## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Assessing the safety and pharmacokinetics of the anti-HIV monoclonal antibody CAP256V2LS alone and in combination with VRC07-523LS and PGT121 in South African women: study protocol for the first-in-human CAPRISA 012B phase I clinical trial |
| **AUTHORS** | Mahomed, Sharana; Garrett, Nigel; Karim, Quarraisha Abdool; Zuma, Nonhlanhla; capparelli, edmund; Baxter, Cheryl; Gengiah, Tanuja; Archary, Derseree; Samsunder, Natasha; Rose, Nicole; Moore, Penny; Williamson, Carolyn; Barouch, Dan; Fast, Patricia; Pozzetto, Bruno; Hankins, Catherine; Carlton, Kevin; Ledgerwood, Julie; Morris, Lynn; Mascola, John; Abdool Karim, Salim |

## VERSION 1 – REVIEW

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| **REVIEWER** | Jaworski Juan Pablo<br>Consejo Nacional de Investigaciones Cientificas y Tecnologicas (CONICET), Argentina |
| **REVIEW RETURNED** | 31-Aug-2020 |

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| **GENERAL COMMENTS** | In the absence of an effective HIV vaccine, new HIV prevention strategies are urgently required. The discovery of broadly neutralizing antibodies (bNAbs) has provided the opportunity to evaluate passive immunization as a potential prevention strategy. This protocol submitted by Mahomed at al (CAPRISA 012B) describe the procedure of a first-in-human phase I clinical trial to test safety, tolerability and PK of anti-HIV CAP256V2LS mAb, used either alone or in double and triple combination with VRC07-523LS and PGT121. CAP256V2LS is a highly potent neutralizing mAb with good coverage against clade C viruses, the dominant HIV clade in sub-Saharan Africa. A couple of bioengineered upgrades introduced to CAP256V2LS mAb extended Ab half-life and improved manufacturability. Finally, preclinical preliminary results support the clinical testing of CAP256V2LS mAb in this particular population.<br><br>NOTE: following Editorial staff advice (email consultation), only the protocol starting at page 5 and ending at page 30 was reviewed.<br><br>Page 5 (Line 44-45). In the Abstract should be mentioned that participants in G3 and G4 are not infected by adding "…to HIV-negative women"<br>Page 5 (L57-58); and page 16 (46-48). Ethics and dissemination. If Ethics Committee and Regulatory Authority approval reference numbers are available, should be provided?<br>Page 8 (L7-8). Highlight and add references indicating that non-pathological implications were associated with administration of anti-Env mAbs with this particular features (e.g. anti-cardiolipin activity) in NHPs. |

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| | Page 8 (L16-26) Regarding the CAPRISA 012 clinical trial program: (i) is there any preliminary information from CAPRISA 012A available to support the doses of PGT121 and VRC07-523LS used in the current trial? Why the increment of higher doses from 10 to 20 mg/kg (VRC07) and 3 to 5 mg/kg (PGT121) comparing CAPRISA 012A and CAPRISA 012B; (ii) in which stage of development/testing is PGT121-LS. If the timing is appropriate, would not be relevant to use PGT121-LS in this phase 1 CT (CAPRISA012B), to have some preliminary data before moving to phase 2 (efficacy) CAPRISA012C?<br>Page 9 (L47-48) mention that participants in G3 and G4 also are not infected by adding "…to HIV-negative women"<br>Page 10 (L14-15) add SC<br>Page 10 (L40-41) discriminate between severe and serious adverse event (SAE)<br>Page 10 (L46-47) "…elimination half-life, clearance, volume of distribution, and area under the concentration decay curve…" add of CAP256V2LS mAb<br>Page 10 (L50) "Change in plasma HIV-1 RNA levels from baseline" add only for groups 1c and 1d.<br><br>TABLE1. When mention that >95 kg as exclusion criteria, explain that it is related to Ab infusion limitations. In addition, I would recommend moving "history of anaphylaxis…" and "evidence of autoimmune disease…" exclusion criteria to 2nd and 3rd places, respectively; so #1,2 and 3 items are all clinical exclusion criteria |

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| **REVIEWER** | Randolph Matthews<br>Merck Research Laboratories, USA |
| **REVIEW RETURNED** | 07-Sep-2020 |

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| **GENERAL COMMENTS** | This is an ambitious protocol that will advance the development of bNAbs as potential therapeutic agents against HIV-1. In addition to safety and tolerability in healthy female study participants, this trial will also assess antiviral efficacy, in preparation for the subsequent Phase 2 trial. The investigators put forth a thorough protocol that will address the stated objectives, which are suitable for this trial. I have only a few comments:<br><br>1. I have some concerns about the use of recombinant hyaluronidase. While the authors note that it has been used in oncology trials, this seems to be the first use in a non-oncologic population. As such, some discussion of potential risks, such as systemic absorption and immunogenicity, should be addressed (even if unlikely). Also, how long is it active in the tissue? Is there a potential of enhancing local tolerability AEs? Does this have toxicities preclinically?<br><br>2. It is not entirely clear whether sequencing will be of the entire genome or only of the antibody susceptibility regions. It appears to be the former, but it is not entirely clear and should probably be addressed in the protocol. Efficacy against common ART mutant strains would be expected, but useful to see here.<br><br>3. It is not clear that sampling via vaginal swab and/or Softcup will address bNAb concentration and/or activity in the female genital tract. It seems that levels are likely to be quite low in vaginal secretions, and more importantly that a lack of bNAb in secretions may not correlate with a lack of protection. I would recommend not including this objective. |

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| | 4. I agree that reactogenicity events are of the utmost importance, but it was difficult to understand how these are being defined. Would pain/tenderness be considered reactogenic? It would probably be good to have strict definitions of what would be included. In addition, there was no mention of photographs to document the reactions, which would be helpful when compiling and comparing AEs. As a very minor point, it is noted on p. 15 that the DSMB will meet "bi-annually", but this should be "semi-annually". |

**VERSION 1 – AUTHOR RESPONSE**

REVIEWER 1 COMMENTS

1. Page 5 (Line 44-45). In the Abstract should be mentioned that participants in G3 and G4 are not infected by adding "…to HIV-negative women"

Response: This has been added as per comment.

2. Page 5 (L57-58); and page 16 (46-48). Ethics and dissemination. If Ethics Committee and Regulatory Authority approval reference numbers are available, should be provided?

Response: Regulatory approval reference numbers (BREC00000857/2019 and SAHPRA:20200123) have been provided in the relevant sections as per comment.

3. Page 8 (L7-8). Highlight and add references indicating that non-pathological implications were associated with administration of anti- Env mAbs with these particular features (e.g. anti-cardiolipin activity) in NHPs.

Response: We thank the reviewer for this comment. The following text and references have been added to the toxicology findings in the protocol paper:
"In non-human primate (NHP) studies, non-pathological implications such as anti-cardiolipin activity were associated with the administration of anti-Env monoclonal antibodies (mAbs). Although these mAbs have polyspecific reactivities to host antigens (27) the immune response of NHPs to therapeutic mAbs is not considered to be predictive of the human response. This is due to the differences at species level. Thus, the ability to compare relative immunogenicity of mAbs in NHPs and humans is low (28)."

4. Page 8 (L16-26) Regarding the CAPRISA 012 clinical trial program: (i) is there any preliminary information from CAPRISA 012A available to support the doses of PGT121 and VRC07-523LS used in the current trial? Why the increment of higher doses from 10 to 20 mg/kg (VRC07) and 3 to 5 mg/kg (PGT121) comparing CAPRISA 012A and CAPRISA 012B; (ii) in which stage of development/testing is PGT121-LS. If the timing is appropriate, would not be relevant to use PGT121-LS in this phase 1 CT (CAPRISA012B), to have some preliminary data before moving to phase 2 (efficacy) CAPRISA012C?
Response: (i) In the last quarter of 2019, an amendment to the CAPRISA 012A protocol to include an additional 10 participants to the original 35 participants was made. These additional 10 participants received an increased dose of study product as no safety concerns were noted at the lower doses. 5 HIV negative participants received PGT121 at 10mg/kg SC one dose and 5 HIV negative participants received VRC07-523LS at 20mg/kg SC one dose. There were no safety concerns at this dose. PK analysis is currently underway. Thus, a higher dose is being evaluated in CAPRISA 012B. Of note,

PGT121 was kept at 5mg/kg in the CAPRISA 012B trial due to the lower concentration of the antibody (50mg/ml), compared to VRC07-523LS and CAP256V2LS at 100mg/ml.
(ii) We agree with the reviewer's comment on the use of PGT121LS. However, this antibody is still in the manufacturing and development phase and will not be ready for use in this trial. If this antibody does become available, we will make an amendment to the protocol and replace the non-LS version following regulatory approvals.
5. Page 9 (L47-48) mention that participants in G3 and G4 also are not infected by adding "…to HIV-negative women"
Response: This has been added as suggested.

6. Page 10 (L14-15) add SC
Response: This has been added as suggested.

7. Page 10 (L40-41) discriminate between severe and serious adverse event (SAE)

Response: In this trial, the severity of AEs (mild, moderate, severe) will be assessed as using the DAIDS Table for Grading the Severity of Adult and Paediatric Adverse Events, Version 2.1, July 2017. An adverse event is reported as a 'Serious Adverse Event' if it meets any of the following criteria: results in death, is life threatening, results in persistent or significant disability/incapacity, requires in-participant hospitalization or prolongs existing hospitalization, is a congenital anomaly/ birth defect, any other important medical condition that requires medical or surgical intervention to prevent permanent impairment of a body function or structure.

To clarify this, the wording in the protocol paper has been corrected as follows:
"Proportion of participants with mild, moderate, and severe adverse events as well as serious adverse events (SAEs) related to the IV or SC administration of CAP256V2LS."

8. Page 10 (L46-47) "…elimination half-life, clearance, volume of distribution, and area under the concentration decay curve…" add of CAP256V2LS mAb

Response: This has been added as suggested.

9. Page 10 (L50) "Change in plasma HIV-1 RNA levels from baseline" add only for groups 1c and 1d.

Response: This has been added as suggested.

10. TABLE1. When mention that >95 kg as exclusion criteria, explain that it is related to Ab infusion limitations.
Response: As suggested, we have added 'due to limitations related to SC antibody administration.' to Table 1.
11. In addition, I would recommend moving "history of anaphylaxis…" and "evidence of autoimmune disease…" exclusion criteria to 2nd and 3rd places, respectively; so #1,2 and 3 items are all clinical exclusion criteria
Response: We have reordered the exclusion criteria in Table 1 as suggested.

REVIEWER 2 COMMENTS
1. I have some concerns about the use of recombinant hyaluronidase. While the authors note that it has been used in oncology trials, this seems to be the first use in a non-oncologic population. As such, some discussion of potential risks, such as systemic absorption and immunogenicity, should be addressed (even if unlikely). Also, how long is it active in the tissue? Is there a potential of enhancing local tolerability AEs? Does this have toxicities preclinically?

Response: We thank the Reviewer for this comment. As of 15 November 2018, HYLENEX® recombinant and other rHuPH20 drug products were administered to 1592 participants enrolled in clinical studies. The individual doses of rHuPH20 ranged from 15 to 96,000 U.

The following paragraph and references have been added to address this:
"SC administration of rHuPH20 was well tolerated in healthy participants, participants with diabetes, rheumatoid arthritis, cancer and dehydration. SC administrations of rHuPH20 alone or in combination with morphine, ceftriaxone, ondansetron, insulin, adalimumab, IgG and hydration fluids was also well-tolerated (34, 35). Most adverse events reported were mild, transient injection site reactions, including erythema, pruritus, tenderness, induration, and paraesthesia. Moderate injection site reactions, occurring less frequently, include burning, erythema, pain, and numbness. Mild-to-moderate headache was also reported (33). Local tissue changes induced by rHuPH20 are reversible within 24-48 hours after administration, without inflammatory or histological changes. Co-administration demonstrated beneficial effects such as improved absorption, increased bioavailability and decreased PK variability (33, 36). rHuPH20 is currently co-formulated with two approved anticancer therapies, trastuzumab and rituximab."

2. It is not entirely clear whether sequencing will be of the entire genome or only of the antibody susceptibility regions. It appears to be the former, but it is not entirely clear and should probably be addressed in the protocol. Efficacy against common ART mutant strains would be expected, but useful to see here.
Response: We thank the reviewer for this comment. We propose a two-pronged approach. Due to the turnaround time of whole genome sequencing, we will use MiSeq sequencing of the V1V2 regions to screen out participants with known resistance mutations. We will also perform full envelope PacBio sequencing of enrolled participants and synthesize representative HIV envelopes for phenotypic validation of their sensitivity to CAP256V2LS, though this will be performed in batches after infusion has occurred. Similarly, we will characterise the genotype and phenotypic characteristics of escape variants. As per the Reviewer's suggestion, we will address the methodology in an updated version of the protocol.

3. It is not clear that sampling via vaginal swab and/or Softcup will address bNAb concentration and/or activity in the female genital tract. It seems that levels are likely to be quite low in vaginal secretions, and more importantly that a lack of bNAb in secretions may not correlate with a lack of protection. I would recommend not including this objective.
Response: We thank the reviewer for this comment and guidance. This objective was added in as part of expanding on existing research that has been conducted by CAPRISA scientists (1). This group previously validated an assay using SoftCup collection of genital tract specimens. They measured the BnAbs in the genital tract from infected women and found superior profiles compared to cervicovaginal lavage samples. In another study, this group showed that both the total immunoglobulins and the HIV-specific antibodies in the SoftCup were up to 100-fold higher in magnitude compared to cervicovaginal lavage (2). By including this objective, we aim to understand the transudation properties of the antibodies given either subcutaneously or systemically. This will allow us to understand the antibody levels and the level of protection conferred based on transudation.

References
1. Archary D, Liebenberg LJ, Werner L, Tulsi S, Majola N, Naicker N, Dlamini S, Hope TJ, Samsunder N, Abdool Karim SS, Morris L. Randomized cross-sectional study to compare HIV-1 specific antibody and cytokine concentrations in female genital secretions obtained by menstrual cup and cervicovaginal lavage. PloS one. 2015 Jul 6;10(7):e0131906.

2. Mkhize NN, Durgiah R, Ashley V, Archary D, Garrett NJ, Karim QA, Karim SS, Moore PL, Yates N, Passmore JA, Tomaras GD. Broadly neutralizing antibody specificities detected in the genital tract of HIV-1 infected women. AIDS (London, England). 2016 Apr 24;30(7):1005.

4. I agree that reactogenicity events are of the utmost importance, but it was difficult to understand how these are being defined. Would pain/tenderness be considered reactogenic? It would probably be good to have strict definitions of what would be included. In addition, there was no mention of photographs to document the reactions, which would be helpful when compiling and comparing AEs.
Response: We thank the reviewer for this comment and the suggestion of photography to document reactions. The following has been added to the reactogenicity assessment section in the protocol paper for clarification:

“Reactogenicity events are 12 common infusion/injection-related signs and symptoms. These are a subset of adverse events and have specific reporting requirements. Reactogenicity signs and symptoms are solicited from the start of the infusion/injection through the 3- day post infusion/injection reactogenicity period.Reactogenicity events may be infusion/injection site reactions (infusion/injection related erythema/redness or induration/swelling), local symptoms (pain, tenderness) or systemic signs or symptoms (increased body temperature, malaise and/or fatigue, myalgia, headache, chills, arthralgia, nausea, and vomiting).”

5. As a very minor point, it is noted on p. 15 that the DSMB will meet "bi-annually", but this should be "semi-annually".
Response: This has been edited in the protocol paper as suggested.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Juan Pablo Jaworski<br>Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Buenos Aires, Argentina |
| **REVIEW RETURNED** | 28-Sep-2020 |

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| **GENERAL COMMENTS** | In this revised version of the manuscript, the authors have addressed all comments |

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| **REVIEWER** | Randolph Matthews<br>Merck Research Labs, Merck & Co., USA |
| **REVIEW RETURNED** | 09-Oct-2020 |

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| **GENERAL COMMENTS** | Thanks to the authors for addressing all comments. Looking forward to seeing the data. Good luck with your trial! |