Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Polymeric nanoparticles to target T-cells in glioma

Synopsis: Glioblastoma (GBM) is an incurable and deadliest primary brain tumour, and its incidence or occurrence has been increasing worldwide, affecting both children and adults. The average survival rate of GBM patients submitted to standard treatments is less than 15 months following diagnosis, making it a public health issue of high relevance. Most GBM patients (<90%) suffer tumour recurrence due to these tumours' aggressive and treatment-resistant behaviour[1,2]. Among the most reasons for the failure of conventional therapies is the presence of a selectively blood-brain barrier (BBB) which controls the transport of molecules to the brain, limiting access to most anti-cancer compounds[3]. Furthermore, the lack of progress in GBM immunotherapies is due to the enrichment of immune suppressors and a paucity of cytotoxic-T cell infiltration[4,5]. Therefore, an effective anti-tumour immune response requires the hijack and activation of T-cells at the tumour site, which remains a challenge for GBM. Polymeric nanoparticles (POs) can overcome the limitation of BBB by allowing their shuttle into the brain and targeting specific cells in the CNS, including tumours[6]. They might work as immunotherapy by modulating T-cell functions against cancer. The project aims to design super-selective poly(ethylene glycol) (PEG)-poly (lactic acid) (PLA) POs-based multi monoclonal antibodies (mAbs), named PolyMutes, to target GBM cells and T-cell engagement to kill tumour cells. To assess the BBB crossing ability of PolyMuTEs decorated with angiopep-2, an in vitro BBB model will be established using transwell inserts and microfluidic devices (Aimbiotech). The BBB model composed of spheroids of GBM cell lines (such as T98G and U87) and endothelial cell line (bEnD3) will be performed. Then, % of POs crossing the BBB will be verified using confocal microscopy (Leica SP8) and fluorometry (Spark Cyto multimode imaging plate reader). To assess and validate the specific targeting of PolyMuTEs to T-cells, the T-cell line HM2 will be used. An in vitro 3D co-culture of GBM spheroids and T-cells will be performed for T-cell engagement with tumour cells. The cytotoxic activation of T-cells will be verified by measuring the interleukins (e.g., IL-2), interferon-gamma (IFNy), tumour necrosis factor-alpha (TNFa), and cytolytic enzymes (e.g., granzyme B) by ELISA, western blot and flow cytometry.

In vitro studies of superselective targeting of PolyMuTEs

Aim	A1. Study the ability of these PolyMuTEs to cross the BBB, biocompatibility, and safety *in vitro* and Evaluation of T-cell activation and cytotoxic profile and GBM targeting involving 3D BBB-glioma models
Deliverables	D1. 3D BBB model in microfluid device (ECs and GBM spheroids) D2. Competent Angiopep2 targeted NPs for BTB crossing. D3. Competent CD3 targeted NPs to T-cell D4. Efficient engagement of cytotoxic T-cell to GBM cells
Milestones	M1. Optimisation 3D BBB model in microfluid device (ECs and GBM spheroids) M2. Controlled superselectivity targeting of NPs M3. Evaluation of cytotoxic effect of T-cell and GBM cell death, *in vitro*

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