



# Combination of the Anti-Angiogenic Adnectin™ BMS-844203 (CT-322) and Temozolomide Provides a Survival Advantage in an Intracranial Glioblastoma Model

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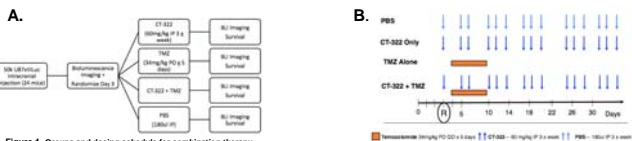
## CT-322 Combines Effectively with Chemotherapy to Inhibit Tumor Growth and Extend Survival

### INTRODUCTION

Current standard of care of glioblastoma, the most malignant form of brain tumor with a median survival just over 15 months, includes maximal safe resection, radiation therapy, and temozolomide chemotherapy. As vascular proliferation is a hallmark of glioblastoma, increasingly anti-angiogenic therapy is also used with recurrent glioblastoma as a salvage therapy with some success in inhibiting bulky tumor growth and reducing tumor-associated brain edema. We tested the hypothesis that upfront combination therapy with TMZ and the anti-angiogenic Adnectin™, BMS-844203 (CT-322), would demonstrate better tumor growth inhibition than either monotherapy alone in a human glioblastoma xenograft model. CT-322, the first Adnectin™ in the clinic, is a potent and specific antagonist of the VEGFR-2 pathway. Adnectins™ are a novel, proprietary class of targeted biologics derived from human fibronectin. The effects of therapy combination of CT-322 plus TMZ were evaluated by MRI imaging and histological evaluation of tumors, including vascular permeability, apoptosis, and microvascular proliferation.

### METHODS

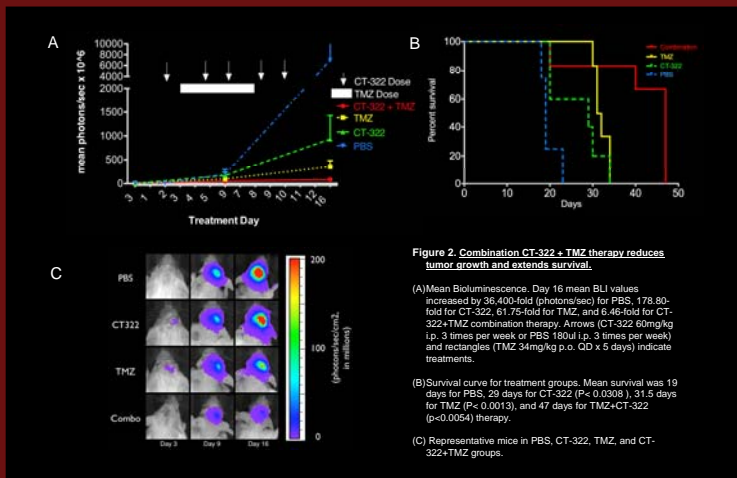
Fifty thousand U87VIII-fluc cells (human GBM cell line expressing firefly luciferase) were intracranially implanted in 24 adult NOD SCID mice. Bioluminescence imaging (BLI) was used at randomization and biweekly time points to follow tumor size. Mice were randomized into vehicle control (Phosphate Buffered Saline (PBS)), TMZ (34mg/kg QD x 5 days), CT-322 (60mg/kg x 3 times per week) and Combination (CT-322 + TMZ) groups of 6 mice. MR imaging was used to characterize treatment effects in select mice. Animals were followed for survival and sacrificed at the first sign of morbidity. A second set of 24 mice were sacrificed for histological analysis at day 7 or 14. Immunohistochemical staining against CD31, Ki67 or cleaved-caspase 3 was performed on paraffin-embedded brain tissue sections.



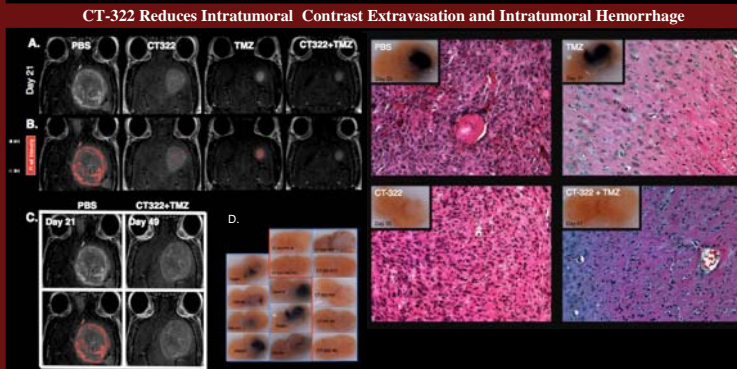
### RESULTS

- A. While TMZ or CT-322 monotherapy inhibits tumor growth relative to PBS, combination therapy (CT-322+TMZ) significantly improves tumor growth inhibition
  - Day 16 mean BLI values grew 179-fold with CT-322 monotherapy, 62-fold with TMZ monotherapy, and 6.5-fold with CT-322+TMZ.
- B. Combination therapy (CT-322+TMZ) significantly extends survival
  - Mean survival of TMZ extended from 31.5 days to 47 days (p<0.015) with addition of CT-322.
- C. CT-322 reduces microvascular proliferation, based on CD-31 staining
  - Qualitative analysis, quantitation to follow
- D. CT-322 reduces intra-tumoral contrast extravasation, independently of tumor size, consistent with vascular normalization
- E. CT-322 reduces intra-tumoral necrosis and hemorrhage

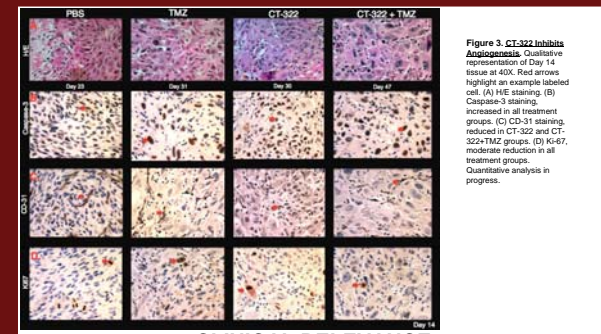
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\* C.E. Sanchez, and A. Sahin Contributed Equally to this Work.



**Figure 2. Combination CT-322 + TMZ therapy reduces tumor growth and extends survival.**  
(A) Mean Bioluminescence. Day 16 mean BLI values measured by 38,408-fold (photons/sec) for PBS, 179.80-fold for CT-322, 61.75-fold for TMZ, and 4.46-fold for CT-322+TMZ combination therapy. Arrows (CT-322 60mg/kg i.p., 3 times per week or PBS 180ul i.p., 3 times per week) and rectangles (TMZ 34mg/kg p.o. QD x 5 days) indicate treatments.  
(B) Survival curve for treatment groups. Mean survival was 19 days for PBS, 22 days for CT-322 (P=0.0008), 31.5 days for TMZ (P=0.0013), and 47 days for TMZ+CT-322 (p<0.0054) therapy.  
(C) Representative mice in PBS, CT-322, TMZ, and CT-322+TMZ groups.



**Figure 4. CT-322 Reduces tumor contrast extravasation, independently from tumor size and reduces gross intratumoral hemorrhage.** (A) Day 21 T1 post-contrast MRI across treatment groups, demonstrating diffusely heterogeneous contrast enhancement in PBS and TMZ but not in CT-322 or CT-322+TMZ groups. (B) Day 21 T1 post-contrast MRI with signal > 11,500 represented in red, representing contrast enhancement, and equivalent windowing across groups. Contrast enhancing tumor makes a large portion of PBS and TMZ treated tumor volumes, compared to a smaller degree of enhancement in the CT-322 tumor and no visible enhancement in the CT-322+TMZ tumor. (C) Similar representation comparing the Day 21 PBS and Day 49 CT-322+TMZ tumors, demonstrating that contrast enhancement is independent of tumor size. (D) Histology blocks at different survival dates in PBS and TMZ groups (left side) and CT-322+TMZ and CT-322 (right side). Gross hemorrhage is clearly visible in all tumors not treated with CT-322.



**Figure 3. CT-322 inhibits Angiogenesis.** Qualitative representation of Day 14 tissue at 40X. Red arrows highlight an example labeled cell. (A) IHC staining for Caspase-3 staining, increased in all treatment groups. (C) CD-31 staining, reduced in CT-322 and CT-322+TMZ groups. (D) Ki67, moderate reduction in all treatment groups. Quantitative analysis in progress.

### CLINICAL RELEVANCE

- The combination of CT-322+TMZ improved survival in mice bearing a human glioblastoma tumor. This provides support for the clinical strategy of using an anti-angiogenic agent as an upfront combined therapy for brain tumors and not solely as a salvage therapy.
- Vascular normalization may underlie the observed effects in animals treated with CT-322. Notably, in this study all animals treated with TMZ alone or PBS alone showed significant intratumoral hemorrhage as tumors increased in size, consistent with reduced vessel integrity. No animal receiving CT-322 demonstrated intratumoral hemorrhage. Furthermore, contrast enhancement was reduced in CT-322 treated animals, also suggesting microvascular stabilization.
- Animals treated with CT-322 and PBS alone demonstrated greater cellularity than mice treated with TMZ+CT-322, reflecting the direct cytotoxic effect of TMZ. However, we found that CT-322 mice lived longer than than PBS treated mice with similar tumor burdens; this may reflect a secondary benefit of anti-angiogenic therapy with CT-322, reduced peritumoral edema and reduced mass effect from a given tumor. Peritumoral edema is known to correlate with poorer survival in higher grade glioma patients.<sup>2</sup>

### CONCLUSIONS

The survival benefit of a combinatorial treatment using CT-322 and TMZ was superior to that of either agent alone. Tumor burden (BLI, MRI) and IHC analysis suggest that tumor growth suppression plus antiangiogenic effects may underlie the observed enhanced survival of the combo treatment.

### REFERENCES

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