EXECUTIVE SUMMARY

Diffuse intrinsic pontine glioma (DIPG) is a devastating brain tumor which affects children and for which there is no effective treatment at the moment. We and others have recently shown that DIPG is characterized by a remarkable degree of intratumoral heterogeneity and consists of distinct genetically and phenotypically heterogeneous subclonal populations. It is well recognized that exosomes mediate the cross-talk among the tumor cells and between the tumor and its microenvironment. We hypothesize that the DIPG subclonal network is sustained through paracrine signaling and that exosomes are involved in this intratumor cross-talk promoting tumorigenesis and tumor progression. To understand the role that exosomes play in DIPG tumorigenesis and determine the mechanistic basis of the exosome-mediated interclonal communication, we aim to: i. determine the speci□c DIPG-exosome signature within the distinct mutational subgroups and within distinct sub-clonal populations; ii. de□ne the exosome-mediated mechanisms of crosstalk between distinct DIPG subclones and determine the functional consequences of such uptake. To address these aims we speci□cally isolated and characterized exosomes derived from different DIPG patient primary-derived cells. We found that exosomes derived from DIPG displays a variable cargo of total protein with a trend for DIPG lines which is higher compared to the exosomes derived from GBM. Electron microscopy showed that the population we isolated is compatible with exosomes with a size ranging between 50-80 nm. Using proteomic analysis we identi□ed □rst a common signature for the DIPG derived exosomes compared to the GBM exososomes and interestingly a peculiar signature related to the different DIPG molecular subgroups. The miRNAs analysis of DIPG-derived exosomes suggested that their miRNAs pro□le is driven by the two main histone H3 variants leading to two distinct oncogenic programmes with distinct potential speci□c therapeutic targets In our project we aim also to investigate the speci□c pro□le of exosomes derived from DIPG subclonal populations and analyze the functional consequences (effect on growth and invasion) of exosomal uptake in co-culture experiments 6/6/2018 Investigating the role of DIPG-derived exosomes in tumor growth and invasion of DIPG subclones. We have successfully transduced one DIPG and one GBM primary derived cell line using 6 different lentiviral vectors expressing each a different □uorescent protein in order to perform long term co-culture experiment and be able to distinguish the dinamics of direct cell-cell interaction via exosomes. So far we demonstrated despite cell transduction, heterogeneity is preserved in the DIPG and GBM cell in terms of morphology, cell growth, migration and invasion. Moreover we have show that labeled exosomes derived from the bulky population are acrively uptaken from a derived subclone. This con□rm our hypothesis that exosomes represent a potential shuttle for exchange of oncogenic signals between glioma subpopulations. Our goal is indeed to better elucidate the mechanisms of cellcell communication that mediate the DIPG growth and invasion. Our future plan in the second year of this project will be i) to validate exosomal miRNAs on additional DIPG cell lines and in patient tissue samples; ii) to validate, by modulation of their expression speci□c miRNAs in DIPG primary derived cell lines; iii) to evaluate the exosomal miRNAs in plasma samples from patients affected by DIPG; iv) to validate speci□c exosomal proteins in tissue patients. We have already collected plasma samples from 8 DIPG and GBM patients and we estimate we will collect 6-8 more during this year. We believe that this study will lead to the identi□cation of potential new targets and the development of new diagnostic/prognostic tools for patients affected by DIPG. Our preliminary data demonstrate that tumor-secreted exosomes can be

ef□ciently used to track the communication among subclonal population in DIPG tumors and the activation of speci□c oncogenic pathways. Dr. Mara Vinci and collaborators have already demonstrated that DIPG is a heterogeneous tumor and that it is characterised by a complex subclonal architecture where distinct subclones co-operate to display the more aggressive phenotype, in particular in terms of migration and invasion (Vinci et al., Nature Medicine 2018, accepted). Her expertise with the collaboration of Dr. Angela Di Giannatale, who is a pediatric oncologist who works with her team on understanding the role of tumor-secreted exosomes in the tumor cross-talk, will ensure that the study will be carried out according to plan. Furthermore the collaboration with 6/6/2018 Investigating the role of DIPG-derived exosomes in tumor growth and invasion other experts (Dr.Putignani and Dr. Levimortera for the proteomic, Dr. Masotti for the miRNA analysis and Prof. Novelli for the FISH study) will enable a complementary synergy fundamental for the success of this project.