Poster ja ettekanne.

Signe Ivask, PhD
ajakirjanduse teadur-lektor, Tartu Ülikool

Millest me räägime?

-milline on mõjus poster?

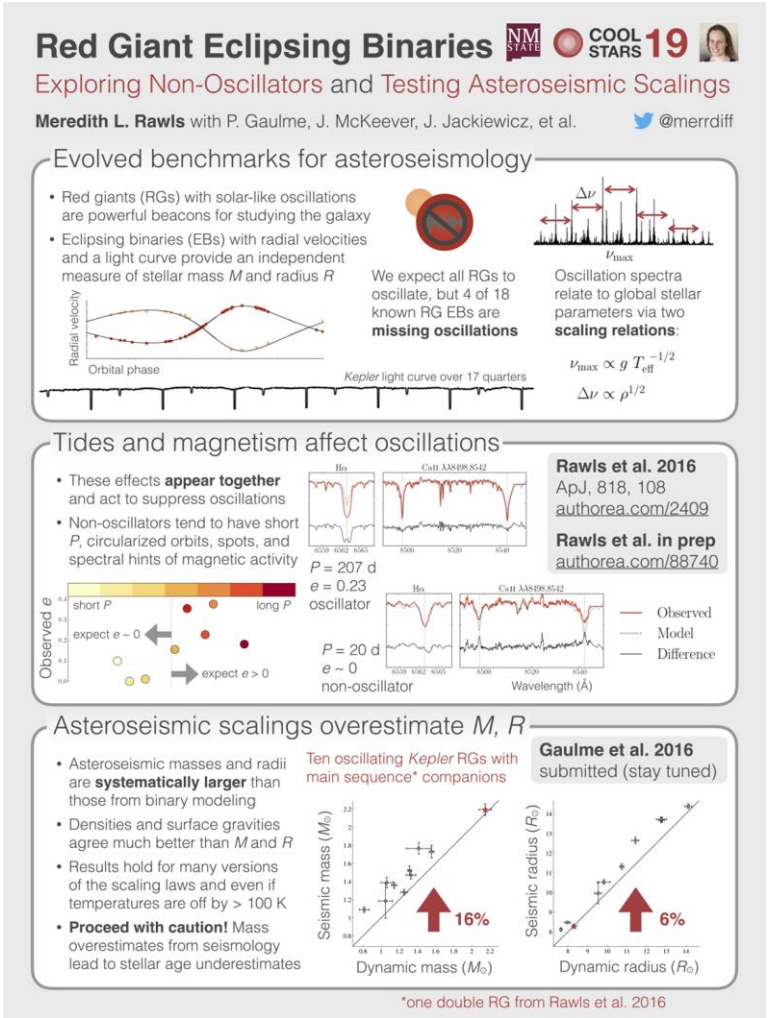
-poster kui õpilase abimees;

-pealkirjastamine, kontekstualiseerimine, valikud;

-kujundus;

-abimehed kujundamisel.

Mõjus poster



Mõjus Poster on...

...selge ja läbimõeldud.

...terviklik.

...haarav ja veenev.

...esitlejale toeks.

...justkui lugu, millel on algus, areng ja lahendus või lõpp.

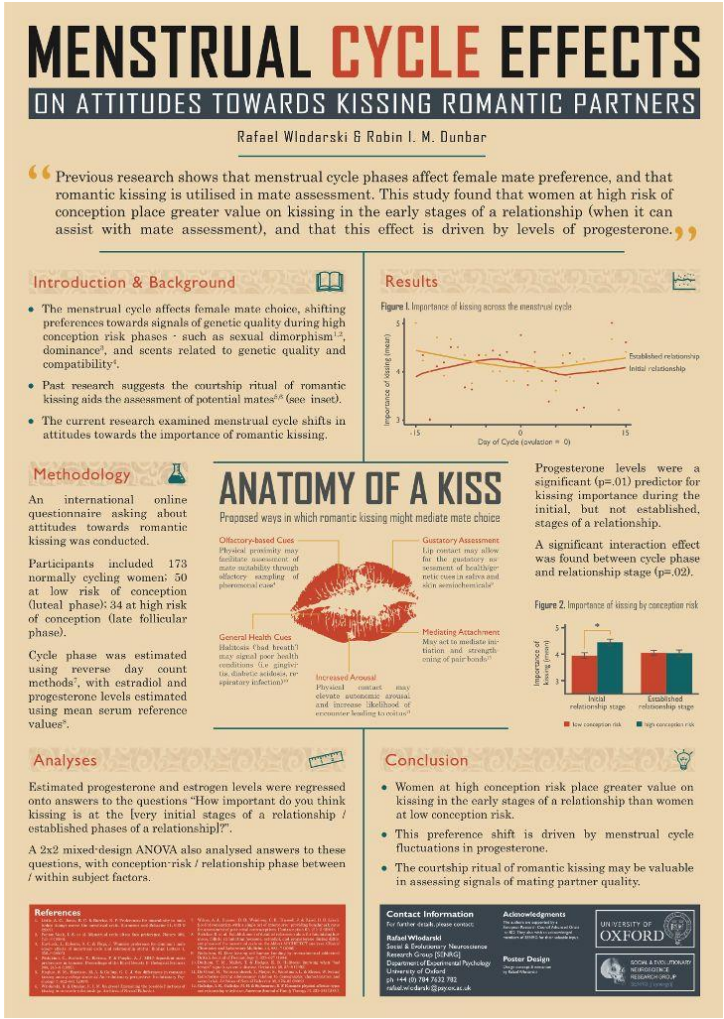
Tähelepanu köitmine

-pealkiri või sissejuhatus või tsitaat;

-anna kuulajale kontekst;

-miks peaks kuulajat sinu uurimus huvitama?;

-keskne küsimus või hüpotees!;



MENSTRUAL CYCLE EFFECTS

ON ATTITUDES TOWARDS KISSING ROMANTIC PARTNERS

Rafael Wlodarski & Robin I. M. Dunbar

"Previous research shows that menstrual cycle phases affect female mate preference, and that romantic kissing is utilised in mate assessment. This study found that women at high risk of conception place greater value on kissing in the early stages of a relationship (when it can assist with mate assessment), and that this effect is driven by levels of progesterone."

Introduction & Background

- The menstrual cycle affects female mate choice, shifting preferences towards signals of genetic quality during high conception risk phases - such as sexual dimorphism[1,2], dominance[3], and scents related to genetic quality and compatibility[4].
- Past research suggests the courtship ritual of romantic kissing aids the assessment of potential mates[5,6] (see inset).
- The current research examined menstrual cycle shifts in attitudes towards the importance of romantic kissing.

Results

Figure 1. Importance of kissing across the menstrual cycle

Methodology

An international online questionnaire asking about attitudes towards romantic kissing was conducted.

Participants included 173 normally cycling women: 50 at low risk of conception (luteal phase); 34 at high risk of conception (late follicular phase).

Cycle phase was estimated using reverse day count methods[7], with estradiol and progesterone levels estimated using mean serum reference values[8].

ANATOMY OF A KISS

Proposed ways in which romantic kissing might mediate mate choice

Progesterone levels were a significant (p=.01) predictor for kissing importance during the initial, but not established, stages of a relationship.

A significant interaction effect was found between cycle phase and relationship stage (p=.02).

Figure 2. Importance of kissing by conception risk

Analyses

Estimated progesterone and estrogen levels were regressed onto answers to the questions "How important do you think kissing is at the [very initial stages of a relationship / established phases of a relationship]?".

A 2x2 mixed-design ANOVA also analysed answers to these questions, with conception-risk / relationship phase between / within subject factors.

Conclusion

- Women at high conception risk place greater value on kissing in the early stages of a relationship than women at low conception risk.
- This preference shift is driven by menstrual cycle fluctuations in progesterone.
- The courtship ritual of romantic kissing may be valuable in assessing signals of mating partner quality.

References

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Acknowledgments

Poster Design

UNIVERSITY OF OXFORD

SOCIAL & EVOLUTIONARY NEUROSCIENCE RESEARCH GROUP

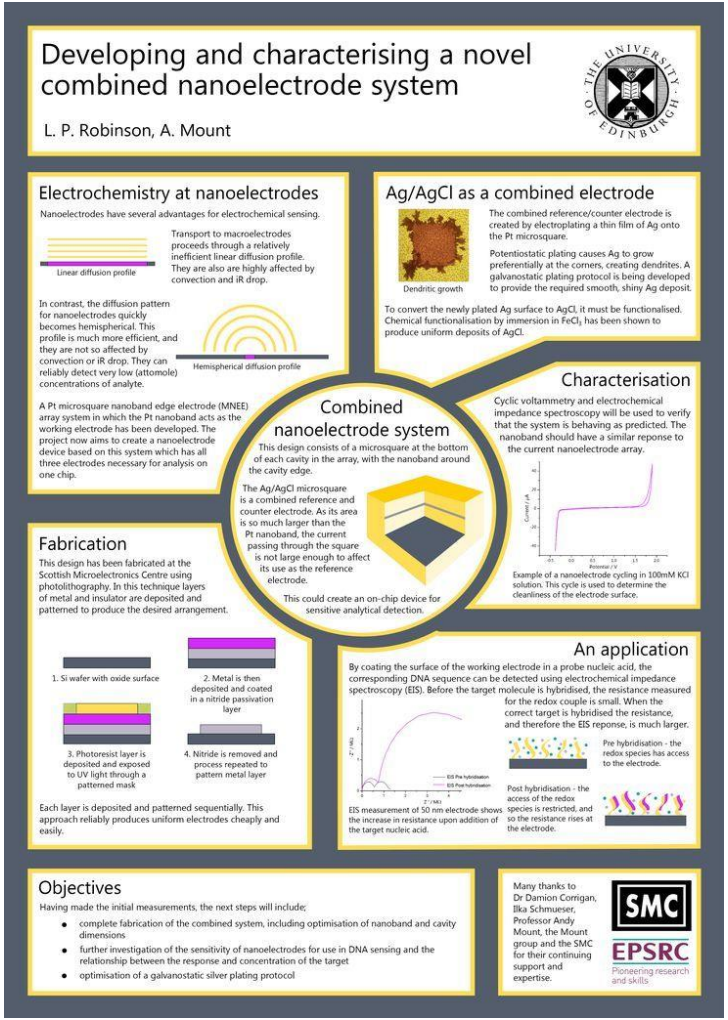
-pilt/visuaal/graafik ütleb rohkem kui tuhat sõna;

-Meetod, valim ja analüüs;

-Tulemused/diskussioon;

-Kokkuvõte;

-70% infograafikuid, 30% juttu.



Poster on õpilase abimees

O⁶-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Resensitizes Breast Cancer Cells to Anti-Estrogen Therapy

Joshua Smith[1], George C Bobustuc[1], Rafael Madero-Visbal[1], Jimmie Colon[1], Beth Isley[1], Jonathan Ticku[1], Kalkunte S. Srivenugopal and Santhi Konduri[1]

[1]Cancer Research Institute of M.D Anderson Cancer Center Orlando [2]Texas Tech University Health Sciences Center, Amarillo, TX

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Abstract

Endocrine therapies using anti-estrogens are least toxic and very effective for breast cancers, however, tumor resistance to tamoxifen remains a stumbling block for successful therapy. Based on our recent study on the involvement of the DNA repair protein MGMT in pancreatic cancer (Clin Cancer Res. 15, 6087, 2009), here, we investigated whether MGMT overexpression mediates tamoxifen resistance. Specifically, we determined whether administration of MGMT inhibitor [O⁶-benzylguanine (BG)] at a non-toxic dose alone or in combination with the anti-estrogens (tamoxifen/fulvestrant) curtails human tamoxifen resistant breast cancer cell growth. Further, we also determined whether BG sensitizes breast cancers to tamoxifen using tamoxifen resistant cells.

MGMT expression was found to be increased in breast cancer cells relative to normal breast epithelial cells. Also, MGMT levels were significantly higher in tamoxifen resistant MCF-7 compared to the parent cells. Silencing of the ER-α expression using a specific siRNA resulted in augmentation of MGMT mRNA and protein levels by 2 fold. We also observed an inverse correlation between MGMT and p53 levels in breast cancer cell lines; moreover, p53 downregulation was accompanied by increased MGMT expression. Other experiments showed that BG alone or BG in combination with tamoxifen or fulvestrast decreased ER-α expression, whereas tamoxifen alone and fulvestrant alone increased and decreased the same respectively. However, all these treatments increased the p21^{WAF1} mRNA and protein expression significantly. BG inhibited tamoxifen resistant breast cancer growth in a dose-dependent manner and it also resensitized resistant breast cancer cells to anti-estrogen therapy (TAM/ICI). These combinations also enhanced the cytochrome C release and the PARP cleavage, indicative of apoptosis. In breast cancer xenografts, BG alone or a combination of BG with tamoxifen or fulvestrant caused significant tumor growth delay and immunohistochemistry revealed that BG inhibited the expression of MGMT, ER- α, ki-67 and increased p21^{WAF1} staining. These findings suggest that MGMT inhibition may provide a novel and effective approach for overcoming tamoxifen resistance.

Introduction

Recent advances in breast cancer research have identified key pathways involved in the repair of DNA damage induced by chemotherapeutic agents. The ability of cancer cells to recognize DNA damage and initiate DNA repair is an important mechanism for therapeutic resistance and has a negative impact on therapeutic efficacy. A number of DNA-damaging alkylating agents attack the nucleophilic O⁶ position on guanine, forming mutagenic and highly cytotoxic interstrand DNA crosslinks. The DNA repair enzyme O⁶-alkylguanine DNA alkyltransferase (AGT), encoded by the gene MGMT, repairs alkylation at this site and is responsible for protecting both tumor and normal cells from alkylating agents. MGMT is expressed constitutively in normal cells and tissues. In breast tumors, MGMT gene expression is elevated and levels are up to 4-fold higher than in the normal breast. Interestingly, it has been shown that tamoxifen accelerates proteasomal degradation of MGMT in human cancer cells. In 1991, Pegg, Moschel, and Dolan observed that O⁶ benzylguanine (BG) inhibited AGT and potentiated the cytotoxicity of both chloroethylating agents and methylating agents. In a series of important observations, they fully characterized the interaction between BG and AGT and its therapeutic impact. They showed that BG binds AGT, transferring the benzyl moiety to the active-site cysteine [29]. The reaction is very rapid and more potent than any other previously known AGT inhibitor. BG is not incorporated into DNA in living cells and reacts directly with both cytoplasmic and nuclear AGT. Because BG is a psuedosubstrate for MGMT which results in the covalent transfer of benzyl group to the active site cysteine, the MGMT protein is degraded after each reaction. This stoichiometric reaction mechanism effectively depletes the AGT content in tumors and the associated repair of alkylation damage. BG is currently undergoing clinical trials in various cancers to increase the efficacy of alkylating agents.

Interestingly, several observations suggest an inverse correlation between the levels of MGMT and p53 tumor suppressor proteins where wild-type p53 suppresses transcription of human MGMT expression. Unfortunately, p53 function is often inactivated or suppressed in human cancers; therefore, restoration of wt-p53 activity is essential for the success of some treatments. However, whether or not this is mediated by suppression of MGMT expression has yet to be determined. To date, the cross-talk between MGMT and ER-alpha (and the link to p53 expression) has not been explored in drug (i.e., tamoxifen) resistant breast tumors. The anti-estrogen tamoxifen is the most commonly used treatment for patients with estrogen receptor positive breast cancer. Although many patients benefit from tamoxifen in the adjuvant and metastatic settings, resistance to this endocrine therapeutic agent is an important clinical problem. The primary goal of present study was to investigate the mechanisms of anti-estrogen drug resistance and to design new therapeutic strategies for circumventing this resistance. The results show that MGMT expression is increased in TAM-resistant breast cancers and inhibition of MGMT by BG significantly improves TAM-sensitivity.

Results

Prolonged Treatment of Tamoxifen Increases MGMT Expression: We developed a tamoxifen resistant MCF-7 cell line by using prolonged treatment of tamoxifen on the parental ER-positive breast cancer cell line, MCF-7. Tamoxifen-resistant MCF-7 cells proliferate at rates similar to the parental MCF-7. Prolonged treatment of tamoxifen onto MCF-7 cells increased MGMT expression compared to parental MCF-7 cells by 2 fold (Fig.1).

Knocking Down ERα Enhances MGMT Expression in Tamoxifen Resistant Breast Cancer Cells: It is not known whether ERα and MGMT transcriptionally regulate each other in tamoxifen resistant breast cancer cells. We therefore investigated whether down regulation of ERα has any effect on endogenous MGMT expression in these cells. As expected, downregulation of ERα using specific siRNA significantly reduced ERα protein levels in these cells. Western blot analysis was performed and the results in the left panel (Fig. 2A) shows that silencing of ERα increases MGMT expression in these cells, and interestingly, the results in the right panel (Fig.2B) show increased MGMT mRNA levels were increased as assessed by qRT-PCR. These data suggest that ERα–mediated signaling functions to repress MGMT gene expression in breast cancer cells.

Transcriptional Regulation Between MGMT and p53: Previously, it was reported that p53 negatively regulates MGMT in breast cancer cells. Therefore, we addressed whether or not silencing the p53 enhances endogenous MGMT transcription. Tamoxifen resistant MCF-7 cells were transfected with either p53 siRNA (p53-KD) (Fig.2C) or MGMT siRNA (MGMT-KD) (Fig.2D) along with Non-specific siRNA (NS). MGMT expression was consistently increased in p53 knock down cells, with different experiments showing a ~ fold augmentation (Fig. 2A) and as expected, knocking down MGMT decreased MGMT transcription where as p53 mRNA levels were unaffected in MGMT knockdown cells (Fig.2D). These results confirm that p53 can regulate MGMT at the transcriptional level.

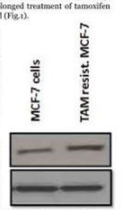


Figure 1. MCF-7 parental and tamoxifen resistant MCF-7 cell pellets were prepared, proteins were isolated and MGMT expression was detected by western blot analysis. Tamoxifen resistant MCF-7 breast cancer cells significantly increased MGMT expression compared to MCF-7 parental cells.

O⁶-Benzylguanine Plays a Dual Role in Tamoxifen Resistant MCF-7 Cells: Contrasting with the experiments above, next, we studied whether or not knocking down MGMT has any effect on ERα transcription. As expected, knocking down MGMT decreased MGMT gene transcripts. However, it was interesting to find that ERα gene transcription was also reduced after MGMT silencing. (Fig.2E). These data demonstrate that BG has the ability to attenuate the not only the MGMT, but also the ERα transcription, indicating a possible dual role for MGMT blockers in these breast cancer cells.

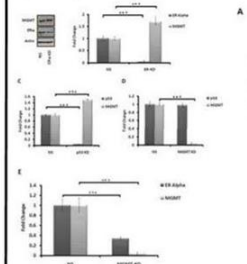


Figure 2. (A) Tamoxifen resistant MCF-7 cells were transfected with ERα siRNA (100nM) (ERα-KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total proteins were isolated and ERα and MGMT expression was determined by western blot analysis. MGMT protein was significantly increased in ERα knock down cells (B) Tamoxifen resistant MCF-7 cells were transfected with ERα siRNA (100nM) (ERα-KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total RNA was isolated and MGMT and ERα transcription was determined by qRT-PCR. MGMT transcription was significantly increased in ERα knock down cells. (C) Total RNA was isolated from non-specific siRNA (NS) (100nM) and p53 siRNA (50 nM) knock down tamoxifen resistant MCF-7 breast cancer cells. MGMT and p53 transcription was determined by qRT-PCR. There is an inverse correlation between MGMT and p53 in tamoxifen resistant breast cancer cells (C & D).

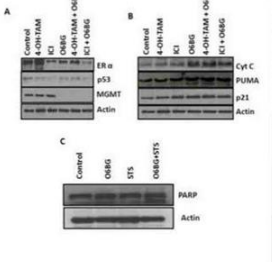


Figure 3. (A) Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μg) and 4Bh post treatment 4-OH-TAM (1μM), ICI (1μM) either alone or in combination with BG. 24h post treatment cells were harvested and proteins were isolated and western blot analysis was performed. (A) ERα, p53 and MGMT expressions (B) Cytochrome C, PCMA and p21 was determined by western blot analysis (C) tamoxifen resistant MCF-7 cells were treated with or without BG for 48h and later treated with staurosporin (5 μM/L) for 6 hrs PARP cleavage was determined by western blot analysis.

O6-Benzylguanine Modulates p53 Down-Stream Targeted Protein Expressions: Encouraged by the results reported, we investigated the effect of combination therapy on endogenous MGMT, p53, and ERα protein expressions. As expected, BG decreased MGMT expression, while combination therapy (4-OH-TAM or ICI combined with BG) significantly decreased both MGMT and ERα expressions. BG alone or in combination with tamoxifen or ICI decreased ER-α expression, whereas tamoxifen alone and ICI alone increased and decreased the same respectively (Fig.3A). p53 expression was slightly altered after ICI treatment. The reduction in p53 expression by ICI alone was reversed when BG was combined (Fig.3A). We investigated the effect of BG on proteins which are involved in cell cycle regulation, apoptosis in tamoxifen resistant breast cancer cells. All these treatments significantly increased the p21^{WAF1} protein expression (Fig.3B). PUMA expression was also increased with these treatments. Hence, PUMA may have translocated to the mitochondria, cytochrome C is released (Fig.3B), and apoptosis was triggered in these cells in presence of combination therapy. PARP cleavage is seen in BG treated cells in presence of staurosporin as an indicative of apoptosis (Fig.3C). Therefore, this data suggest that BG promotes cell cycle arrest and can induce apoptosis by modulating p53 function.

O6-Benzylguanine Modulated Transcriptional Targets in Tamoxifen Resistant Breast Cancer Cells: The effect of combination therapy on endogenous MGMT mRNA levels ws aso studi. Quantitative real-time PCR (qRT-PCR) resulted that anti-estrogens (TAM/ICI) increased the MGMT expression while the combination therapy decreased it compared to control levels. ERα transcription was decreased compared to controls with all these treatments (Fig.4A). Surprisingly, p21 and PUMA mRNA was significantly increased in the presence of combination treatments (Fig.4B &C). These results suggests that p53 mediated target gene transcription was affected by the drug combinations in breast cancer cells (Fig. 3 & 4).

O6-Benzylguanine Enhances p21 Transcriptional Activity in Tamoxifen Resistant Breast Cancer Cells: In order to investigate the effect of BG on p53 function, we performed luciferase reporter assays. Tamoxifen resistant MCF-7 breast cancer cells were transfected with p21 luc promoter construct in presence or absence of BG (target gene of p53). These results clearly demonstrate that BG significantly enhanced p21 transcriptional activity by 4-5 fold in these cells (Fig.4D).

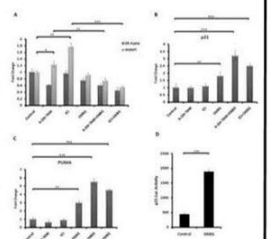


Figure 4. Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μg) for 48h and later 4-OH tamoxifen and ICI (1μM) was either alone or in combination with BG and 24h later cells were harvested and total RNA was isolated. (A) MGMT and ERα (B) p21 transcription (C) PUMA transcription was determined by qRT-PCR. 4-OH tamoxifen and ICI enhances MGMT transcription. BG induced PUMA and p21 transcription. (D) Tamoxifen resistant MCF-7 breast cancer cells were transfected with p21-luc construct and 6h later treated with BG and 24h later cells were harvested. p21 transcriptional activity was significantly increased by BG in these cells.

O6-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Increase Resistant Breast Cancer Cell Sensitivity to Anti-Estrogen Therapy (TAM/ICI): Detailed necropsy revealed that all the mice had tumors in the breast. The data summarized in Table 1 show the daily BG alone or in combination with twice weekly tamoxifen/ICI significantly decreased median tumor volume and weight as compared with that seen in tamoxifen/ICI treated and control mice. The combination of BG with tamoxifen or ICI produced the greatest decrease in median tumor volume as compared with control mice (83.99 mm³, 9.33 mm3 (TAM+BG), respectively; p< 0.0001); (83.99 mm³, 31.60 mm³ (ICI+BG), respectively; p<0.0001). Tumor weight was also significantly reduced in mice treated with combination therapy as compared with control mice (81.23 mg, 22.30 mg (TAM+BG), respectively, p<0.0005; (81.23 mg, 51.57 mg (ICI+BG), respectively, p<0.0005). (Table.1). Body weight was not changed among all treatment groups as compared with control mice. No visible liver metastases were present (enumerated with the aid of a dissecting microscope) in all treatment groups.

Histology and IHC Analysis: We next determined the *in vivo* effects of BG (alone or in combination) with tamoxifen/ICI, Tumors harvested from different treatment groups were processed for routine histological and IHC analysis. Tumors from mice treated with BG alone or in combination with tamoxifen/ICI exhibited a significant decrease in MGMT, ERα, ki-67 as compared with tumors treated with tamoxifen/ICI alone or control group. p53 expression was not much altered in these treatment groups. In sharp contrast, the expression of p21 was significantly increased in tumors from mice treated with BG either alone or in combination with tamoxifen/ICI. The images were analyzed by ImageJ (NIH) and MGMT, ERα, p53, p21 and ki-67 expressions were quantified by the ImmunoRatio plugin. (Fig.5).

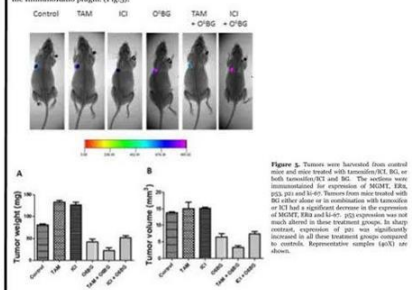
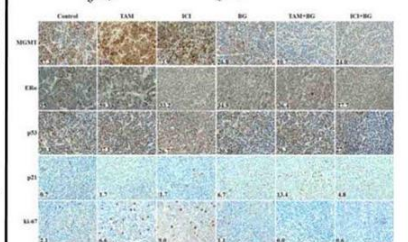


Figure 5. Tumors were harvested from control mice and mice treated with tamoxifen/ICI, BG, or both tamoxifen/ICI and BG. The sections were immunostained for expression of MGMT, ERα, p53, p21 and ki-67. Tumors from mice treated with BG either alone or in combination with tamoxifen or ICI had a significant decrease in the expression of MGMT, ERα and ki-67. p53 expression was not much altered in these treatment groups. In sharp contrast, expression of p21 was significantly increased in all these treatment groups compared to controls. Representative samples (40X) are shown.



Conclusions

1. In the present study, we observed that prolonged treatment with anti-estrogens causes drug resistance by inducing the DNA repair protein O⁶-methylguanine DNA methyltransferase (MGMT).
2. Decreasing the expression of MGMT by exposing breast cancer cells to BG sensitized these cells to anti-estrogen therapy (tamoxifen and ICI 182,780).
3. We also observed that combination therapy of anti-estrogens and MGMT blockers not only overcame the MGMT derived drug (tamoxifen and ICI) resistance but also increased the efficacy of anti-estrogen therapy by decreasing estrogen receptor expression and restoration of the functional activity of p53 in tamoxifen-resistant breast cancer cells.
4. Combination therapy inhibited tamoxifen resistant tumor growth *in vivo*.

Acknowledgements

We would like to thank the Florida Department of Health, Bankhead-Coley Cancer Research Program 09BS10 for their funding of this project.

Halva postri bingo.

Different parts of poster don't line up	Boxes within boxes	Zigzag reading order	More than three **typefaces**	Long-winded title
Gradient fills in coloured boxes	Big blocks of text	Photographic background	Unlabelled error bars on graphs	Pixelated pictures
More than five colours	Institutional logos bookending title	Free space	ALL CAPITALS	Text with shadows, outlines, or bevels
Abstract	Underlined text	Comic Sans	3-D graphs	Checking tablet or phone during presentation
Tables showing data that could be in a graph	Poster does not fit on poster board	Comic Sans (it's that annoying)	Objects almost touching or overlapping	Tiny, unreadable type

By Zen Faulkes, betterposters.blogspot.com

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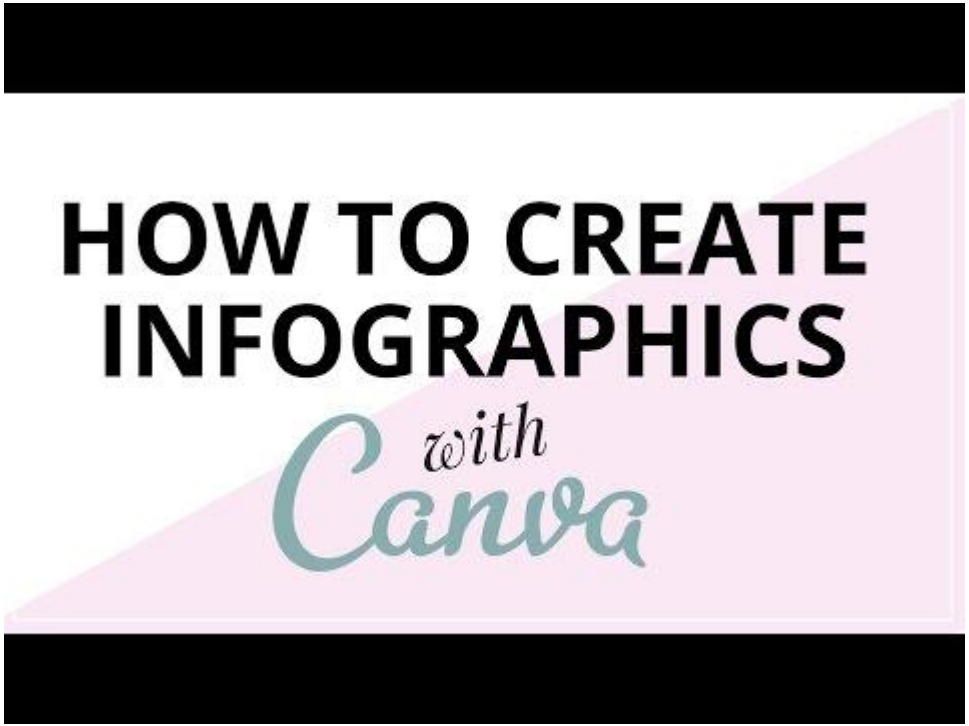

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AGA!

Signe Ivask, Tartu Ülikool. 2020