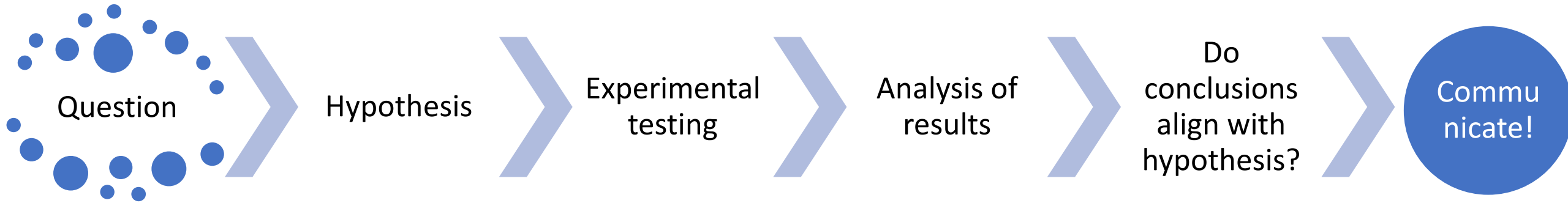
Laavanya Sankaranarayanan
Duke Genetics PhD Student
NCSSM Miniterm 2019

Ted Espenschied, *The Inside Track*, 2018. Fluorescent Micrograph.


Question
Hypothesis
Experimental testing
Analysis of results
Do conclusions align with hypothesis?
Commu nicate!

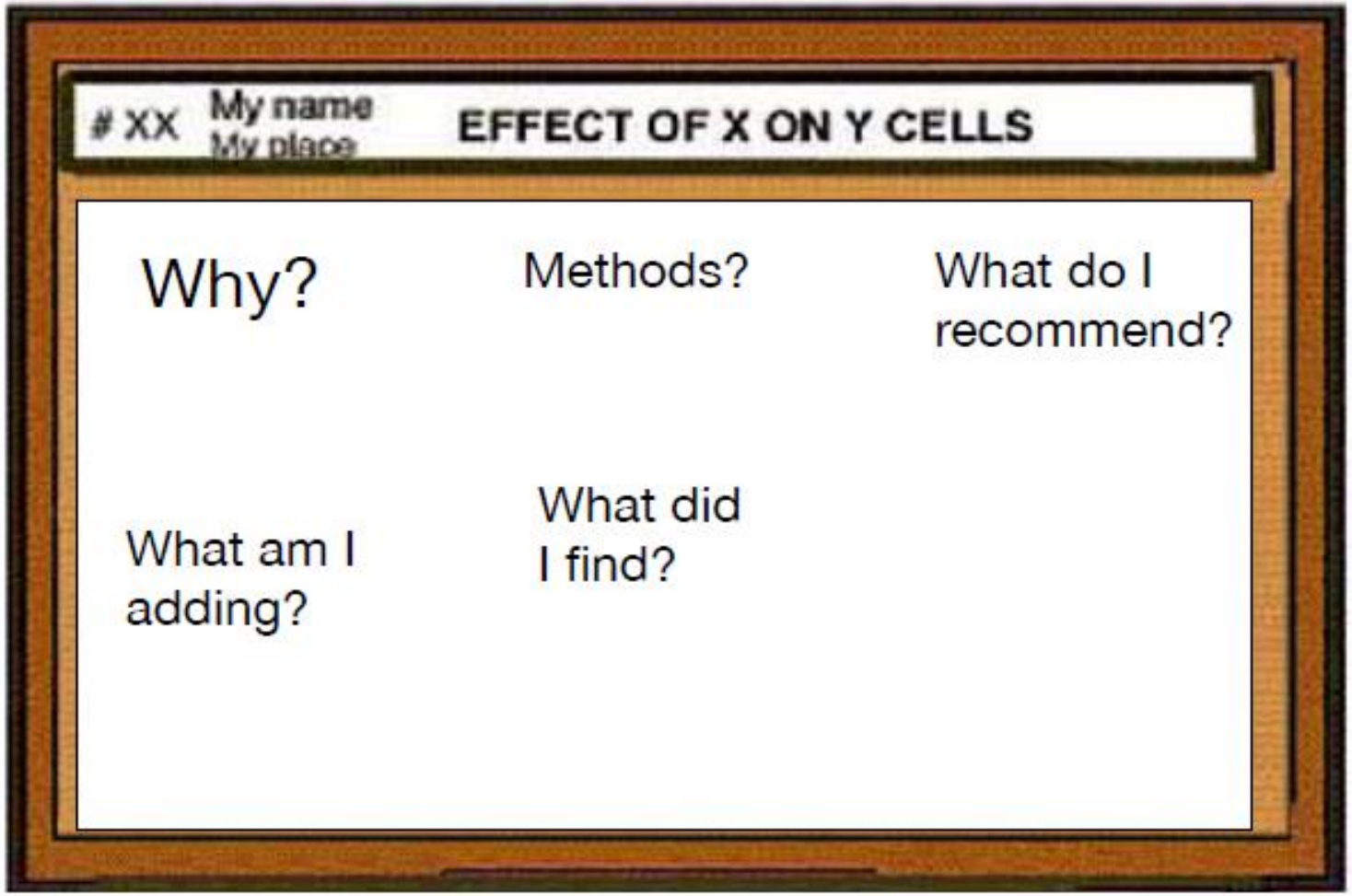
What is a poster?

Why?

An advertisement of your hard work

Kool, wow!, check this out!, you must be smart!

What



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Content

- Title
- Authours
- Background
- Questions
- Results
- Future Directions
- Acknowledgement + Funding
- Contact

A research poster is

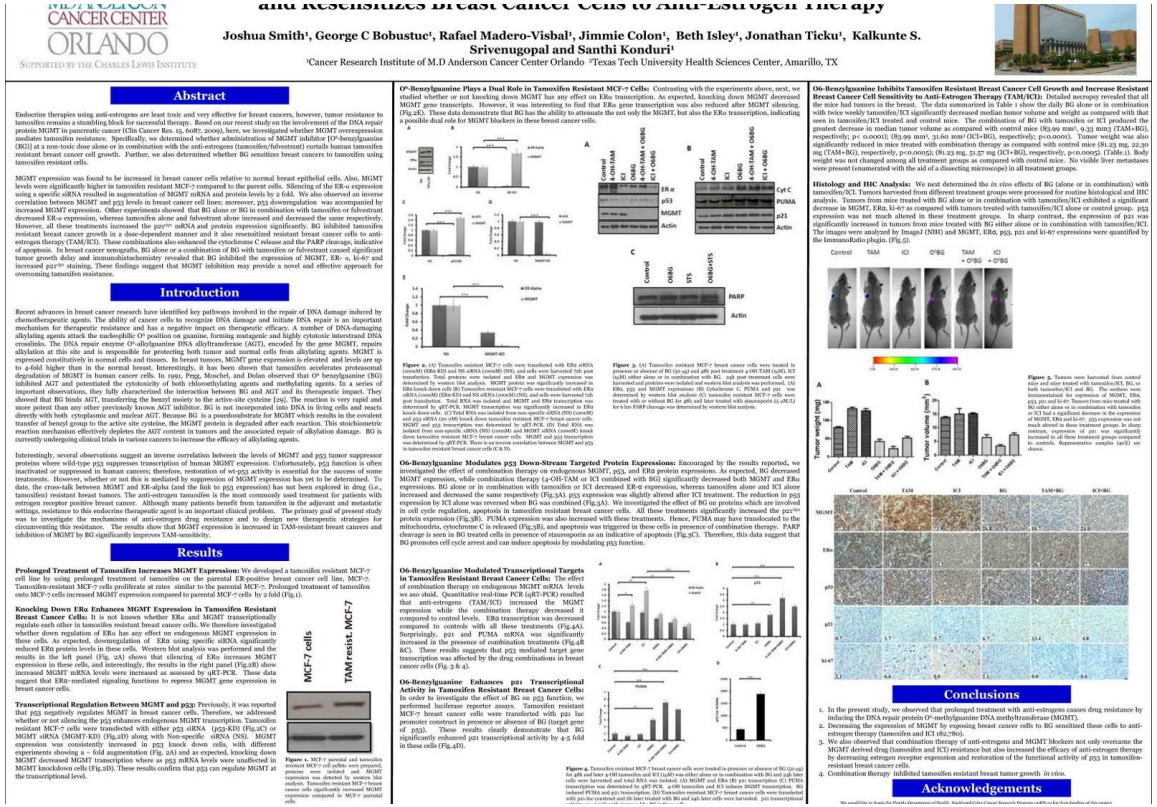
A summary of the project

Visually engaging

Highlighting the context of your work through methods and future directions

A logical presentation of work that can be understood even if you weren’t around to talk about it

What do you think?



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]

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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 (O^6-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 fulvestrant decreased ER-α expression, whereas tamoxifen alone and fulvestrant alone increased and decreased the same respectively. However, all these treatments increased the p21^{WAF} 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^{WAF} 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 O^6 position on guanine, forming mutagenic and highly cytotoxic interstrand DNA crosslinks. The DNA repair enzyme O^6-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 O^6 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 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-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, protein 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.

O^6-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 tamoxifen resistant MCF-7 breast cancer cells 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). (E) Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μg) and ERα gene transcription was determined by qRT-PCR. ERα transcription was significantly decreased in BG treated cells.

Figure 3. (A) Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (10 μg) and 4OH-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, 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 (1 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^{WAF} 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 we also studied. 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 induces 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

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 mm^3, 9.33 mm3 (TAM+BG), respectively; p< 0.0001); (83.99 mm^3, 31.60 mm^3 (ICI+BG), respectively; p<0.0001). Tumor weight was also significantly reduced in mice treated with combination therapy as compared with control mice (81.25 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).

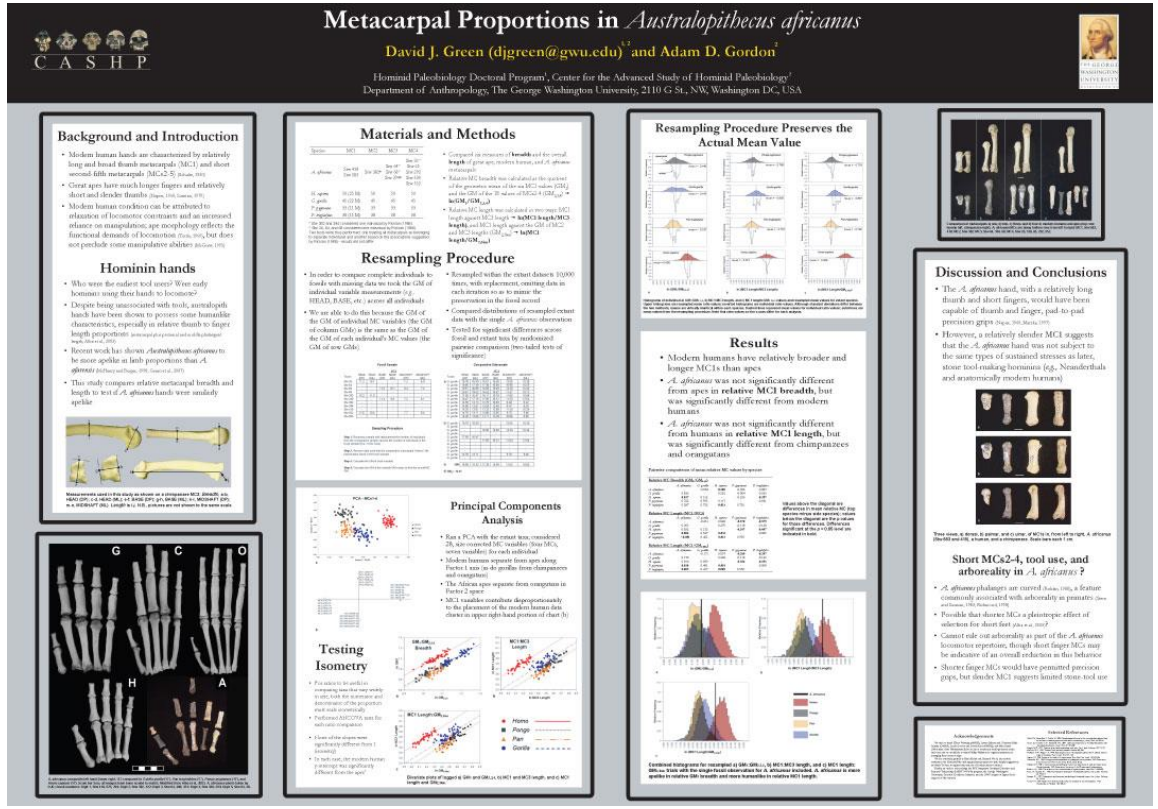
Figure 5. Tumors were harvested from control mice and mice treated with tamoxifen/ICI, BG or both tamoxifen/ICI and BG. The sections were immunostained for expression of MGMT, ERα, p53, p21 and ki-67. Tumors from mice treated with BG either alone or in combination with tamoxifen or ICI had a significant decrease in the expression of MGMT, ERα and ki-67 as compared with tumors from mice treated with tamoxifen/ICI alone or control. 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 O^6-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 breast tumor growth *in vivo*.

Acknowledgements

We would like to thank the Florida Department of Health, Bankhead-Coley Cancer Research Program (09B02) for their funding of this project.



Metacarpal Proportions in *Australopithecus africanus*

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Department of Anthropology, The George Washington University, 2110 G St., NW, Washington DC, USA

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Background and Introduction

- Modern human hands are characterized by relatively long and broad thumb metacarpals (MC1) and short second-fifth metacarpals (MC2-5) (Napier, 1962).
- Great apes have much longer fingers and relatively short and slender thumbs (Napier, 1963; Susman, 1979).
- Modern human condition can be attributed to relaxation of locomotor constraints and an increased reliance on manipulation; ape morphology reflects the functional demands of locomotion (Tuttle, 1969), but does not preclude some manipulative abilities (McGrew, 1981).

Hominin hands

- Who were the earliest tool users? Were early hominins using their hands to locomote?
- Despite being unassociated with tools, australopith hands have been shown to possess some humanlike characteristics, especially in relative thumb to finger length proportions (pollex:longest digit or pollex:digital length; Susman et al., 1984).
- Recent work has shown *Australopithecus afarensis* to be more apelike in limb proportions than *A. africanus* (McHenry and Berger, 1998; Green et al., 2005).
- This study compares relative metacarpal breadth and length to test if *A. africanus* hands were similarly apelike

Measurements used in this study as shown on a chimpanzee MC3 (dmidc3, a.b. =BAO (OP); c-d =BAO (BL); e-f: BASE (DP); gh=BASE (ML); i= MIDSHAFT (DP); m is MIDSHAFT (BL). Length is c-d; BASE pictures are not shown to the same scale.

Materials and Methods

- Compared on measures of **breadth** and the overall **length** of gross apes, modern human, and *A. africanus* metacarpals
- Relative MC breadth was calculated as the quotient of the geometric mean of the six MC1 values (GM$_{MC1}$) and the GM's of the 20 values of MC2-5 (GM$_{MC2-5}$) → **ln(GM$_{MC1}$/GM$_{MC2-5}$)**
- Relative MC length was calculated as two ratios: MC1 length against MC2 length → **ln(MC1 length/MC2 length)**, and MC1 length against the GM of MC2 and MC3 length (GM$_{MC2-3}$) → **ln(MC1 length/GM$_{MC2-3}$)**

Resampling Procedure

- In order to compare complete individuals to fossils with missing data we took the GM of individual variable measurements (e.g., HEAD, BASE, etc.) across all individuals
- We are able to do this because the GMs of the GM of individual MC variables (the GM of column GMs) is the same as the GM of the GM of each individual's MC values (the GM of row GMs)

Resampling Procedure Preserves the Actual Mean Value

Results

- Modern humans have relatively broader and longer MC1s than apes
- *A. africanus* was not significantly different from apes in **relative MC1 breadth**, but was significantly different from modern humans
- *A. africanus* was not significantly different from humans in **relative MC1 length**, but was significantly different from chimpanzees and orangutans

Principal Components Analysis

- Ran a PCA with the extant taxa, considered 28, size-corrected MC variables (nine MCs, seven variables) for each individual
- Modern humans separate from apes along Factor 1 axis (to do gorillas from chimpanzees and orangutans).
- The African apes separate from orangutans in Factor 2 space
- MC1 variables contribute disproportionately to the placement of the modern human data cluster in upper right-hand portion of chart (b)

Testing Isometry

- For axiom to be applied to comparing fossil to ape/modern to infer, both the systematic and determinate of the proportion that exists systematically
- Performed ANCOVA tests for each ratio comparison
- If axes of the slopes were significantly different from 1 (isometry)
- If both met, the modern human p-average was significantly different from the apes'

Bivariate plots of logged ln GM+ and GM$_{MC2-5}$ (c), ln(MC1 and MC3 length, and c), MC1 length and GM$_{MC2-3}$.

Discussion and Conclusions

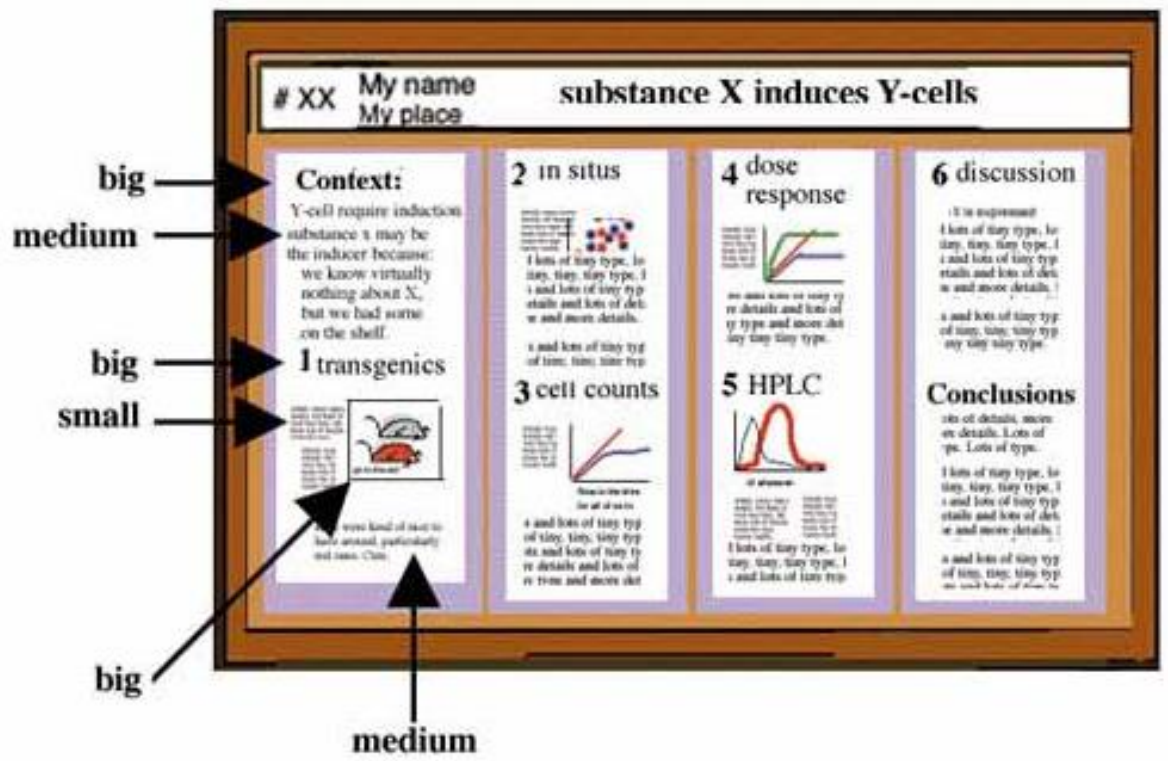
- The *A. africanus* hand, with a relatively long thumb and short fingers, would have been capable of thumb and finger, pad-to-pad precision grips (Napier, 1960; Marzke, 1997).
- However, a relatively slender MC1 suggests that the *A. africanus* hand was not subject to the same types of sustained stresses as later, stone tool-making hominins (e.g., Neanderthals and anatomically modern humans).

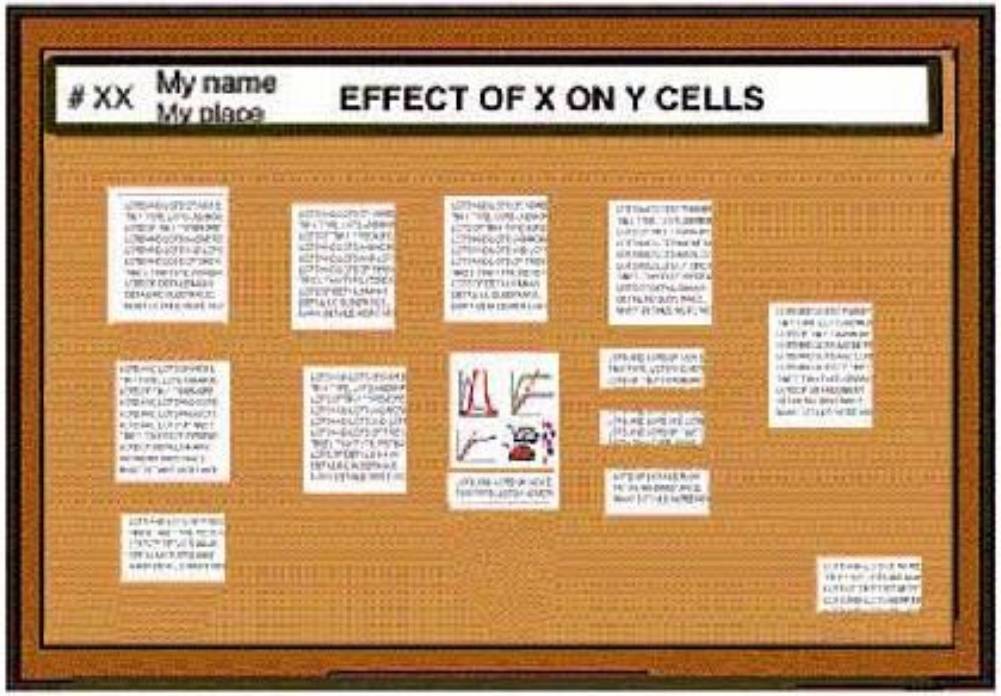
Short MCs2-4, tool use, and arboreality in *A. africanus*?

- *A. africanus* phalanges are curved (Tobias, 1981), a feature commonly associated with arboreality as primates (Susman and Stern, 1983; Richmond et al., 2001).
- Possible that shorter MCs a pleiotropic effect of selection for short feet (Lovejoy et al., 2009).
- Cannot rule out arboreality as part of the *A. africanus* locomotor repertoire, though short finger MCs may be indicative of an overall reduction in this behavior.
- Shorter finger MCs would have permitted precision grips, but slender MC1 suggests limited stone tool use

Conditional histograms for metacarpal a) GM+ GM$_{MC2-5}$, b) MC1 MC3 length, and c) MC1 length: GMs are from with the single-fossil observations for *A. africanus* included. *A. africanus* is more apelike in relative GM+ breadth and more humanlike in relative MC1 length.

Visual Grammar





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Use a graphic hierarchy that visually reflects the relative importance of elements.

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Use a text-heavy, publication-style format.

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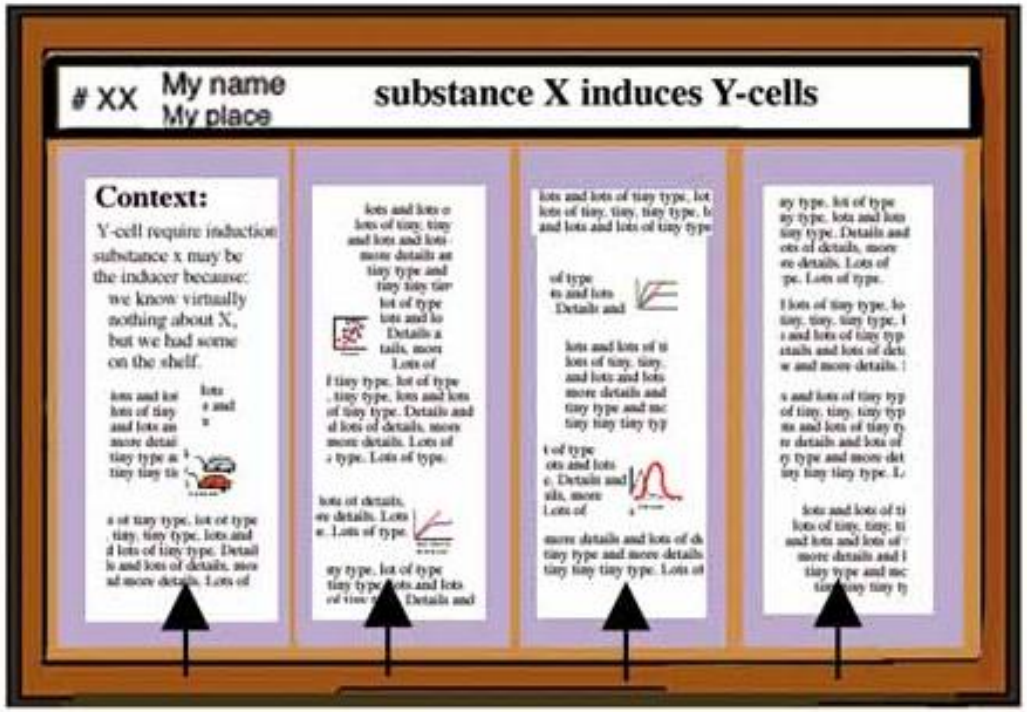
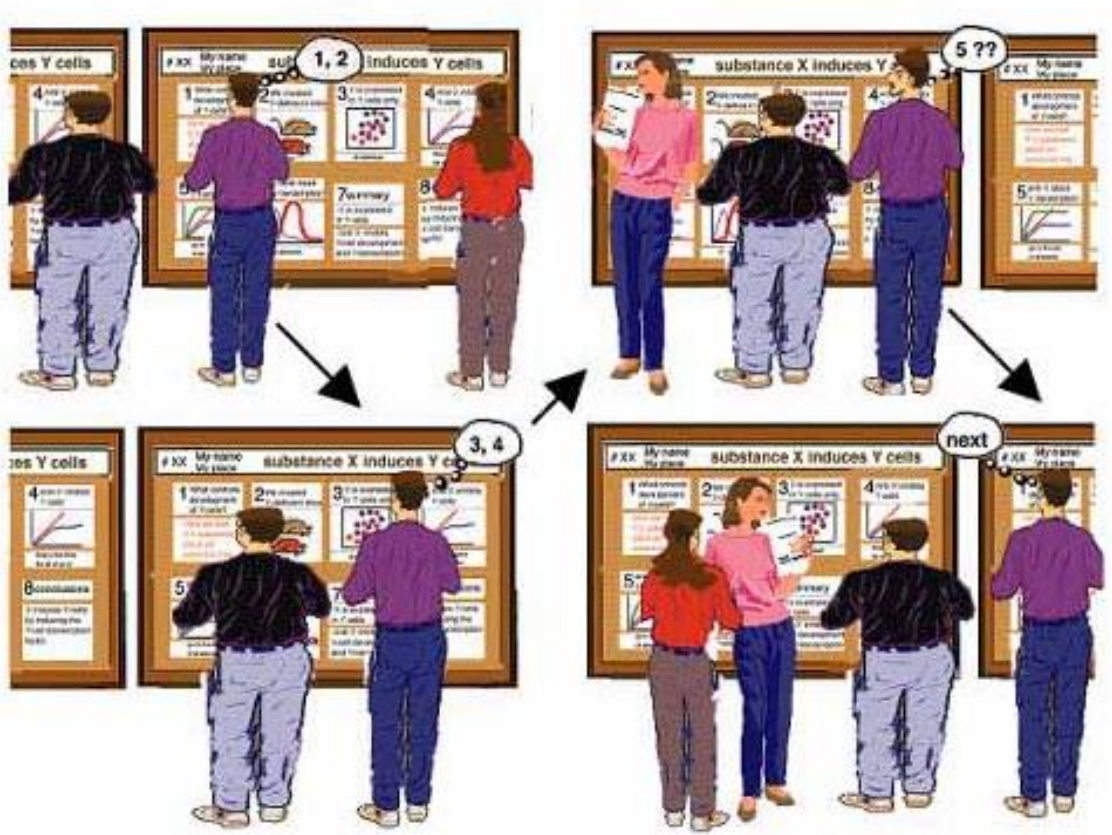
Guiding with columns





Do this ...

Use a columnar format. It allows readers to read the entire poster as they procedd from left to right.

... not this

Use a row-oriented layout. This format moves readers past your poster very quickly.

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Keep in mind

USE A VISUAL GRAMMAR TO GUIDE READERS TO THE IMPORTANT PARTS OF YOUR POSTER.

USE A COLUMN FORMAT TO MAKE YOUR POSTER EASIER TO READ IN A CROWD.

USE ORGANIZATION CUES TO GUIDE READERS THROUGH YOUR POSTER.

USE "READER GRAVITY" WHICH PULLS THE EYE FROM TOP TO BOTTOM AND LEFT TO RIGHT (WHEILDON 1995).

USE HEADINGS INTELLIGENTLY TO HELP READERS FIND YOUR MAIN POINTS AND KEY INFORMATION.

BALANCE THE PLACEMENT OF TEXT AND GRAPHICS TO CREATE VISUAL APPEAL.

USE WHITE SPACE CREATIVELY TO HELP DEFINE THE FLOW OF INFORMATION.

White space is your friend. :)

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Elements of storytelling

Elements of scientific storytelling

- Set the stage
- Purpose
- Where’s the action?
  - Expectations!
  - Plot twists?
- Conclude
- What can we expect in the future – a sequel!

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- Set the stage
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Virulence QTLs and Genome-wide Recombination Rates in *Cryptococcus*

Cullen Roth[1,3], Sheng Sun[2], R. Blake Billmyre[2], Debra Murray[3], Joseph Heitman[2], Paul M. Magwene[1,3]
1) University Program in Genetics and Genomics, 2) Department of Molecular Genetics and Microbiology, 3) Department of Biology, Duke University

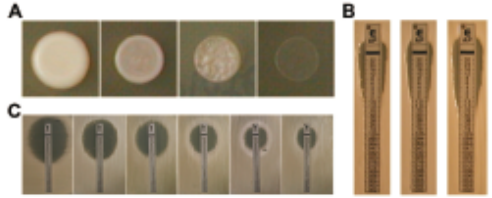
Introduction

Cryptococcal disease affects ~223,100 people, causing approximately 180,000 deaths, annually[1]. Environmental isolates of *Cryptococcus deneoformans*, vary in their pathogenicity, ranging from benign to hyper-virulent.

Goal: Identify the genetic basis underlying the variation in virulence-related traits, (high temperature tolerance and resistance to anti-fungal drugs, amphotericin B and fluconazole) using quantitative trait locus (QTL) mapping in *C. deneoformans*.

Strains XL280a, XL280α, and 431α were crossed to generate haploid segregants via a-α bisexual reproduction (N = 39) and α-α unisexual reproduction (N = 55). Approximately 87,000 single nucleotide polymorphisms (SNPs) were identified across these segregants using short-read sequencing[2].

Variation of Virulence Traits

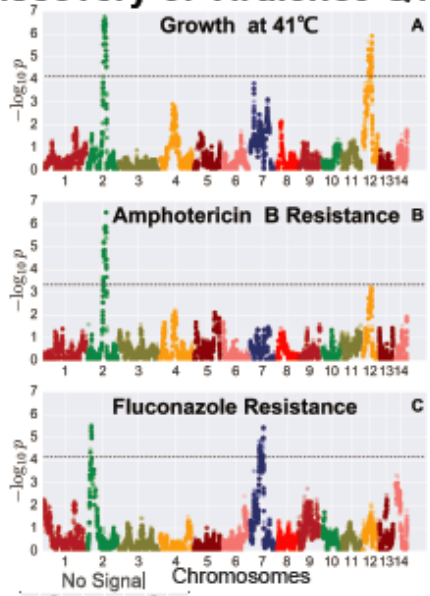


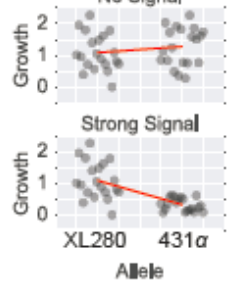
(A) The variation in growth at 41°C on YPD plates is shown for 4 segregants. (B) For amphotericin B resistance, E-strips were used to calculate the minimum inhibitory concentration (MIC). A similar assay was used to calculate (C) the MIC for fluconazole resistance. The results of 6 segregants are shown.

High Density Recombinant SNP Data

From top to bottom, the SNP density for chromosome 3 (length ~2.1Mb) calculated as the number of SNPs per 10kb (Total: 9779 SNPs), the haplotypes for 5 segregants from the bisexual cross and 5 segregants from the unisexual cross. Haplotypes are inferred from SNP data and displayed as blue if inherited from XL280a and XL280α or orange if inherited from 431α. The position of the centromere is displayed in black.

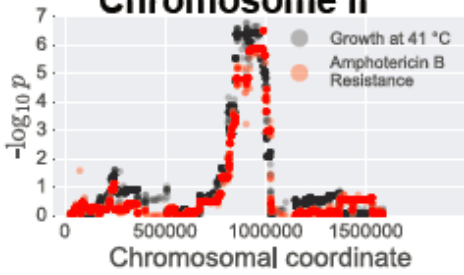
Discovery of Virulence QTL





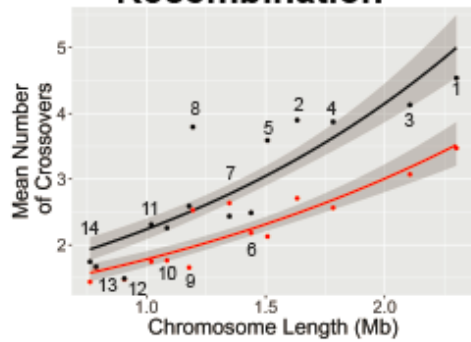
A marker regression framework (left) was used to identify QTL for (A) growth at 41°C (chromosomes II and XII), (B) amphotericin B resistance (chromosome II), and (C) fluconazole resistance (chromosome II and VII). Horizontal, dotted lines indicate genome-wide significance thresholds established via permutation tests.

Pleiotropic QTL on Chromosome II



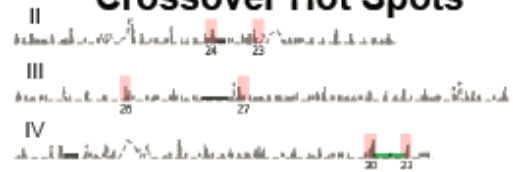
A QTL implicated in high temperature growth and amphotericin B resistance. A total of 48 genes lie in the QTL, two (*HRT1* and *KTR3*) are known to play a role in temperature sensitivity[3,4] and one (*SSK1*) is known to cause amphotericin B sensitivity in *C. neoformans*[5].

Genome-wide Recombination



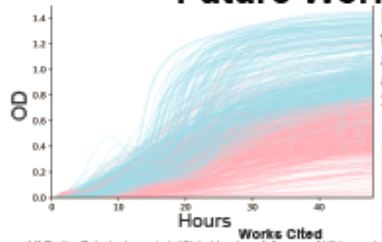
Genome-wide recombination rates were estimated using Poisson regression. This model predicts an obligatory ~ 0.98 crossovers per chromosome for offspring from the α-α unisexual crosses (red) and ~ 1.30 crossovers per chromosome for offspring from the a-α bisexual crosses (black). The expected number of crossovers increases by a ratio of ~1.768 per Mb increase in chromosome size (slopes of red and black lines). Shaded regions are 95% confidence intervals of the regression estimates. Numbers indicate chromosomes.

Crossover Hot Spots



The crossovers along chromosomes II, III, and IV are shown. Each chromosome was overlaid with 50kb bins to identify crossover hot spots (highlighted red) using the expected number of crossovers from our analysis above. The number of crossovers within hot spots are labeled underneath. The *MAT* locus and centromeres are displayed as green and black bars, respectively.

Future Work



High temperature growth data across 48 hours at 30 (blue) and 37°C (pink).

Acknowledgments
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Poster workshopping