GRAPHENE QUANTUM DOTS CONJUGATED WITH BORTEZOMIB ANALOGUES IMPROVE DRUG DELIVERY AND EFFICACY IN AN *IN VITRO* MODEL OF MULTIPLE MYELOMA

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Introduction: Multiple myeloma is one of the most common hematologic cancer, caused by the accumulation of malignant plasma cells in the bone marrow. Although multiple myeloma remains incurable, new treatments are offering several lines of therapy and prolonged control of disease, characterized by further improvements in patient outcomes. Among emerging treatments, proteasome inhibitors were recognized as potential targets for the treatment of haematological malignancies. Proteasome plays a pivotal role in the intracellular protein turnover and regulation of cell cycle progression and its inhibition leads to protein overload, endoplasmic reticulum stress, and ultimately death of multiple myeloma cells. Among proteasome inhibitors, bortezomib was the first one approved for clinical use for treatment of multiple myeloma; it is a peptide boronic acid that reversibly inhibits the β5catalytic subunit of the 20S proteasome and in some cases the immunoproteasome. Our research group has been working in the past years trying to identify new lead compounds that may block the proteasome (both the constitutive and the immunoproteasome) starting from the chemical structure of bortezomib. However, delivery of these compounds to myeloid cells might be difficult and in these regards, nanomaterials might be of help.
Among the different classes of nanomaterials, graphene quantum dots (GQD) are recognized as ideal candidates for cancer diagnosis and treatment thanks to their physical, chemical and biological properties. Indeed these nanoparticles are small, non-toxic, biocompatible, and are endowed with stable strong intrinsic fluorescence and the many reactive groups on their surface allow their multimodal conjugation with various functional groups and biologically active molecules making them ideal nanocarriers for the delivery of therapeutic agents into cancer cells.
The aim of this study was to evaluate whether graphene quantum dots conjugated with bortezomib analogues could improve the internalization of these new drugs in human multiple myeloma cells.

Materials and methods: MM1.S (dexamethasone-sensitive) and MM1.R (dexamethasone-resistant) cell lines were obtained from the American Type Tissue Culture Collection (ATCC). These cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and antibiotics. Cells were treated with graphene quantum dots conjugated with different bortezomib analogues at several concentrations (5, 10, 15, 20μM), depending on the IC50 of each compound. After treatment (from 10 minutes up to 24hours) treated cells were observed at the microscope to identify fluorescence from GQD and then used to assess their efficacy on both the constitutive- and immune-proteasome activity.

Results: GQDs improved the internalization of bortezomib analogues inside either MM.1S and MM.1R and demonstrated an increase in the accumulation of ubiquitinated protein aggregates in the cytoplasm through confocal fluorescent microscope and in the expression of p62SQSTM1protein by western blot analysis.

Discussion and conclusions: Proteasome inhibitors are one of the most important drugs emerged in the last years for the treatment of multiple myeloma although there are still toxicity and off-side problems. The use of novel inhibitors with a more targeted activity and conjugated with GQDs that can be addressed to specific cell types might overcome these problems. The data obtained in this in vitro experiment are promising and suggest a further investigation in other models.
In the last years target therapy is catching researchers' attention thanks to its purpose to minimize systemic toxicity and undesirable side effects, typically associated with conventional chemotherapy. GQD-based system delivery can represent a new milestone in the search of compounds that can specifically target cancer cells and deliver the proper drug.