

INSTITUTE FOR CLINICAL
RESEARCH EDUCATION



Designing a Poster for the Dean's Summer Research Program

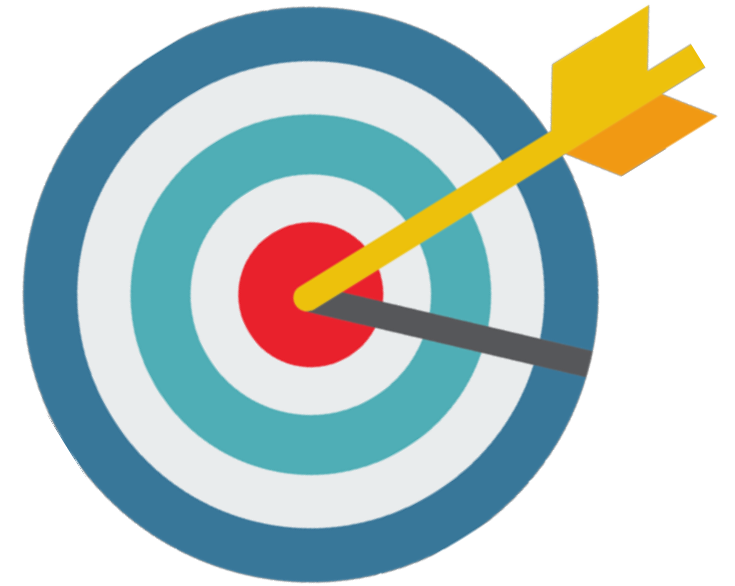
Colleen Mayowski, EdD, MLIS

Donald B. DeFranco, PhD

July 18, 2017

Learning objectives:

1. Describe how posters are used and what they should accomplish.
2. Identify strategies for designing effective posters for the Dean's Summer Research Program.
3. Distinguish characteristics of effective and ineffective posters.





Posters are a visual medium.



Prepare

- If you have a name tag, wear it in an easily visible place
- If you have business cards, bring them
- Have a way to store other people's cards
- Handouts are a good idea
- Eat in advance (don't try to snack at the same time)
- Take the initiative – don't passively stand by
- Be aware of interested bystanders and loop them in

Practice!

Practice a 2 minute presentation – example:

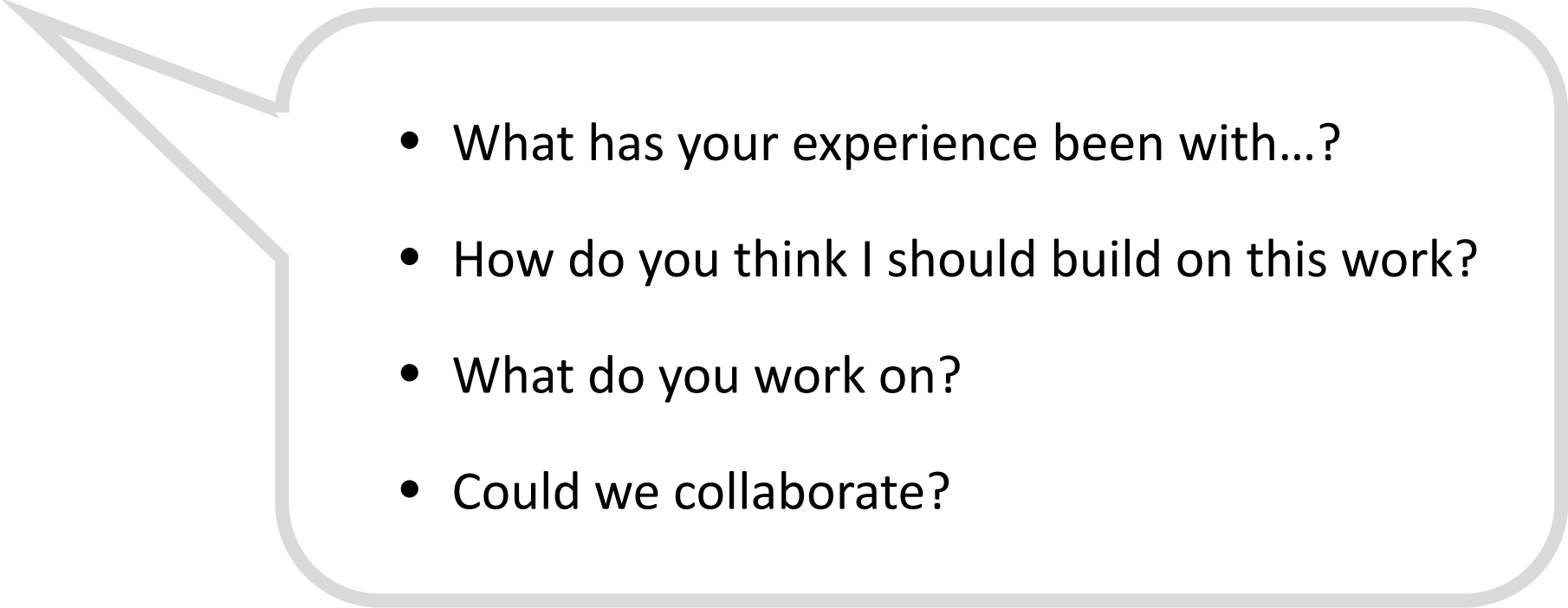
[https://www.youtube.com/watch?v=NBa0cnsRMUQ.](https://www.youtube.com/watch?v=NBa0cnsRMUQ)



Start the conversation...

- Hi, can I give you a tour of my poster?
- My name is _____. I'm at the University of Pittsburgh...Where are you from?
- As you may know...this is an important topic because...
- We were interested in...
- So what we did was...
- And we found that (point to bullet 1; point to figure 1)...

THEN open up the conversation...

- 
- What has your experience been with...?
 - How do you think I should build on this work?
 - What do you work on?
 - Could we collaborate?

A poster is not a research paper stuck to a board.



Clinical Trials with Multiple Outcomes: A Statistical Perspective on their Design, Analysis, and Interpretation

Stuart J. Pocock, PhD

Medical Statistics Unit, London School of Hygiene & Tropical Medicine, London,
United Kingdom

ABSTRACT: This article tackles both practical and statistical issues in the handling of multiple outcomes in clinical trials, with relevance to trial design, analysis, and reporting. Specific topics illustrated by examples include: the advantage of prespecifying priorities amongst outcomes and analyses, corrections for multiple significance testing and their limited value, problems with adverse event data, the use of a single global test of significance for clinically related outcomes, the use of a combined outcome for clinical event data, and the value of exploring interrelationships amongst outcomes. The problems in handling multiple outcomes are enhanced by trials being too small, dichotomous attitudes (is the trial "positive" or not?), obsession with p -values, and the manipulative instincts of human nature. While predeclarations of priorities in analysis and reporting of multiple outcomes are important in suppressing distortive claims, it would be unfortunate if too inflexible an approach suppressed unpredictable findings from being seriously considered. *Controlled Clin Trials* 1997;18:530-545 © Elsevier Science Inc. 1997

KEY WORDS: Clinical trials, multiple outcome measures

INTRODUCTION

In many disease conditions, the patients response to treatment can have several different aspects. While a small minority of large simple trials may focus on a single outcome measure, e.g., mortality, in most circumstances this would be an oversimplification of the diversity of patient responses. Hence, most trials entail the recording of multiple outcome measures which might cover such diverse aspects as clinical events, symptoms, physiologic measurements, blood tests, side effects, and quality of life. The problem is that one then runs the risk of data dredging and distortive reporting with a post hoc emphasis on the most statistically impressive findings. This problem is enhanced by:

1. The limited size of most clinical trials, which leaves the findings unclear for many of the outcome measures.

Address reprint requests to: Prof. Stuart J. Pocock, Medical Statistics Unit, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK.
Received March 27, 1996; revised June 26, 1996; accepted February 10, 1997.

Controlled Clinical Trials 18:530-545 (1997)
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Remember, it's a
visual medium.
Graphics should dominate
Text should be minimal



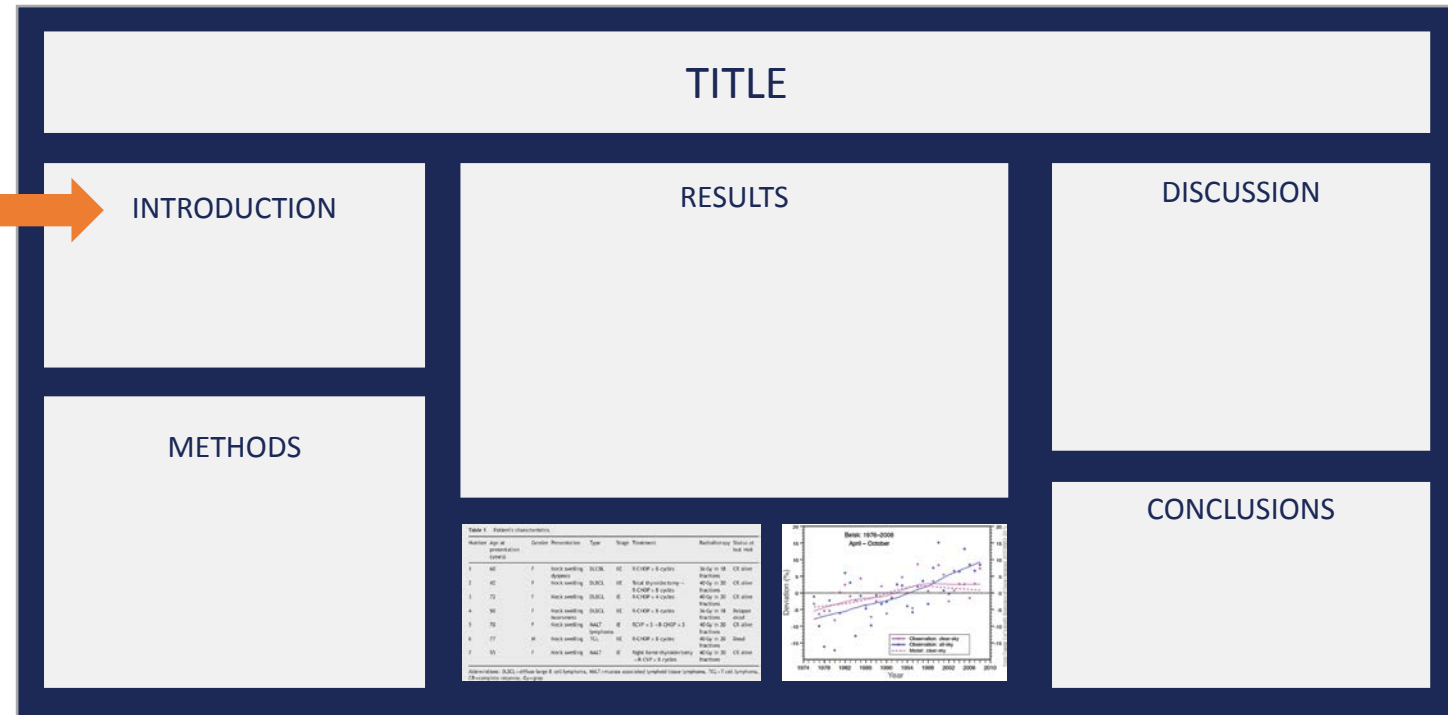
Titles

- Blah title: “Blood Pressure Control in Elderly Patients at Risk for Stroke”
- Better title: “Diuretic Treatment Decreases Stroke Rate in the Elderly”
- Cute title: “Why Bring That Up? Advances in Treating Seasickness”
- Point size: 90-120, bold. Subtitle: 72

Headers

Make your section headings do some heavy lifting.

Point size:
36-48



Fonts

Use fonts that are easy to read: Point size: 24-28 for body text



serif font
(includes perpendicular
lines on the terminal ends
of letters)



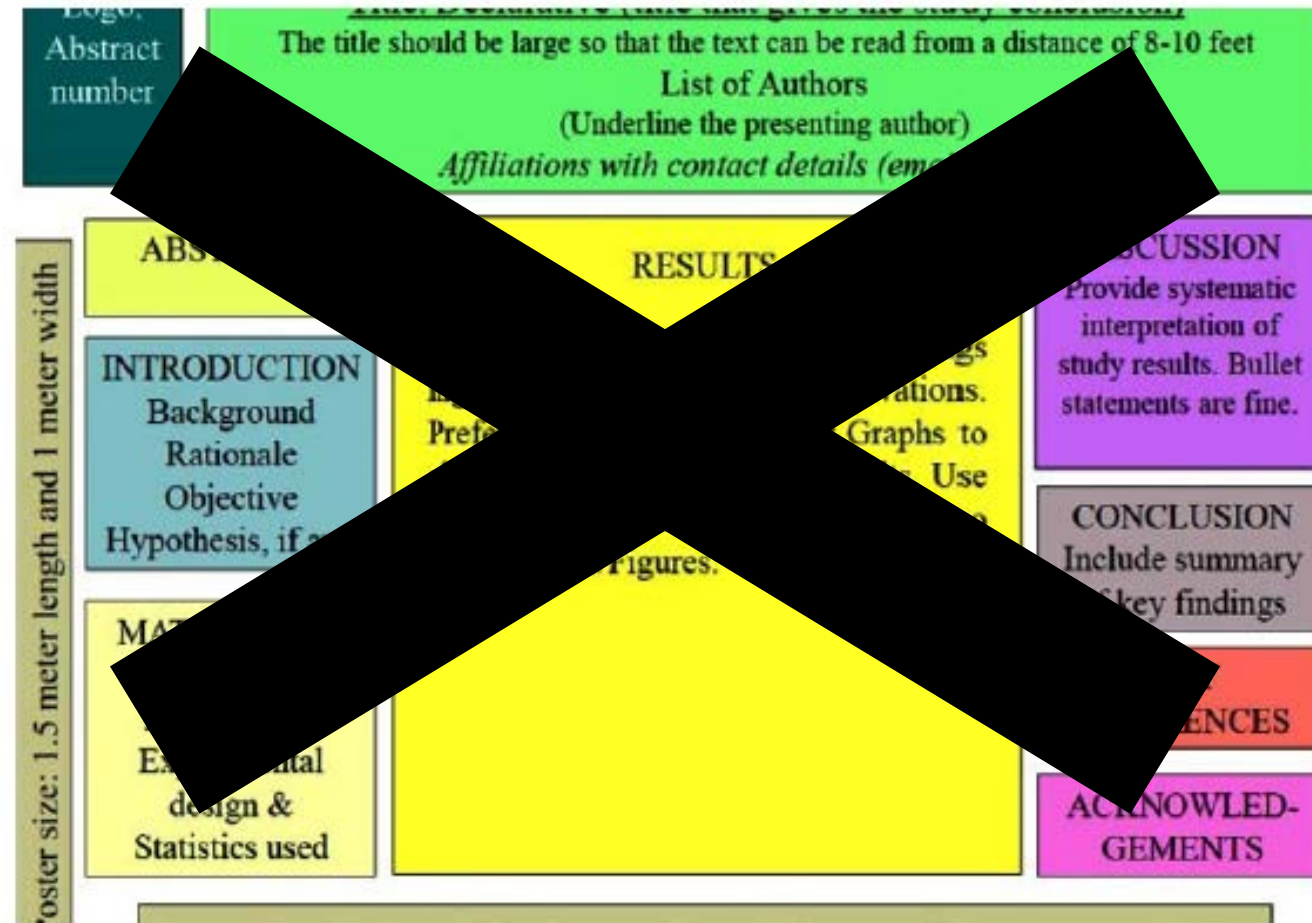
sans serif font
(does not include
perpendicular lines
on the terminal ends of letters)



ornate font
(avoid using in
body of poster)

<http://library.wlu.edu/services/oversized-printing/#designtips>

Colors: The Rainbow Stops Here



PHARMACOTHERAPY FOR HAZARDOUS DRINKING IN WOMEN WITH HIV: A PILOT RANDOMIZED CLINICAL TRIAL

RL. Cook, X. Hu, K.M. Weber, D. Mai, M. Karki, K. Thomas, B. Brumback,
M. Rathore, K.J. Bryant, M. Young, M. Cohen

Department of Epidemiology and Department of Medicine, University of Florida (Gainesville)
Cook County Health & Hospital Systems (Chicago), Georgetown University (Washington, DC), University of Florida (Jacksonville)

Background

Hazardous alcohol consumption is associated with poor health outcomes in women living with HIV, but there is little evidence on whether reduction in drinking can improve HIV care outcomes.

Purpose

To determine the acceptability, feasibility, and potential efficacy of a pharmacologic intervention to reduce alcohol consumption in a community-based sample of women with HIV.

Methods

- WHAT-IF? Pilot Study (Will having alcohol treatment improve functioning?)
- Double-blind, randomized trial recruited women with HIV who reported current, hazardous drinking (>7 drinks/week or ≥4 drinks per day).
- Women recruited from WIHS Cohort Study participants in Chicago and Washington DC, and from a hospital-affiliated clinic in Jacksonville, FL.

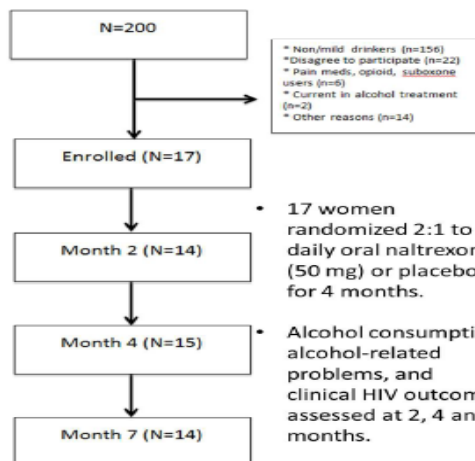


Table 1. Characteristics of women enrolled in WHAT-IF clinical trial pilot study.

	Naltrexone (n=10)	Placebo (n=7)
Age (years; mean,SD)	48 (6.4)	50 (9.3)
Race, black (n, %)	9 (90%)	7 (100%)
Any drug use (Current)	6 (60%)	6 (86%)
alcohol (drinks/week)	33 (4.5)	48 (15.4)
Mean (sd)		
PeTH >0	10 (100%)	7 (100%)
Audit>=16 (n, %)	6 (60%)	4 (57%)

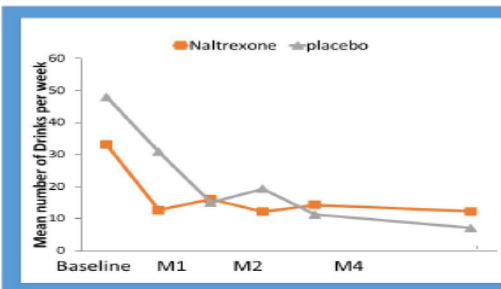


Fig 1. Mean number of drinks per week in women who received naltrexone (n=10) or placebo (n=7). Overall decreases in drinking significantly different from baseline at all timepoints ($p<0.05$).

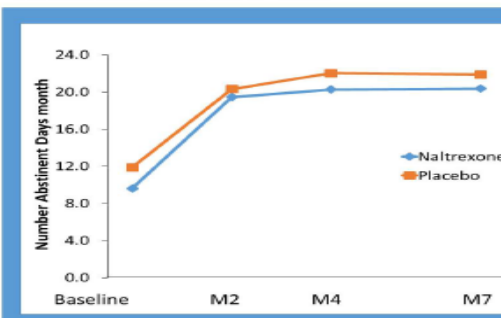


Fig 2. Mean number of abstinent days in the past 30 days. Overall decreases in drinking significantly different from baseline at all timepoints ($p<0.05$).

Results

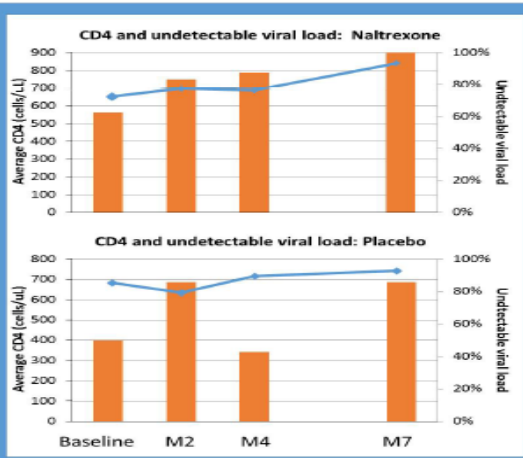


Fig 3. HIV clinical outcomes CD4 counts (orange bars) and HIV viral suppression (blue line) in women randomized to naltrexone or placebo.

Conclusions

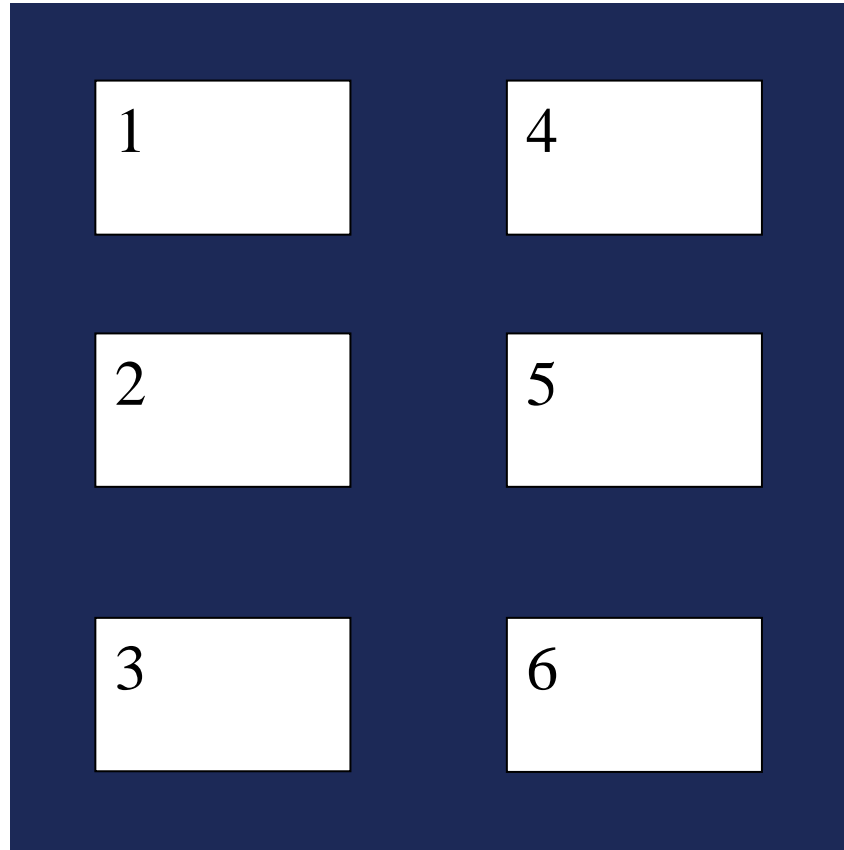
- Women with HIV were able to significantly reduce their drinking, and the reduction in drinking corresponded with improvements in HIV-related clinical and behavioral outcomes.
- Naltrexone and placebo had similar effects, although drinking reduction appeared greater in naltrexone group at 1 month.
- Larger, Phase III with > 200 women ongoing – scheduled to finish recruitment in 2016

Acknowledgements

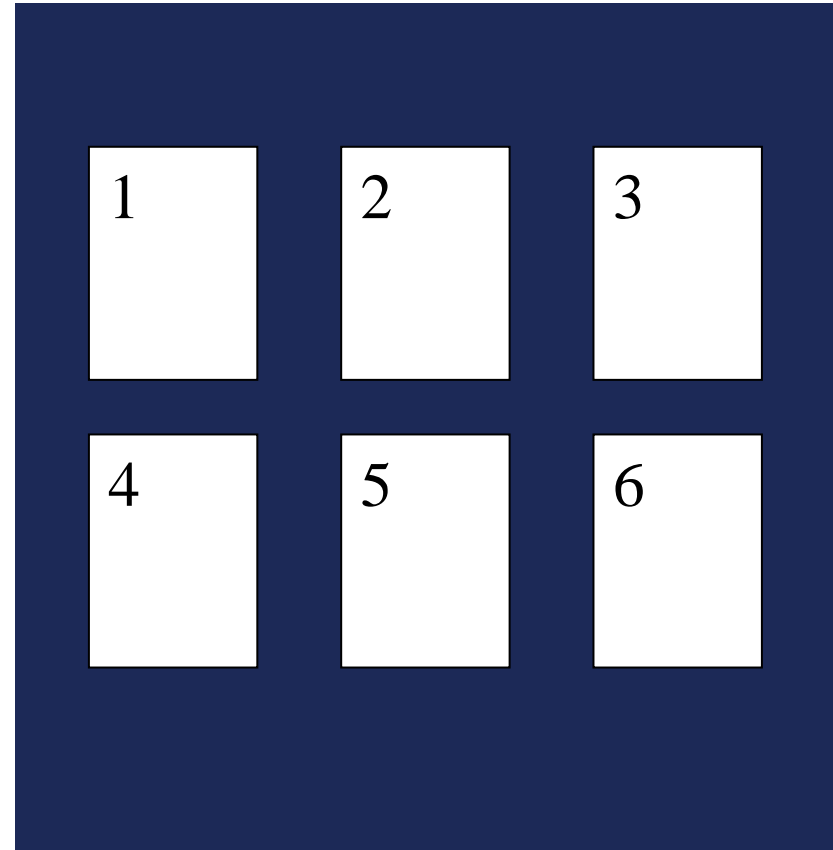
This work was supported in part by NIAAA grant R01 AA018934 and NIAAA grant U24 AA022002.
www.sharc-research.org



Top to bottom, then left to right.



NOT



Title [Make title impactful and descriptive]

Names of Creators

Professor's name / class / organizational affiliation or funding body

Be informative & succinct

- Use bullet points to illustrate your main points.
- Use a serif font if creating a thick block of text.

Milk bone consumption increases with seasonal change [descriptive title]

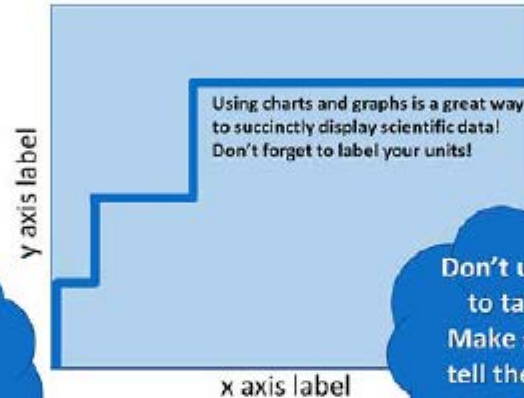


FIGURE 2: *Blue chart*

Check & double-check

- Before printing, do NOT forget to proof your poster!
- There is nothing more embarrassing than misspelling "Science" or "Human" or even your own name!



FIGURE 1: *Beagle at rest*

If you use an image you did not create, provide an attribution in the caption.

Let the organization of the poster tell a story

- Think of your poster as a story.
- The plot progresses from the left top of the poster to the right bottom.



FIGURE 3: *Beagle pre-bark*

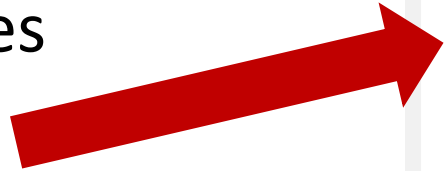
Use high resolution images when enlarging to avoid "pixelation."

Don't forget to include your references!

Even cite class notes if you use a direct quotation or if you grab an image from a professor's PowerPoint.

Don't reinvent the wheel

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Estrogen Receptor Beta's Role in Prostate Epithelial Cells

Department of Pharmacology and Chemical Biology, University of Pittsburgh



BACKGROUND

Prostate cancer is the most frequently diagnosed non-skin cancer in the United States and the third leading cause of cancer deaths. It is more likely to occur in men with a family history of prostate cancer and men of African American descent. African American men also have more than two times the mortality rate compared to Caucasian men. At present, there is no cure for the advanced castration-resistant form of the disease.

Several studies have looked at the cancer microenvironment to evaluate its role in supporting cancer cells as they proliferate. Research has shown that the beta isoform of estrogen receptor (ER β) appears to promote prostate tissue homeostasis and limit prostate cancer development and progression. However, results from a recent study suggest that this protective function may not be as beneficial in African Americans. In an adjusted multivariate analysis, ER β expression in African American men correlated with poor clinical outcome (Elmageed et al, 2013). The aim of this study was to categorize African American prostate cancer cell lines and test ER β functionality.

HYPOTHESIS

- ER β is less functional in African American males with prostate cancer and therefore less protective, leading to higher mortality rates.

METHODS

- We used four different cell lines, two Caucasian American (CA; DU145, C4-2) and two African American (AA; E006AA-PAR, E006AA-HT).
- To check functionality of ER β , we treated each cell line with ER β ligands (5 α -Androstane-3 β ,17 β -diol, and WAY20070) and ran RT-qPCR on E-cadherin, a known ER β target gene.
- Next, we plated 300,000 cells in 6cm-well plates and allowed to grow until 100% confluence. We then performed a scratch assay with treatments to quantify the effects of ER β on migration.

RESULTS



Figure 1: E-cadherin expression in AA cell line E006AA-PAR. ER β specific ligand, adiol, up-regulates E-cadherin, an epithelial cell marker known to be controlled by ER β activation, in the African American prostate cancer cell line E006AA-PAR.

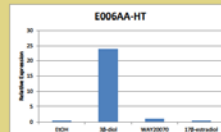


Figure 2: E-cadherin expression in AA cell line E006AA-HT. ER β specific ligand, adiol, up-regulates E-cadherin, in E006AA-HT cell line. E006AA-HT is derived from E006AA-PAR, and is more tumorigenic in vivo.

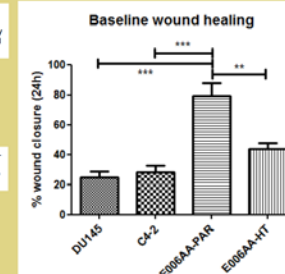
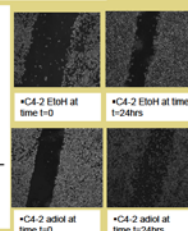
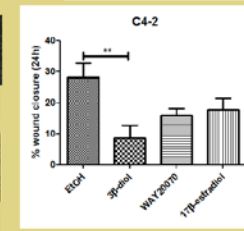
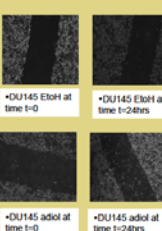
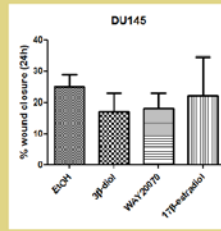
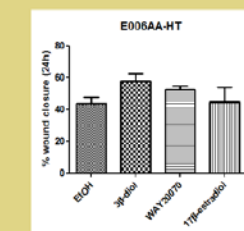
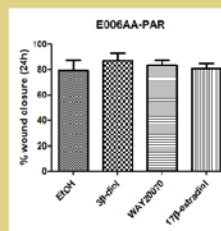


Figure 3: African American cells have greater cell motility than Caucasian cells. Vehicle treatment of cells shows that E006AA-PAR cells have greatest migratory ability. ** p-value = 0.01, *** p-value = 0.001



Figures 4 and 5: Cell motility assessed given different treatments for CA cell lines. ER β specific ligands, adiol and WAY, limits migration. ** p-value = 0.01



Figures 6 and 7: Activation of ER β in African American cells has a minimal effect on cell motility. Migratory ability of African American prostate epithelial cell lines not inhibited by activation of ER β .

SUMMARY

- ER β ligands up-regulate E-cadherin.
- For the E006AA-PAR and E006AA-HT cell lines, there was greater E-cadherin up-regulation with 3 β -diol treatment.
- Treatment with 3 β -diol and WAY led to less wound healing compared to control in the DU145 and C4-2 cell lines.
- Activation of ER β in African American cell lines did not inhibit migration.
- Migration in the African American cells (at least 80% wound healing) occurred at a much higher rate than in the Caucasian cell lines (maximum of 30% wound healing).

CONCLUSIONS

- These findings indicate that activation of ER β in the African American cell lines does not inhibit epithelial to mesenchymal cell transition as observed in the Caucasian cell lines.
- There may be some underlying biological differences that regulate ER β functionality and this could contribute to the racial disparity of prostate cancer that we observe.
- More work on the biological contribution can help in improving diagnostic or therapeutic strategy to enhance the efficacy of endocrine therapy for African American men.

REFERENCES

•Abd Elmageed ZY, Moroz K, Srivastav SK, et al. High circulating estrogens and selective expression of ER β in prostate tumors of Americans: implications for racial disparity of prostate cancer. *Carcinogenesis*. 2013;34(9):2017–2023

ACKNOWLEDGEMENTS

•Teresa Liu
•Krystle Frahm



Modification of Antenatal Glucocorticoid Effects by Statins

Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pa.



Introduction

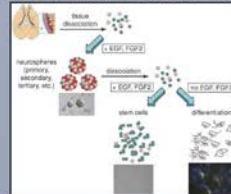
Pre-term labor is a serious, life threatening health concern that affects about 12% of pregnancies. Synthetic glucocorticoids (GCs) such as dexamethasone (Dex) are given to pregnant women at risk for pre-term labor to accelerate development and reduce respiratory distress and the risk of intraventricular hemorrhage in premature babies. GCs act primarily through the glucocorticoid receptor (GR) to alter expression of select target genes. Prior work in the lab has demonstrated an effect of GR to enhance both differentiation and maturation along an oligodendrocyte lineage in primary embryonic (E14.5) mouse neural stem/progenitor cell (NSPC) cultures.

Statins are HMG-CoA Reductase inhibitors that reduce cholesterol biosynthesis. Many intracellular signaling pathways are influenced by reduced cholesterol levels; these include gene expression responses to various hormones and growth factors. For example, statins reduce the expression of various pro-inflammatory genes in pregnant mice. In the brain they have been shown to increase neurogenesis after a traumatic brain injury and enhance oligodendrocyte proliferation and differentiation.

The impact of statins on GC signaling has been revealed in various model systems. For example, statins increased the anti-inflammatory effects of GCs in asthmatic patients. The impact of statins on GC effects in the developing brain has not been examined.

Purpose

To examine the impact of statins on GC signaling in the brain. We hypothesize that statins alter GR-regulated transcription in NSPCs and thereby impact hormone effects on proliferation and differentiation.



Methodology

Procedures

NSPC cultures were derived from the cerebral cortex of mice at E14.5 and used after three passages (P3) to enrich for neural stem cells. Simvastatin or pravastatin (or vehicle as a control) were added to NSPCs 20hr prior to Dex (or vehicle) addition at concentrations (1, 10, 25 μ M) to evaluate differential effects on GR responses.

Data Analysis

After treatment, RT-PCR was used to measure expression of established GR target genes, adenosine deaminase (ADA) and hypoxia inducible factor 3a (HIF-3 a) mRNA. Fold change (differences) in expression were assessed by running a 2-tailed-t test of the experimental groups versus the control. Results were analyzed in NSPCs from males only (n=3) or in both male and female cells (n=4)



Results

There was a trend for pravastatin to enhance and simvastatin to repress Dex induction of ADA gene expression at high doses (i.e. 25 μ M) in male NSPCs only, while the lowest dose (1 μ M) of pravastatin tested reduced Dex induction of ADA gene expression. These same trends were observed when all groups were compared suggesting that statin effects were not influenced by gender. In contrast, at all doses tested (i.e. 1, 10, 25 μ M) both simvastatin and pravastatin showed a trend towards repressing Dex induction of HIF-3a gene expression. This effect attained significance ($p < 0.05$) following t-test analysis at 1 pravastatin and 25 μ M simvastatin in male NSPCs and at 10 and 25 μ M simvastatin and pravastatin in combined male and female cells.

Conclusions

We observed different gene-specific and perhaps gender specific mRNA expression trends when combining Dex with statins.

Low concentrations of pravastatin may repress Dex induction of ADA as compared to Dex alone while at higher concentrations Dex induced ADA expression may be enhanced. There are possible inducing effects of simvastatin on ADA at intermediate doses while suppressive effects are seen at both low and high dosages as compared to Dex alone.

When examining statin effects on Dex induction of HIF-3a we observed repression at all concentrations of both pravastatin and simvastatin. Results were significant at all doses of pravastatin and at high doses of simvastatin.

These results are the first demonstration of statin effects on GC-regulated transcription in stem cells. These results have clinical implications for potential future combination drug therapy with statins and Dex in pregnant women, since dose-dependent effects of statins on specific GR target genes could impact the function of NSPCs in the developing fetus. With additional analysis of GR target genes relevant to NSPC function (e.g. proliferative capacity, cell fate) statin doses may be identified that limit the adverse neurodevelopmental effects associated with antenatal synthetic GCs.

Acknowledgement

This research was funded by NIH Training Grant T35DK065521-10 (Program Director Kleymen, Thomas R.)

References

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The impact of androgen deprivation on a benign prostatic hyperplasia (BPH) cell line

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Introduction

The etiology of benign prostatic hyperplasia is poorly understood. Recent evidence suggests that epithelial-mesenchymal transition (EMT) and anti-apoptotic signaling may contribute to BPH. According to this perspective, EMT plays a role by facilitating the movement and trans-differentiation of epithelial prostate tissue into mesenchymal-like cells, and dysregulation of apoptosis contributes to hyperplasia. 5- α -reductase inhibitors (SARIs) promote prostate involution in BPH and are used to treat BPH. Surprisingly, data suggest that androgens inhibit EMT and promote apoptosis, via a Cox-2 and TGF- β dependent pathway [DeFranco Lab].

Abstract

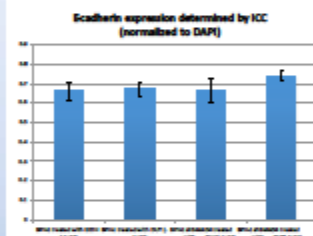
We investigated the impact of dutasteride, a 5- α -reductase inhibitor (SARI), treatment on BPH-1 cells (an established BPH cell line) and BPH-1 shSMAD4 cells (BPH-1 cells ablated of SMAD4) to confirm previous data suggesting that androgen deprivation promoted EMT (epithelial-mesenchymal transition) and that this effect was TGF- β mediated. Motility assays and immunocytochemistry (E-cadherin mouse anti-rabbit IgG) failed to show a significant difference between the treatment and control arms.

Methods

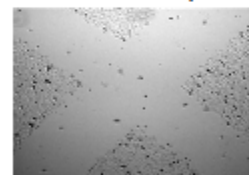
Cell motility was assayed using a scratch (wound-healing) assay. Motilities of two cell lines: BPH-1 and BPH-1 shSMAD4 (BPH-1 ablated of SMAD4), were assayed over a period of 24 hours, during treatment with dutasteride (.25uM) or control EtOH (.25uM). The "wound" was created with a p200 pipette tip. Movement of cell front was determined at four points per well and then averaged.

E-cadherin expression was assayed via immunocytochemistry. BPH-1 and BPH-1 shSMAD4 cells were plated onto coverslips at 100,000 cells per 6-well, and treated with .1uM dutasteride or .1uM EtOH (control). E-cadherin antibody was normalized against DAPI nuclear stain. All conditions were set up in triplicate. Measurements were taken from four areas on each coverslip and averaged. Two-sample comparisons were performed using Student's t-test. P values of less than 0.05 were considered significant.

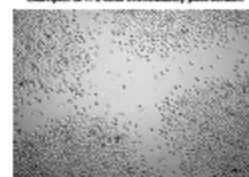
Results



Scratch assay



Example: BPH-1 cells immediately post-scratch



BPH-1 cells 24 hours post-scratch

Conclusions

These results are unexpected. Previous data showed a marked decrease in E-cadherin mRNA expression when BPH-1 cells were exposed to dutasteride. This response was attenuated in BPH-1 shSMAD4 cells, suggesting that the repression of E-cadherin expression is TGF- β mediated. No such difference was found in protein expression when assayed by immunocytochemistry. A variety of factors could be responsible for these differences. A repeat of the qPCR along with Western blotting might shed light on the discrepancies. The cell motility "scratch" assays exhibited a high degree of variance. This could be attributed to proliferation confounding motility. An invasion assay could be a way to eliminate proliferation as a confounding factor.

Acknowledgements

Marcia Lewis and Emily Harrington provided invaluable help and training for this project.

practice



Inorganic Biochemistry of Iron Proteins

Jared J. Heymann, Claire J. Parker Siburt, Katherine D. Weaver,
and Alvin L. Crumbliss

Duke University – Department of Chemistry – Durham, NC



Purpose:

To study iron protein
biochemistry from the
perspective of the iron
Protein = Ligand

The Iron Paradox

Iron is needed for nearly
every living cell

Iron is toxic and can
produce reactive oxygen
species & must be
controlled

Iron Abundance in Humans

45-55 mg/kg in humans
70% in Red Blood Cells (Hemoglobin)
0.1% in Transferrin

Turnover of transferrin iron is ~30 mg / 24 hours with 80% of
this Fe being transported to the bone marrow for hemoglobin
synthesis
Bacteria can also target Tf as a source of iron
Andrews (1999) N Engl J Med 341, 1986-1995

Proteins act as the
1st & 2nd coordination
shell of iron and can
modulate the kinetics
and thermodynamics
of reaction.

Techniques:

Spectroelectrochemistry
UV-Visible Spectroscopy
Fluorescence Spectroscopy
Difference Spectroscopy
Stopped-Flow Kinetics
SUPREX

TRANSFERRIN

A mechanistic study of the
iron release by receptor-bound
transferrin using spectroelectrochemistry

FERRIC BINDING PROTEIN

Role of a synergistic anion on modulating iron
uptake in a bacterial transferrin by pathogenic
bacteria: A study in kinetics and thermodynamics

HEMOGLOBIN

Effects of subunit cross-linking on
hemoglobin oxidation states determined
by spectroelectrochemistry

How is Fe³⁺ removed
from Tf when K_d = 10⁻²⁰?
Hypothesis: When transferrin
binds to a receptor, the
reduction potential shifts into a
biologically relevant range.

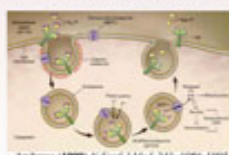
Transferrin

Spectroelectrochemistry utilizes a short pathlength
created by an OTTE cell, to measure the variations in
visible spectra as the analyte is oxidized or reduced by
an externally applied potential. This technique is ideal for a
biological analyte because only a small sample volume
is required.

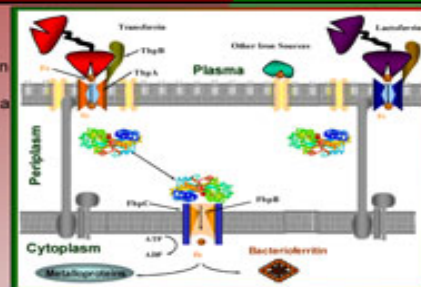
Optically Transparent Thin Layer Electrode

Heterogeneous reactions
are complicated because the
metal can be buried in the
protein and proteins diffuse
slowly to the electrode
surface. Mediators are used
to act as electron shuttles.

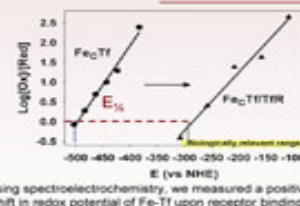
Iron loaded Tf binds to the human receptor and is
taken into the cell by endocytosis. Tf releases
iron inside in the endosome where the conditions
are acidic (Andrews, 1999). However, the chemical
mechanism is unclear. The reduction potential of
Fe-Tf in the plasma (pH 7.4) and in the endosome
(pH 5.8) is too low for biological reducing agents.



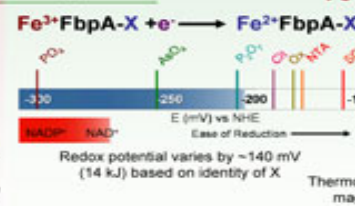
The transferrin receptor is capable of shifting the reduction potential into the range
accessible by biological reducing agents, allowing for a redox mechanism of Fe release.
Transferrin not only supplies iron to mammalian cells, but has been identified as a target
for pathogens to mechanistically steal iron from their host.



Tf can be scavenged by receptors on the bacterial surface
BpA is a nodal point in this iron acquisition process.
**Structural Fe binding site similarity
between Tf and BpA**



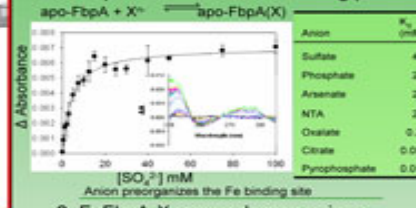
Using spectroelectrochemistry, we measured a positive
shift in redox potential of Fe-Tf upon receptor binding.



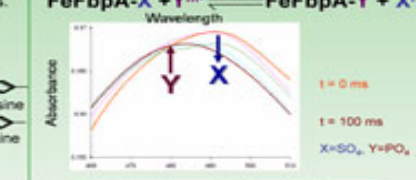
Iron transport can occur by a redox or non-redox mechanism in the periplasm. The
thermodynamic stability and reduction potential are both varied by the identity of the
synergistic anion. Kinetically labile exchange is possible in the diverse anionic
conditions of the periplasm.

Like Tf, BpA requires a synergistic anion to facilitate tight
iron binding, which may play a role in ease and rate of Fe
uptake by the bacteria.

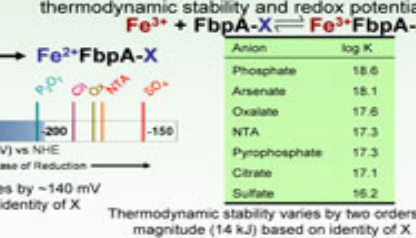
1. BpA acts as an anion binding protein



2. FeBpA-X can exchange anions



3. Anion identity modulates both thermodynamic stability and redox potential

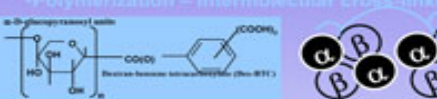


Chemically modified Hb

- Pyridoxalation
- Pegylation
- Conjugation to polysaccharides & proteins



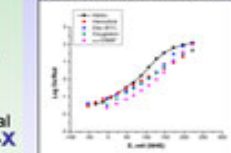
•Polymerization ~ Intermolecular cross-linking



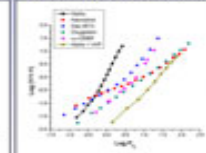
•Intramolecular cross-linking



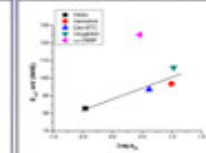
Combined Nernst Plot



Combined Hill Plot



E_{1/2} vs Log P₅₀



Sample	E _{1/2} mV (NHE)	Oxidation P ₅₀	Log P ₅₀	Oxygenation P ₅₀
HbA ₀	83	1.3	-0.455	2.28
Hemolink	97	0.7	0.994	0.71
Dex-BTC	94	0.9	0.618	1.49
OxygD ₂ bin	106	0.9	1.028	1.11
aa-DBSF	125	1.0	0.461	1.56

Implications

- Reengineering redox center not necessary
- Drive for autoxidation not thermodynamic
- Structural modifications perturb kinetics by altering exposure of heme cavity

Modified Hb Conclusions

Oxygen Transport

- Loss of cooperativity
- Lower oxygen affinity
- T-state stabilization

Anaerobic Reduction Potentials

- Loss of cooperativity
- E_{1/2} potential increased vs HbA₀
- Normal physiological range
- Decreased tendency to form metHb

Joseph M Volpe, Lindsay G Cowell, and Thomas B Kepler

1 Introduction

The defining characteristic of adaptive immunity is the somatic diversification of its antigen receptor genes. The genes for the T-cell receptor (TCR) and immunoglobulin (Ig) are formed by a process known as V(D)J recombination, in which transcribable genes are composed by the combinatorial joining of gene segments from two or three classes, depending on the specific locus involved. Detailed knowledge of how specific broadly neutralizing HIV antibodies are formed or of the differences in composition between self and non-self antigen receptors is elusive but important for research in vaccine design and autoimmune disorders. Understanding the details of the composition of specific Ig and TCR and the specific processes they underwent during development can facilitate these research endeavors. Thus, we have developed a web-based software tool that analyzes antigen receptor sequences to identify the V, D and J gene segments used, as well as the recombination sites, point mutations and N- and P-nucleotides introduced. We demonstrate the functionality of the software here with an analysis for differential biases within large samples of Ig classified as autoreactive or not.

2 SoDA – (Somatic Diversification Analysis)

SoDA¹ is an implementation of a 3-dimensional algorithm for aligning multiple contiguous DNA sequences to a master antigen receptor sequence in order to determine:

- 1: the most likely gene segment composition
- 2: the rules for combining those segments during the formation of an antigen receptor

Rules for recombination include:

- > Identifying recombination sites: some gene segments may undergo exonuclease activity, resulting in a loss of nucleotides in the coding junction.

...AGATCTGACGACACG V segment

- > Identifying n-nucleotide additions: the enzyme TdT can introduce non-templated nucleotides into the coding junctions.

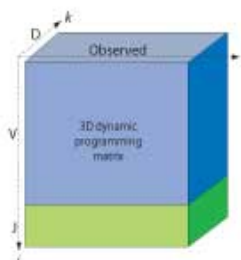
... ATCTGACGACACG ATTGAGGATATTGT ...
V segment n's D segment

- > Identifying somatic mutations: in B-cells, receptor genes undergo somatic hypermutation, whereby random point mutations are introduced in the gene segments

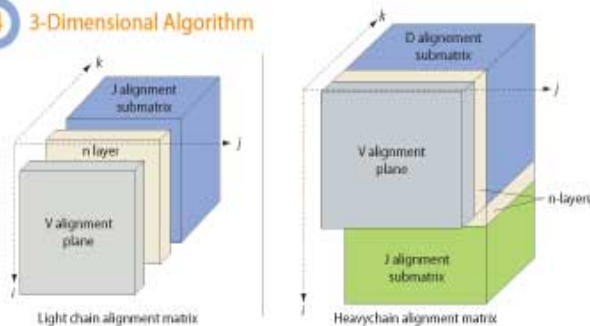
...CTCTTACGACACG...ATTGAGGATAGTGT...

3 Why is SoDA unique?

Other bioinformatics solutions already exist (JOINSOLVER⁶ and V-QUEST⁷) for approaching this problem, so why build another one? The approach for SoDA is unique in that it relies on dynamic programming, which guarantees an optimal solution. But, unlike typical alignment software, it implements a 3-dimensional algorithm to find it's solution. Formally, SoDA seeks to maximize the likelihood function $P(G, M|T)$, where G = set of gene segments, M =rules for combining those gene segments, T = target sequence, and MIG = T .



4 3-Dimensional Algorithm



Considering exonuclease activity in the solution adds a constraint to the alignment algorithm: from any point in one gene segment alignment, the traceback path must be able to jump to any point in the contiguous gene segment alignment. For both light and heavy chains, the V alignment is computed in a single plane. Then, the alignment for each contiguous sequence that follows is computed in a 3-dimensional matrix where the alignments run perpendicular to the direction of the alignments for the previous gene segment alignment. This enables the traceback path to jump from any point in one alignment, to any point in the contiguous alignment, thus accounting for the exonuclease constraint. TdT enzymatic activity is captured by the traceback path through the special n-layers.

5 Testing the Algorithm and Results

We tested SoDA using 120 artificial recombination sequences generated by a program that simulates V(D)J recombination. We created 30 sequences at each of four mutation rates: 2.5%, 5%, 10%, and 15%. SoDA proved to be the most reliable in predicting the correct gene segment composition.

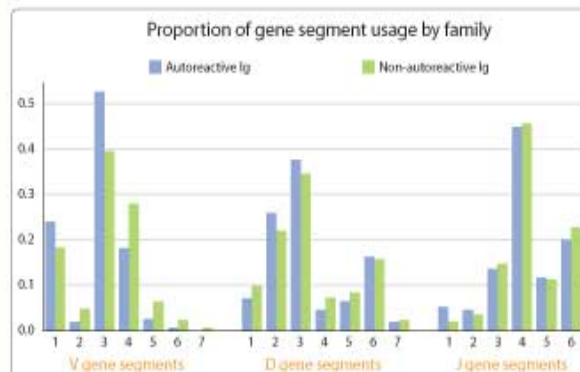
	2.5% mutation			5% mutation			10% mutation			15% mutation		
	SoDA	JS	VQ	SoDA	JS	VQ	SoDA	JS	VQ	SoDA	JS	VQ
V correct	30	30	30	30	26	29	30	25	29	30	23	28
D correct	28	26	16	28	28	10	27	25	18	22	19	16
J correct	30	29	29	30	30	28	29	28	20	28	30	28
VQ correct	28	26	16	28	25	8	27	20	15	22	15	15

SoDA was designed to find the most likely gene segments as well as the most likely rules that created that rearrangement. So, we tested SoDA against 30 rearrangements from Genbank. Below is the CDR3 region of an Ig rearrangement that arguably shows how SoDA's approach finds the best possible explanation for the gene segments and the rules for combining them:

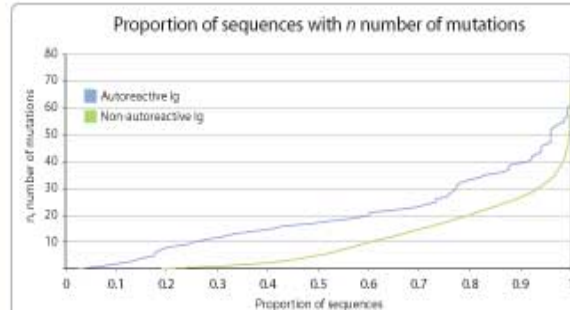
[illegible]

6 Characterizing Autoreactive vs. Non-autoreactive Antibodies

With SoDA, we are able to quantitatively characterize Ig sequences, specifically looking at gene segment usage, CDR3 length, n-nucleotide addition, and mutations. To do this, we downloaded and analyzed approximately 9,450 non-autoreactive Ig and 154 autoreactive Ig sequences from Genbank. Sequences were distinguished and divided into these two groups through keyword searches. Clonal duplicates were removed from each set using an automated procedure that compared several criteria in the sequences.



The differences in segment usage between autoreactive and non-autoreactive Ig are contrasted here. The differences in V-gene segment usage are statistically significant.



This graph shows the number of mutations present in the evaluated sequences for proportions of the sequences. At each proportion, autoreactive Ig contain more mutations than non-autoreactive sequences.

Try out SoDA on the web: <http://dulci.org/soda>

References

1. Volpe JM, and Cowell LG, and Repler TS. SoDA: implementation of a 3D alignment algorithm for inference of antigen receptor recombinations. *Bioinformatics*. 2006;22(4):478-44.



Abstract

A 49-year-old man with rheumatoid arthritis (RA) and gout treated for 4 years with infliximab, methotrexate and prednisone (5 mg daily) presented with a painful, swollen left arm. He had a suppurative right elbow ulcer after a gout flare and trauma one year earlier. The ulcer recurred and persisted despite antibiotics and intravenous steroids, biopsy was negative for bacteria. Three weeks prior to presentation nonpainful edema with two large distinct, deeply erythematous plaques developed on his left forearm with peripheral satellite papules. Right elbow biopsy taken 3 weeks earlier showed numerous elongated, beaded mycobacteria. Left arm tenderness ensued. On presentation there was fever to 102.5°F, substantial left extensor forearm fluctuance, and a swollen, tender right index finger PIP joint. No cardiac murmurs were present. No history of travel, swimming or aquarium exposure. WBC was 6000/mm³ and ESR 13. Left forearm aspirate yielded 57,500 WBC with 80 PMNs and 4+ AFB on smear. Extensive purulence and necrotizing fasciitis were found at surgery, requiring debridement of left extensor and flexor forearm fascia and nonviable muscle. All intraoperative specimens grew *Mycobacterium kansasii* with rifampin MIC 0.12 µg/ml; bacterial and fungal cultures were negative. Blood cultures and echocardiogram were negative. Two weeks later debridement of the right index finger also grew *M. kansasii*. Treatment with rifampin, ethambutol, and isoniazid and VAC dressings followed by skin grafts resolved the infection. Methotrexate 7.5 mg weekly and prednisone 6 mg daily without infliximab were resumed 6 months after presentation. Approximately 40 cases of musculoskeletal infection with *M. kansasii*, mostly septic arthritis in compromised hosts or rheumatologic disease, have been described. This is the first reported case of necrotizing fasciitis due to *M. kansasii* and is notable for its subacute presentation and association with infliximab therapy.

Introduction

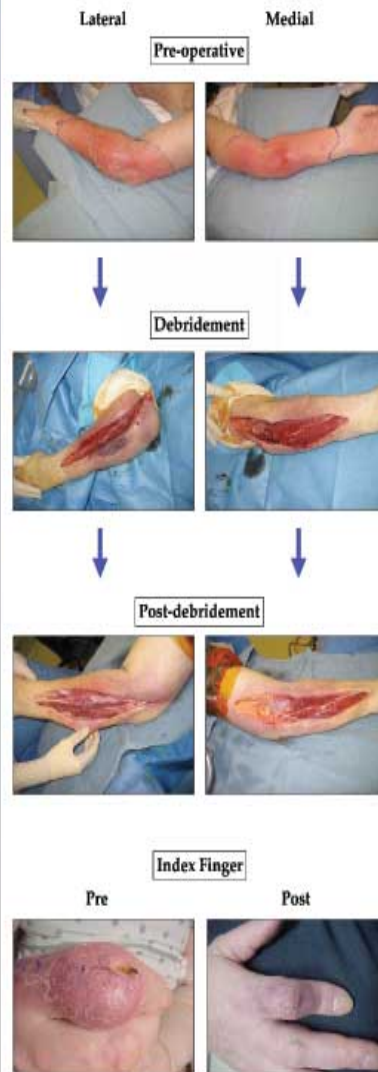
Mycobacterium kansasii is classified as a slow growing mycobacterium that most often causes pulmonary disease that clinically resembles tuberculosis. It is generally considered to be minimally contagious although it is abundant in the environment. In the United States *M. kansasii* disease occurs most commonly in the Midwestern and Gulf Coast states yielding rare case reports of extrapulmonary disease. The most common extrapulmonary disease is septic arthritis, generally affecting the upper extremities. *M. kansasii* musculoskeletal disease appears to be associated with intraarticular steroids as well as conditions and medications that may lead to an immunocompromised state.

Clinical Course

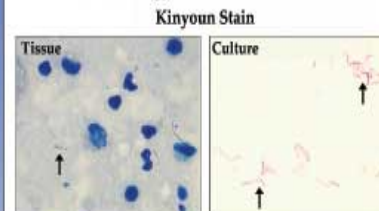
This case involves a 49-year-old man with rheumatoid arthritis, systemically treated for 4 years with infliximab (injections every 5 weeks), methotrexate (7.5 mg weekly) and prednisone (5 mg daily) who developed a relatively indolent left upper extremity soft tissue infection. This was preceded one year earlier by a traumatic suppurative right elbow ulcer approximately that healed but later became chronic despite antibiotics and intravenous steroids. Biopsy was negative for bacteria. Three weeks prior to presentation nonpainful edema with two large distinct, deeply erythematous plaques developed on his left forearm with peripheral satellite papules. Right elbow biopsy taken three weeks earlier showed numerous elongated, beaded mycobacteria. Left arm tenderness ensued.

On presentation there was fever to 102.5°F, substantial left extensor forearm fluctuance, and a swollen, tender right index finger PIP joint. No cardiac murmurs were present. No history of travel, swimming or aquarium exposure. WBC was 6000/mm³ and ESR 13. Left forearm aspirate yielded 57,500 WBC with 80 PMNs and 4+ AFB on smear. Due to the severity of the appearance and the progressive nature of the process, he was taken to the operating room. Extensive purulence and necrotizing fasciitis were found at surgery, requiring debridement of left extensor and flexor forearm fascia and nonviable muscle. All operative specimens grew *M. kansasii* with rifampin MIC 0.12 µg/ml; bacterial and fungal cultures were negative. Blood cultures and echocardiogram were also negative. Two weeks later debridement of the right index finger yielded tissue that also grew *M. kansasii*.

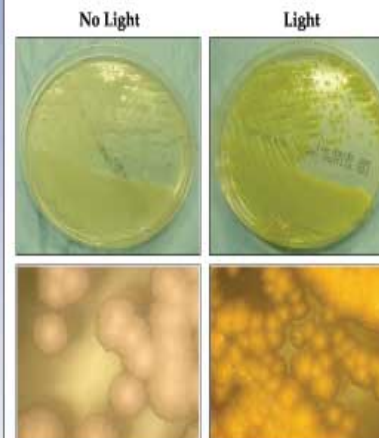
Surgical Findings



Microbiology Methods and Results



Photochromogen: light exposure → yellow pigment



Microbiology Timeline

Day 1: Tissue received. 4+ AFB were seen on direct Kinyoun Stain (Becton, Dickinson and Co, Sparks, MD). Plated directly to liquid and solid mycobacteriological media at room temperature and 37°C.

Day 4: Liquid Mycobacteria Growth Indicator Tube (MGIT) (Becton, Dickinson) at 37°C was positive for acid-fast bacilli. MGIT subbed to selective mycobacteriological media, Mitchinson 7H11 agar (Becton Dickinson).

Day 11: Original solid media positive for a rough, buff colony. Kinyoun stain positive. Culture yielded an atypical mycobacterium whose microscopic characteristics at 1000x were large-sized acid-fast rods with a cross-banding appearance (arrows). Identification as *M. kansasii* made by microscopic and macroscopic morphology, growth rate, pigment production and DNA sequencing. The isolate was rifampin sensitive (MIC 0.12 µg/ml; ARUP, Salt Lake City, UT).

Summary

Mycobacterium kansasii is an acid fast organism that generally causes pulmonary disease and is antigenically similar to *M. tuberculosis*. While an uncommon cause of extrapulmonary infections in humans, the most common manifestations of *M. kansasii* are arthritis and tenosynovitis. There are several risk factors associated with articular *M. kansasii* infections including immunosuppressive medications, rheumatologic conditions such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA), psoriasis, AIDS, diabetes mellitus and intraarticular corticosteroids (1,2).

M. kansasii is environmentally contracted, with the most likely source being tap water, where it has been found to survive for up to a year. (3) This organism is one of the so-called slow growing mycobacteria, and is characterized by being a photochromogen – that is it acquires a yellow pigmentation upon exposure to light. In fact, this bacterium was initially known as the “yellow bacillus” due to this phenomenon.

M. kansasii musculoskeletal infection generally follows a very indolent course, with a mean time to diagnosis of 14 - 17 months. Laboratory studies such as elevated leukocyte count and ESR are not helpful and PPD is often negative. (1,2) While rare, septic arthritis and tenosynovitis are the most common extrapulmonary infections caused by this organism. A literature search did not reveal any cases of necrotizing fasciitis caused by *M. kansasii*. Other atypical mycobacteria that have been reported to cause necrotizing soft tissue infections include *M. ulcerans*, *M. marinum*, and *M. haemophilum*. *M. ulcerans* (Buruli ulcer) is the most commonly reported, with rapid growth of cases in Africa, Australia, India, South America and other tropical regions. Buruli ulcer is characterized by large, well circumscribed necrotic areas of the deep dermis and panniculus.

This patient had multiple predisposing risk factors for mycobacterial infection including RA, gout, intralesional steroid injections, methotrexate, low dose prednisone and infliximab, a systemic tumor necrosis factor inhibitor. After surgical debridement and vacuum-assisted closure accompanied by 12 months of treatment with isoniazid, ethambutol, and rifampin his wounds are well healed with no signs of recurrent infection. Reasonable control of his arthritis was achieved with low dose prednisone and methotrexate resumed 6 months after surgery. Infliximab has not been restarted. This is the first reported case of necrotizing fasciitis due to *M. kansasii* and is notable for its subacute presentation and association with infliximab therapy.

Medial forearm after treatment



Literature Cited

- Bernard L, Vincent V, Lortholary O et al. *Mycobacterium kansasii* septic arthritis: French retrospective study of 5 years and review. *Clin Infect Dis* 1999;29:1405-60.
- Nakamura T, Yamamura Y, Tsuruta T et al. *Mycobacterium kansasii* arthritis of the foot in a patient with systemic lupus erythematosus. *J Intern Med* 2001;250:498-9.
- Jaynes, DH. Water: the natural habitat of *Mycobacterium kansasii*? *Tubercle* 1979;60:77-81.



Southern Flounder Exhibit Temperature-Dependent Sex Determination

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Introduction

Southern flounder (*Paralichthys lethostigma*) support valuable fisheries and show great promise for aquaculture. Female flounder are known to grow faster and reach larger adult sizes than males. Therefore, information on sex determination that might increase the ratio of female flounder is important for aquaculture.

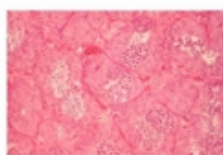
Objective

This study was conducted to determine whether southern flounder exhibit temperature-dependent sex determination (TSD), and if growth is affected by rearing temperature.

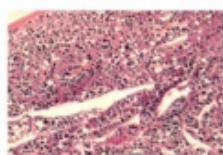
Methods

- Southern flounder broodstock were strip spawned to collect eggs and sperm for *in vitro* fertilization.
- Hatched larvae were weaned from a natural diet (rotifers/*Artemia*) to high protein pelleted feed and fed until satiation at least twice daily.
- Upon reaching a mean total length of 40 mm, the juvenile flounder were stocked at equal densities into one of three temperatures 18, 23, or 28°C for 245 days.
- Gonads were preserved and later sectioned at 2-6 microns.
- Sex-distinguishing markers were used to distinguish males (spermatogenesis) from females (oogenesis).

Histological Analysis

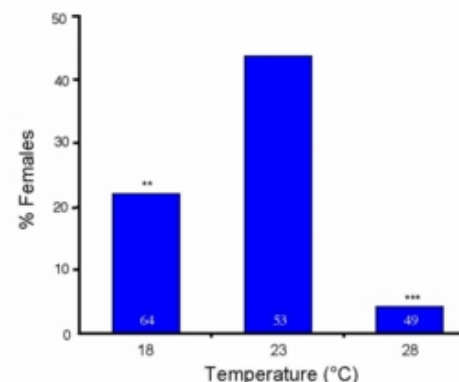


Male Differentiation



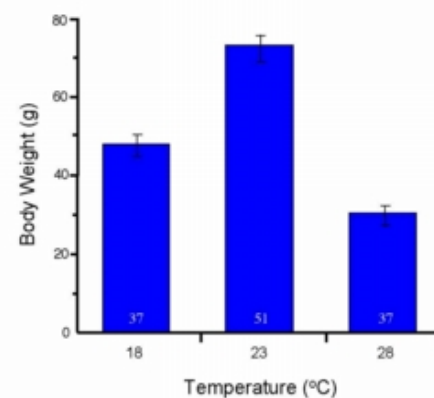
Female Differentiation

Temperature Affects Sex Determination

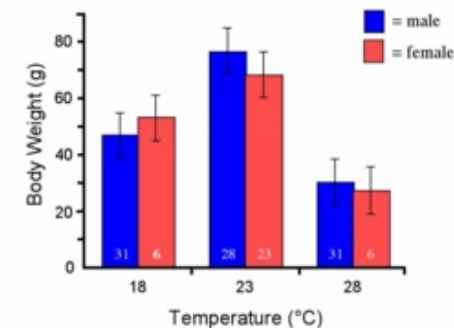


(**P < 0.01 and ***P < 0.001 represent significant deviations from a 1:1 male:female sex ratio)

Rearing Temperature Affects Growth



Growth Does Not Differ by Sex



Results

- Sex was discernible in most fish greater than 120 mm long.
- High (28°C) temperature produced 4% females.
- Low (18°C) temperature produced 22% females.
- Mid-range (23°C) temperature produced 44% females.
- Fish raised at high or low temperatures showed reduced growth compared to those at the mid-range temperature.
- Up to 245 days, no differences in growth existed between sexes.

Conclusions

- These findings indicate that sex determination in southern flounder is temperature-sensitive and temperature has a profound effect on growth.
- A mid-range rearing temperature (23°C) appears to maximize the number of females and promote better growth in young southern flounder.
- Although adult females are known to grow larger than males, no difference in growth between sexes occurred in age-0 (< 1 year) southern flounder.

Acknowledgements

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