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.

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 LDL1-BRCA2- in response to DX1 and combination treatment (absent in LDL1-BRCA2-).
- Rad51 foci observed in both U251 cell lines, suggested another mechanism possible for U251-Null accumulation of P53BP1 foci.

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 olaparib treatment (top), and relative pADPr fluorescence intensity following 4 and 24 hour incubations with DX1 (bottom).

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.

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.

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.

References


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