Decoding and overcoming Temozolomide resistance through CRISPR/Cas technologies

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Supplementary material

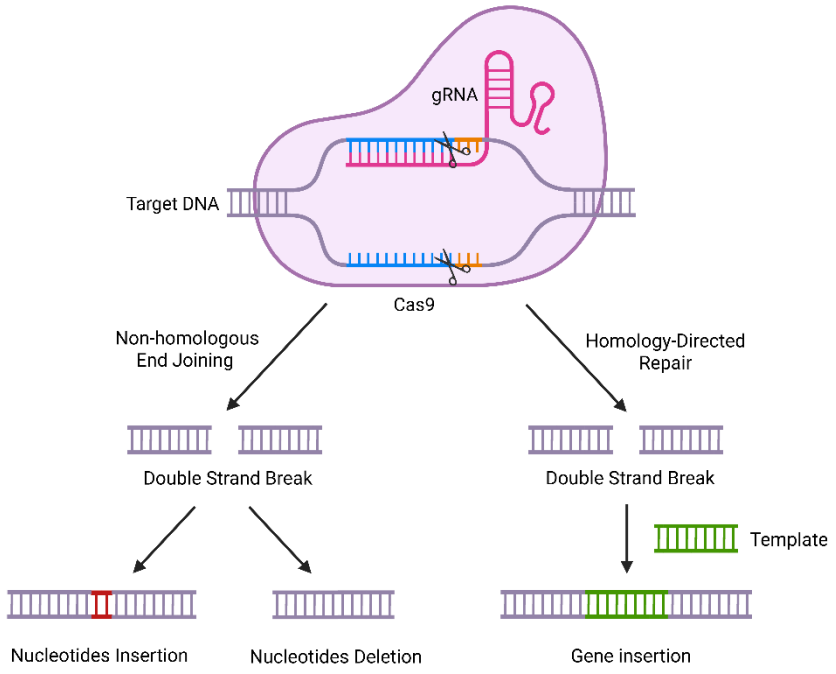

Figure S1. CRISPR/Cas9 mechanism in genome editing. The gRNA directs Cas9 to a complementary DNA sequence, where Cas9 introduces a double-strand break. The cell repairs the cleavage either by non-homologous end joining, which

commonly produces mutations, or by homology-directed repair, which can use a donor template for precise sequence modification. gRNA (guide RNA). Adapted from Guan et al., 2016 and Marques *et al., 2026*. Created with BioRender.com.

Table S1. Inclusion and exclusion criteria. **Key**: TMZ (Temozolomide).

Inclusion criteria	**Exclusion criteria**
1. Studies using CRISPR/Cas or related CRISPR/Cas-based gene editing systems. 2. Studies involving brain tumours. 3. CRISPR/Cas systems utilized to investigate, map, or modulate mechanisms of TMZ resistance/sensitivity or 4. CRISPR/Cas used to generate *in vitro* (3D/organoid) or *in vivo* brain tumour models. 5. Original research articles or protocols with experimental data.	1. Studies focusing exclusively on brain tumour biology (*e.g.,* tumorigenicity, stemness, proliferation, and invasion) without assessment of therapeutic response (TMZ) or novel model validation. 2. Studies focusing exclusively on chemotherapeutic drugs or therapies other than TMZ. 3. CRISPR/Cas interventions where the genetic modification is completely unlinked to TMZ therapeutic outcomes or modelling dynamics (*e.g.,* used only as co-treatment or for gene-editing therapy). 4. Brain tumour models generated by methods other than CRISPR/Cas (*e.g.,* chemical or viral induction only). 5. Reviews.

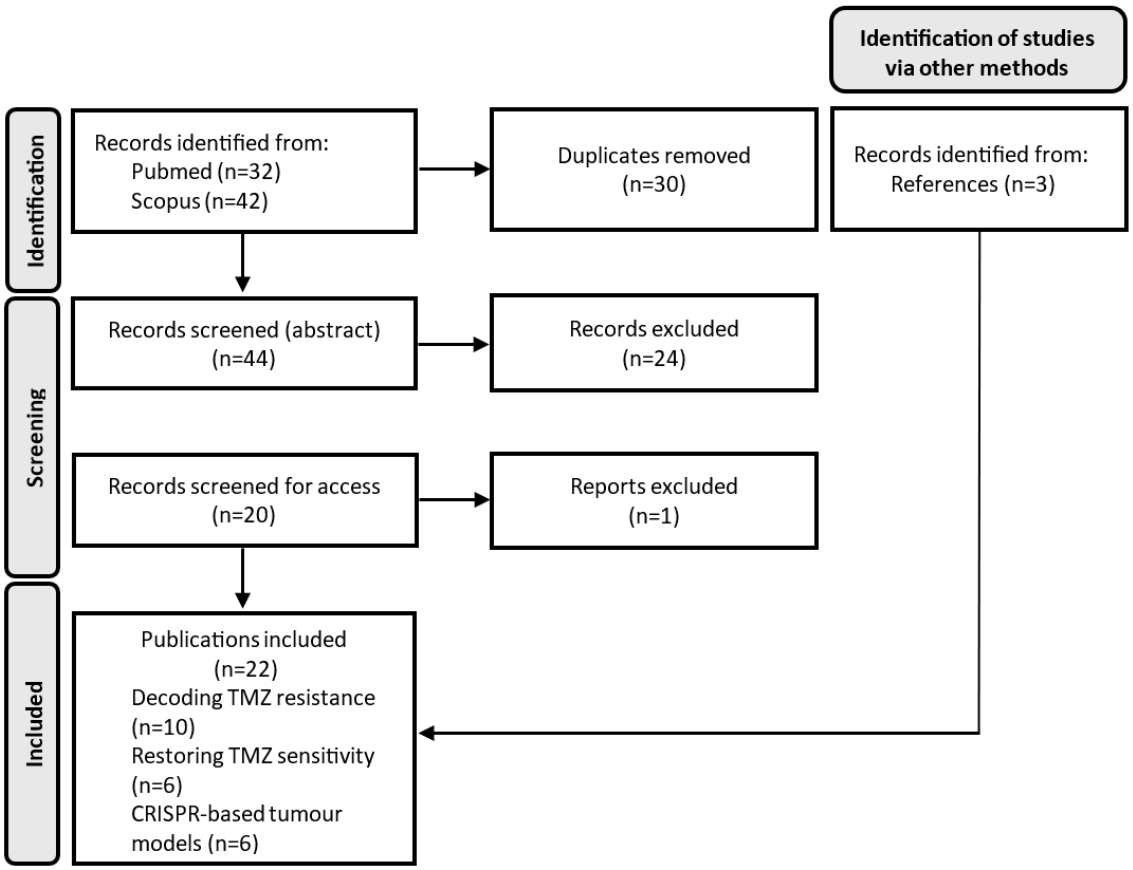

Figure S2. PRISMA flow diagram.

References

[1] Guan L, Han Y, Zhu S, Lin J. Application of CRISPR-Cas system in gene therapy: Pre-clinical progress in animal model. DNA Repair (Amst) 2016;46:1–8. https://doi.org/10.1016/j.dnarep.2016.07.004.

[2] S. Marques B, Vitorino C, V. Ventura F. CRISPR Applications in HIV Management - Prevention, Diagnosis, Monitoring and Treatment. Curr HIV/AIDS Rep 2026;23. https://doi.org/10.1007/S11904-025-00769-6.