An ENT2-dependent, cell-penetrating, and DNA-damaging autoantibody crosses the blood-brain barrier to target brain tumors

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Background

- Some autoantibodies penetrate live cell nuclei and have therapeutic potential.
- 3E10 is a lupus anti-DNA autoantibody that localizes to DNA in tumor environments and penetrates cells via the ENT2 nucleoside transporter.
- Inside the nucleus, 3E10 interferes with the DDR and kills HR-impaired cancer cells while sparing normal cells (**Fig 1**).

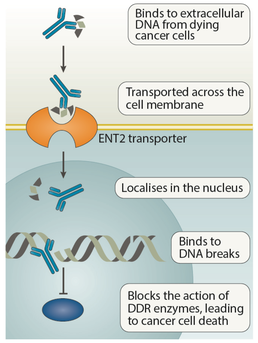


Fig 1. 3E10, ENT2, and the DDR.

Rationale for use of 3E10 against brain tumors

- PTEN loss is common in primary and metastatic brain tumors, yields defects in DNA repair, and predicts vulnerability to 3E10.
- ENT2 is expressed in brain endothelial cells (BECs), and 3E10 has previously delivered a linked heat shock protein to ischemic brain.
- We hypothesize that DX1, an optimized and humanized fragment of 3E10 (**Fig 2**), will cross the BBB to target brain tumors (**Fig 3**).

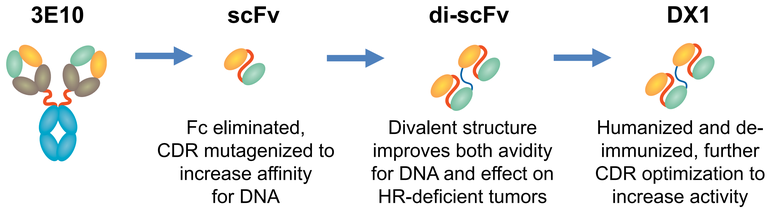
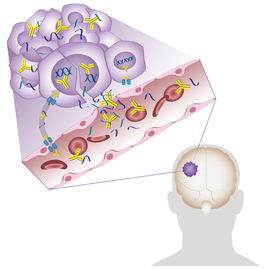


Fig 2: Evolving 3E10 into DX1 (Deoxymab or PAT-DX1).

Fig 3: Hypothesized mechanism of brain tumor-targeting by 3E10. 3E10/DX1 binds to DNA released by necrotic tumor cells and crosses the BBB through both local breaks and ENT2 in BECs.



Results

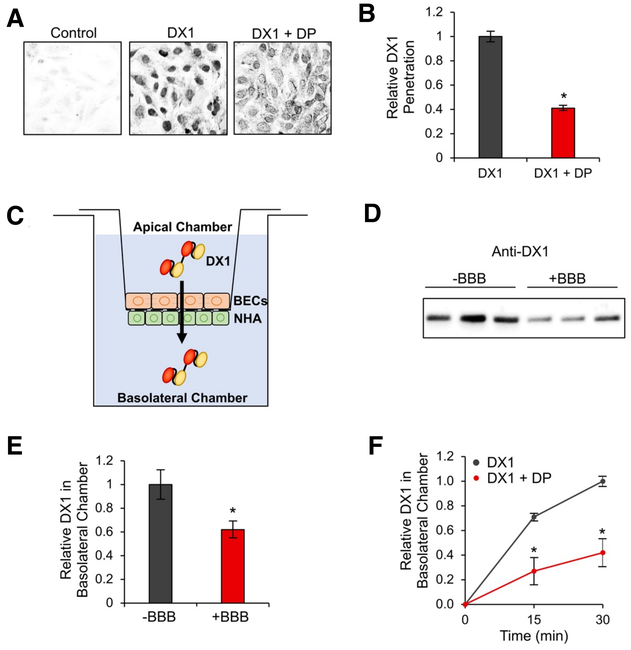


Fig 4. DX1 crosses a Transwell filter model of the BBB.

(**A,B**) An ENT2 inhibitor, dipyridamole (DP), blocks DX1 penetration into hCMEC/D3 BECs.

(**C,D,E**) Anti-DX1 western blots of basolateral chamber contents in Transwell filters (+/-hCMEC/D3 BBB) show that DX1 crosses an intact BBB model.

(**F**) DP inhibits DX1 crossing of the BBB model, consistent with ENT2-dependent transport.

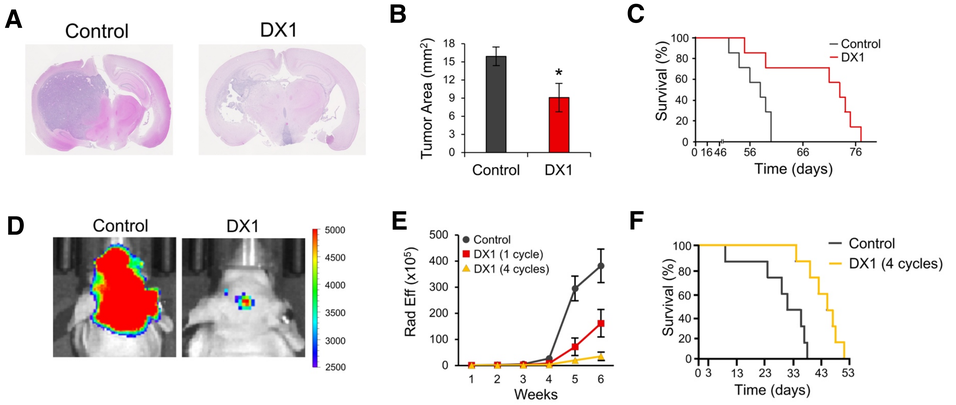


Fig 5. DX1 suppresses orthotopic PTEN-deficient GBM and breast cancer brain metastases.

(**A,B,C**) Orthotopic PTEN-deficient GBM tumors were established by inoculation of patient-derived glioma stem-like cells into the brains of immunodeficient mice. Mice were treated with tail vein injection of control (PBS) or DX1 (20 mg/kg) 3X/week. H&E stain for GBM area measurement at 9 weeks post-inoculation demonstrated suppression of tumor growth by DX1 (**A,B**). DX1 increased median survival to 73 days compared to 58 days in control mice (P=0.02) (**C**).

(**D,E,F**) Breast cancer brain metastases were generated in immunodeficient mice by intracardiac injection of luciferase-expressing 231-BR cells. Mice were treated with tail vein injection of control (PBS) or DX1 (20 mg/kg) 3X/week for 1 or 4 weeks. IVIS images at week 5 and radiance efficiency plots show suppression of brain metastases by DX1 (**D,E**). DX1 increased median survival to 45 days compared to 31 days in control mice (P<0.002) (**F**).

Summary/Key Points

- DX1 is a re-engineered anti-DNA autoantibody that inhibits the DDR, is synthetically lethal to HR-deficient cancer cells, and crosses the BBB in an apparent ENT2-dependent manner
- Studies to date demonstrate single agent activity of DX1 in multiple mouse models of HR-deficient cancer, now including brain tumors, with no evidence of off-target toxicity.
- DX1 has potential applications in cancer therapy as a single agent, as a molecular delivery vehicle, and in combination with synergistic DDR inhibitors and/or DNA-damaging agents.