BAMBINO GESÙ CHILDREN'S HOSPITAL

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USING IMAGING MASS CYTOMETRY TO IDENTIFY NEXT GENERATION IMAGING BIOMARKERS IN PHGG AND DIPG

Translational
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EXECUTIVE SUMMARY

Pediatric neuro-oncology has been transformed by a number of key studies that, by employing different sequencing approaches and molecular profiling, have been able to better delineate the different pediatric central nervous system (CNS) tumors and separate them into distinct molecular, locational and clinico-pathologcal entities, identifying key biological drivers, potential cell of origin and new insights into their tumor heterogeneity. A missing element to be considered in the spectrum of pediatric CNS tumors, is an in depth understanding of the tumor microenvironments directly into the patient tissue samples which takes into account the different cell types, their functional states, their inter-relationships in the context of the topographical and architectural structure of the tissue itself.

Gaining such additional information would not only enlarge our knowledge on the biology of pediatric CNS tumors in relation for example to the immune response and to the blood brain barrier niches, but it would also provide direct links between molecular alterations and cellular functions and more importantly, identify novel clinically relevant tumor biomarkers.

Recent advances in mass cytometry have demonstrated that the same principles of the CyTOF technology, where more than 35-40 metal-tag antibodies are simultaneously used to recognize extracellular and intracellular markers, can be successfully applied to patient tissue slices with the *Imaging Mass Cytometry (IMC)*, providing high-dimensional single-cell data, for a truly comprehensive investigation of the tissue ecosystem. Such a highly innovative technology has been exploited, for example in breast cancer, to provide single-cell atlas of cancer and immune cells, distinguishing distinct tumor ecosystems in different patients and informing on their prognosis and therapeutic treatments.

We *hypothesize* that by setting up a platform for IMC, specifically designed for pediatric CNS tumors, we will be able to generate an innovative approach to finely dissect the complexity of their specific microenvironments and their inter and intra-tumor heterogeneity to ultimately identify *next generation imaging biomarkers*.

In particular, we believe that such approach will be of great value and should be prioritized for pHGG and DIPG. Those are highly heterogeneous cancers where both homotypic and heterotypic cellular relationships are present. Therefore tumor classification and future patient stratification should be done in the light of the tumor ecosystem as whole. Moreover, for those tumors, only small biopsy samples are often available. Thus, the multiplexing high-dimensional imaging capability offered by imaging mass cytometry is ideal to maximize the use of precious patient tissue samples for diagnostic and prognostic purposes.

Based on this, our specific aims are the: ***1***. Development and validation of a specific *tumor* and *brain microenvironment* IMC antibody panel for pHGG and DIPG; ***2.*** Identification of IMC specific *next generation imaging biomarkers* for pHGG and DIPG.

To achieve these aims, we will initially use retrospectively collected patient tumor tissue samples (formalin fixed paraffin embedded tissue slices) from 5 patients covering hemispheric, midline and pontine locations and comprising 4 molecular subgroups such as Histone WT, H3.3G34R, H3.3K27M and H3.1/ACVR1 mutants. For 2 of the patients, multiregion and longitudinal samples are also available.

We will obtain and customize a panel of 37 metal-tag antibodies to specifically recognize tumor cells, normal brain cells, specific mutational status, proliferation as well as elements of the BBB, extracellular matrix and potential immune cells. After a preliminary validation step, the IMC brain and tumor microenvironment antibody panel will be used to stain patient tissue slices and from those, 4 regions/slice will be processed by IMC on the Hyperion Imaging System. This will allow to capture images that are similar to immunofluorescence ones but useful to visualize, with sub-cellular resolution, the expression of all the markers of the panel, altogether for the same regions. The images will be subject to computational analysis by using specialized software and pipelines to obtain a multi-dimensional readout of all the cell types, cell functional states, cell-cell interactions for a comprehensive map of the tumor *in situ*.

The preliminary data provided demonstrate that we have precious tissue samples to conduct the study for which we have already built part of the panel that we aim to finalize with this application. We have already customized a specific metal tag-antibody for the detection of the H3.3G34R while the H3K27M conjugation is ongoing. We have validated the conjugated antibodies by immunofluorescence and demonstrated that we can use them for IMC analysis. Moreover, we have the expertise to apply the computational analysis required to detect cell types and the different morphologies, to identify and quantify marker expression for a variety of cell types and to extract a number of single cell features. We also took advantages of the statistical analysis and of the dimensional reduction analysis useful for the identification of cell populations, histological neighborhoods and significant interactions between cell groups.

This approach will allow the identification of new "*next generation imaging biomarkers*" for pHGG and DIPG that, with the expansion of specific IMC antibody panel, beyond this project, could be easily translated and extended to other pediatric CNS cancers.

We believe we have a team with the right expertise which covers the biology and clinical aspects of the proposed project, the specific expertise in the field of pHGG and DIPG heterogeneity, the technical expertise required, the specialized facilities for the IMC processing as well as the computational skills to handle the multi-parametric and high-dimensional data which will be obtained.
This is a highly innovative project which, if funded, will have a great impact on future clinical practice as it will offer a full read through of pHGG and DIPG patient tissues enabling the identification of new diagnostic and prognostic biomarkers.