OFFICE OF ACQUISITIONS
NATIONAL CANCER INSTITUTE

REQUEST FOR PROPOSAL NUMBER: N02RC91002-56

Amendment No.: 1

Date of Issuance: 10/21/2008

The above numbered Request For Proposal (RFP) is amended as set forth below. The hour and date specified for receipt of Offerors remains unchanged: 10/30/2008.

Offerors MUST acknowledge receipt of the amendment prior to the hour and the date specified in the solicitation or as amended, by separate letter, telegram, or Electronic Mail which includes a reference to the RFP and Amendment number(s). For your convenience, the Proposal Intent Response Form is provided in SECTION J - List of Attachments of this RFP, for this purpose.

FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERORS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER.

This Amendment revises the RFP as stated below:

RFP Number : N02RCM91002-56
Amendment 1
OFFICE OF ACQUISITIONS
NATIONAL CANCER INSTITUTE

REQUEST FOR PROPOSAL NUMBER: N02RC91002-56

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The above numbered Request For Proposal (RFP) is amended as set forth below. The hour and date specified for receipt of Offerors remains unchanged Offerors MUST acknowledge receipt of the amendment prior to the hour and the date specified in the solicitation or as amended, by separate letter, telegram, or Electronic Mail which includes a reference to the RFP and amendment number(s). For your convenience, the Proposal Intent Response Form is provided in SECTION J - List of Attachments of this RFP, for this purpose.

FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERORS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER.

This Amendment revises the RFP as stated below:

AMENDMENT ONE (1)

OFFICE OF ACQUISITIONS
National Cancer Institute

REQUEST FOR PROPOSAL NUMBER: N02-RC-91002-56
AMENDMENT No. 1
Date of RFP Issuance: 09/30/2008
Date of Amendment Issuance: 10/21/2008

The above numbered Request for Proposal (RFP) is amended as set forth below. The hour and date specified for receipt of Offerors remains unchanged, 3:30 PM eastern prevailing time on October 30, 2008.

Offerors MUST acknowledge receipt of the amendment prior to the hour and the date specified in the solicitation or as amended, by separate letter, telegram, or Electronic Mail which includes a reference to the RFP and

Amendment number (s). For your convenience, the Proposal Intent Response Form is provided in SECTION J - List of Attachments of this RFP, for this purpose.

FAILURE OF YOUR ACKNOWLEDEMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERORS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER.

This Amendment revises the RFP as stated below:

**Question:**
1. Will the Government provide a single selected producer cell line clone, or will several clones to be tested?

**Answer:**
A single packaging clone will be provided.

**Question:**
2. Will the description of the producer cell line be provided, including growth characteristics (medium type, serum or serum-free, cell population doubling time, cell densities, etc.)?

**Answer:**
Yes, producer clone information, medium requirements and growth data can be provided.

**Question:**
3. What method is to be used for the retroviral titer estimation?

**Answer:**
As there is no selectable marker in our vector, titer is assessed by transduction of human PBL and transgene expression is measured accordingly. The contractor will provide back to NCI aliquots of supernatant generated at various times in the production process and titer will be assessed by NCI unless determined otherwise.

**Question:**
4. What is the timeframe for observation and harvesting of the titer peak?

**Answer:**
The packaging clone constitutively express retroviral vector. Maximum titer is reached once the cells become confluent.

**Question:**
5. How long can the producer cell line can be propagated in culture (passage number limitation)?

**Answer:**
Once the cells become confluent, vector is harvested every day followed by a medium exchange. We have harvested vector over a one week period without a detectable loss in transduction efficiency. In addition, minimal cell loss, as determined by cell sloughing, has been observed over the same one week period. The absolute passage number limits have not been determined; however, the cells can be passaged 10-20 times without noticeable loss in vector titer as measured by transduction efficiency.

**Question:**
6. What are the specifications for the initial retroviral supernatants from pilot vector production run (minimal acceptable titer)?

**Answer:**
Specifications would be demonstrated sterility and transduction efficiency within 20% of the original packaging clone during the time of selection. Selection data could be provided to establish target values.

**Question:**
7. What pore size is to be used for the 18L batch filtration (0.45 micron filter)?

**Answer:**

It is assumed the contractor has an established production process for both retroviral and lentiviral vectors that could support Phase I/II clinical trials. The process could be assessed for comparability and product quality in a pilot production run. However, our objective is to minimize any process development. Filtration is required as a clarification step and 0.45 micron filters could be sufficient, but optimization is likely required. Alternatively, step-filtration or a modified version of that process could be utilized. Product titer as measured by transduction efficiency would be compared to demonstrated transduction levels generated during clone selection at NCI.

**Question:**
8. Before freezing of the filtered harvest, are any additives or protectors needed in the final formulation for better stability?

**Answer:**
No, the vector supernatant is collected, clarified and filled into bags immediately after each harvest and then frozen at -80C.

**Question:**
9. Does the Contractor have to generate and characterize the MCB and WCB (master and working cell bank) of the 293T cells to be used for the Lentivirus production or will it be provided by the government fully certified and ready for use in GMP manufacturing?

**Answer:**
The contractor must have fully validated master and working cell banks sufficient for clinical lentiviral vector production.

**Question:**
10. Is the optimal ratio between the 4 plasmids to be used in the transient transfection already known or to be tested and established?

**Answer:**
It is assumed that the contractor has an optimized lentiviral vector production process requiring minimal to no process development. Additionally, it is assumed that the contractor has the necessary packaging plasmids and the NCI would only be providing the packageable genome.

**Question:**
11. Are there any preferences or limitations for the transfectant to be used in the transient transfection?

**Answer:**
The transfection reagent (transfectant) must be suitable for clinical production of Phase I/II clinical material and ideally is animal-origin free and made under or tested as a GMP reagent.

**Question:**
12. What are the specifications for the 20L clinical batch production (by p24 capsid protein ELISA or by titer)?

**Answer:**
Specifications would include viral-associated and total p24 as well as transduction efficiency within 20% of our pre-clinical evaluation. Data would be provided by NCI to establish target values.

**Question:**

13. What is the target in the downstream concentration and diafiltration of the 20L clinical bulk harvest: copy number of the transgene, p24, titer, other?

**Answer:**
Maximum concentration and diafiltration should not exceed 10X as assessed by viral-associated and total p24 as well as transduction efficiency.

**Question:**
14. Any final formulation studies needed before freezing the product?

**Answer:**
During diafiltration, the vector will be medium exchanged into a serum-free medium to be specified by NCI.

**Question:**
15. Can we use a subcontractor for the GLP Biosafety testing or is it required to be performed at the same location as where it is manufactured? It seems that we can use a subcontractor, but we want to make sure.

**Answer:**
A GLP biosafety testing subcontractor can be used.

**Question:**
16. Article B.3.a. (p. 4). The base and options go from 1 - 5 vectors, each. This is challenging to cost since 5 vectors cost more than one and lenti will cost differently than retrovirus. Can you be more specific as to what is required here?

**Answer:**
The SOW allows for 1-5 projects in the base contract and three additional options for 1-5 projects. The cost is per project and can either be a retroviral or lentiviral vector product depending on NCI requirements. The response should detail costs for separately for either a retroviral or lentiviral product and then an averaged cost.

**Question:**
17. Article F.2.a (p. 10). "Deliveries" - Does "completion" in the Delivery Schedule section of the table mean that manufacturing is completed or does it mean that the product has all the testing completed successfully and therefore can be released for use and delivery.

**Answer:**
Completion implies product has been manufactured and passed all biosafety testing, all necessary reports have been generated, and the actual clinical product and reports have been delivered to NCI.

This Amendment revises the Statement of Work as stated below:

Statement of Work for the Biosafety Testing of GMP Master Cell Banks and Retroviral Vectors for NCI clinical trials.

A. Acronyms

The following acronyms used in this contract are hereby defined as follows:

293 Human 293 cell line
ADA Adenosine deaminase
B19 Parvovirus B19
cGMP Current good manufacturing practices
GLP Good laboratory practices
CMV Cytomegalovirus
E1A Adenovirus E1A oncogene
EBV Epstein-Barr Virus
ECO RCR Ecotropic replication-competent retrovirus
ELISA Enzyme-Linked ImmunoSorbent Assay
GaLV RCR Gibbon-Ape Leukemia Virus Replication Competent Retrovirus
HBV Hepatitis B virus
HCV Hepatitis C virus
HIV 1 / 2 Human Immunodeficiency Virus Type 1/2
HHV 6 Human Herpesvirus 6
HHV 7 Human Herpesvirus 7
HHV 8 Human Herpesvirus 8
HTLV I / II Human T-cell Lymphotropic Virus Type I/II
LAL Limulus Amebocyte Lysate
LCMV Lymphocytic Choriomeningitis virus

PCR Polymerase chain reaction
PG-4 Feline PG-4 cell Line
RCL Replication-competent lentivirus
RCV Replication-competent Virus
S+L- assay Sarcoma positive/Murine leukemia virus negative cell line used in assaying for RCR.
SV40 Simian vacuolating virus 40
TEM Transmission Electron Microscopy
VSVg Vesicular stomatitis virus G protein

B. The Contractor shall produce and perform biosafety testing on a maximum of 5 retroviral or lentiviral gene therapy products. This contract contains three (3) options for additional quantities. If the Government exercises an option pursuant to the Option clause of this contract, the maximum quantity of retroviral or lentiviral gene therapy products produced and tested will be increased in increments of five (5) up to a total maximum of twenty (20). A retroviral product is comprised of two components: 1) production and certification of a master cell bank; and 2) production and certification of a retroviral vector supernatant. A lentiviral product is comprised of a single component: 1) production and certification of a lentiviral vector supernatant. For retroviral vector production, the Government will supply to the Contractor retroviral vector producer cell line clones and the nucleotide sequence of the retroviral vector; for lentiviral vector production GMP-quality plasmid DNA and the nucleotide sequence of the lentiviral vector will be provided. The Contractor shall develop Standard Operating Procedures (SOP's), Quality Assurance (QA) practices, qualify production components, prepare batch records, and the issue final reports containing the certificates of analysis for the stated product or assay. All manufacturing and biosafety testing shall comply with current Points to Consider and 21CFR regulatory guidelines as stipulated by the Food and Drug Administration (FDA). All manufacturing and testing shall be in compliance with current requirements for cGMP Phase I/II FDA Submissions.

C. Requirements for Production of a Retroviral Master Cell Bank:

1. Using a selected cell clone provided by the Government, the Contractor shall expand this clone for preparation of the MCB. Producer clone information, medium requirements and growth data will provided by the Government for the cell clone. The selected clone shall be expanded and 100 vials of between 0.5 - 5 X 107 cells/vial shall be cryopreserved. Certification shall include the tests listed below and shall not be initiated until the titer of the retroviral vector derived from the Master Cell Bank has been approved by the Surgery Branch/NCI. Pilot vector production runs may be undertaken by the Contractor in an attempt to optimize the production of retroviral vector supernatant if requested by the Surgery Branch. If a pilot production run is required, aliquots of supernatant from the pilot production shall be supplied to the Government for testing. Certification of the MCB shall not be initiated until the potency (titer) of the retroviral vector derived from the Master Cell Bank has been approved by the Government. The retroviral titer from the MCB must be within 20% of that obtained when the PG13 packaging clone was initially selected. If the titer is less than 80% of the original, the Government may elect to not accept the MCB. With the exception of the sterility requirement, the Government will reimburse the Contractor for the costs of all production and testing initiated at the time of discontinuation of the study if the Master Cell Bank fails one or more of the certification tests. If the Master Cell Bank does not meet the sterility requirement, the Contractor shall prepare a new Master Cell Bank at no additional cost to the Government.

2. The Contractor shall conduct certification assays in accordance with FDA guidelines. MCB certification tests shall include:

a. General Sterility. This test shall include aerobic and anaerobic culture for bacterial and fungal contamination. The MCB must fully meet or exceed the requirements of 21 CFR 610.12.

b. Mycoplasma. This test shall include broth, agar, and cell culture. The MCB must meet the recommended procedure for mycoplasma testing according to the Draft Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, Attachment #2 (1993).

c. In-Vitro Adventitious Virus Assay. This test shall include amplification on three cell lines and hemadsoprtion with three species of red blood cells.

d. Bovine Virus Assay. This test shall include amplification with permissive cell line and immuno-fluorescence antibody detection.

e. Porcine Virus Assay. This test shall include amplification with permissive cell line and immuno-fluorescence antibody detection.

f. In-Vivo Virus Assay This test shall include assay in guinea pigs, adult mice, suckling mice, and embryonated chicken eggs.

g. Mouse Antibody Production with LCMV Challenge. Inoculated animals shall be evaluated for development of antibodies against 16 common adventitious murine viruses (Ectromelia virus, Hantaan virus, Mouse K virus, Lactate dehydrogenase elevating virus, Lymphocytic choriomeningitis virus, Mouse adenovirus, Mouse cytomegalovirus, Mouse encephalomyelitis virus type II, Mouse hepatitis virus, Epizootic diarrhea virus of infant mice, Pneumonia virus of mice, Polyoma virus, Reovirus type 3, Sendai virus, Mouse thymic virus).

h. Transmission Electron Microscopy. Fixed specimens shall be evaluated by TEM.

i. GALV RCR. 1% of the cells and 5% of the supernatant from the MCB shall be tested for GALV RCR by extended S +L- assay using the 293 and PG-4 cell amplification and detection system.

j. ECO RCR, One (1) vial of the MCB shall be tested for ecotropic RCR by amplification in NIH3T3 cells followed by focus forming assay on D56 cells, marker rescue or a reverse transcriptase assay in conjunction with an XC plaque assay.

k. Iso-Enzyme Analysis. Cells shall be evaluated for species origin by analysis of the ADA isoenzyme.

l. Human Virus Panel. PCR analyses shall be performed for detection of the following viruses of human origin: EBV, CMV, HTLV I / II, HIV 1 / 2, HBV, HCV , HHV 6, HHV 7, HHV 8 and B19

m. PCR for E1A and SV40. PCR analyses shall be performed for detection of E1A and SV40 large T antigen

D. Production and Biosafety Testing of Retroviral Vector Supernatants

1. Following certification of the MCB, initial retroviral vector supernatant production shall be initiated. Conditions for production shall be based on the pilot production run conditions approved by the Government or predetermined production conditions agreed upon by the Government prior to production. An aliquot of the MCB shall be expanded sufficiently to collect a large amount of supernatant from the retroviral vector producer cells. The minimum volume for satisfaction of this production agreement is 10 liters; however, an attempt should be made to produce up to 18 liters of product. The product shall be harvested, filtered, frozen and maintained at < -70o C in aliquots 50-100 grams/ unit (volume to be defined by Government). If the titer of the production is within 20% of that obtained when the MCB was approved and the Government elects to not accept the production, the Contractor will be reimbursed for the preparation charges of the supernatant. With the exception of the sterility requirement, the Government will reimburse the Contractor for the costs of all production and testing initiated at the time of discontinuation of the study if the supernatant fails one or more of the certification tests. If supernatant does not meet the sterility requirement, the Contractor shall prepare a new supernatant at no additional cost to the Government.

2. Aliquots of supernatant from the production run shall be supplied to the Government for titer determination. Certification of the supernatant shall include the tests listed below and shall not be initiated until the titer of the production has been approved by the Government. The Contractor shall conduct certification assays in accordance with FDA guidelines. Supernatant certification tests shall include:

a. Sterility. This test shall include aerobic and anaerobic culture for bacterial and fungal contamination. The supernatant must fully meet or exceed the requirements of 21 CFR 610.12.

b. Mycoplasma. This test shall include broth, agar, and cell culture. The MCB must meet the recommended procedure for mycoplasma testing according to the Draft Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, Attachment #2 (1993).

c. In-Vitro Adventitious Virus Assay. This test shall include amplification on three cell lines and hemadsoprtion with three species of red blood cells.

d. GALV RCR. 108 post-production cells and 5% of the supernatant from the final harvest shall be tested for GALV RCR by extended S+L- assay using the 293 and PG-4 cell amplification and detection system.

e. Endotoxin. An aliquot of the final harvest shall be evaluated for endotoxin by the LAL gel clot method. The value shall be considered acceptable if the result is less than or equal to 0.3 EU/ml.

f. General Safety. This testing shall be performed in accordance with 21 CFR 610.11. The supernatant shall meet the safety requirements of 21 CFR 610.11.

E. Production of a Lentiviral Vector Supernatant

1. Conditions for lentiviral vector production shall be based on predetermined production conditions agreed upon by the Government. A basic production consists of transient transfection of human embryonic kidney (293T) cells with 4 lentiviral plasmids encoding the following lentiviral genetic elements: 1) gag/pol; 2) rev; 3) the genomic DNA encoding the desired transgene; and 4) VSVg envelope for pseudotyping. The vector produced shall be harvested and subjected to concentration and diafiltration prior to filling at 50-100 grams/unit (to be specified prior to production) and freezing at <-70oC. A typical lentiviral clinical grade production is 20 liters. An aliquot from the production run shall be sent to the Surgery Branch for titer determination. With the exception of the sterility requirement, the Government will reimburse the Contractor for the costs of all production and testing initiated at the time of discontinuation of the study if the supernatant fails one or more of the certification tests. If supernatant does not meet the sterility requirement, the Contractor shall prepare a new supernatant at no additional cost to the Government.

RFP NOTE: Currently, all lentiviral productions are transient.

2. Aliquots of supernatant from the production run shall be supplied to the Government for titer determination. The Contractor shall be responsible to conduct certification assays based on current FDA guidelines. Certification of the supernatant shall include the tests listed below and shall not be initiated until the titer of the production has been approved by the Government. The Contractor shall conduct certification assays in accordance with FDA guidelines. Supernatant certification tests shall include:

a. Sterility. This test shall include aerobic and anaerobic culture for bacterial and fungal contamination. The supernatant must fully meet or exceed the requirements of 21 CFR 610.12.

b. Mycoplasma. This test shall include broth, agar, and cell culture. The MCB must meet the recommended procedure for mycoplasma testing according to the Draft Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, Attachment #2 (1993).

c. In-Vitro Adventitious Virus Assay. Assays shall include amplification on three cell lines and hemadsoprtion with three species of red blood cells. RCL 108 post-production cells and 5% of the supernatant from the final harvest shall be tested for VSVg RCL testing by serial transfer and ELISA assay for p24 antigen or an alternative cGMP-compliant RCL assay. The vector stock shall be tested at a limit of sensitivity of 1 infectious unit per milliliter, and the test shall include a known positive control. Each inoculum must be free of RCV prior to use in animals.

e. Endotoxin. An aliquot of the final harvest shall be evaluated for endotoxin by the LAL gel clot method. The value shall be considered acceptable if the result is less than or equal to 0.3 EU/ml.

f. General Safety. This testing shall be performed in accordance with 21 CFR 610.11. The supernatant shall meet the safety requirements of 21 CFR 610.11.

g. PCR for E1A and SV40. PCR analyses shall be performed for detection of E1A and SV40 large T antigen.