UNDERSTANDING THE MECHANISMS OF CROSSING, DELIVERY AND TARGETING OF HYDROGEL-NANOSTRUCTURES FOR BRAIN THERANOSTICS

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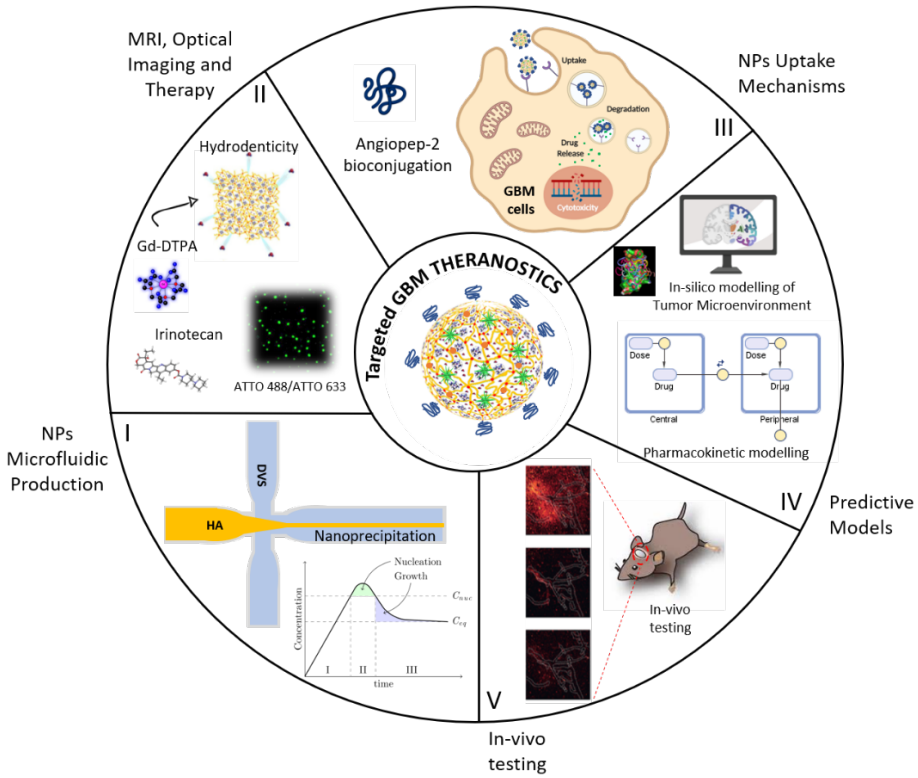
Most of the Central Nervous System (CNS) pathologies and in particular Brain Tumours are characterized by very poor patient prognosis and poorly effective clinical outcomes. Indeed, the delivery of diagnostic and therapeutic compounds to the brain is severely hampered by very peculiar biological barriers involving the Blood to Brain Barrier (BBB), an altered extracellular space and an increased interstitial fluid pressure among others, that strongly impair the transport of active species to the target brain tumor cells. In this regard, nano-formulated active agents for the precision treatment of brain tumours offer several advantages over conventional medicine such as improved drug solubility, enhanced stability and blood circulation time, increased bioavailability, improved BBB crossing and site-specific targeting with the consequent reduction of systemic toxicity that overall improve the pharmacokinetics of these compounds and thus, their efficacy. However, despite the extensive research in this field demonstrated the great potential of precision medicine to achieve an earlier diagnosis and effective therapy of brain tumors, the clinical translation of nano-formulated therapeutics is very limited with less than 10 approved nanoformulations for the whole cancer diagnosis and therapy area. The reasons underlying this limited clinical success, regard the poor understanding of the mechanisms involved in the in-vivo biodistribution of nanoparticles, especially at the tumor site, the lack of information about transport properties of these systems in the tumor and the unclear interaction that they have with tumor cells even at a molecular level.

With the aim of studying the interaction occurring between nanomaterials and the biological systems, the nano-biointeractions, as phenomena determining their biodistriution and in-vivo fate, this PhD project starts with the design and development of a fully biocompatible and theranostic nanovector with the use of FDA approved materials, that is able to provide improved therapeutic and imaging function simultaneously for the precison treatment of Glioblastoma multiforme (GBM), one of the most aggressive malignant brain tumours. Once produced, the impact of different surface properties of the vector (pegylation, anti-fouling polymers, peptide functionalization, antibodies) on the interaction with tumor cells in-vitro (uptake rate, cellular distribution, cellular degradation) and with the tumor in-vivo (plasma stability, biodistribution, BBB crossing, tumour accumulation) will be studied. Finally, nanoparticle interaction with biological systems will be evaluated down to the molecular level by means of tools evaluating the energetical, chemical, and biological interaction between different components of the system and in-silico pharmacokinetic models will be used to study local barriers to transport and evaluate the transport properties of the developed vector in such a complex system.

To the purpose, starting from the consolidated expertise of our research group in the microfluidic production of polymeric nanoparticles encapsulating Gadolinium (Gd) chelates (the most common contrast agent in clinical Magnetic Resonance Imaging) with improved MRI properties by the effect of Hydrodenticity[1-3], a new vector co-encapsulating imaging and therapeutic active compounds has been developed and characterized during the first year. The co-encapsulation of Gd-DTPA and the standard NIR dye ATTO633 has conferred multimodal imaging properties to the vector and the co-encapsulation of Gd-DTPA and the chemotherapeutic drug Irinotecan a theranostic function[4].
The use of microfluidics has offered the possibility of tuning product composition and properties by process parameter adjustments, has guaranteed uniformity of the product, providing improved space-time yields (product formed per reactor volume and time), accelerating the potential of clinical translation of the formulation[5].
Furthermore, during the second year, the surface of the nanoparticles has been diversely functionalized to study the impact of different surface properties on the nano-biointeractions. First, the surface of nanoparticles has been pegylated in a one-step microfluidic process to modulate the phenomenon of protein corona formation on the surface of nanoparticles once in plasma circulation, which represents the first biological barrier to overcome for the successful brain delivery[5]. The effect of this modification on protein surface adsorption and uptake by tumor cells has been evaluated and compared with the effect provided by other anti-fouling polymers (on-going). Secondly, the surface conjugation with the peptide Angiopep-2, a peptide able to double target endothelial cells of the BBB and GBM cells

has been explored. At conjugation, an improved uptake both on standard tumor cell line and patient derived cells with respect to bare particles has been demonstrated. As a consequence of the improved transport properties of the Angiopep-2 functionalized vector inside the cell, an improved therapeutic effect of the encapsulated drug irinotecan has been observed[4].

During the third year, the mechanism of internalization of Angiopep-2 functionalized particles has been demonstrated. As a perspective, the impact of different ligands and different superficial ligand density will be studied. In-vivo experiments will allow to understand nanoparticle biodistribution and pharmacokinetics and assess particle targeting ability and brain accumulation. Results of the experiments will be used to validate pharmacokinetics models whose results could be confirmed with more confidence.



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Angela Costagliola di Polidoro, PhD student XXXIV cycle, May 2021