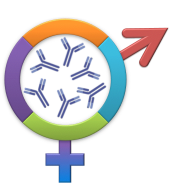
Title	  **SOP 09 - Blood sampling and gene expression analysis**
Document type	SOP
Version N.	2.0
Date	October 7th 2019

	NAME	**DATE**
PREPARED BY	Loris De Cecco	June 05th 2019
REVIEWED BY	Rosalba Miceli	July 22nd 2019
APPROVED BY		
PURPOSE : Describe blood sampling procedure, RNA isolation and gene expression analysis in G-DEFINER		

Abbreviations

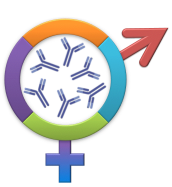
Acronym	**Description**
G-DEFINER	Gender Difference in sidE eFfects of ImmuNotherapy: a possible clue to optimize cancEr tReatment
INT	Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
PI	Principal investigator
SOP	Standard Operating Procedure

Related documents

Document type	**Title**

Appendices:

Document type	**Title**

Title	  **SOP 09 - Blood sampling and gene expression analysis**
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1. Scope and applicability

This SOP applies to the collection of blood specimens at baseline (before treatment start) for extraction of RNA and gene expression analysis in the G-DEFINER study. The main purpose for this sampling protocol is to ensure that blood samples from patients included in G-DEFINER are processed in a consistent manner at all study sites.

2. Glossary/definitions

None

3. Responsibilities

PIs at G-DEFINER study sites: responsible for implementing SOP at own study site. It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

4. Procedure

In G-DEFINER, blood specimens will be collected at baseline (before treatment start) during treatment at second infusion and at the first immune related adverse event grade ≥ 3.

Collection and processing will be done according to this SOP. All samples will initially be biobanked at the sampling hospital, and transferred to the Unit "Piattaforma di Biologia Integrata" at the Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Milan, Italy, upon agreement.

4.1 Equipment/reagent requirements list

- Blood collection system
- Personal protective equipment; gloves, laboratory coat, protective glasses
- Blood collection tube: PaxGene (Qiagen)
- Freezer -20°C/-80°C for long-term storage

4.2 Blood collection and storage

Instructions:

- The collection of blood should be obtained from an existing arterial or venous line, or venipuncture.
- Collect 3 ml blood.
- Arterial or venous whole blood is collected into a room temperature (18-25o C) PAXgene Blood RNA Tube
- Allow at least 10 seconds for a complete blood draw to take place, fill the blood collection tube until the black mark on the tube indicating the correct amount of blood to be drawn.
- After blood collection, gently invert the PAXgene Blood RNA tube 8-10 times.
- Incubate the PAXgene Blood RNA tube for at least 2 hours at room temperature.
- The PAXgene Blood RNA Tubes can be stored at −20˚C and below. If tubes are to be kept at temperatures below −20˚C, freeze them first at −20˚C for 24 hours, then transfer them to −70˚C or −80˚C.
- See section 4.6 for instruction of dates registration.

NB: If the PAXgene Blood RNA tube is the only tube to be collected, draw blood into a "discard tube" prior to using the PAXgene Blood RNA tube. Otherwise, the PAXgene Blood RNA tube should be the last tube drawn.

4.3 RNA Isolation (at INT)

- RNA will be isolated using the PAXgene Blood RNA kit (Qiagen) according to manufacturer's manual.
- RNA concentrations will be measured by Qubit flourimeter and quality check will be performed by TapeStation (Agilent)
- See section 4.6 for instruction on dates registration in the study database.

4.4 RNAseq analysis (at INT)

RNAseq analysis will be performed at Platform of Integrated Biology (INT).

- See section 4.6 for instruction on dates registration in the study database.

4.5 Blood Sample Shipment

Instructions:

- Samples has to be shipped in dry ice
- Each center can use his preferred courier; however the courier should ensure delivery in 2/3 days
- Before shipment a mail should be sent to dr. Loris De Cecco (loris.dececco@istitutotumori.mi.it) with the list of samples (in Excel).
- Avoid shipments on Thursday/Friday

4.5.1 Packaging instruction

Samples should be packaged in order to comply with IATA regulations (see http://www.iata.org for further information).

All samples, independent of their infectious characteristics, should always follow a triple packaging system:

- Properly labeled, outer rigid packaging with minimal dimensions.
- Watertight, secondary packaging with absorbent material (shocks and/or leaks)
- Watertight inner packaging [e.g. the blood collection tube(s), cryovial(s)]

NB: National authorities (country of origin/destination) and courier companies have the possibility to incorporate stricter exceptions on the IATA general rules.

Contact person	Dr. Loris De Cecco E-mail: loris.dececco@istitutotumori.mi.it
Full address	Platform of Integrated Biology Fondazione IRCCS Istituto Nazionale dei Tumori, AmadeoLab Via Amadeo 42 20133 Milan Italy

4.6 Registration of the dates in the database

- Register in the study database the following dates: date of blood collection, date of freezing, date of defrost, date site lab received blood sample, date of DNA isolation, date of analysis.

- The paper DCFs "CRF 07 - Study specific blood and stool samples" may be used to collect data for a later entry in the study database.

5. Dissemination

The SOP will be disseminated according to the G-DEFINER SOP of SOPs 1.1, and the PI at each site will ensure implementation of the SOP.

6. Document history

Version No.	Date	Reviewer	Details of changes
0.1	July 3rd 2019	Loris De Cecco	First version
1.0	July 22nd 2019	LDC / RM	Added ml blood to be collected (3 ml) and 3 times of collection (T0,1,2) instead of T0 only
2.0	October 7th 2019	RM	Added section 4.6: Registration of dates on the database