Effect of glioblastoma tumour microenvironment on the modulation of the immune system

**INTRODUCTION**

- Glioblastoma (GBM) is considered the most lethal primary brain tumour in adults.
- The average survival of patients is about 14 months.
- Patients are treated with radiotherapy and chemotherapy, with concurrent and adjuvant temozolomide after surgical resection [1].

- Glioblastoma tumour microenvironment (TME) is characterized by being highly immunosuppressive and having low immunogenicity, highlighting it as a 'cold tumour'.
- The migration of immune system cells and access to tumour antigens is an important aspect. This is hindered by the increased stiffness of the extracellular matrix (ECM).

**OUR APPROACH**

Our approach consists of generating different coculture conditions to recreate a physiological environment similar to in vivo conditions and apply that settings in the future into an organ-on-a-chip cell culture model, that represent the real physiology of the tumour.

To carry this objective it is very important to find the optimal conditions for all populations.

To optimize the conditions, peripheral blood mononuclear cells (PBMCs) and tumoural cells (U-251 MG) were seeded together and separately in 24-well plates and different factors were studied.

To observe that, viability tests were performed with propidium iodide (PI) and calcein AM (CAM) stains.

A study of cytokine secretion by the different populations in the culture conditions that were considered more physiological was also carried out.

**RESULTS**

**EFFECT OF THE CULTURE MEDIUM**

**SYNERGISTIC EFFECT OF STIMULI (INTERLEUKIN 2 + U-251 MG)**

**CYTOKINES SECRETED BY THE DIFFERENT MICROENVIRONMENTS**

- TGF-β
- IL12
- TNF-α

**EFFECT OF INTERLEUKIN 2**

**FUTURE INSIGHTS**

The future perspectives of our research are to make a translation to 3D models and in addition to identify in a more specific way the interaction of the immune system with the tumour microenvironment by performing flow cytometry and seeing which immune populations are secreting the studied cytokines.

**CONCLUSIONS**

- It has been proven that the most optimal medium for co-culture is RPMI.
- Immune system cells that have been subjected to cytogenic stress have a lower viability than PBMCs freshly extracted from fresh blood.
- Immune cells that receive an external stimulus, from cytokines or other cell populations, are able to maintain their high viability for up to 24 hours longer than those that are isolated from any type of stimulus.

**ACKNOWLEDGEMENTS**

After the studies carried out, it has been proven that the most optimal medium for co-culture is RPMI.

- The presence of tumor cells (U-251 MG) induces increased secretion of TNF-α and IL-12.
- Tumor cells alone secrete detectable amounts of TGF-β.
- The tumour microenvironment (TME) has a complex influence and interaction with the PBMCs.

---

**BIBLIOGRAPHY**
