European Society for Immunodeficiencies
Mijnbouwstraat 23, Delft, the Netherlands
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# ESID End of fellowship report

## 1. Fellowship details

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| First name: | Alice | Last name: | Valagussa |
| Type of fellowship: | Short Term Fellowship | | |
| Start date: | August 1st 2022 | End date: | November 3rd 2022 |
| Hosting institution: | Università degli Studi di Milano - Italia | | |
| Supervisor: | Dr. Sven Kracker and Dr. Monica Beltrame | | |

## 2. Summary of the work done during the fellowship
(max.400 words)

Polikoderma with Neutropenia (PN) is a disease characterized by poikiloderma, genodermatosis, pachyonychia, hyperkeratosis, bone anomalies and neutropenia, predisposing to myelodysplasia. Different disease causing homozygous or compound heterozygous loss of function (LOF) mutations in the USB1 gene have been already described.

A patient presenting skin manifestations, osteopenia, immunologically with low memory B cell count and moderate neutropenia was genetically investigated. By whole-exome sequencing, a de novo variant was identified in the USB1 gene.

We plan to study the consequences of the de novo variant in the zebrafish model of PN established by the hosting laboratory/Institute (Larizza et al. in Nature Scientific Reports – 2015). We took advantage of the know-how in analyzing zebrafish models and the available tg(mpx:GFP) zebrafish line in the collaborating laboratory/institute for the subsequent proposed experiments:
i. to characterize the effect of the de novo USB1 variant;
ii. to characterize whether the USB1 de novo variant possess functional activity.

## 3. New skills aquired during the fellowship
(max.200 words)

The collaboration with “Dipartimento di Bioscienze, Università degli Studi di Milano – Italy” allowed me to acquire different skills. I followed both both teoric and practical training, that were followed by two exams in order to test my competence in handling zebrafish. Between the skills, there are counting and manipulation of adult zebrafish, zebrafish sex recognition, mating set-up, embryo collection and manipulation. I also observed the squeezing and euthanasia procedure. Furthermore, injection into one- or two-cell-stage zebrafish embryos RNA, picture acquisitions (fluorescence microscopy) at 2dpf or 5dpf followed by analysis with Fiji 2.3.0/1.52q software. Finally, Alcian blue and Alizarina Red stainining protocol.

# 4. Your professional plan for the near future and how the fellowship impacted this plan (max 400 words)

Currently, I am carrying out my doctorate at the Imagine Institute – Paris, financed by the "Hematology Oncology and Biotherapies" doctoral school and under the supervision of Dr. Sven KRACKER. I am part of the Human Lymphohematopoiesis Laboratory directed by Dr. Isabelle ANDRE, collaborating closely with the Biotherapy department headed by Pr. Marina CAVAZANNA. This already allows me to reach my short-term goal: being part of an excellent laboratory, surrounded by researchers available to share their knowledge and critical opinion. In addition, thanks to the established collaboration with the Dipartimento di Bioscienze, Università degli studi di Milano, I acquired the expertise to work on zebrafish and as such novel laboratory techniques. Working in the same laboratory that established the morpholino based usb1-deficient zebrafish model I am currently using for experiments, allowed me to be directly in contact with people who have a long-standing expertise in this field. Regular and fruitful discussions on data I obtained, as well as practical suggestion, were essential to obtain the promising results. I am looking forward to go back to the hosting laboratory to perform the last experiments in order to finalize this part of my research project. Being able to perform the replicates of the experiments using the same instruments, as well as the support of the hosting team, are certainly crucial to reach this important goal in the shortest possible time period.

## 5. Results obtained from your fellowship project. Please, mention any publications or meeting communications derived (if applicable, max 800 words)

The collaboration with “Dipartimento di Bioscienze, Università degli Studi di Milano – Italy” has the aim to investigate the functional consequences of a de novo USB1 variant in a zebrafish model of Polikoderma with Neutropenia (PN). This model was established by the hosting laboratory (Larizza et al., 2015). A plasmid containing the full-length human wild-type USB1 cloned in the pCS2+ poly(A) vector was provided by the Dipartimento di Bioscienze, Università degli Studi di Milano. Site-directed mutagenesis were performed to introduce into the plasmid the de novo mutation or another already described mutation included as a control in our assays. A plasmid containing mRFP1 cloned in the pCS2+ poly(A) vector provided by the hosting laboratory was used as injection control. Embryos were dechorionated and anaesthetized, before observations and picture acquisitions. Neutrophil count, pigmentation and area of the tail were evaluated at 2 dpf (days post fertilization) and quantified with Fiji 2.3.0/1.52q software. Alcian blue and Alizarina Red staining was performed to identify cartilage structures and bones, respectively, as previously described (Walker & Kimmel, 2006).
To reproduce previously published results (Larizza et al., 2015), splice-blocking morpholino A (SMO-A) 0.6 pmol/e was injected into one- or two- cell embryos. As described, most SMO-A morphants at 2 dpf appeared smaller than controls (e.g. uninjected and Std-MO embryos), presenting pericardial oedema, decreased pigmentation of the skin and myeloid lineage defects. Massive skeletal defects in SMO-A morphants were detected at 5 dpf. For the characterizing whether the de novo USB1 variant possess functional activity, 300pg/e of RNAs encoding different USB1 proteins were injected in conjunction with SMO-A. In a second line of experiments, injection into one- or two-cell-stage embryos RNA encoding different USB1 proteins were performed.

## 6. Any other comments

(max.200 words)

Due to experimental circumstances I prolonged my stay in the hosting laboratory until November 2022.