Employment of Spatial Omics and Quinomics Technologies For the Advancement of Mitochondrial Targeted Therapeutics

Michael A. Kiebish PhD[1], Niven R. Narain PhD[1,2], Juan Aristizabal-Henao PhD[1], Sylwia Stopka PhD[1], Srada Karmacharya MS[1], Megan Cox PhD[1], Abbas Khojasteh MD[3], Lawrence Recht MD[3], Eliana Barriocanal Casado PhD[4], Stephane Gesta PhD[1], Alba Pesini PhD[4], Catarina Quinzii MD[4], Vijay Modur MD PhD[1]

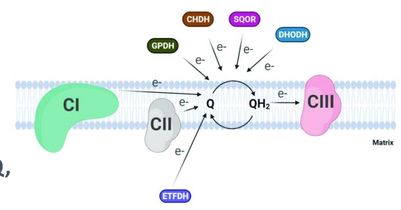
[1]BPGbio, Framingham, MA USA, [2]Departments of Dermatology & Cutaneous Surgery and Biochemistry and Molecular Biology, Miller School of Medicine, University of Miami, Miami, FL USA,* [1]Neurology and Neurological Sciences, Stanford University, Stanford, CA USA, *[4]Columbia University New York, NY USA

ABSTRACT

Therapeutic development in diseases rooted in mitochondrial dysfunction and dysregulation are pharmacologically burdened by numerous challenges stemming from heterogenous presentation and variable metabolic phenotype across tissues and cells. Recent advances in omic technologies now allow for the interpretation of single cell metabolomics and lipidomics in tissue samples resulting in the spatial resolution of adaptive metabolic changes across tissues and cells, allowing for profound insight into therapeutic efficacy of pharmaceutical agents. Further, investigation of key drivers of redox state, ie quinome metabolites (quinomics) both spatially and with high resolution bioanalytical platforms further integrates the mechanistic interplay between mitochondrial efficiency and pathological ROS generation. Herein, we utilized the Bruker TIMSTOF Flex workflow to assess spatial metabolomics/lipidomics/quinomics as well as high resolution LC MS/MS metabolomics/lipidomics workflows to investigate the therapeutic MOA of BPM31510. BPM31510 (a novel lipid nanodispersion of ubidecarenone) is highly stable, possesses unique biophysical properties, and delivers supraphysiological concentrations of oxidized CoQ10 under IV administration. Notably, the formulation demonstrates potent metabolic activity and potentiation of mitochondrial OXPHOS and controlling ROS production, conferring actionable therapeutic potential across multiple disease phenotypes. Current investigations have focused on spatial omic/quinomics analysis in BPM31510 treated mice/rats for tissue distribution (as well as quinomics in a Phase 1 clinical trial) and subsequent metabolomic adaption in oncology and mitochondrial disease models (to include CoQ deficiency models). Due to the inherent biophysical, bioenergetic, pharmacokinetic, and pharmacodynamic properties of BPM31510, as well as its ability to achieve high bioavailable concentrations of CoQ10, there is broad applicability in the treatment of mitochondrial diseases. Furthermore, engaging adaptive spatial omic, metabolomic, and quinomic workflows demonstrated bioactivity, delivery to the mitochondria and homeostatic changes in energy metabolism that allows for an enhanced tool in drug discovery and development.

Figure 1: CoQ10 and Challenges of Developing CoQ10 as a Therapeutic

CoQ10 plays an integral role in mitochondrial metabolism, ROS generation, and maintaining the electron gradient



Challenges in Development of CoQ10 as a Therapy

- Poor oral bioavailability (<0.1%)
- Lack of pharmaceutical cGMP grade product/formulations for IV, subQ, topical, or oral formulations
- Low pK/PD ratios to effectuate mitochondrial function at tissue level
- A true lack of the translational potential of the molecule in mainstream medicine and pharmaceutical ecosystem

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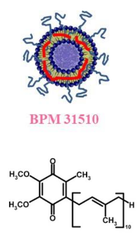
Figure 2: Development of BPM31510 For Delivery of Supraphysiological Concentrations of CoQ10



- Proprietary & **stable** formulation
- Contains **CoQ10** and nanoparticle lipid membrane to modify mitochondrial metabolism
- A **bio-membrane** -like capsule
- Enrichment in **mitochondria**
- Well-tolerated with a **favorable safety profile**
- Potentially mitigates treatment toxicity from chemo or radiotherapy

Figure 3: 4% CoQ10 is the Optimal Formulation For Stability and Delivery

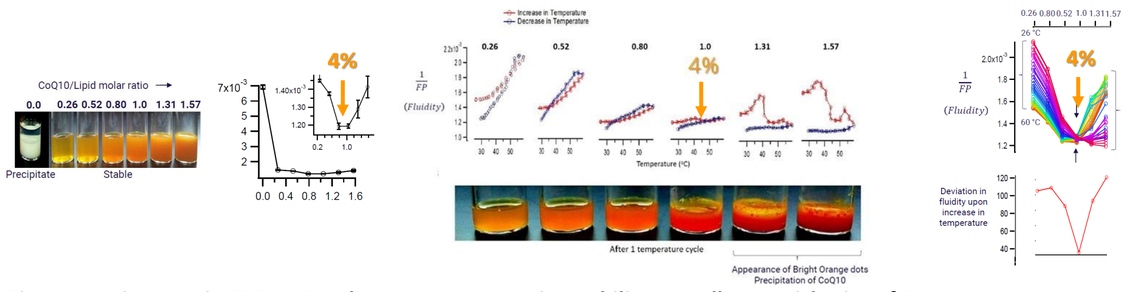


Figure 3: 4% CoQ10 in BPM31510 demonstrates superior stability as well at particle size of 40-60 nm

Figure 4: BPM31510 Enriches CoQ10 in Mitochondria

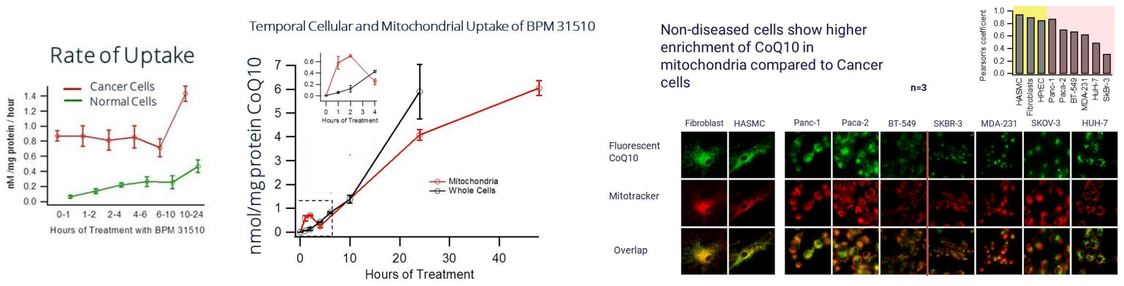
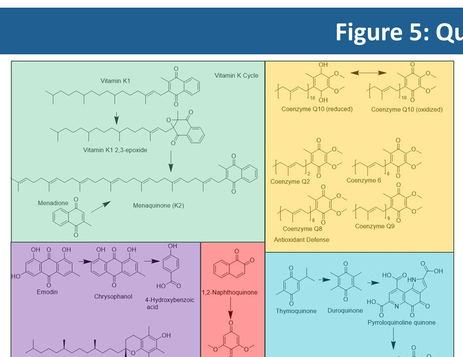
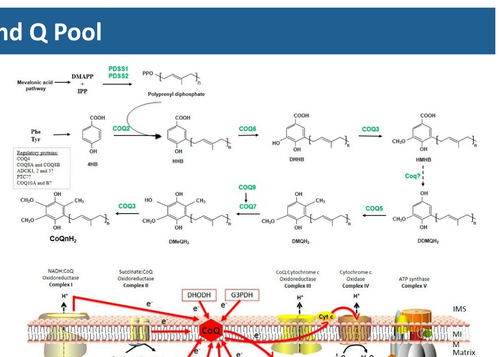


Figure 4: BPM31510 demonstrates enhanced cellular and mitochondrial uptake

Figure 5: Quinomics and Q Pool





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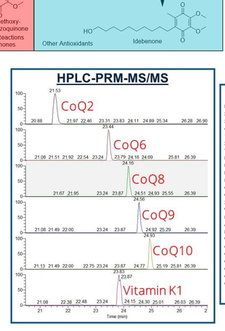

Metabolite Name	Formula	Lmk	m/z	Adduct
Pyrroloquinoline quinone	C14H6N2O8	72909-34-3	329.00486	[M+H]+
2,6-dimethoxy-1,4-benzoquinone	C8H8O4	26547-64-8	169.05008	[M+H]+
2,3-dihydroxy-1,4-benzoquinone	C6H4O4	615-94-1	141.01878	[M+H]+
4-Hydroxybenzoic acid	C7H6O3	99-96-7	137.02385	[M-H]-
1,2-naphthoquinone	C10H6O2	524-42-5	159.04459	[M+H]+
2-Methoxy-1,4-naphthoquinone	C11H8O3	2348-82-5	189.05516	[M+H]+
Thymoquinone	C10H12O2	490-91-5	165.09154	[M+H]+
Hexadione	C10H12O2	58-27-5	173.06022	[M+H]+
Duroquinone	C10H12O2	527-17-3	165.09154	[M+H]+
Idebenone	C19H30O5	58186-27-9	339.21715	[M+H]+
Emodin	C15H10O5	518-82-1	269.04505	[M-H]-
Coenzyme Q2	C19H26O4	606-06-4	319.19003	[M+H]+
Chrysophanol	C15H10O4	481-74-3	253.05013	[M-H]-
2,6-Di-tert-butyl-P-benzoquinone	C14H20O2	719-22-2	221.15414	[M+H]+
Vitamin K1 2,3-epoxide	C31H46O3	25486-55-9	465.33691	[M+H]+
D-alpha-tocopheryquinone	C29H50O3	7559-04-8	447.38581	[M+H]+
Alpha-Tocopherol	C29H50O2	59-02-9	431.38689	[M+H]+
Menaquinone (K2)	C31H46O2	863-61-6	451.33721	[M+H]+
Vitamin K1	C31H46O2	12001-79-5	451.35759	[M+H]+
Coenzyme Q6	C39H54O4	1065-31-2	608.4079	[M+NH4]+
Coenzyme Q8	C49H74O4	2284-40-3	746.5651	[M+NH4]+
Coenzyme Q9	C54H82O4	303-97-9	812.6557	[M+NH4]+
Coenzyme Q10 (reduced)	C59H92O4	992-78-9	862.72305	[M+NH4]+
Coenzyme Q10	C59H90O4	303-98-0	880.7183	[M+NH4]+
Coenzyme Q10 (deuterated (d9))	C59D9H81O4	n/a	889.77478	[M+NH4]+

30 compounds and growing

Quinomics: The simultaneous and comprehensive analysis of quinone metabolites, including co-enzyme Q10, its precursors, breakdown products, and related compounds impacting the Q pool

Figure 6: Assessment Of Quinones Following BPM31510-IV Treatment in Advanced GBM Patients

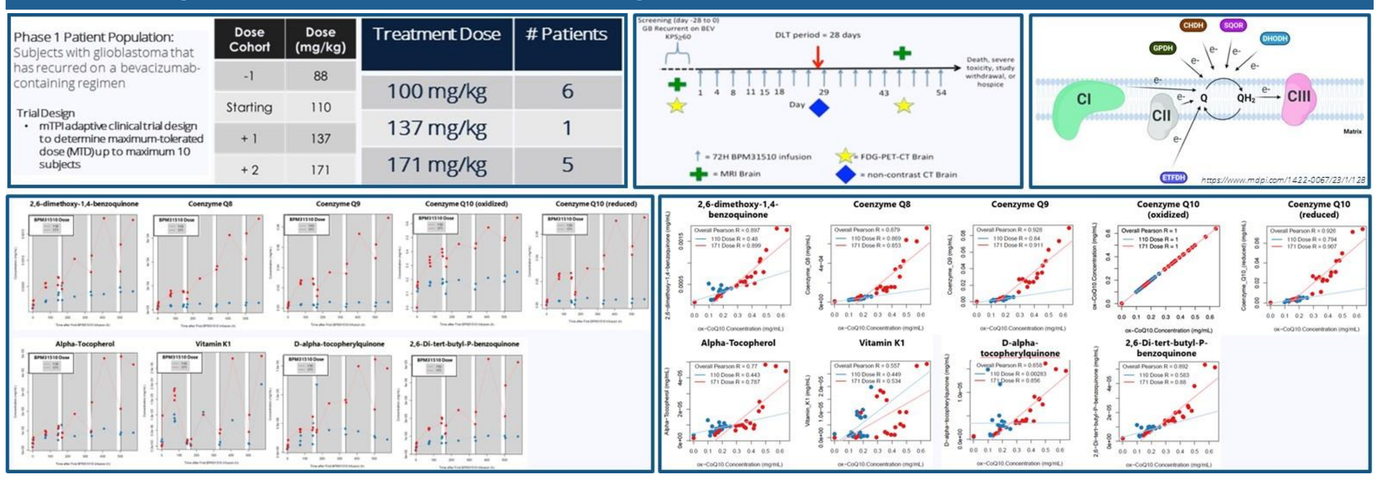

Figure 6: BPM31510 clinical trial quinomics analysis reveal dose dependent impact on quinone pharmacodynamics

Figure 7: Spatial OMIC Imaging To Achieve Assessment of Single Cell Metabolism In Vivo and Mitochondrial Fingerprinting

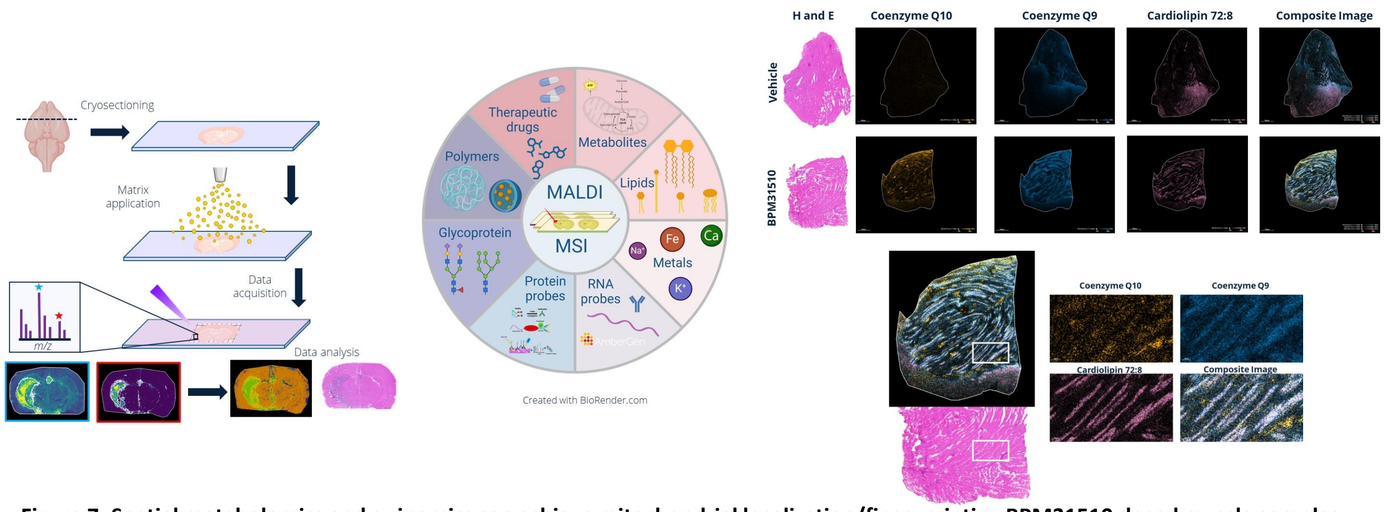

Figure 7: Spatial metabolomics and quinomics can achieve mitochondrial localization/fingerprinting BPM31510 dosed muscle samples

Figure 8: Spatial Omic and Quinomic Analysis in Experimental Glioblastoma Model Using BPM31510

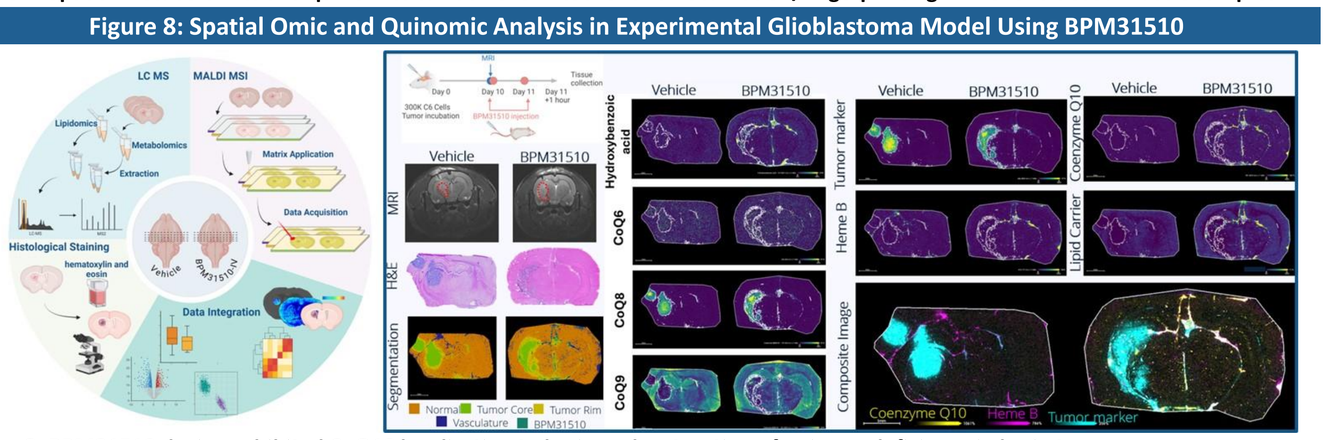

Figure 8: BPM31510 dosing exhibited CoQ10 localization to brain and restoration of quinone deficiency in brain tumor

Figure 9: Spatial Metabolomic Assessment of Brain/Tumor in Rats Treated with BPM31510 (TCA and Glycolysis Impact)

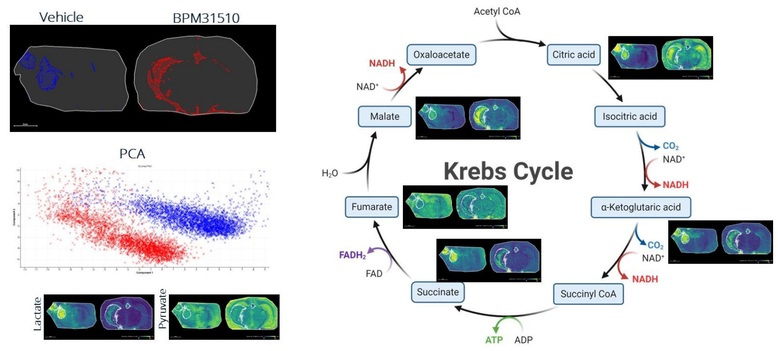

Figure 9: BPM31510 treatment demonstrated metabolic effect across the blood brain barrier on glycolytic metabolites as well as critical TCA metabolites (differentially in tumor vs brain) due to its mitochondrial localization and regulation in the electron transport system

Figure 10: BPM31510 Exhibits Superior Tissue Distribution Compared to Oral CoQ10 in a Two-Week Study

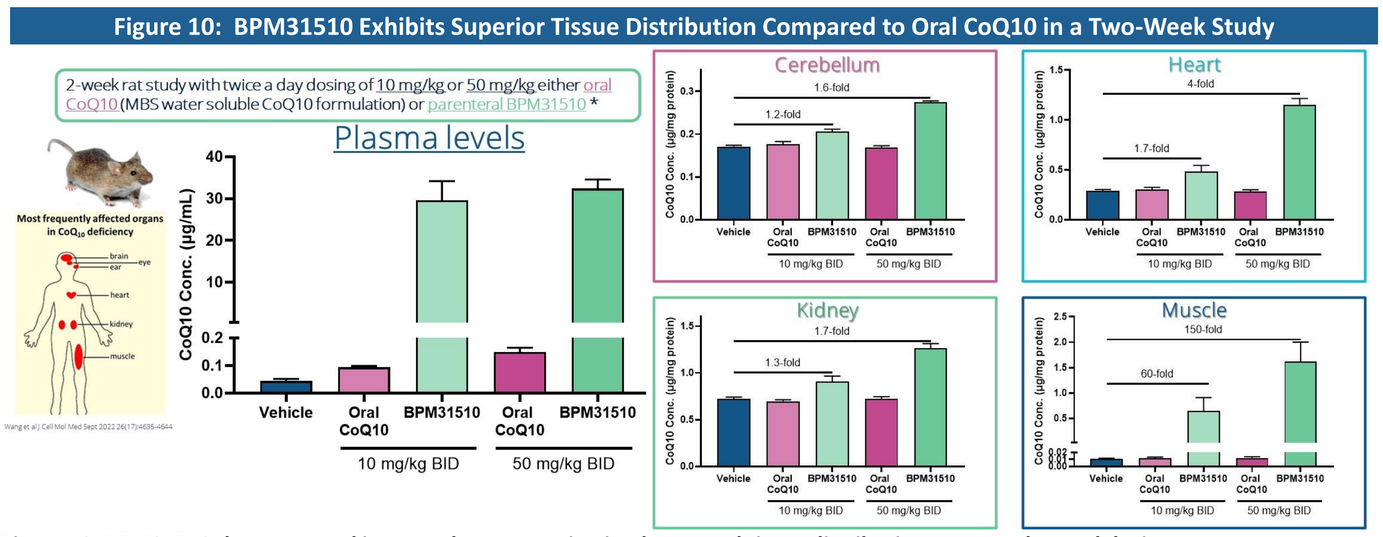

Figure 10: BPM31510 demonstrated increased concentration in plasma and tissue distribution compared to oral dosing

Figure 11: Treatment of CoQ2 Mutant Fibroblasts to Restore CoQ10 and ATP Levels in BPM31510 Treated Cells

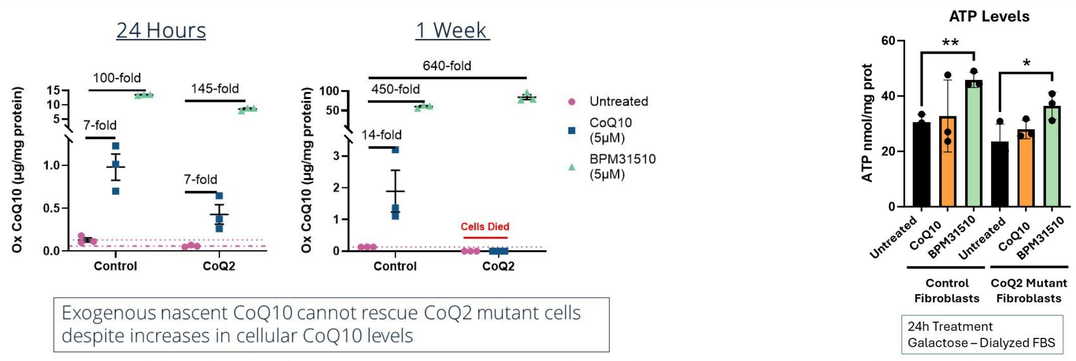

Figure 11: BPM31510 exhibited a significant increase in CoQ10 uptake and impact in mitochondrial ATP production compared to CoQ10 in human CoQ2 mutant fibroblast cells, which have severe metabolic CoQ deficiency

Conclusions

- **CoQ10 inherently has poor solubility, stability, bioavailability, and cellular uptake, thus BPM31510 (nanoparticle formulation of oxidized CoQ10) was developed to create a stable formulation with a well-tolerated safety profile that had superior bioavailability and uptake into mitochondria.**
- **To investigate the effect of BPM31510 (a highly bioavailable form of CoQ10 which is being developed for oncology and mitochondrial diseases) we created a bioanalytical and spatial quinomics platform that provided pharmacodynamic assessment as well as a spatial metabolomic workflow to characterize metabolism.**
- **BPM31510 demonstrated clear effects on mitochondrial metabolism (as demonstrated by spatial metabolomics and mitochondrial ATP generation) and superior tissue distribution than CoQ10 without optimized formulation.**
- **Utilizing quinomics and spatial OMICS technologies allowed for the comprehensive metabolic characterization of BPM31510 for the treatment of oncology and mitochondrial disease indications, demonstrating its potent therapeutic and mechanistic activity in enhancing mitochondrial function compared to oral CoQ10.**