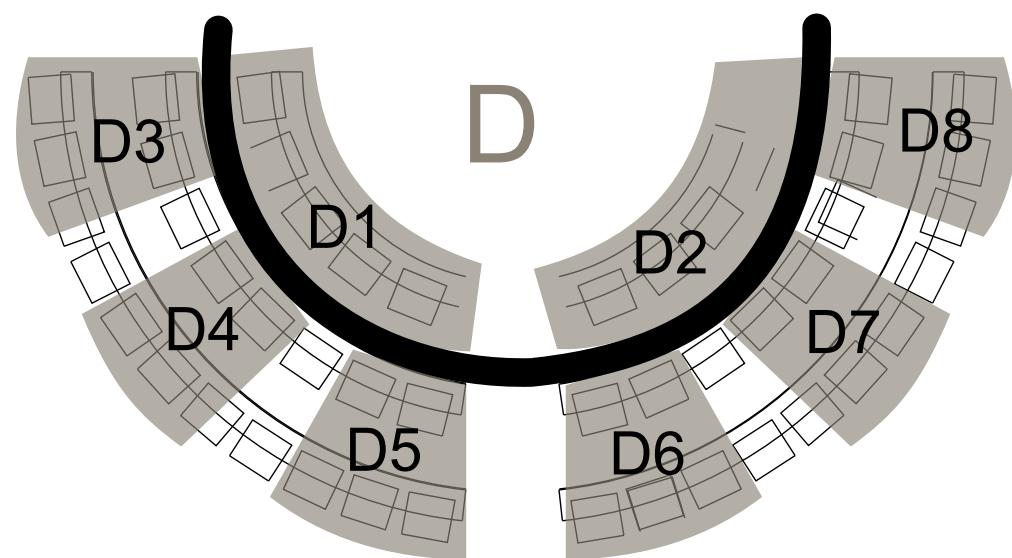


G115



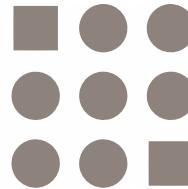
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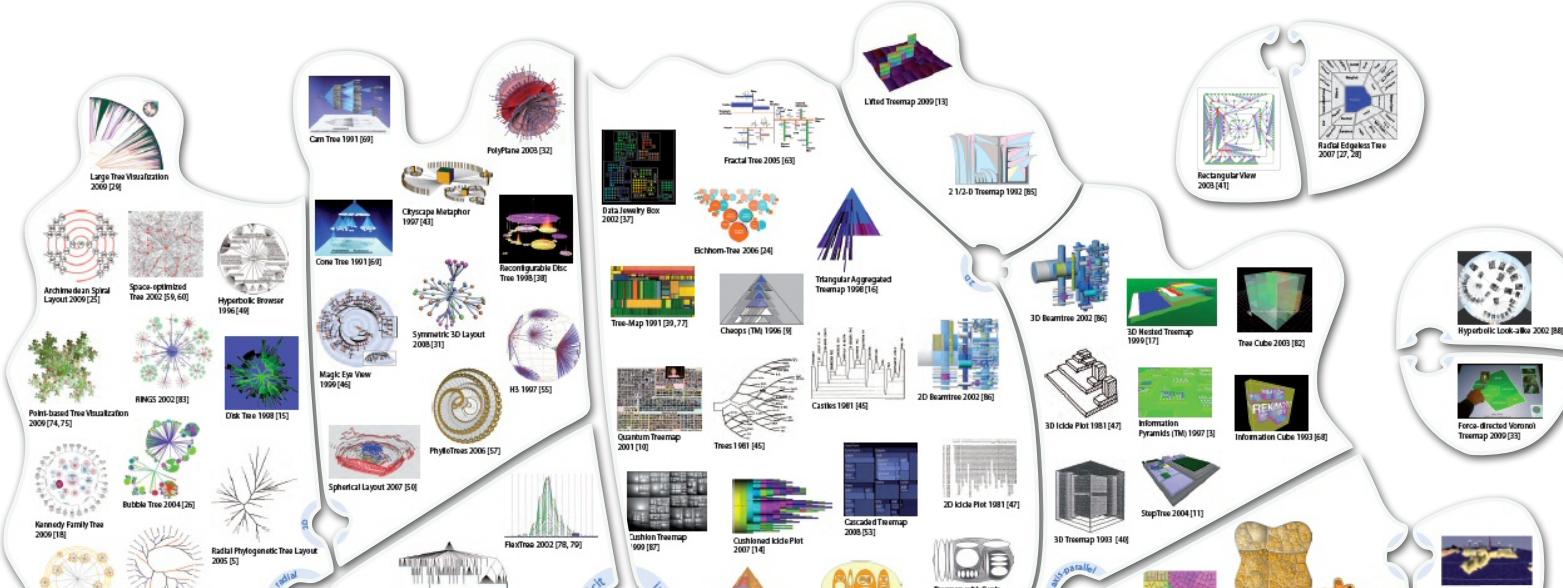
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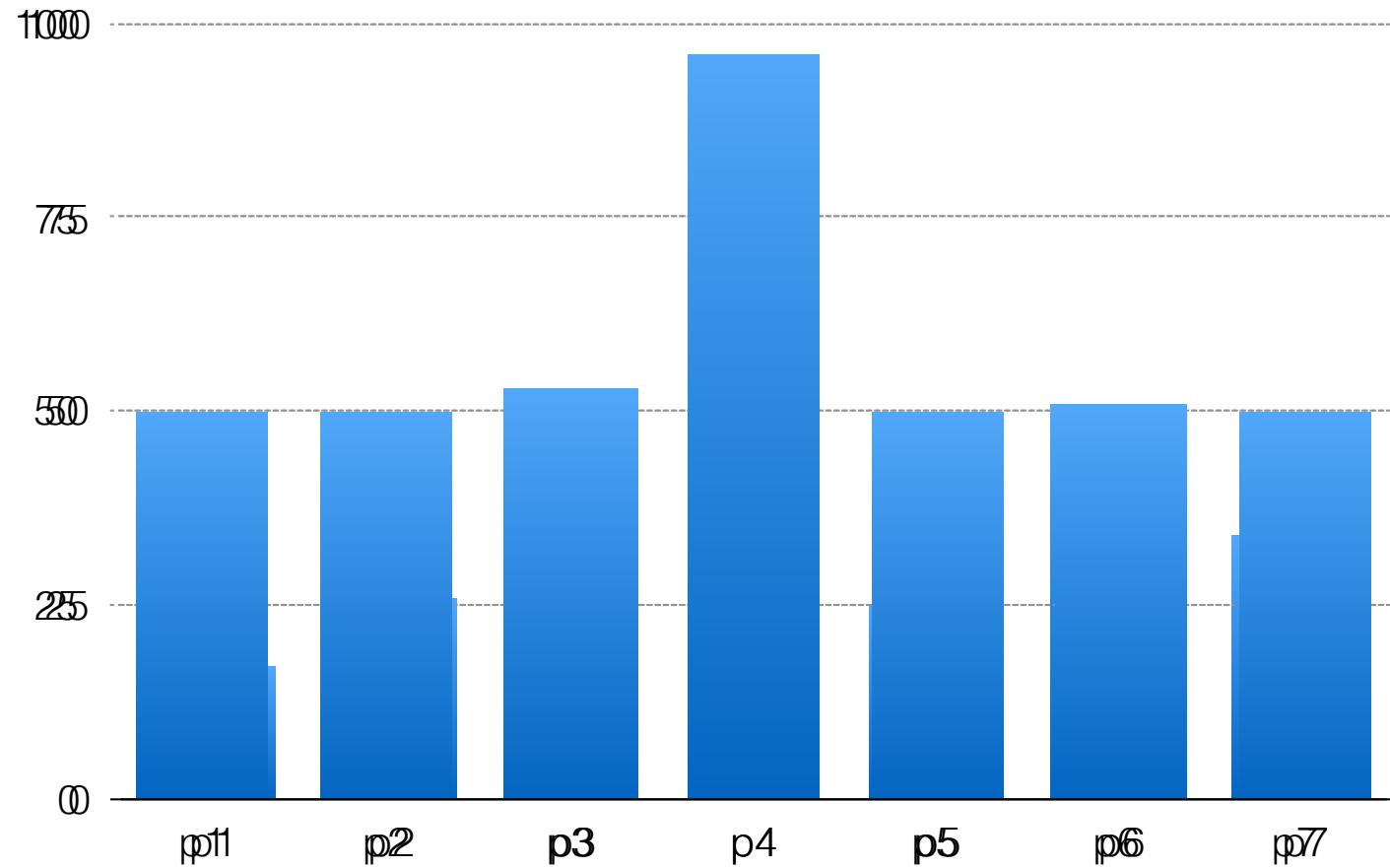
Vis Exploration

Hendrik Strobelt / Hanspeter Pfister



Feedback

- Our project is about X, but we are assigned to Y
- How much preliminary feedback will we get as we develop our project visualizations?
- The connection between two rooms does not work well.
- Why are we doing lectures like this from now on? Completely pointless
- It is useful especially before our team project starts to avoid possible problems



We want you to create a successful and creative final project.

Goal of Vis Exploration

- Get to know about the classics in Visualization.
- Why?
 - be fluent in visualization language to communicate with other visualization experts and creating ideas.
 - be able to judge, if your new idea is novel

Activity

Find consensus in your team on which you think are the three most representative visualizations for your assigned field.

[5 min]



Activity

Take all your printouts and get together in your expert groups and collect ideas about your topic area.

Each group will be lead by one of our TFs. [15 min]

A

Geographical Data /
Maps

B

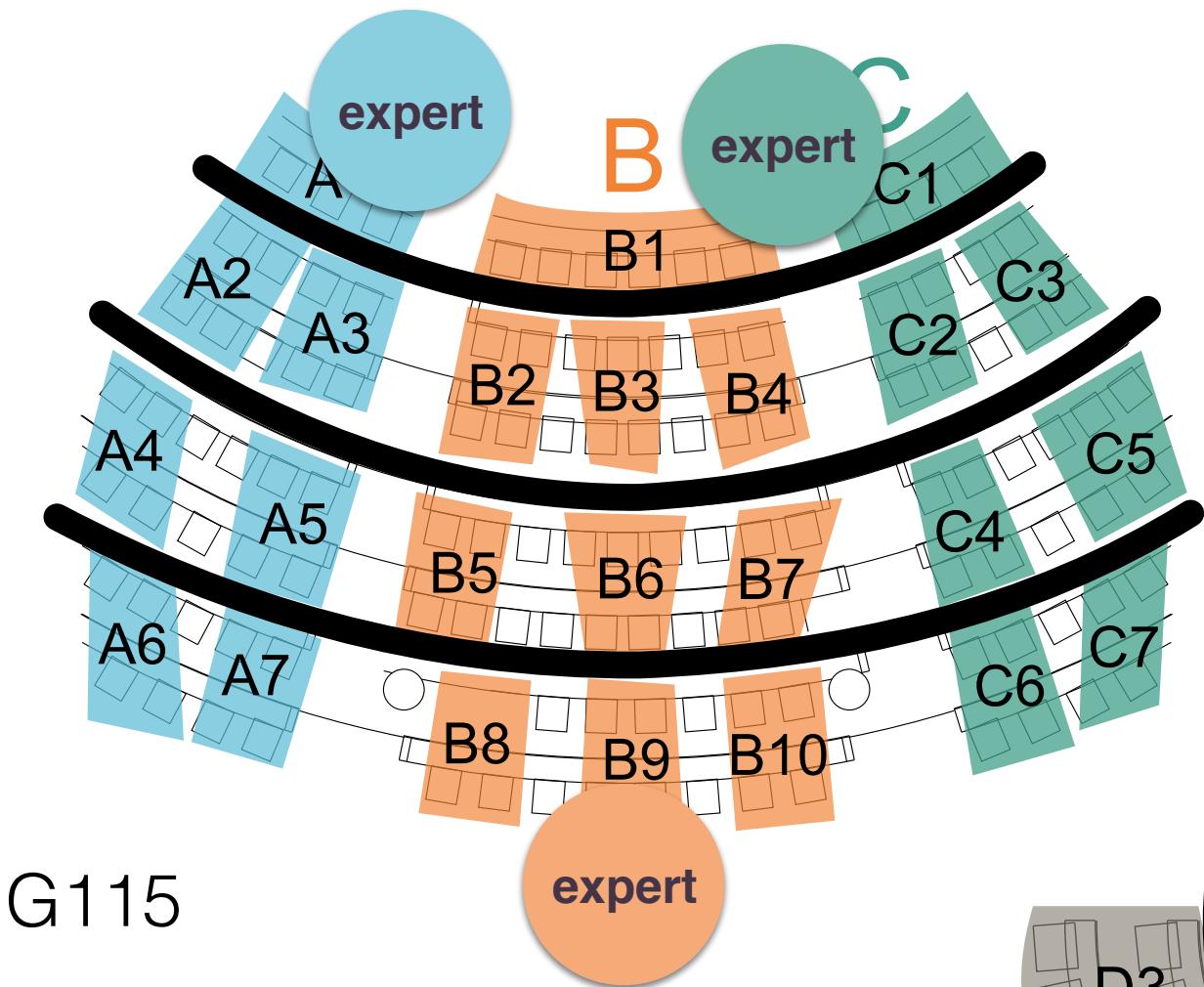
Trees & Networks

C

Visualization for Text

D

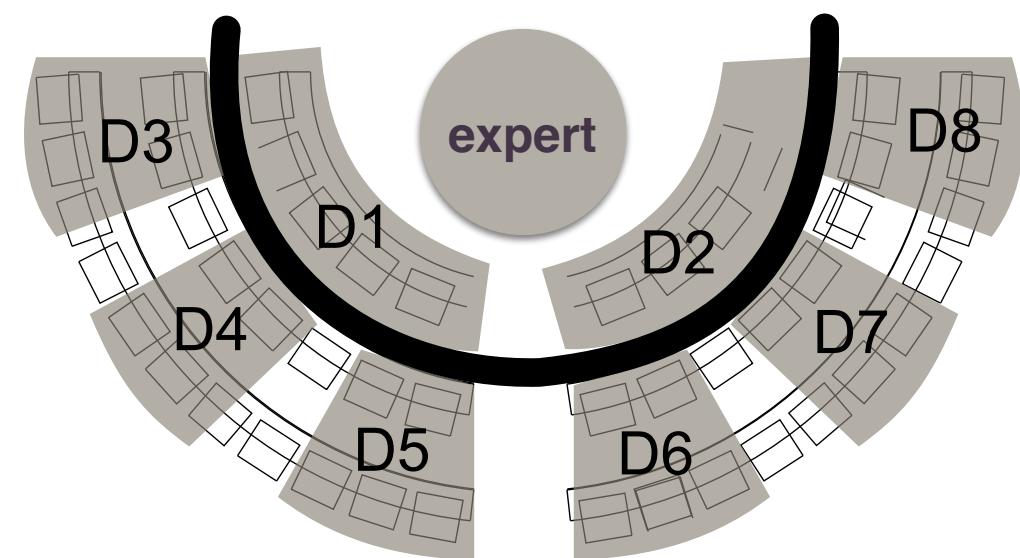
Vis for High
Dimensional Data



G115

**Please get together
at the marked places**

G125



Your poster has to convey a message - you have two options.

1) Project-related Poster

- explain your project idea in a compact way
- explain how the visualizations would be included in your vis design

2) Vis Exploration Poster

- give an overview of important visualizations for your field
- explain each technique

Activity

Create a sketch of your poster on a sheet of paper that represents your team's selection of methods. Abstract your printouts by wireframes.

You can use the input from the expert group to modify your selection of visualizations.

[5 min]

What is a good poster?

- Text should be large enough to be seen 5ft away
- The pieces should be organized in a way that leads the viewer through the display. [Important info first]
- Keep it simple and brief. viewers should “get it” in 30 seconds. [Tip: detailed information on handout]
- Organize your material and edit your content to eliminate distracting visual noise.
- Use of bullets, numbering, and headlines make it easy to read
- Add small captions to images.

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

Joshua Smith¹, George C Bobustuc¹, Rafael Madero-Visbal¹, Jimmie Colon¹, Beth Isley¹, Jonathan Ticku¹, Kalkunte S. Srivenugopal and Santhi Konduri¹

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



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 in 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 fulvestrant decreased ER- α expression, whereas tamoxifen alone and fulvestrant alone increased and decreased the same respectively. However, all these treatments increased the p21^{kip} mRNA and protein expression significantly. BG inhibited tamoxifen resistant breast cancer growth in a dose-dependent manner and it also resensitizes 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^{kip} 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-methylguanine-DNA alkyltransferase (AGT), encoded by the gene MGMT, repairs alkylation at this site and is responsible for protection both of normal and tumor cells from alkylating agents. MGMT is expressed constitutively in most cells and tissues. In breast tumors, MGMT expression is elevated and levels are up to 4-fold higher than in the normal breast. Interestingly, it has been shown that tamoxifen accelerated proteosomal degradation of MGMT in human cancer cells. In 1991, Peggi, Moschel, and Dolan observed that O⁶-benzylguanine (BG) inhibited AGT and potentiated the cytotoxicity of both chlorinating agents and methylation 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 pseudosubstrate 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- α (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 on tamoxifen- τ cells increased MGMT expression compared to parental MCF-7 cells by a fold (Fig. 1).

Knocking Down ER α Enhances MGMT Expression in Tamoxifen Resistant Breast Cancer Cells: It is not known whether ER α and MGMT transcriptionally regulate each other in tamoxifen resistant breast cancer cells. We therefore investigated whether down regulation of ER α has any effect on endogenous MGMT expression in these cells. As expected, downregulation of ER α using specific siRNA significantly reduced ER α protein levels in these cells. Western blot analysis was performed and the results in the left panel (Fig. 2A) shows a silencing of ER α 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 ER α -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 4-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 cells were grown in presence or absence of 10 nM tamoxifen for 14 days. 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 ER α transcription. As expected, knocking down MGMT decreased MGMT gene transcripts. However, it was interesting to find that ER α 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 ER α transcription, indicating a possible dual role for MGMT blockers in these breast cancer cells.

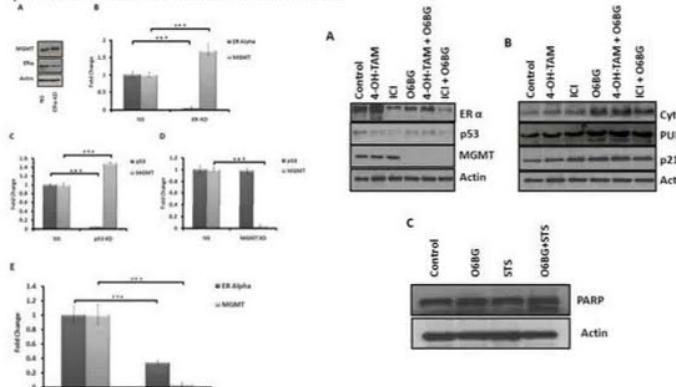


Figure 2. (A) Tamoxifen resistant MCF-7 cells were transfected with ER α siRNA (100nM) (ER α -KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total proteins were isolated and ER α and MGMT expression was determined by Western blot analysis. (B) ER α mRNA (ER α -KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total RNA was isolated and ER α mRNA expression was determined by qRT-PCR. (C) Tamoxifen resistant MCF-7 cells were transfected with p53 siRNA (20 nM) and MGMT siRNA (20 nM) and cells were harvested 72h post transfection. Total proteins were isolated and ER α and MGMT expression was determined by Western blot analysis. (D) ER α , p53 and MGMT expressions (E) Cytochrome C, PUMA and p21 was determined by Western blot analysis (F) Tamoxifen resistant MCF-7 cells were treated with or without BG for 48h and later treated with staurosporin (5 μ M/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 ER α protein expressions. As expected, BG decreased MGMT expression, while combination therapy (4-OH-TAM or ICI combined with BG) significantly decreased both MGMT and ER α 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 with ICI (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^{kip} 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 indicator of apoptosis (Fig.3C). Therefore, this data suggest that BG promotes cell cycle arrest and can induce apoptosis by modulating p53 function.

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 (8.21 mg, 22.30 mg (TAM+BG), respectively; p < 0.0005); (8.23 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 treatment histological and IHC analysis. Tumors from mice treated with BG alone or in combination with tamoxifen/ICI exhibited a significant decrease in MGMT, ER α , 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, ER α , p53, ki-67 expressions were quantified by the ImmunoRatio plugin. (Fig.5).

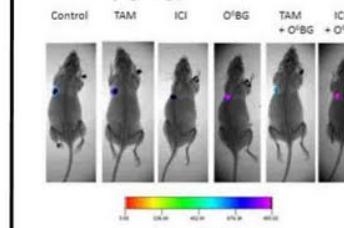
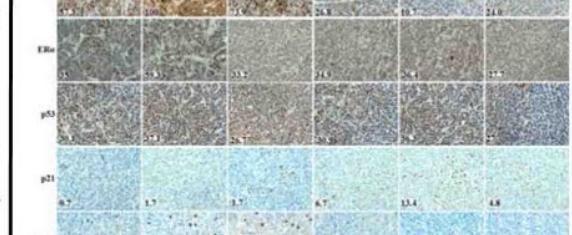


Figure 3. (A) Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μ g) and 48h post treatment 4-OH-TAM (10 μ M), ICI (1 μ M) either alone or in combination with BG. 24h post treatment cells were harvested and total proteins were isolated and ER α and MGMT expression was determined by Western blot analysis. (B) Cytochrome C, PUMA 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 μ M/L for 6 hrs PARP cleavage was determined by Western blot analysis.

Figure 4. Tumors were harvested from control mice and mice treated with tamoxifen/ICI/BG, on both tamoxifen/ICI and BG. The sections were immunostained for expression of MGMT, ER α , p53, p21 and ki-67. Tumors from mice treated with tamoxifen/ICI and BG had a significant decrease in expression of MGMT, ER α 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 control. Representative samples (40X) are shown.



Conclusions

- In the present study, we observed that prolonged treatment with anti-estrogen causes drug resistance by inducing the DNA repair protein O⁶-methylguanine DNA methyltransferase (MGMT).
- Decreasing the expression of MGMT by exposing breast cancer cells to BG sensitized these cells to anti-estrogen therapy (tamoxifen and ICI 182,780).
- We also observed that combination therapy of anti-estrogens and MGMT and ICI induces MGMT transcription, BG induced MGMT and ER α transcription.
- Combination therapy inhibited tamoxifen resistant breast tumor growth *in vivo*.

Acknowledgements

We would like to thank the Florida Department of Health, Roskilde-Coley Cancer Research Program (RCCRP)-for their funding of this project.

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

Joshua Smith¹, George C Bobustue¹, Rafael Madero-Visbal¹, Jimmie Colon¹, Beth Isley¹, Jonathan Ticku¹, Kalkunte S. Srivenugopal and Santhi Konduri¹

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



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 (*J Clin Cancer Res.* 16: 6087-6093, 2006), 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 tamoxifen (ICI) could sensitize human tamoxifen resistant breast cancer cell growth to tamoxifen using tamoxifen resistant cells.

Posters rarely need abstracts

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 cells. Interestingly, silencing of the ER α expression in these cells also observed an inverse correlation between MGMT expression and ER α . This was accompanied by decreased ER α expression and increased MGMT expression. However, administration of tamoxifen or fulvestrant decreased ER α expression, whereas tamoxifen alone and fulvestrant alone increased and decreased the same respectively. However, all three treatments increased the p21^{kip1} 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. By breast cancer micrographs, 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^{kip1} 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 methyltransferase (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 at low levels and are upregulated by various cellular stresses. MGMT levels are upregulated prosomatically by benzylguanine (BG) treatment. In a series of experiments, we have shown that BG has a rapid and potent effect on MGMT expression which results in the covalent transfer of benzyl group onto the AGT enzyme, thereby depleting the AGT activity and inhibiting the repair of alkylating agent-induced damage. BG is currently undergoing c

Text dissolves into intimidating, boring gray

Interestingly, several proteins where wild-type inactivated or suppressed the success of some treatments. However, whether or not this is mediated by suppression of MGMT function has yet to be determined. To date, the cross-talk between MGMT and ER α (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 α -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 on MCF-7 cells increased MGMT expression compared to parental MCF-7 cells by fold (Fig.1).



Knocking Down ER α Enhances MGMT Expression in Tamoxifen Resistant Breast Cancer Cells: It is not known whether ER α and MGMT transcriptionally regulate each other in tamoxifen resistant breast cancer cells. We therefore investigated whether down regulation of ER α has any effect on endogenous MGMT expression in these cells. As expected, downregulation of ER α using specific siRNA significantly reduced ER α protein levels in these cells. Western blot analysis was performed and the results in the left panel (Fig. 2A) shows that silencing of ER α 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 ER α -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.3C) or MGMT siRNA (MGMT-KD) (Fig.3D) along with Non-specific siRNA (NS). MGMT expression was consistently increased in p53 knock down cells, with different experiments showing a \sim 4-fold augmentation (Fig. 3A) and as expected, knocking down MGMT decreased MGMT transcription where as p53 mRNA levels were unaffected in MGMT knockdown cells (Fig.3B). These results confirm that p53 can regulate MGMT at the transcriptional level.

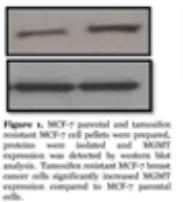


Figure 1. MCF-7 parental and tamoxifen resistant MCF-7 cell pellets were isolated and MGMT expression was detected by western blot analysis. Tamoxifen resistant MCF-7 breast cancer cells significantly increased MGMT expression compared to MCF-7 parental cells.

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 ER α transcription. As expected, knocking down MGMT decreased MGMT gene transcripts. However, it was interesting to find that ER α gene transcription was also reduced after MGMT silencing (Fig.4A). These data demonstrate that BG has the ability to attenuate the not only the MGMT, but also the ER α transcription, indicating a possible dual role for MGMT blockers in these breast cancer cells.

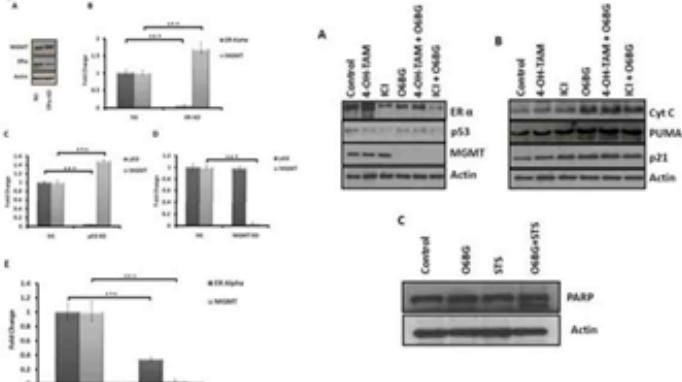


Figure 4. (A) Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (10 μ M) and with/without treatment a-OH-TAM (1 μ M). ICI (10 nM) alone or in combination with BG and total RNA were isolated and ER α and p53 mRNA levels were determined by qRT-PCR. (B) Total RNA was isolated from ER α knock down MCF-7 cells treated with BG and with/without treatment a-OH-TAM (1 μ M). ICI (10 nM) alone or in combination with BG and total RNA was isolated and MGMT mRNA levels were determined by qRT-PCR. (C) Total RNA was isolated from ER α knock down MCF-7 cells treated with BG and with/without treatment a-OH-TAM (1 μ M). ICI (10 nM) alone or in combination with BG and total RNA was isolated and MGMT and p53 transcription was determined by qRT-PCR. There is an inverse correlation between MGMT and p53 in tamoxifen resistant breast cancer cells (A, B).

O⁶-Benzylguanine Modulates p53 Down-Stream Targeted Protein Expressions: Encouraged by the results reported, we investigated the effect of combination therapy on endogenous MGMT, ER α , and p53 protein expressions. As expected, we observed MGMT expression, while combination therapy (4-OH-TAM or ICI combined with BG) significantly decreased both MGMT and ER α expressions. ER α 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^{kip1} 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 indicator 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 was also studied. Quantitative real-time PCR (qRT-PCR) revealed that anti-estrogens (TAM/ICI) increased the MGMT expression while the combination therapy decreased it compared to control levels. ER α 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 data suggest that BG may increase the transcriptional activity of p53 in tamoxifen resistant breast cancer cells.



Figure 4. Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (10 μ M) and with/without treatment a-OH-TAM (1 μ M) and ICI (10 nM). Total RNA was isolated and MCF-7 cells were transfected with p53 construct and 48 h later cells were harvested. p53 transcription was determined by qRT-PCR. (B) Tamoxifen resistant MCF-7 breast cancer cells were transfected with p21 construct and 48 h later cells were harvested. p21 transcription activity was significantly increased by BG in these cells.

O⁶-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Increase Resistant Breast Cancer Sensitivity to Anti-Estrogen Therapy (TAM/ICI): Detailed necropsy revealed that all the harvested 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, 31.60 mm³; TAM+BG, respectively; p < 0.0001; 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 < 0.0001; ICI+BG, respectively; p < 0.0001) (Table 1). Body weight was not changed among all treated mice as compared with control mice. No visible liver metastases were present in any of the mice examined (examined under microscope) in all treatment groups.

Crammed!

Histology and IHC Analysis: We also analyzed 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, ER α , 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, ER α , p53, p21 and ki-67 expressions were quantified by the ImmunoRatio plugin. (Fig.5).

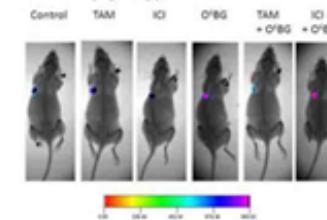


Figure 5. (A) Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (10 μ M) and with/without treatment a-OH-TAM (1 μ M). ICI (10 nM) alone or in combination with BG and total RNA were isolated and ER α and p53 mRNA levels were determined by qRT-PCR. (B) Tumors harvested from mice treated with BG alone or in combination with tamoxifen/ICI were fixed in formalin, paraffin embedded and sectioned. Sections were stained with hematoxylin and counterstained with hematoxylin. MCF-7 cells were used as positive control. (C) PUMA and p21 was also measured in tamoxifen resistant MCF-7 cells treated with BG alone or in combination with tamoxifen/ICI. Total RNA was isolated and p21 and p53 mRNA levels were determined by qRT-PCR. (D) Tumors harvested from mice treated with BG alone or in combination with tamoxifen/ICI were fixed in formalin, paraffin embedded and sectioned. Sections were stained with hematoxylin and counterstained with hematoxylin. MCF-7 cells were used as positive control. (E) Tumors harvested from mice treated with BG alone or in combination with tamoxifen/ICI were fixed in formalin, paraffin embedded and sectioned. Sections were stained with hematoxylin and counterstained with hematoxylin. MCF-7 cells were used as positive control. (F) Tumors harvested from mice treated with BG alone or in combination with tamoxifen/ICI were fixed in formalin, paraffin embedded and sectioned. Sections were stained with hematoxylin and counterstained with hematoxylin. MCF-7 cells were used as positive control.

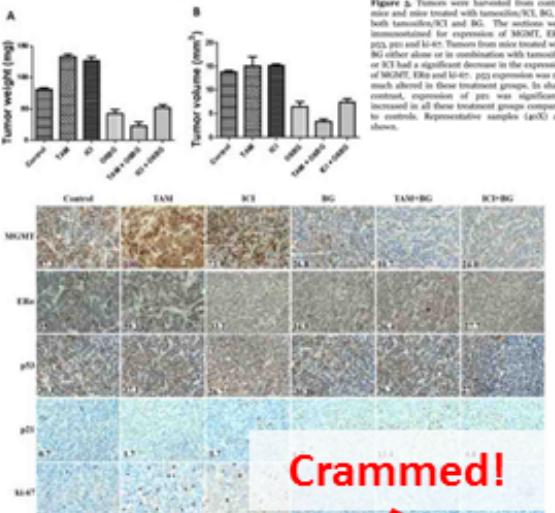


Figure 5. Tumors were harvested from control mice and mice treated with BG alone or in combination with tamoxifen/ICI. The sections were immunostained for expression of MGMT, ER α , p53, p21 and ki-67. Tumors from mice treated with BG alone or in combination with tamoxifen/ICI showed a significant decrease in MGMT, ER α and ki-67. Tumor weight was not much altered in these treatment groups. In sharp contrast, the expression of p21 was significantly increased in all these treatment groups. Representative samples (A-E) are shown.

Crammed!

Conclusions

- 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).
- Decreased the expression of MGMT by exposing breast cancer cells to BG sensitized these cells to anti-estrogen therapy (tamoxifen and ICI).
- We also observed that combination therapy of anti-estrogen and MGMT blockers not only overcome the MGMT derived drug resistance (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.
- Combination therapy inhibited tamoxifen resistant breast tumor growth *in vivo*.

Acknowledgements

We would like to thank the Florida Department of Health, Biostatistic/Cancer Research Program-mDRS for their funding of this project.

Alignment and Whitespace

- most important things of first
- use a grid to keep items aligned and straight
- use a column format to split space (3-4 for landscape, 2 for portrait)
- Limit use of boxes and lines (visual clutter), instead use GP: proximity



The “white space” in this image is 40% of the image area.

Remember C.R.A.P. ?

Contrast

Making elements different increases understanding.

Repetition

Repeat visual elements to create strong unity.

Alignment

Nothing should be placed arbitrarily. Placement illustrates relationships between elements.

Proximity

Related items should be placed together.

Activity

Exchange your draft with your neighbor.

Use a marker to highlight good and improvable design parts.

[5 min]



Text in Your Poster

- should be concise
- name each technique appropriately
- explain **which** information is encoded
- explain **how** information is encoded

The History of Donuts

Homer Simpson



....

E1

group number

The History of Donuts

Homer Simpson



....

E1

Red fields are required.

Activity

Create your poster and use:

- title and authors
- proper alignment and whitespace
- storytelling principles

You can also make use of the source:
tiny.cc/cs171-poster



Poster Session on Thursday

CS
171


Goal of Vis Exploration

- Get to know about the classics in Visualization.
- Why?
 - be fluent in visualization language to communicate with other visualization experts and creating ideas.
 - be able to judge, if your new idea is novel

Poster Session

- You can iterate over your poster if you like (take your current version home or get a new sheet).
- A photo of your poster has to be submitted to Canvas **latest on Thursday 11:59pm** (it's part of your project grading)
- Please be in time (2:30pm) to hang up your poster at the designated location.
- We will hand out questionnaires that you should fill and submit with your homework.

Poster Session

- For each Vis field, the poster creators should stand at their poster for presentation. All others should walk around and explore (and fill in questionnaire).

		Presenting	Exploring
2:40 - 3:00	Geographical Data / Maps	A	B C D
3:00 - 3:20	Trees & Networks	B	A C D
3:20 - 3:40	Vis for High Dimensional Data	C	A B D
3:40 - 4:00	Visualization for Text	D	A B C

Poster Session (DCE)

- Please submit your poster until Friday 11:59pm
- We will provide a link to all posters on Saturday.
- Please fill in the questionnaire and submit it with your homework until Monday.



This Thursday...

- Poster Presentation
- Reading: How to present a poster in class
<http://www.owlnet.rice.edu/~cainproj/presenting.html>



Next Tuesday...

- Evaluation/Innovation / Innovation in D3
- Reading: TBD



Project (due Monday)...

- Project Plan, Poster Questionnaire