Precision medicine approaches to Fanconi anemia oral cancer personalized prevention/treatment

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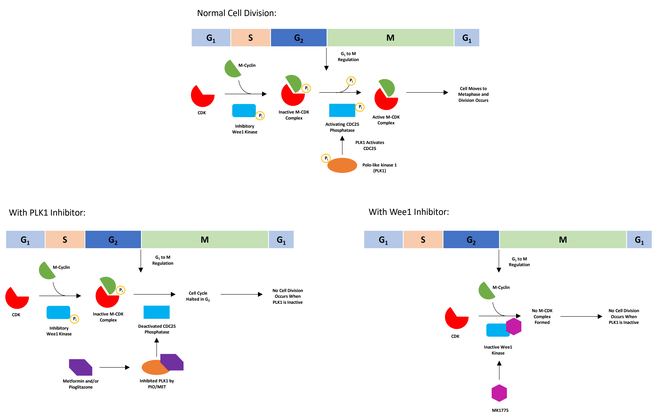
INTRODUCTION

Fanconi anemia (FA) is a genetic disorder which leads to bone marrow failure and cancer, attributable to mutations in DNA repair mechanisms. FA is also a solid tumor-prone disease, affecting patients decades earlier and at a rate several hundred-fold higher than the general population, necessitating regular cancer surveillance. To better understand post-bone marrow transplant (BMT) oral SCC tumors and assist in development of preventative, survival-enhancing therapies for FA patients, we examined the effects of Actoplus Met components, metformin/pioglitazone, polo-like kinase 1 (PLK 1) inhibitor, GSK461364, and Wee1 kinase inhibitor, MK1775, all agents of high interest in Fanconi anemia head and neck cancer treatment, on the proliferation of Fanconi-derived oral carcinoma cells. Actoplus Met is a current agent under study in an NCI sponsored trial (clinicaltrials.gov NCT02917629). MK1775 may act more effectively under states of p53 deficiency but also may synergize with other downregulators of mTOR making it particularly attractive for use with metformin. Development of an adjunct therapy or preventative survival-enhancing therapy with a minimal side effect profile will benefit this population in which chemotherapy and radiation are particularly damaging.



MATERIALS & METHODS

HNSCC Fanconi Anemia Cell Lines

We tested pioglitazone, metformin, and , for growth inhibition in Fanconia Anemia head and neck cancer cell lines, including our FA1 SCC cell line (established from a post bone marrow transplant FA patient with a T2N2bM0 oral SCC grown as an adherent monolayer culture). Additional cell lines include VU-1131-T2.8 and VU1365-T (oral squamous cell carcinomas).

MTT

Cells were plated at $5x10^3$ cells/well in 96-well plates and treated 24h later with chemopreventives. MTT was added 24, 48, 72, and 120 hrs after treatment and allowed to incubate at 37^o for 4hrs. Crystals were solubilized and the absorbance read at 560nm.

RESULTS

Fig. 1: Metformin decreases cell proliferation at high concentrations. MTT analysis shows metformin at physiologically achievable concentrations results in no change in cell proliferation and cytotoxicity.

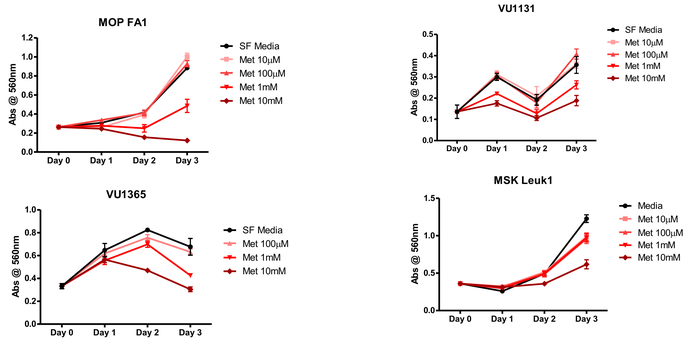


Fig. 2: Pioglitazone decreased cell proliferation in a dose dependent manner. MTT analysis shows decreases in cell proliferation with pioglitazone treatment at achievable serum concentrations.

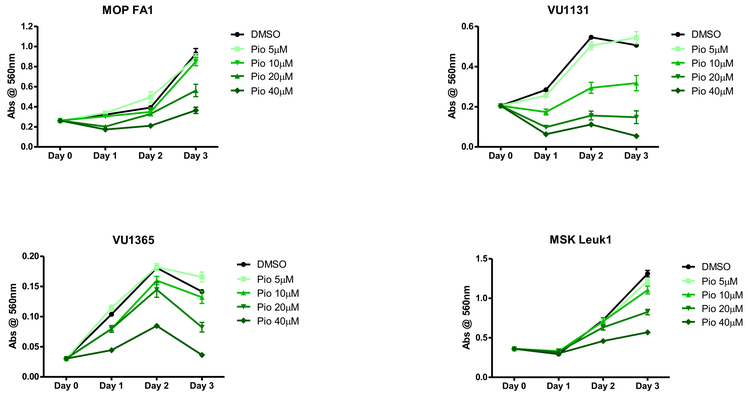


Fig. 3: GSK461364 did not significantly alter cell proliferation in FA squamous carcinoma cells. GSK did not significantly increase or decrease cell proliferation in the cell lines at the concentrations tested.

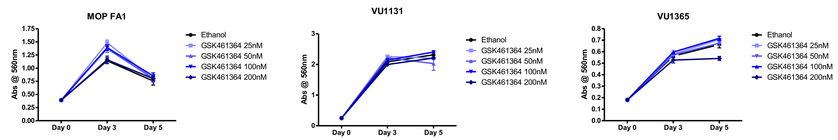


Fig. 4: MK1775 decreases FA squamous carcinoma cell proliferation in a dose dependent manner. MK1775 is effective at decreasing cell proliferation at physiologically relevant concentrations.

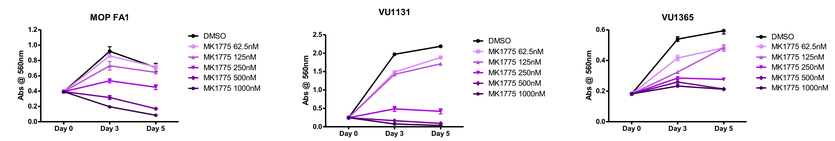
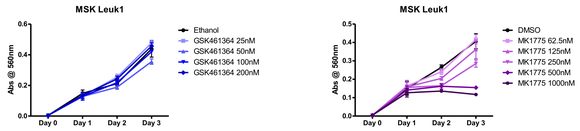


Fig. 5: MSK Leuk1 control cells reflect effects seen in FA squamous carcinoma cell lines. MK1775 is effective at decreasing cell proliferation at physiologically relevant concentrations.



CONCLUSIONS

- Cell proliferation is not decreased with metformin treatment at concentrations achievable in serum.
- Our concentrations of metformin equate to a ≥8mg/L (~62 microM) serum concentration, (human peak plasma concentrations range from 1-1.5mg/L) (~8-12 microM serum concentration). Consequently, increasing the dosing of metformin to elicit decreased cancer cell growth in these cell lines is not an informative option.
- Pioglitazone (5, 10, 20, 40 μM) decreases cell proliferation compared to solvent controls.
- GSK 461364, a PLK1 inhibitor did not significantly alter cell proliferation at concentrations tested.
- MK1775, a Wee Kinase inhibitor, decreased cell proliferation in a dose dependent manner.
- Our results are an early demonstration that difficulties experienced with genotoxic chemotherapy in Fanconi-associated squamous cancer treatment may be ameliorated with approaches involving reuse of diabetes agents with/without targeted therapies.

ACKNOWLEDGEMENTS

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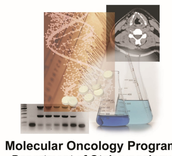
fanconi.org/research



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