find the role of the dose of nevirapine in evolution of drug resistance in minor viral quasispecies in children initiating therapy.

Specific Aims:

1. To identify NNRTI-associated mutations among minor viral quasispecies following initiation of the nevirapine-based therapy with the standard dose escalation strategy among children.
2. Assess plasma nevirapine levels among these children, and correlate with evolution of drug resistance to NNRTI.

8. Proposed Methodology (***Not more than 2-3 pages***)

(i) Subjects

- Inclusion Criteria
    1. Children of the age group 2-18 years.
    2. ART-naïve with no maternal antenatal exposure to ART,
    3. Currently ready to initiate therapy with nevirapine as part of the ART regimen.
- Exclusion Criteria
    1. HIV-2 infection

(ii) Type of subjects:

- Males and / or Females : Both
- Volunteers : Yes / **No**
- Patients : **Yes** / No
- Vulnerable subjects : **Yes** / No

If yes, does it include Pregnant women / Children / elderly / Fetuses / Prisoners / destitute / Service providers / Terminally ill / Others (Specify)… **Children*……***

(iii) Sample size and the basis for the same

Previous studies indicate that up to 40% of infected infants exposed to maternal nevirapine can develop NNRTI-resistance[8]. If we expect our proportion resistance to be 40%, assuming an alpha of 0.05 and a power of 80%, our required

sample size is 24. As this is a longitudinal study, we may expect 10% loss-to-followup, thus our target sample size will be 28 children.

(iv) Trial Subject's age group : **0 – 5 years / 6- 9 years / 10-19 years** /
20+ (Specify…….) / >65 years

(v) Following selection of subjects as listed above, describe the methodology to be followed in the proposed study in detail – please be SPECIFIC and BRIEF, describe preferably in steps.

HIV-infected children attending the infectious diseases clinic at St John's hospital, and eligible for initiation of ART will be invited to participate in the study after obtaining informed consent from the caregivers. Assent will be obtained from children older than 8 years of age.

Patients will be asked to visit the clinic in the following scheduled visits: Baseline (when initiating the therapy), Week 01, Week 02, Week 04, Week 08, and Week 12. At initiation of ART, close monitoring is usually required, especially if the regimen consists of zidovudine, which can occasionally cause drug-induced severe anemia. Hence these visits and hemoglobin monitoring are part of routine care for these children.

The following extra laboratory tests will be done during these visits. These tests would require extra sample of about 4 ml blood.

	Baseline	WK01	WK02	WK04	WK08	WK12
Viral load and genotyping	X	X	X	X	X	X
Serum trough nevirapine level	X	X	X	X	X	X

Viral load and genotyping: Viral RNA will be extracted from stored plasma using QIAamp viral RNA extraction kit (Qiagen, Germany). Partial reverse transcriptase (RT) regions of the polymerase (*pol*) gene will be amplified using RT-PCR with random primer followed by conventional nested PCR using the primers. Next-gen sequencing techniques will then be applied. Frequency of different mutations at positions L100, K101, K103, V106, V108, Y181, Y188 and G190 will be enumerated.
Nevirapine plasma levels: Plasma concentration of nevirapine will be determined using a validated HPLC method with UV detection.

(vi) If the research subjects are to undergo any procedures during their participation in the study (like collection of blood/urine/feces, venepuncture, Xrays, intubation, special diet, drugs administered with dose, others) please specify the procedures.

Yes, patients will undergo venepuncture. Blood is drawn as part of routine care for those newly initiated on ART, and there will be additional sample of 4 ml drawn at the same time.

(vii) State any potential or known hazards of the procedure listed in the above clause. How does the investigator intend to overcome this aspect?

Potential risks will include pain or discomfort. To minimize this, only trained personnel skilled in the technique of venepuncture will be used to minimize pain. The procedure is done under sterile conditions to reduce risk of infection.

(viii) Does the study include any procedure involving radioisotopes or irradiation? If so, give details.
No.

(ix) If placebos are used, justify the use of the same. What precaution will be taken to ensure the safety of the patient given placebo? Is any standard treatment withheld?
No.

(x) State the statistical analysis proposed to be used?

This is a descriptive study and frequencies of different drug resistance mutations that develop at the different time points tested will be described. Statistical analysis of the data will include descriptive analysis and differences between groups (based on age, adherence levels, CD4 counts, viral load) will be analyzed by Student's t test or chi-square tests. Univariate and multivariate regression analyses will be used to evaluate the relationship between the variables and presence of new drug resistance mutations. All p values will be two-sided, and a value of <0.05 will be considered as significant.

(xi) Duration of the study - **2 years**

(xii) Which consultant / investigator(s) are responsible for conducting the study?

Dr. Anita Shet, M.D., Pediatrics
Dr. Kayur Mehta, M.D., Pediatrics

9. Study Design : (tick the single most appropriate one)

- Case Control study
- Community based trial / intervention
- Secondary Data analysis
- Clinical Trial (Hospital / Clinic)
- Family follow-up study
- **Descriptive study [√]**
- Cross Sectional Study
- Cohort Study
- Record Review
- Vaccine Trial
- Surveillance / Monitoring

- Validational Study / Diagnostic tests
- Others (Specify)

10. Determination of Risk : (Tick all that applies):

Does the research involve:

- Human exposure to radioactive agents? - No
- Human exposure to infectious agents? - No
- Investigational new drug? - No
- Investigational new device? - No
- New treatment regime? - No
- Use of new vaccines? - No
- Observation of public behavior? - No
- Fetal tissue or abortus? - No
- Pathological or diagnostic clinical specimen only? (Mention source: **Blood)**

  - Yes
- Existing data available via public archives source? - No
- Existing data available from co-investigator? - No

11. What is the Investigator's personal experience with the proposed study, patient management and the associated techniques involved? (***Give details of the preliminary work by the investigator, wherever appropriate)***

The investigators have performed several similar studies previously. The team has experience in both laboratory and field studies all of which involve collection of blood samples. The investigators have worked with drug resistance before and have observed a high level of NNRTI-related mutations among patients attending HIV clinics in Bangalore. All these patients had initiated nevirapine using the dose escalation strategy. Similar experience has also been noted in children; a cross-sectional study on children aged 1-16 years at St. John's Hospital also confirms the presence of a high proportion of K103N mutation in children who were in virological failure [*Shet et al; Ped Inf Dis J, in press*].

12. Will any sample collected from the patients be sent :

- To extramural institutions within India - **No**
- To any other place outside India - **No**

(if so, please specify)

13. In case the samples are preserved for future tests, how long will they be preserved and how is the use of such samples controlled?

Samples will be preserved for another 2 years apart from study time. The access and use of the samples will be restricted to the principal investigator.

14. Informed Consent

a. How will the informed consent be obtained and by whom? (Please specify PI / Co-PI/ Nurse / Counselor / Research staff / Any other……) **Nurse/PI/Co-PI**

b. Is the informed consent written / oral with a witness / audio-visual? **Written**

15. Is the form of consent with the explanatory note (Patient Information Sheet – PIS) appended? **Yes**
(The PIS should be in understandable, simple language and should include the research aspect of the study; Sponsor of study; Purpose and procedures; risks and discomforts; Benefits; Compensation for participation or for study related injury; Alternatives to participation and right to withdraw; Confidentiality of records; Contact information; Statement that consent is voluntary)

16. Please state whether subjects will have to bear expenses related to any of the following and state how the expenses would be met:

(i)	Cost of Medicine	Nil
(ii)	Cost of Investigations	Will be met from existing projects.
(iii)	Cost of travel	Nil
(iv)	Any other costs (Specify)	**NIL**

17. Will the subjects receive financial benefit / other material benefit as a result of participation in this study? Please specify. **NIL**

18. Is there a compensation for injury (due to the study) given by Sponsor / Investigator / Insurance / any other means? If yes, please specify. **Not applicable**

19. Conflict of interest:

(i) Is there any conflict of interest involving the study team? (financial / non-financial) **No**

Please mark as applicable and **explain** if the response if YES:

- I or my immediate family members own or control shares of the concerned company — **Yes / <u>No</u>**
- I or my immediate family members have a proprietary Interest in the product that I have contracted to test (Financial interest also includes interest in a company that would benefit from the approval of the test product) — **Yes / <u>No</u>**

(ii) I will notify the sponsor and the IERB if there are any changes in the information disclosed herein that occur

during the clinical trial and for one year following completion of the trial **Yes / No**

(iii) Is the Clinical Trial Agreement enclosed? **Yes / No/NA**

20. a) How many ongoing trials are you currently involved in? **One**

b) How much of time (on a daily / weekly basis) can you allot for the present study? **4-5 hours /week**

21. In case of chart review / retrospective studies, please mention as to how the identify of the patient is de linked and how confidentiality is maintained? **NA**

22. Confidentiality: ***Please mark on (Yes / No)***

a) Is the information recorded in such a manner that subjects can be identified from the information provided directly or through identifiers liked to the subject? **Yes**
However this information is available only to the principal investigator and immediate research team.

b) Does the research deal with sensitive aspects of the subject's behavior such as sexual behavior, alcohol use or illegal conduct such as drug use? **Yes / No**

c) Could the information recorded about the individual, if got to be known outside the research:

(i) Place the subject at risk or criminal or civil liability? **Yes / No**

(ii) Damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc? **Yes / No**

d) Is confidentiality maintained in handling of data by staff? **Yes / No**

23. Risk assessment: do you consider this research (***Check one)***

(iii) Greater than minimal
(iv) Not more than minimal risk **[√]**
(v) No risk
(vi) Only part of the diagnostic test

(**Minimal risk is a** "***a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or test. For eg., the risk of drawing a small amount of blood from a healthy individual for research purposes is not greater than the risk of doing so as a part of routine physical examination"***

24. Is the proposal funded? **Yes / <u>No</u>**

If yes, the sponsor's name :……..

25. Is the proposal being submitted for funding? **<u>Yes</u> / No**

If yes, name of funding agency: Collaborative Initiative for Paediatric HIV Education and Research (CIPHER) - Paediatric Research Grant Programme

Declaration by the Principal Investigator:

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious of fraudulent statement or claims may subject me to criminal, civil or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports as and when required.

Date : **Signature of PI:……**

Checklist:

Sl.No.	Documents	Yes	No
1	Covering letter	√	
2	Project proposal	√	
3	Patient Information Sheet	√	
4	Informed Consent Form / Assent Form in English	√	
5	Informed Consent Form/ Assent Form in regional languages with back translations *		NA
6	Clinical Trial protocol *		NA
7	Investigator's brochure for recruiting subjects *		NA
8	Copy of advertisements / Information brochures		NA
9	Questionnaire (if any)		NA
10	Clearance certificate from DCGI / DGFT *		NA
11	Insurance Certificate *		NA
12	Copy of the Clinical Trial Agreement *		NA
13	CV's of the PI / Co-PI / Co-investigators / Guide (if they are from outside St. John's)		NA
14	Soft copies of the above documents	√	
15	IERB processing Fees		NA

*** Documents for Clinical Trials only**