PUBLIC NOTICE PRELIMINARY MARKET CONSULTATION IN PREPARATION FOR THE INDICATION OF A PROCEDURE FOR THE AWARDING OF THE SUPPLY OF GMP GRADE LENTIVIRAL VECTORS (LVV) FOR THE APPLICATION OF PHASE 1 AND 2 CLINICAL TRIALS AS PART OF THE PROJECT "CREATION OF A NATIONAL CENTER FOR RNA AND GENE THERAPY" - CUP E83C22003170001.

The Ospedale Pediatrico Bambino Gesù (hereinafter, for brevity "**contracting authority**") announces that, for the preparation of the contract and for the carrying out of the related procedure relating to the supply in question, it intends to carry out a specific preliminary market consultation pursuant to the articles 77 and 78 of the Legislative Decree. n. 36/2023.

The contracting authority therefore invites all interested parties to participate in the "consultation" by providing the contributions deemed necessary. In particular, the aspects governed by the documentation attached to this notice are the subject of the contribution.

It will be the responsibility of the subjects interested in this notice to highlight the contributions for which they reasonably believe there are aspects worthy of protection of secrecy from a technical and commercial point of view.

It is represented that:

- the needs and the tools to meet them can be found in the attached documentation;
- the indicative total cost for the acquisition of the supply and services can be estimated approximately at an amount equal to €2.500.000 excluding VAT;
- the contracting authority will evaluate any reasonable alternative solutions proposed in the context of the contributions provided;
- the contracting authority will proceed with the purchase through a procedure that will be identified pursuant to Legislative Decree. n. 36/2023, if at the end of the investigation it finds the existence of the relevant conditions and therefore does not consider any reasonable alternative solutions proposed to be viable.
- finally, the contracting authority remains available to provide further information that economic operators may request in compliance with the principles of transparency and equal conditions.

Participation in this "*consultation*" does not give rise to any expectations or rights towards the contracting authority and the contributions made do not give the right to any compensation or reimbursement. The assignment of the supply covered by this "*consultation*" is subject to any subsequent and separate procedure carried out pursuant to and for the purposes of the Legislative Decree. 36/2023.

This "*consultation*" does not, therefore, represent an invitation to propose an offer, nor does it bind the contracting authority in any way towards the interested parties.

The contracting authority may at its sole discretion interrupt, suspend or revoke this "*consultation*", as well as interrupt the consultation of one or more of the interested parties, at any time.

The contracting authority reserves the right to use what is collected as part of this "*consultation*" for the planning and carrying out of the procurement procedure, "*provided that it does not have the effect of distorting competition and does not lead to a violation of the principles of discrimination and transparency*" (art. 77, paragraph 2, Legislative Decree no. 36/2023).

The form of the contributions is free (it is preferable that the files consist of electronic documents rather than scans of paper documents) and may include the documentation referred to in the art. 77 paragraph 2 of the Legislative Decree. n.36/2023.

Contributions must be received by 17/5/2024 to the addresses roberto.dipinto@opbg.net and giacomo.disalvo@opbg.net. The email must contain the following wording in the subject: "PUBLIC NOTICE PRELIMINARY MARKET CONSULTATION PREPARATORY FOR THE INDICATION OF A PROCEDURE FOR THE AWARDING OF THE PRODUCTION OF GMP GRADE LENTIVIRAL VECTORS (LVV) FOR THE APPLICATION OF CLINICAL STUDIES PHASE 1 AND 2 - sending contribution". The contracting authority will not take into consideration contributions that are not submitted in the manner described and within the deadline indicated.

For any further information relating to the methods of participation in this consultation, it is possible to use the aforementioned electronic address (for information of an administrative nature and for information relating to the technical specifications and other elements of a technical/economic nature).

Rome,
2/5/2024

The President
Tiziano Onesti

TECHNICAL DOCUMENT PRELIMINARY CONSULTATION

1) REQUIREMENT

The supply in question is necessary for the Ospedale Pediatrico Bambino Gesù to carry out clinical trial activities for the hospital itself.

In particular, production activities of minimum high quality (HQ) or GMP grade plasmids are required, as well as production activities of GMP grade Lentiviral Vectors (LVV) for the application of clinical trials phase 1 and 2.

2) EXPECTED COSTS

The expected costs for the supply and services in question are estimated at an amount equal to 2.500.000 euros (excluding VAT).

Public Tender technical requirements for LVV manufacturing

Pediatric Hospital Bambino Gesù (OPBG) requests the activation of two services:
A) High Quality (HQ) or GMP grade production Lentiviral transfer plasmid containing the gene of interest (GOI);
B) GMP grade Lentiviral Vector (LVV) manufacturing for clinical trial phase I/II application.
For both services A and B, the final products and the ancillary requests have to be provided within 13 months starting from the signature of the agreement.

A. GOI Transfer plasmid HQ/GMP manufacturing:

High Quality production is the minimum grade required for plasmid manufacturing. The requirements for HQ grade manufacturing are listed below:

- Documentation control, deviation, CAPA and change management;
- Quality oversight of processes;
- Materials traceability and sourced from approved suppliers;
- Process equipment appropriately qualified and subject to a maintenance program;
- All Quality Control testing performed using qualified analytical methods;
- Quality agreements for outsourced activities.

Starting from GOI third generation lentiviral transfer plasmid research grade (RG), supplied by OPBG, are required the following services:

a. Research Grade plasmid production for process confirmation/verification. Plasmid derived from this run will be used by the supplier and the spared material to be provided to OPBG.
b. Manufacturing of GMP E. coli Master cell bank (MCB) up to 200 vials. Characterization, release and stability plan of the MCB, following the analytical/release testing listed in table n°1.
c. HQ/GMP grade production of Transfer plasmid to be used in Step B, and to provide to OPBG for the spared amount (at least 500 mg). Characterization/release, and stability plan for the plasmid, following the analytical/release testing listed in table n°2.

B. GMP grade LVV manufacturing:

It is required GMP grade production of third generation Lentiviral vector (LVV) containing the transfer vector with GOI provided by OPBG. According with the supplier production process, the starting DNA plasmid material used for LVV manufacturing will be the GOI transfer vector produced in step A, and the off-the-shelf (OTS) lentiviral third generation packaging plasmids, HQ/GMP grade, provided by the supplier. The envelope packaging plasmid (env) has to encode for the G glycoprotein of the Vesicular Stomatitis Virus (VSV-G).

For GMP grade LVV manufacturing are required the following services:

a. LVV research grade production for process confirmation and feasibility study. The purified RG LVV derived from this run will be filled, and the vials will be provided to OPBG.

i. Go/No-go decision: OPBG will test LVV vector derived from this run to evaluate whether to proceed with the next phases of the project.

b. **Provision of HQ OTS Lentiviral packaging plasmid required for the GMP LVV production.**
   i. **Additional amount of OTS VSV-G** packaging plasmid it is required for OPBG use. HQ/GMP grade plasmid amount to be provided to OPBG: 500 mg.

c. **GMP grade LVV production, characterization, release and stability plan following the analytical/release testing as detailed in table n°3.**
   i. **The minimum quantity required, expressed as total transducing unit (TU), of drug product (DP, purified LVV), is 5E10 tot. Total TU is intended as the amount of LVV to be filled for the clinical trial Phase I/II, which does not include the needed LVV quantity for** QC tests and stability plan.
   ii. **The final concentration of LVV drug substance (DS) will be determined after go/no go decision, but the minimum concentration that will be required is 1x10^8 TU/mL.**
   iii. According to the supplier's **Media Fill Qualification (MFQ), DP filling volume will be determined after go/no go decision. The most suitable filling container** for OPBG internal process is the cryobag.

Table 1. Analytical methods for the characterization, release (*Time point 0) and stability plan of MCB.

Assay		Time point (month)					
		0*	12	24	36	48	60
Bacterial Characterization	Bacterial cell count	+	+	+	+	+	+
	Colony morphology	+	+	+	+	+	+
Bacterial host strain identity	Gram staining	+					
	Confirmation of species (16S rDNA)	+	+	+	+	+	+
Bacterial host strain purity	Microscopic observation	+					
	Bacteriophages detection	+					
	Purity Test- Plating method	+					
Plasmid Characterization	Antibiotic sensitivity	+	+	+	+	+	+
	Plasmid retention rate	+	+	+	+	+	+
	Plasmid copy number	+	+	+	+	+	+
Plasmid Identity	Restriction Digest	+	+	+	+	+	+
	Full Sequencing	+		+			+

Table 2. Analytical methods for the characterization/release (*Time point 0) and stability plan of HQ/GMP grade plasmid.

Assay		Time point (month)									
		0*	3	6	9	12	18	24	36	48	60
Physio-chemical Property	Appearance	+	+	+	+	+	+	+	+	+	+
	pH	+	+	+	+	+	+	+	+	+	+
Concentration	Concentration by Agarose Gel Electrophoresis	+	+	+	+	+	+	+	+	+	+
Identification	Identity by Restriction Digest	+									
	Full Sequencing	+									
Purity	Purity (A_{260}/A_{280})	+	+	+	+	+	+	+	+	+	+
	Plasmid Percentage Supercoiled	+	+	+	+	+	+	+	+	+	+
Safety	Sterility	+				+		+	+	+	+
	Endotoxin Determination	+									
	Mycoplasma Testing	+									
Residual Impurities	Residual host-cell RNA	+									
	Residual host-cell DNA	+									
	Residual *E. coli* host-cell Protein	+									

Table 3. Analytical methods for the production, release (*Time point 0) and stability plan of GMP grade Lentiviral Vector.

Assay	Material Tested	Time Point (month)								
		0*	3	6	9	12	18	24	36	48
Bioburden	Bulk Drug Substance	+								
Mycoplasma	Bulk Harvest	+								
In vitro adventitious agents	Bulk Harvest	+								
Replication competent Lentivirus	Bulk Harvest (supernatant and cells) / DS	+								
Lentiviral infectious Titer	Bulk Harvest/ Drug substance/ Filled DS	+	+	+	+	+	+	+	+	+
Lentiviral Titer determination by p24 capsid	Drug substance/ Filled DS	+	+	+	+	+	+	+	+	+
Genome Sequencing	Drug substance	+								
Residual HCP	Drug substance	+								
Residual HCD	Drug substance	+								
Residual plasmid DNA	Drug substance	+								
Residual E1A DNA	Drug substance	+								
Residual Endonuclease	Drug substance	+								
Residual SV40 Large T DNA	Drug substance	+								
Endotoxin	Drug substance/ Filled DS	+				+				+
Appearance	Filled DS	+	+	+	+	+	+	+	+	+
Osmolality	Filled DS	+								
pH	Filled DS	+				+		+	+	+
Sterility	Filled DS	+		+		+		+	+	+
Bacteriostasis/Fungistasis	Filled DS	+								
Determination of extractable volume	Filled DS	+								