# Abstract 3

## MEMBRANE BLUE DUAL PROTECTS RETINAL PIGMENT EPITHELIUM CELLS AND GANGLION CELLS CULTURED IN BOTH PHYSIOLOGIC CONDITIONS AND IN THE PRESENCE OF UVB THROUGH THE MODULATION OF THE MITOCHONDRIAL FUNCTION AND OF THE REDOX BALANCE

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#### Purpose:

We examined the effects of Membrane Blue-Dual (MBD) with/without polyethylene glycol (PEG) on both human retinal pigment epithelial (ARPE-19) and retinal ganglion (RGC-5) cells cultured in the presence/absence of ultraviolet B (UVB) treatment on viability, mitochondrial function, oxidants/antioxidants, proliferation/migration and apoptosis.

#### Methods:

In ARPE-19/RGC-5 cells either treated or not with UVB, the effects of MBD with/without PEG were evaluated by specific assays for viability, mitochondrial membrane potential and mitochondrial reactive oxygen species (mitoROS) release. Annexin V was used to detect apoptosis, whereas trypan blue and the scratch assay were used for proliferation/migration evaluation. Finally, through Western blot, we analyzed the superoxide dismutase (SOD) 2 and the nuclear protein ki67 expression.

### **Results:**

In both physiologic condition and in the presence of UVB, MBD with/without PEG increased viability, mitochondrial membrane potential, proliferation and migration of both ARPE-19 and RGC-5 cells. In general, the effects of MBD with PEG were higher than those caused by MBD without PEG. In particular, in the presence of UVB, we could find a greater improvement of cell viability, mitochondrial membrane potential and SOD2 expression and a stronger reduction of mitoROS release and apoptosis in both ARPE-19 and RGC-5 cells treated with the dye additioned with PEG. Examples of effects of MBD on ARPE-19 are shown in attached figure.

#### Conclusions:

Our results suggest that in particular MBD with PEG is a safe and effective dye for vitro-retinal surgery through the modulation of mitochondrial function and the oxidants/antioxidants balance. The data we obtained may add new knowledge about the use of MBD and could be relevant for the clinical use.



Figure shows the effects of MBD with PEG (a, c, e) and without PEG (b, d, f) on ARPE-19 cells. In a and b, mitochondrial membrane potential In c and d, mitochondrial ROS release In e and f, SOD2 expression The results are the mean  $\pm$  SD of repeated experiments