Prevention of Base Excision Repair by TRC102 (Methoxyamine) Potentiates the Anti-Tumor Activity of Pemetrexed *in vitro* and *in vivo*

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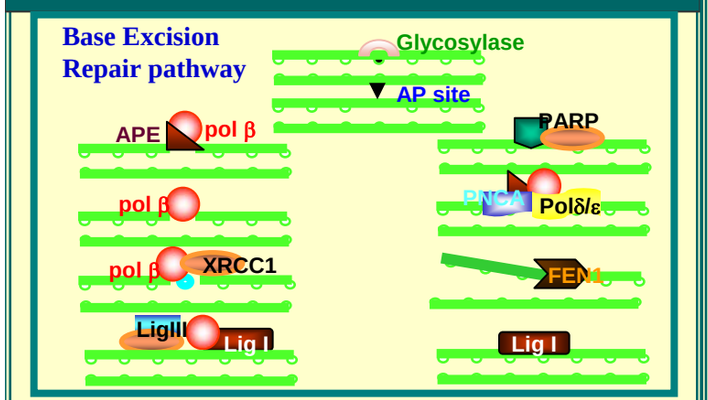
ABSTRACT

PURPOSE: TRC102 (methoxyamine) reverses resistance to alkylating agents by inhibiting base excision repair (BER; a mechanism of DNA repair), thereby increasing DNA strand breaks and potentiating the anti-tumor activity of alkylating agents without additional toxicity. Based on these data, TRC102 is currently being studied in combination with temozolomide in a Phase 1 trial. We hypothesized that nucleotide imbalances caused by the anti-folate pemetrexed would also produce AP sites recognized and repaired by BER, and that inhibition of BER by TRC102 would therefore improve the anti-tumor activity of pemetrexed. **METHODS:** Pemetrexed-induced apurinic/apyrimidinic (AP) sites and BER inhibition were quantified using an AP site assay *in vitro*. Single and double DNA strand breaks were quantified by the Comet assay *in vitro*, apoptosis was quantified by Annexin V staining, and anti-tumor activity was assessed in an *in vivo* xenograft study of subcutaneously implanted human lung, colorectal and breast cancer cells. **RESULTS:** AP sites increased proportionally with dose in pemetrexed–treated H460 lung cancer cells *in vitro*. TRC102 reduced the number of available AP sites in pemetrexed-treated cells (by 60-80%), indicating successful inhibition of BER. TRC102 treatment increased DNA strand breaks (2 fold increase versus treatment with pemetrexed alone) and apoptosis. TRC102 increased the activity of pemetrexed *in vivo* (tumor growth delay of 2 days in mice bearing H460 or A549 lung cancer xenografts treated with 150mg/kg pemetrexed alone versus 9 days in mice treated with 150mg/kg pemetrexed + 4mg/kg TRC102; p<0.05); *in vivo* systemic toxicity was not increased and TRC102 alone had no effect *in vitro* or *in vivo*. TRC102 also increased pemetrexed activity on HCT116 colorectal and MDA-MB-468 breast cancer xenografts. Moreover, the combination selectively up-regulated the BER proteins, uracil DNA glycosylase and polymerase β, which provides strong evidence that DNA damage induced by the drug combination induces BER. **CONCLUSION:** TRC102 effectively inhibits BER in cancer cells treated with pemetrexed. Inhibition of BER by TRC102 results in an increase in DNA strand breaks and apoptosis, and improved anti-tumor activity versus treatment with pemetrexed alone. Given its preclinical safety and efficacy profile, clinical study of TRC102 combined with pemetrexed is warranted.

Base Excision Repair Pathway



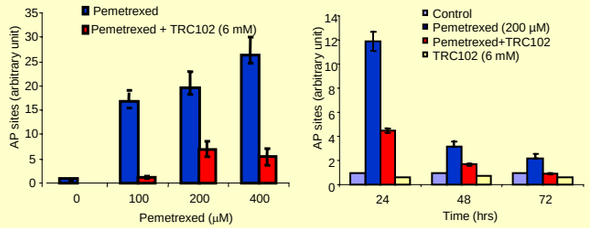
Hypotheses:

1) Pemetrexed inhibits several key enzymes in the de novo pathways of pyrimidine and purine biosynthesis, leading to nucleotide pool imbalances, which favor the Incorporation of mismatched bases to initiate base excision repair (BER).

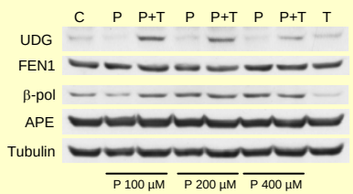
2) TRC102 blocks BER and enhances cytotoxicity of pemetrexed.

RESULTS

AP Sites Detected in H460 Cells After Treatment with Pemetrexed and TRC102



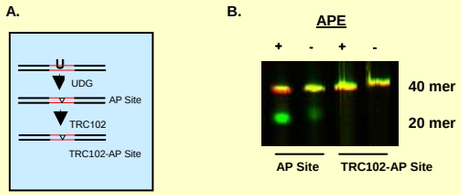
BER Protein Levels in H460 Cells Before and After Treatment with Pemetrexed or the Combination



C = Control
T = TRC102
P = Pemetrexed

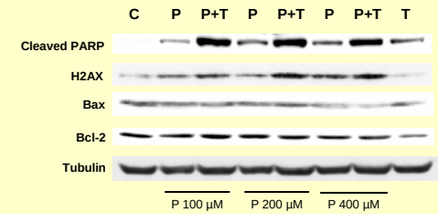
Cells were treated with TRC102 at 6 mM and collected at 24 hr

TRC102 Bound AP Sites are Refractory to the Repair by AP Endonuclease



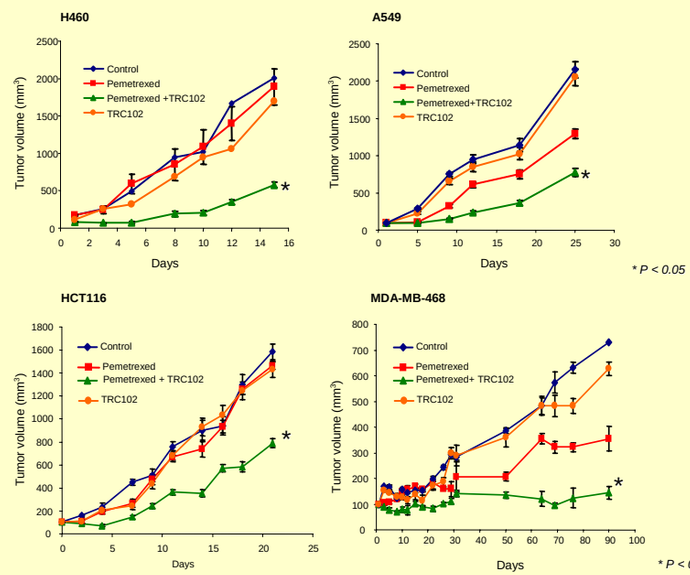
A. Schematic diagram indicate the preparation of a position specific oligonucleotide substrates containing an AP site or an TRC102 bound AP site.
B. APE has the ability to cleave the AP site but not the TRC102 bound AP site, indicating that TRC102 bound AP site is refractory to the repair by APE.

The Combination of Pemetrexed and TRC102 Enhances DNA Double Strand Breaks and Apoptosis that is Independent of the Bcl2 Pathway



Cells were treated with TRC102 at 6 mM and collected at 24 hr

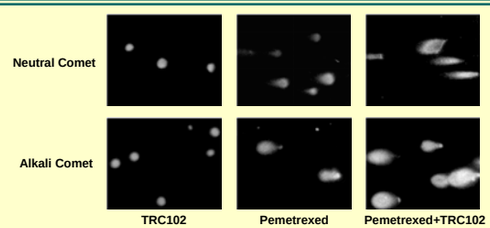
TRC102 Enhances Antitumor Effect of Pemetrexed in Nude Mice Carrying Human Tumors



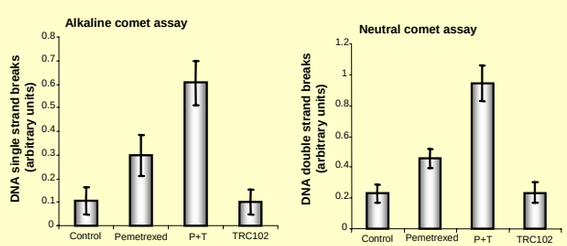
* *P* < 0.05

* *P* < 0.05

Neutral Comet / Alkali Comet



DNA Strand Breaks Measured by Comet Assay in H460 Cells 4 hours after Treatment



SUMMARY

1) **Pemetrexed induces the incorporation of abnormal bases that are removed by DNA Glycosylases as part of base excision repair (BER), thereby producing AP sites.**

2) **TRC102 binds to AP sites to efficiently block BER and increase DNA strand breaks.**

3) **Inhibition of BER by TRC102 enhances pemetrexed antitumor activity against several human solid tumors, including lung, breast and colorectal cancers.**