HSP90 Inhibition as a Dual Therapeutic Strategy for Glioblastoma and Brain Tumour-Related Epilepsy

Eva McParland[1], Ms Zoe Hoysted[2], Dr Kate Connor[2]

[1] School of Medicine, Trinity College Dublin, Dublin [2] Discipline of Physiology, School of Medicine, Trinity College Dublin, Dublin.

Background

Glioblastoma (GBM) is the most aggressive brain cancer (glioma) in adults. Median survival remains 14–18 months and five-year survival around 5%, despite maximal surgical resection, radiotherapy, and temozolomide (chemotherapy). Major contributors to treatment resistance include infiltration beyond visible margins and cellular heterogeneity. Brain tumour-related epilepsy (BTRE) affects up to 90% of low-grade and nearly 50% of GBM patients, profoundly impacting quality of life[1]. BTRE arises through excess glutamate, reduced GABA signalling, and neuroinflammation. Standard antiseizure medications often fail due to distinct BTRE resistance mechanisms and drug interactions with anticancer therapies[2]. To address this clinical need, this research interrogates a chaperone protein called Heat Shock Protein 90 (HSP90). HSP dysregulation has not only been linked to tumour proliferation, but also to drug-resistant epilepsy. Nevertheless, the role of HSPs in BTRE remains unexplored. Establishing this link between HSP and BTRE has the potential to identify a novel therapeutic target capable of addressing both tumour progression and seizure burden[3].

Objective

This study aimed to investigate whether HSP90 inhibition reduces glioma-associated seizures and/or slows tumour progression in GBM.

Methods

1. Gene expression patterns of heat-shock proteins were analyzed in publicly available GBM samples via Ivy GAP and GlioVis databases.
2. Cell viability assays (MTT) measured HSP90 inhibitor impact alone and combined with cannabidiol (CBD) on CT2A murine glioma cells at 24, 48, and 72 hours. (Figure 1)
3. Bliss synergy analysis assessed drug interactions between the heat shock inhibitors and CBD [4].
4. Local field potential recordings in acute mouse brain slices (400 µm) monitored 4-aminopyridine-induced ictal-like activity in medial entorhinal cortex before, during, and after KUNB31 (1 µM) treatment, followed by washout.

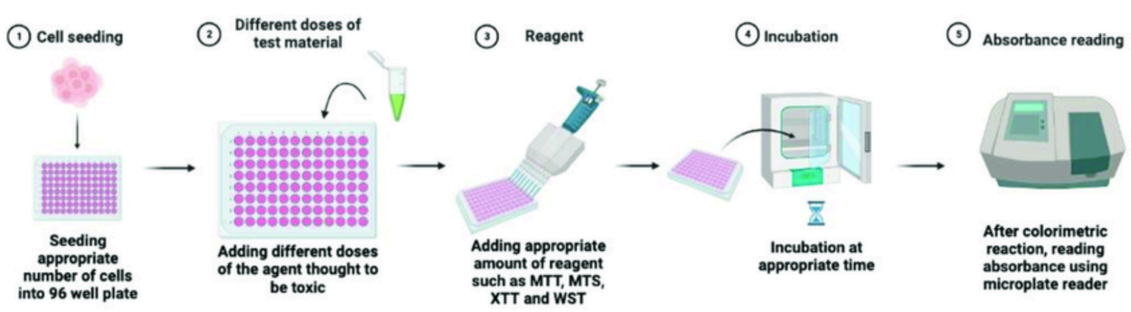

Figure 1: Cell viability assay workflow

Results

HSP90B1 expression correlated with poorer survival in human GBM (HR=0.61, 95% CI 0.42–0.88; p=0.0084). Expression was also examined in relation to various gene states.

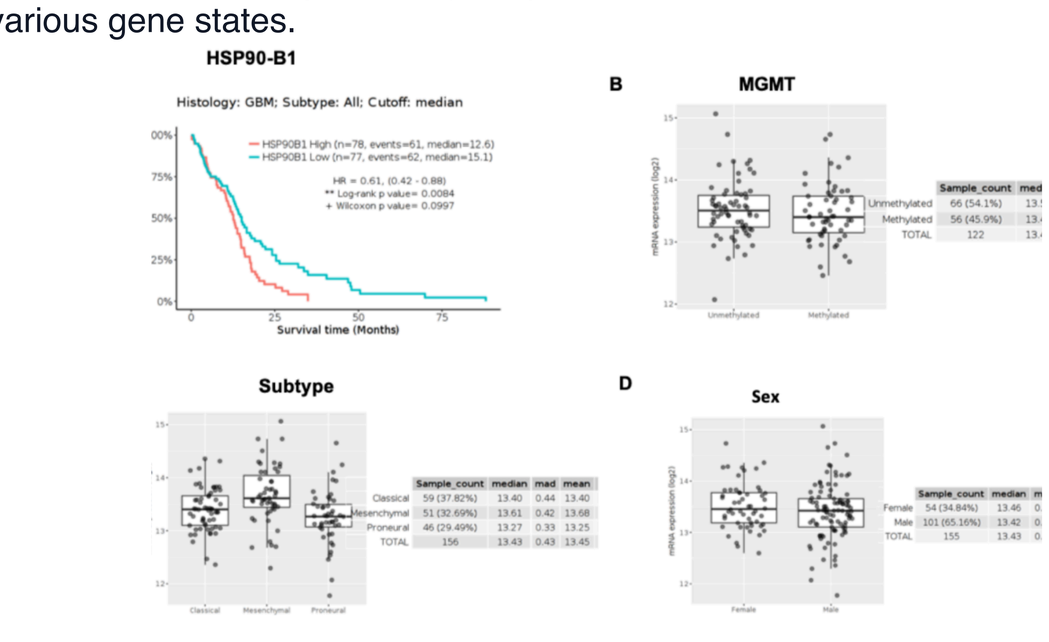

Figure 2: *HSP90B1 expression and clinicopathological associations in human glioblastoma. (A) Kaplan–Meier survival analysis comparing patients with high (n = 78) and low (n = 77) HSP90B1 expression, stratified using the median expression value. (B) HSP90B1 mRNA expression according to MGMT promoter methylation status, comparing unmethylated (n = 66) and methylated (n = 56) tumours. (C) HSP90B1 mRNA expression across classical (n = 59), mesenchymal (n = 51) and proneural (n = 46) GBM transcriptional subtypes. (D) HSP90B1 mRNA expression according to patient sex, comparing female (n = 54) and male (n = 101) patients. .*

Results

HSP90 inhibition significantly reduced CT2A glioma cell viability, with strongest effects at 72 hours (onalespib+CBD: p=0.0006). Bliss analysis indicated predominantly additive rather than synergistic interactions (scores: 3.542 at 24h, 0.835 at 72h). Preliminary electrophysiology showed KUNB31 reduced ictal event duration and magnitude.

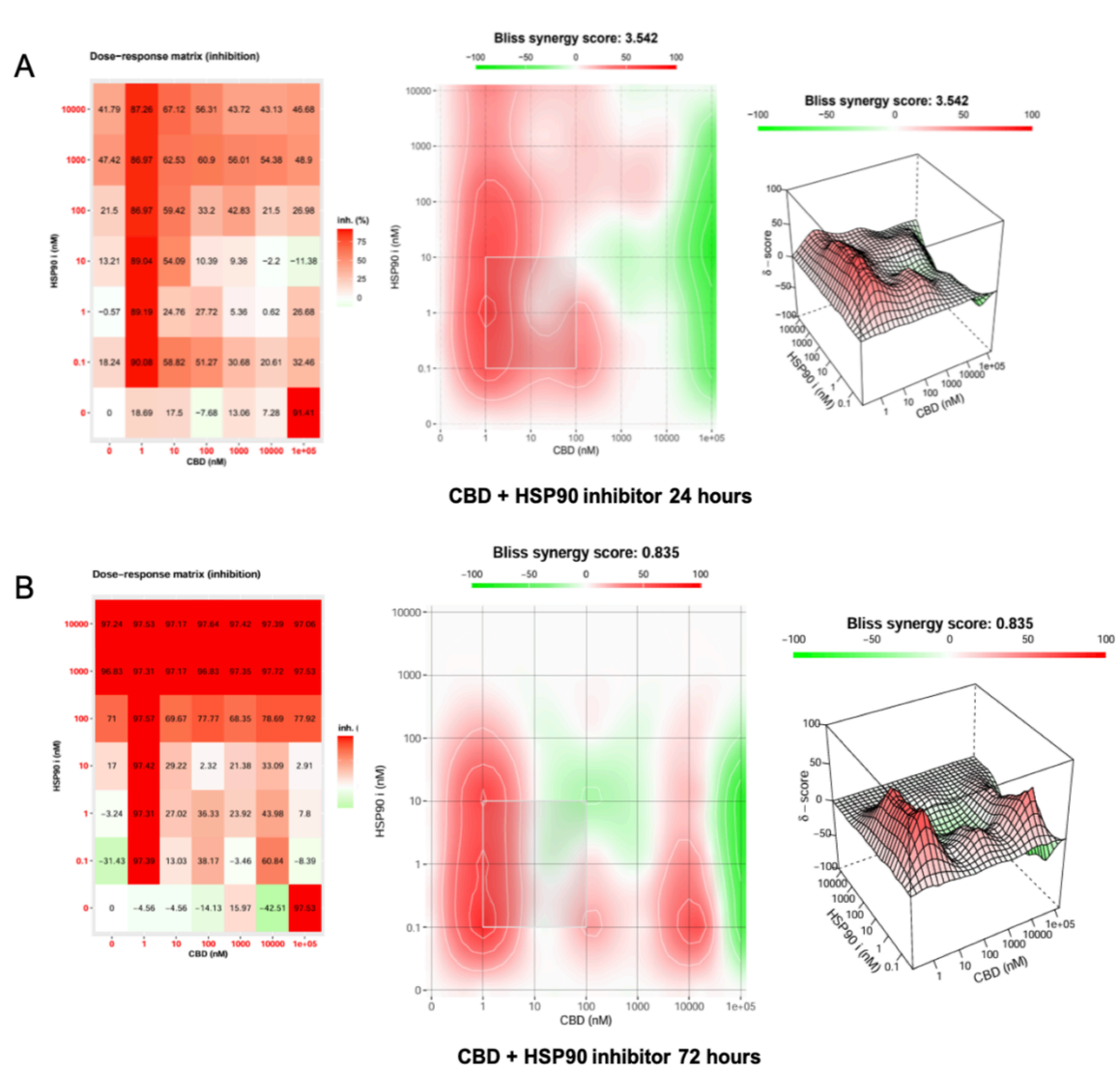

Figure 3: Bliss synergy assay of combined HSP90 inhibition and CBD treatment in CT2A glioma cells. CT2A cells were treated with increasing concentrations of the HSP90 inhibitor and cannabidol (CBD), and drug interactions were assessed using the bliss independence model at 24 hrs (top) and 72 hrs (bottom). Dose-response matrices on the left show percentage inhibition across individual drug combinations. Positive synergy scores indicate synergy.

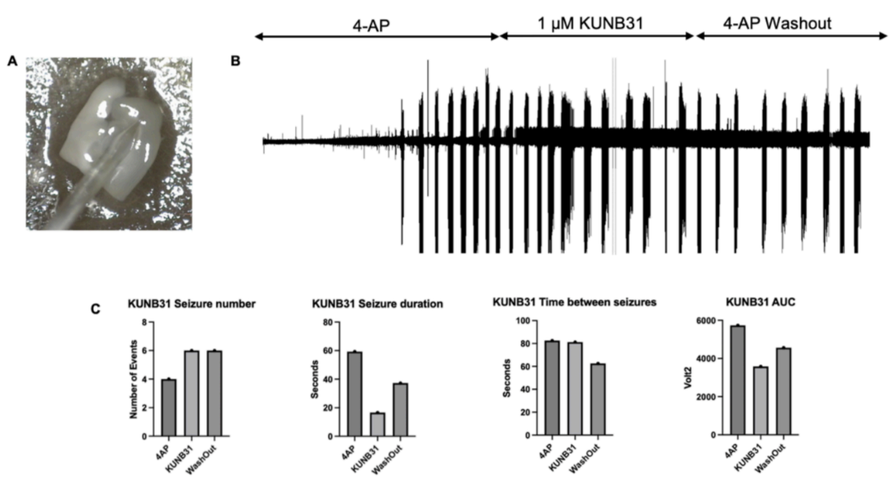

Figure 4: Effect of KUNB31 on 4-AP octal-like activity in acute mouse brain slices. (A) Representative image showing placement of the recording electrode in the mEC. (B) Representative local field potential (LFP) recording illustrating 4-AP-induced spontaneous ictal-like activity. (C) Quantification of electrophysiological parameters during 4-AP baseline, treatment with KUNB31. Data is presented descriptively from preliminary recordings

Conclusions

- Findings provide preliminary evidence that HSP90β may represent a dual therapeutic target in GBM, potentially influencing both tumour viability and seizure activity.
- Critically, HSP90B1 expression predicts poorer survival in GBM datasets.
- Selective HSP90β inhibition with KUNB31 reduced glioma cell viability and ictal-like activity duration.
- Further validation with additional biological replicates and tumour bearing tissue is required. Future investigations may involve strategies with epigenetic agents to target glioma cell heterogeneity.

Acknowledgements

The authors acknowledge funding from the Laidlaw Undergraduate Leadership and Research Programme.

References

[1] You et al., 2021; [2] Vacher et al., 2021; [3] Hossain et al., 2021., [4] Ianevski et al., 2017