Deoxymab-1, a Re-engineered DNA-Damaging Lupus Autoantibody, Shows Promise in the Treatment of DNA Repair-Deficient Malignancies

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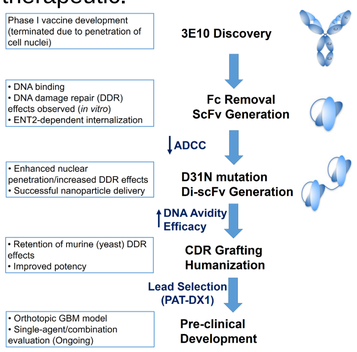
Background

A lupus autoantibody, 3E10, has previously been demonstrated to penetrate cells and localize to nuclei. 3E10 is synthetically lethal to homology-directed DNA repair (HDR) deficient cells, and spares repair-proficient cells and tissues. It is thought that accumulation of DNA breaks in HDR-deficient cells is responsible for associated cell death. To date, no evidence of off-target toxicity has been observed following 3E10 administration, rendering 3E10 attractive as a future therapeutic.

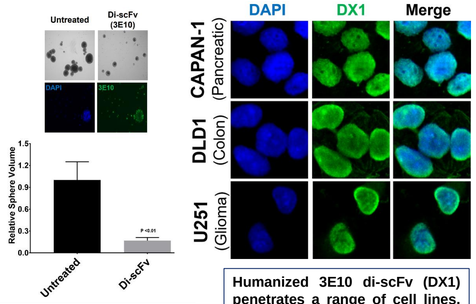


Chronological milestones of Deoxymab-1 (PAT-DX1) evolution from a whole antibody to a di-scFv

The present work reports the *in vitro* and *in vivo* biological effects of PAT-DX1 (hereafter referred to as DX1). We compare DX1 *in vitro* and *in vivo* performance to another DNA damage repair agent, olaparib (a PARP inhibitor), and evaluate their synergism in a panel of *in vitro* models.

DX1 Penetrates a Range of Cell Lines

Previously, 3E10 di-scFv (murine) was found to penetrate a range of cell lines. For example, 3E10 penetrated GS5 glioblastoma stem cells and suppressed sphere growth **(left panel)**.



3E10 di-scFv penetrates and inhibits GS5 growth *in vitro* (P <0.01)

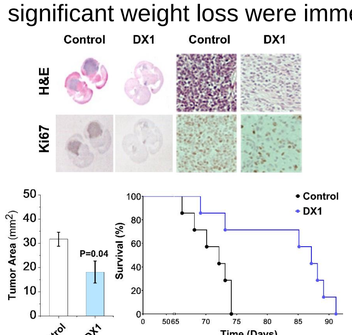
Humanized 3E10 di-scFv (DX1) penetrates a range of cell lines. Confocal micrographs of protein L (green) immunodetection of DX1 and DAPI (blue) counterstain.

Following humanization, DX1 retained the ability to penetrate cells and localize to cell nuclei.

Our previous work had indicated *in vivo* efficacy of murine di-scFv. Hence, DX1 was assessed in an *in vivo* model, and a panel of *in vitro* cell lines.

Assessment of DX1 *in vivo* Efficacy

An orthotopic GBM patient-derived xenograft (PDX) model was dosed with either a control vehicle (PBS), or DX1 (20 mg/kg three times weekly). All subjects were monitored for weight loss, and signs of cytotoxicity. Any subjects exhibiting neurological symptoms or significant weight loss were immediately euthanized.

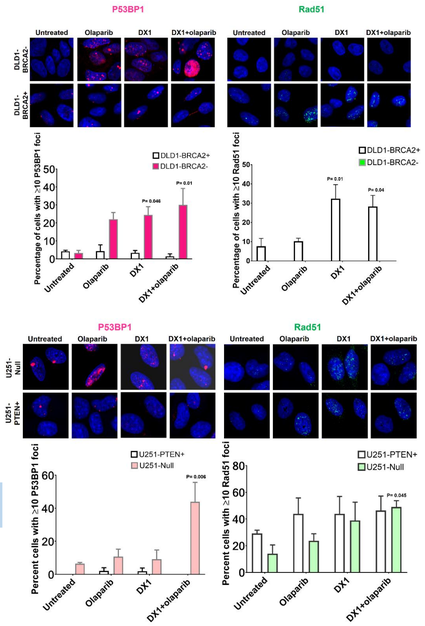


Treatment with DX1 reduces tumor growth and improves survival outcomes in a GBM PDX model. Representative macroscopic and microscopic evaluation of DX1 effects in a GBM PDX model using H&E, Ki67 (positive cells are brown).

- DX1 treatment improved survival outcome (n=7)/reduced tumor size (n=3).
- Based on the readout from the *in vivo* study, *in vitro* assessment of DX1 was performed to inform potential combination strategies.

The Effect of DX1 on Foci Accumulation

In the present work, the impact of DX1 (10 µM), olaparib (5 nM), and combination treatment was assessed in a panel of HDR-proficient and deficient cell lines.

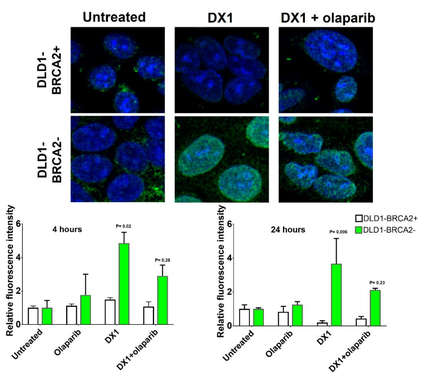


- The percentage of P53BP1-positive cells increased in HDR-deficient cells following 24 hour DX1 and combination treatment(s).
- Percent Rad51-positive cells increased in DLD1-BRCA2+ in response to DX1 and combination treatment (absent in DLD1-BRCA2-).
- Rad51 foci observed in both U251 cell lines, suggested another mechanism possible for U251-Null accumulation of P53BP1 foci.

The influence of DX1 on P53BP1 and Rad51 foci formation in a panel of cell lines Representative overlay images of P53BP1 (pink, **left)** and Rad51 foci **(green, right)** with DAPI **(blue)** counterstain for paired DLD1 **(top)** and U251 **(bottom)** cells treated for 24 hours.

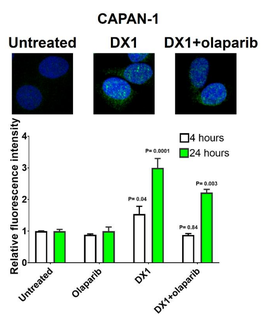
The Effect of DX1 on PARP Activity

To evaluate the mechanism contributing to increasing foci-positive cells for combination treatments, DX1 effects on pADPr levels were assessed as a marker of PARP activity.



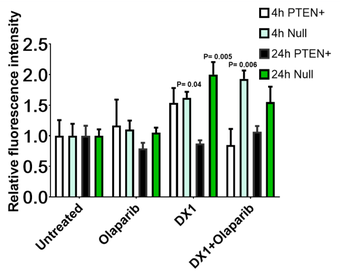
DX1 increases pADPr levels Representative overlays of pADPr **(green)** and DAPI **(blue)** staining following 24 hour DX1, and DX1 and olaparib treatment **(top)**, and relative pADPr fluorescence intensity following 4 and 24 hour incubations with DX1 **(bottom).**

Previous work with the murine di-scFv had indicated that 3E10 does not impact PARP1 expression in DLD1-BRCA2-deficient and proficient cell lines.



pADPr staining in CAPAN-1 cells Representative overlay images of pADPr (green) and DAPI (blue) staining following a 24 hour treatments **(top)**, and corresponding pADPr intensity following 4 and 24 hour treatments **(bottom)**.

Similar to DLD1-BRCA2- increased pADPr fluorescence observed with DX1/combinations in CAPAN-1 cells.

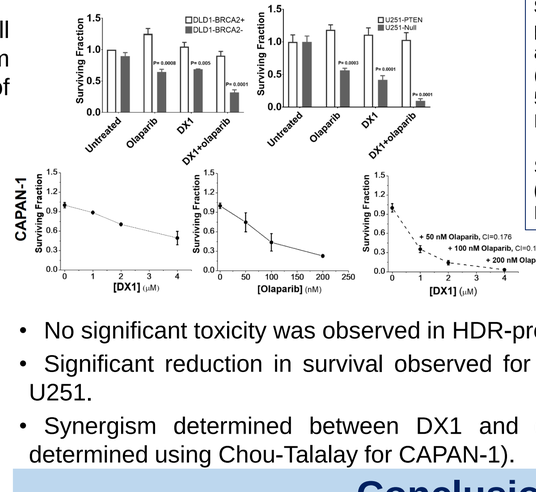


Increased pADPr intensity observed in BRCA2- DLD1 cells (DX1 versus untreated). Similar observations with PTEN-Null U251 and BRCA2-deficient CAPAN-1 cells.

Relative pADPr fluorescence (to control) for immunostained U251-PTEN+ and U251-Null following 4 and 24 hour treatments.

DX1 is Synthetically Lethal to HDR-Deficient cells

The impact of DX1, olaparib, or combination treatment on cell survival was investigated in a panel of cell lines.



Surviving fraction of DLD1 BRCA2-proficient and deficient cells (n=6) **(top, left)** and PTEN-proficient and deficient U251 cells (n=5) **(top, right)** treated with control media, 5 nM olaparib and 10 µM DX1, or a DX1/olaparib combination for seven days.

Surviving fraction of CAPAN-1 cells (n=3) **(bottom)** at various doses of olaparib and DX1 as single treatments, or in combination.

- No significant toxicity was observed in HDR-proficient cells.
- Significant reduction in survival observed for DLD1-BRCA2- and PTEN-deficient U251.
- Synergism determined between DX1 and olaparib (see combination indices determined using Chou-Talalay for CAPAN-1).

Conclusions

- DX1 shows great promise as a therapeutic option in the management of HDR-deficient cancers.
- Significant single-agent reduction in GBM tumor size and histological changes were noted following single-agent DX1 treatment in the pre-clinical PDX model.
- Evidence of synergy with olaparib indicated *in vitro*.
- DX1 potentially modulates PARP function, as evidenced by pADPr levels- further work is ongoing to probe the exact mechanism.

Ongoing & Future Work

- Currently work is ongoing to establish DX1 intracellular targets.
- Evaluation of DX1 efficacy in additional HDR-deficient pre-clinical models where previous *in vitro* data has indicated synthetic lethality.
- Establishing the sequence of events following cellular internalization of DX1, and the contributory mechanism responsible for observed *in vivo* growth suppression.
- Future work will also attempt to enhance olaparib BBB bioavailability, and investigate efficacy with DX1 in combination.
- *In vitro* PARP activation/inhibition assays & the impact of DX1 on PARP expression.

References

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