Supplementary Figure legends

Supplementary Figure 1: Analysis of recurrent tumors after HSV-TK/Ganciclovir treatment (A) Representative MRI (T2 RARE) images are shown. valGCV was given for 3 weeks and naïve animals were left untreated. Solid tumor regions are marked with dashed lines. (B) Western blot analysis for some key GBM markers in lysates derived from tumor cells of recurrent versus primary tumors (2 different animals each group; S= suicide gene therapy group)

Supplementary Figure 2: MRI of recurrent tumors after HSV-TK/valGCV treatment (A) MRI (T2 RARE) images of the apparently cured animal after long-term treatment with valGCV. **(B)** MRI (T2 RARE) images of two distant recurrences near the brain stem. 'Week' refers to the period passed after the start of valGCV treatment. Yellow arrowheads point towards the hazy contour of the tumor mass.

Supplementary Figure 3: No significant difference between cyclic and continuous administration of valGCV in terms of survival benefit. (A) Representative MRI (T2 RARE) images are shown. The solid tumor regions are marked with dashed lines. (B) Kaplan-Meier survival analysis shows no difference between the cyclic and continuous treatment group.

Supplementary Figure 4: Immunhistochemical/Molecular analyses of recurrent versus primary tumors. (A) Immunohistochemical stainings with antibodies for olig2, pMAPK and c-MET. (B) FISH with an EGFR/chromosome 7 probe in pink and green respectively

Supplementary Figure 5: RNA-seq data analysis of TK-valGCV treated xenografts compared to valGCV only controls. (A) Assessment of species-specific origin of the sequence reads. Graphical output from FastQ Screen after mapping fastq files of all the samples against several reference genomes. Reads that were mapped to the rat genome were less abundant than the human and the level of contamination was consistent among all the samples except VGCV_5, which was subsequently excluded from the downstream analyses. Color code for human is blue, rat is marigold and red refers to multiple hits. (B) Principal component analysis (PCA) of normalized RNA-seq data. Component 1 (PC1, x axis) represented 55.0% and PC2 (y axis) represented 18.0% of total variation in the data. TK/valGCV in red and valGCV in cyan.

Supplementary Figure 6: Transcriptional changes in xenografts after 3-month prodrug (TK+valGCV) treatment. (A) Heatmap of differentially expressed genes. (B) Volcano plot showing the significantly (padj<0.05) upregulated (blue) and downregulated (red) genes in the TK+valGCV group compared to the valGCV-only group. The arrow points to EGFR (violet) on the graph. (C, D) GSEA enrichment plots. The green curve shows the running Enrichment Score for the gene set reflecting its highest value positioned on extreme values (red dashed line). Vertical bars represent the gene contributing to the gene set and its position is relative to the defined ranked list. The ranked list metric plot indicates the gene correlation with the biolocal state. Genes showing high correlation with TK+valGCV group display high positive values on the left side (D) whereas genes showing high correlation with valGCV control group display low negative values on the right side (C). Panel C has been obtained using Gene Ontology – Biological Process gene sets and panel D using KEGG pathways gene sets.