



MELCAYA

NOVEL HEALTH CARE STRATEGIES FOR MELANOMA IN CHILDREN,
ADOLESCENTS AND YOUNG ADULTS

Grant Agreement: 101096667

D3.1 Standardized protocol for tumour tissue processing



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Document Information

Deliverable number:	D3.1
Deliverable title:	Standardized protocol for tumour tissue processing
Deliverable version:	1.0
Work Package number:	WP3
Work Package title:	Histological, computational and molecular pathology for melanoma subtypes classification
Due Date of delivery:	31.05.2024
Actual date of delivery:	30.07.2024
Dissemination level:	PU – Public
Type:	R – Document, report
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Project name:	Novel health care strategies for melanoma in children, adolescents and young adults
Project Acronym:	MELCAYA
Project starting date:	01.12.2022
Project duration:	48 months
Rights:	MELCAYA consortium

Document history

Version	Date	Beneficiary	Description
0.1	12.06.2024	UNIFI	First draft
0.2	20.06.2024	NIO-PIB	Revised draft
0.3	10.07.2024	HCB	Revised draft
0.4	18.07.2024	FCRB	Revised draft
0.5	24.07.2024	UNIFI	Revised draft
1.0	30.07.2024	FCRB	Final version

Executive Summary

The goal of this deliverable is to define the parameters and steps that each collaborator must follow to ensure the production of standardized samples for morphological, immunohistochemical and molecular analyses planned in the MELCAYA project. This document will include a detailed description of how the standardized cutting protocol for the retrospective study cohort of formalin fixed and paraffin embedded (FFPE) samples was created. The purpose of this deliverable is to establish a standardized protocol for the cutting of FFPE inclusions of MELCAYA cases. Creating this protocol is crucial to ensure the quality and reliability of the data from the analyses conducted within the project, thereby guaranteeing the quality of the obtained results.

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Acronyms & Abbreviations

Term	Description
FFPE	Formalin fixed and paraffin embedded
CAYA	children, adolescents, and young adults
TME	Tumour Microenvironment
H&E	Haematoxylin and eosin
IHC	Immunohistochemistry

1 Introduction

A key goal of the MELCAYA project is to develop structured synoptic templates for melanoma pathology, aiming to standardize melanoma reporting for children, adolescents, and young adults (CAYA) patients. To introduce a novel hybrid taxonomy, this task includes conducting mutational analyses and transcriptomic profiling, independently validating melanoma methylation array profiling, developing a methylation subtype classifier for melanoma subclasses, characterizing tumour microenvironment (TME) by immunohistochemical analysis and correlating somatic, transcriptomic, and methylation profiles with demographic, clinical, and morpho-phenotypic data.

In order to recruit a retrospective cohort to perform the analyses described above, 171 CAYA cases were screened and retrospectively selected by the MELCAYA pathology board:

- Daniela Massi UNIFI (Florence, Italy)
- Lluçia Alos, HCB (Barcelona, Spain)
- Stephan Forchhammer, UT (Tübingen, Germany)
- Anna Szumera-Ciećkiewicz, NIO-PIB (Warsaw, Poland)
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- Sylvie Fraitag, Responsable de la Dermatopathologie Département de Pathologie Hôpital Necker-Enfants Malades (Paris, France)
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- Sokol Sina, Anatomia Patologica Azienda Ospedaliera Universitaria Integrata (Verona, Italy)
- Valerio Gaetano Vellone, U.O.C. Anatomia Patologica IRCCS Istituto Giannina Gaslini (Genova, Italy)

Revision of haematoxylin and eosin (H&E) stained samples collected from different clinical centres of the consortium was performed during the MELCAYA pathology virtual review sessions by the MELCAYA pathologist's board. Cases which had an H&E slide that was deemed of inadequate quality were excluded. A standardized template was developed to systematically compile all histopathological and clinical data for each sample. All collected data for the enrolled patients were consolidated into a unified file.

A total of 10 sessions were held during this first reporting period. During these sessions the classification criteria for the cases have been discussed and agreed by the MELCAYA pathology board. The pathology board evaluated the H&E digital slide of each case based on immunohistochemistry (IHC) data and the relevant clinical information available. The reviewed cases were then divided in two groups based on MELCAYA pathology board concordance diagnosis: (1) CAYA melanoma or (2) atypical melanocytic tumour requiring further molecular assessment for final diagnostic classification. Among the 171 screened cases, 114 cases with at least one FFPE inclusion have been selected for WP3 analysis.

2 Standardized tissue processing protocol

2.1 Centralization of FFPE blocks

To ensure maximum standardization in sample preparation the MELCAYA consortium agreed to centralize representative blocks for each case at UNIFI. To facilitate the transfer of FFPE material between institutions, Material and Data Transfer Agreement (MDTA) forms were redacted. These forms include: names and addresses of the transferring and receiving institutions, signatures of the laboratory managers from each institution and a brief description of the material and data being transferred by each institution, along with the procedures that will be performed on these materials or data. Once both parties have signed the MDTA, the materials can be transferred between institutions via postal service or private delivery companies.

To date, we centralized 66 FFPE inclusions from the following centers:

- University Hospital of Tübingen (n = 21)
- Maria Skłodowska-Curie National Research Institute of Oncology (n = 9)
- Hôpital Necker-Enfants Malades (n = 15)
- University of Florence, Florence (n = 21)

2.2 Tissue quality control (QC)

After collecting the FFPE inclusions from various institutions, the the quality control (QC) process was started. The pathologist, in collaboration with the laboratory technician, evaluated each FFPE inclusion to determine if the material was adequate and of acceptable quality for immunohistochemical and molecular analyses. Assessments included thickness, tumor content percentage, and the presence of healthy or necrotic tissue. Tissue samples with over 50% tumor content will be processed for both immunohistochemical and molecular analyses.

Based on these criteria, the inclusions will be categorized into three groups:

- **Group 1:** inclusions with sufficient material for both immunohistochemical and molecular analyses.
- **Group 2:** inclusions with adequate material for only immunohistochemical analyses.
- **Group 3:** inclusions with insufficient material for any analyses.

3 Standardized cutting protocol

Slides preparation involves sequential cutting from the FFPE block resulting in:

1. 1 slide of 3 μm stained with H&E
2. 10 slides of 3 μm for immunohistochemical analysis
3. 5 slides of 7 μm for methylation analyses
4. 5 slides of 3 μm for WES, SNP arrays and RNAseq
5. 1 quality control slide of 3 μm stained with H&E to verify if the lesion is still present in FFPE block*

**If the material is insufficient for all the intended cuts, the quality control H&E slide will still be prepared after step 2 or 3.*

Always proceed first by cutting the sections for IHC before the thick section for molecular analyses. Each section has to be placed alone and in the centre of a glass slide. Electrostatic TOMO slides must be used for the IHC sections and non-electrostatic slides for the H&E and molecular analysis.

4 Conclusions

Collected samples are undergoing a quality control process of FFPE material to determine the quantity of material available for each FFPE inclusion. Once the centralization of the blocks at UNIFI is completed, we will define which cases will be suitable for all the planned analyses.

In the event that the FFPE material will not be sufficient for all planned analyses, the samples cutting flow will follow the subsequent prioritization:

1. DNA methylation;
2. Immunohistochemical analyses;
3. Whole-exome sequencing (WES), SNP arrays and RNA sequencing (RNAseq)