BPM31510: Targeting the tumor microenvironment (TME) via mitochondrial-mediated ROS production

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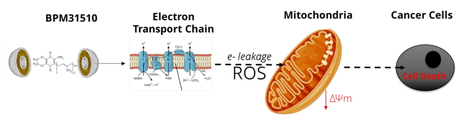
Introduction

- BPM31510 is a CoQ10-lipid nanoparticle conjugate in which oxidized CoQ10 has been incorporated into a mixture of mitochondria compatible lipids, enabling delivery of supraphysiological concentrations of CoQ10 to the mitochondria.
- BPM31510 has been evaluated in a human Phase I dose escalation trial (NCT01957735, NCT03002935, NCT02486055). BPM31510 has a favorable safety and tolerability profile.
- BPM31510 induces ROS through high intra-cellular levels of ubidecarenone in aggressive cancer phenotypes through induction of mitochondrial ROS.
- The tumor microenvironment (TME) is a complex ecosystem surrounding a tumor including e.g., immune cells, blood vessels, fibroblasts, and signaling molecules.
- BPM31510 was assessed in pre-clinical *in vivo* and *in vitro* models to provide evidence for the impact of BPM31510 on the TME.
- Currently, the clinical efficacy of BPM31510 is being evaluated in an ongoing first-line phase 2b study in glioblastoma in combination with radiotherapy and temozolomide to assess the potential for mechanistic synergy of multiple ROS-inducing mitochondrial mediated regimens.

Mechanism of Action of BPM31510

- Mitochondria produce 90% of the endogenous reactive oxygen species (ROS) in the cell.
- Dysregulated redox homeostasis is a hallmark of cancer cells creating a vulnerability to ROS levels above a protective threshold with subsequent cell death.
- This opens a therapeutic window for ROS-inducing agents such as BPM31510.



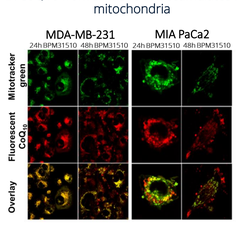
- BPM31510 promotes electron slippage within the Electron Transport Chain (ETC)
- Leaked electrons react with molecular oxygen forming superoxide and subsequently ROS.
- ROS accumulation and oxidative stress make mitochondria dysfunctional resulting in loss of membrane potential and activation of cell death pathways.

BPM31510 shows broad *in vitro* potency through induction of ROS

A. BPM31510 *in vitro* cytotoxicity across multiple cancer types

Cell Line	Indication	IC_{50} (μM)
MIA PaCa2	Pancreatic Cancer	137
MDA-MB-231	Breast Cancer	155
HUH-7	Liver Cancer	209
U-87 MG	Glioblastoma	310
SkBr-3	Breast Cancer	369
U-251 MG	Glioblastoma	392
LnCap	Prostate Cancer	440
Panc-1	Pancreatic Cancer	455
BT549	Breast Cancer	464
PC-3	Prostate Cancer	787
MCF-7	Breast Cancer	922

B. CoQ10 from BPM31510 accumulates in mitochondria



C. BPM31510 induces ROS

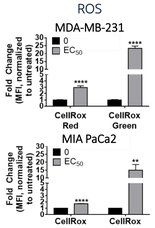
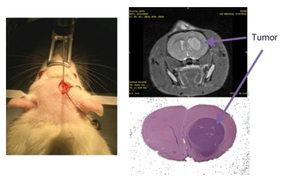


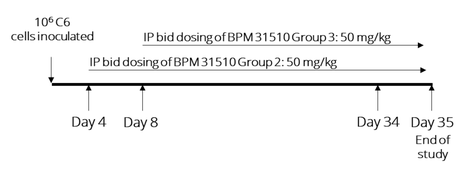
Figure 1. *In vitro* potency of BPM31510. **(A)** BPM31510 IC_{50} values were determined for a panel of 11 cancer cell lines using CellTiter-Fluor (Promega) viability assay. 72h post treatment with BPM31510 (0-1000 μM dose-response curve, three biological replicates. **(B)** Fluorescently tagged CoQ10 (red) from BPM31510 (200 μM) colocalized with the mitochondria (MitoTracker; green) in MDA-MB-231 and MIA PaCa-2 cells after 24h and 48h exposure. **(C)** MDA-MB-231 and MIA PaCa2 cells were treated with EC_{50} BPM31510 for 24h and incubated with the cell permeant fluorogenic probes CellRox Green (mitochondria-nuclear specific) and CellRox Red (cytosol specific). Reactive oxygen species (ROS) was measured in MDA-MB-231 (top) and MIA PaCa2 (bottom).

BPM31510 demonstrates efficacy in an orthotopic syngeneic glioma model

A. Stereotactic implantation of C6 glioma cells in brain of rat



B. Overview of study design



C. BPM31510 exerts efficacy in an aggressive orthotopic glioma model

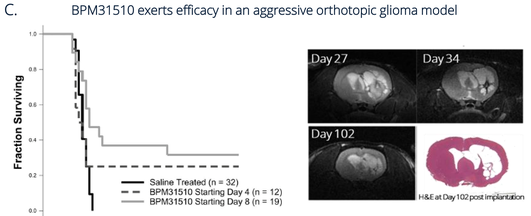
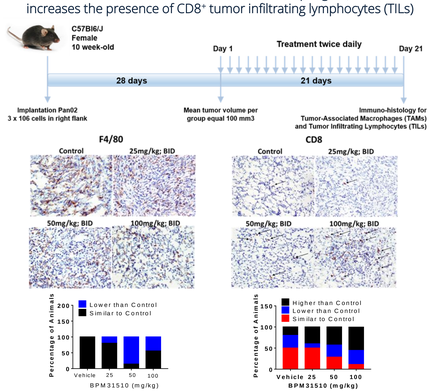


Figure 2. C6 allograft glioma model. Wistar rats received either saline or 50 mg/kg BPM31510 IP, twice daily, five days per week, starting at either 4 (n=12) or 8 (n=19) days after implantation of 10^6 C6 glioma cells into the right striatum. **(A)** Stereotactic implantation procedure of C6 glioma cells in rat brain and **(B)** overview of study design. **(C)** Survival of rats treated either with saline (n=32) or BPM31510. Over 25% of BPM31510 treated rats survived to the end of experiment (at least 75 days) compared to 0% of PBS treated controls ($P < 0.01$, log rank statistic). No significant difference was noted between the BPM31510 treated groups starting at 4- or 8-days. Serial MRI of a long-term survivor (Day 27, Day 34, and Day 102 post-implantation) demonstrating persistent effects even after treatment was withdrawn. Lower right panel is a coronal plane H&E stained section of the same long-term survivor demonstrating a cystic cavity with no obvious tumor present..

BPM31510 exerts potent anti-tumor effects in immune-competent syngeneic murine cancer models and increases cytotoxic TILs

A. BPM31510 decreases tumor associated macrophages (TAMs) and increases the presence of $CD8^+$ tumor infiltrating lymphocytes (TILs)



B. BPM31510 increases cytotoxic TILs and enhances the ratio of $CD8^+$ to regulatory T cells

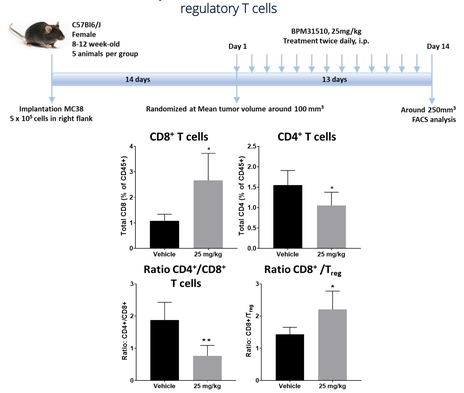


Figure 3. BPM31510 effects in immune-competent mouse models of pancreatic and gastric cancer. **(A)** C57BL/6 mice were implanted with $3X10^7$ Pan02 pancreatic cancer cells with different doses of BPM31510. At a mean tumor volume of 100 mm^3, animals were randomized into four groups and treated with vehicle control or BPM31510 (25, 50, 100 mg/kg, BID) for 21 days. For immune cell analysis, tumors were removed and subjected to IHC analysis for Tumor Associated Macrophages (TAMs) using F4/80 or CD8 T cells (CD8). All slides were subjected to a pathological scoring. Scores were relative to control slide (from the control group) which demonstrated the best level of intensity. Arrows indicate the presence of TAMs or T cells. **(B)** Frequencies of tumor infiltrating immune cells were characterized by injecting female C57BL/6 mice in the flank with MC38 tumor cells ($5x10^5$/animal). When the tumors reached ~100 mm^3, animals were randomized into two groups of vehicle (saline) or BPM31510 (25 mg/kg .i.p. daily) and treated for 13 days. Tumors were collected at ~250 mm^3, processed to a single cell suspension, stained for surface expression of CD45/CD3/CD4/CD8/FoxP3 and subjected to FACS analysis. Statistical analysis were performed using Welch's t test. * $P<0.05$; ** $p< 0.01$.

BPM31510 immune potentiating properties by promotion of cytotoxic T cells

A. BPM31510 positively affects the frequency of viable T cells

B. BPM31510 increases proliferation and cytolytic potential of activated cytotoxic T cells

C. BPM31510 increases IL-2 and IFN-γ secretion while decreasing levels of IL-10

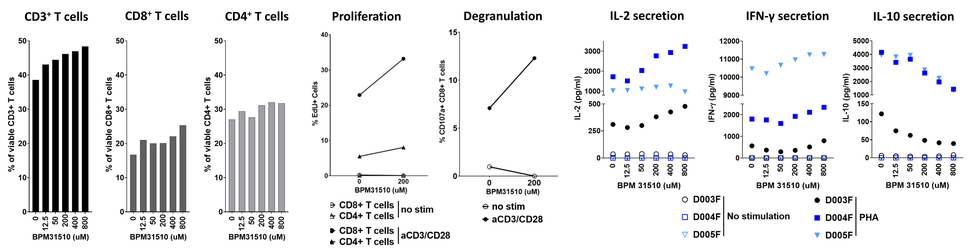


Figure 4. BPM31510 effects on immune cells *in vitro*. **(A)** Frequency of T cell subpopulations within αCD3/CD28 bead stimulated or unstimulated PBMCs concurrently treated with BPM31510 (0-800 μM) evaluated by flow cytometry. 24h post treatment, PBMCs were stained for CD3/CD8, or CD3/CD4, as well as Annexin V/7AAD. Data depicted are representative of 10 healthy donors tested. **(B)** Proliferative and cytotoxic potential of T cells was assessed by incubating PBMCs with αCD3/CD28 beads while concurrently treating with BPM31510 (200 μM). Proliferation was assessed by EdU incorporation. Briefly, after PBMC incubation in presence of stimulant and BPM31510 for 72h, 10 μM of EdU was added for the final 18h and stained with Invitrogen Alexa Fluor 488 piclyl azide followed by staining with antibodies for CD3/CD8, or CD3/CD4 and analysed by flow cytometry. Cytotoxic potential of $CD8^+$ T cells was evaluated by measuring CD107a expression on surface of T cells. PBMCs, stimulated and incubated with 200 μM of BPM31510 for 24h in presence of monensin (0.7 μM/ml) and brefeldinA (1 μM/ml). Cells were harvested, fixed, and stained for surface markers CD3 and CD8 and analyzed by flow cytometry. Data are representative of 3 donors tested. **(C)** Levels of cytokines IL-2, IFN-γ, and IL-10 in supernatants of healthy donor PBMCs treated with BPM31510. Briefly, healthy donor PBMCs were treated with or without PHA and various concentrations of BPM31510 (0-800 μM). 24h post treatment, cytokines were measured according to the manufacturer's protocol for R&D Quantikine ELISA kits specific to each cytokine. Shown are representative data from three out of eight independent donors tested.

BPM31510 attenuates activation induced immune checkpoint expression on T cells

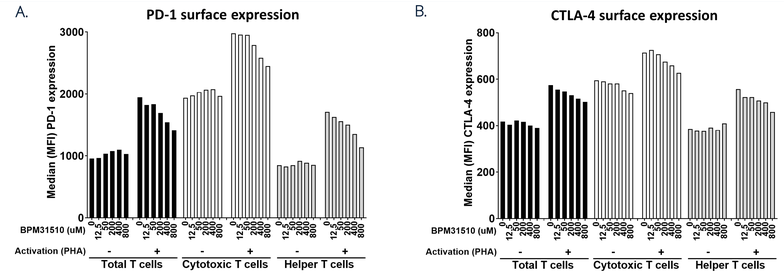


Figure 5. Inhibitory receptor surface expression on T cells within PBMCs treated with BPM31510 for 24h. Expression of immune checkpoint receptors were measured by staining cells with phenotypic markers for CD3/CD8, or CD3/CD4 in combination with antibodies against PD-1 or CTLA-4. Live cells were identified as 7AAD negative lymphocytes followed by T cell phenotype characterization of total $CD3^+$ T cells, cytotoxic T cells, or helper T cells, as indicated below plot. PD-1 **(A)** or CTLA-4 **(B)** cell surface expression was measured as mean fluorescence intensity on live T cells. Data are representative of 3 donors tested.

CONCLUSIONS

- BPM31510 demonstrated potency in various cancer cell models *in vitro* and *in vivo* through the increase of mitochondrial ROS (Dadali T et al., Sci Rep 11, 5749; 2021).
- BPM31510 demonstrated robust efficacy in a preclinical syngeneic model of GBM. Treatment significantly increased long-term survival compared to the untreated control group in a C6 allograft glioma rat model (Sun J et al., Scientific reports, 10(1); 2020).
- BPM31510 showed potent anti-tumor effects in immune-competent syngeneic cancer cell models. Tumors had an increase in cytotoxic T cells with an increase in cytotoxic T cell/regulatory T cell ratio as well as decrease in tumor associated macrophages.
- *In vitro*, BPM31510 increased the frequency of viable cytotoxic and helper T cells and augmented their function (increased proliferation, cytolytic potential, IL-2, IFN-γ, and decreased in IL-10) demonstrating direct immuno-modulatory effects.
- BPM31510 attenuates activation induced immune checkpoint expression.
- BPM31510 is currently being evaluated in a first-line Phase 2b study of BPM31510 in glioblastoma in combination with radiotherapy and temozolomide.