Presentation Skills Training

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Communicating Ideas – to persuade, inform, or entertain

Presentations

- Showcase your research findings or sell your ideas
- Rapid communication to an interested group
- Communication followed by discussion
- Conferences (formal research presentation)
- Job interviews
- Pitches
- Formal research talks as part of your course

Posters

- Summarize research concisely and attractively to help publicize it and initiate discussion
- Typically brief text with tables, graphs, pictures in large (A0/A1) format
- Generally presenter stands by poster to aid additional discussion

KNOW YOUR AUDIENCE

Good presentations and posters:

Interesting, inspiring, thought provoking, educational.

Poor presentations and posters:

Dull, off-putting, confusing, uninformative.

A good presentation or poster will:

- communicate the importance of what you want to say
- clearly state what you did, why you did it, why it’s important
- prompt others to ask questions and give feedback

Tips for Good Presentations

What to know about presentations:

- Time-limited
- Unidirectional (audience can't go back)
- Speaker sets pace of delivery
- Audience are multi-tasking (looking and listening)
- Heavy reliance on visual communication

You are telling the audience a story, rather than them reading one. This means we need to think about the following:

- Narrative
  - Introduction / motivation
  - Information
  - Analysis / discussion
  - Summary / conclusions
- Who is your audience?
- Clear, precise communication (spoken rather than written)
- Quality of visual illustrations (figures, etc.)

Preparing your slides

- How long do you have?
- General rule (as a guide) have half the number of slides as you have minutes
- You need at least one minute per slide
- A strong narrative with **one or two key points** or results is key

Start to organize your talk by creating a slide deck of half the number of slides to minutes then give each slide a purpose.

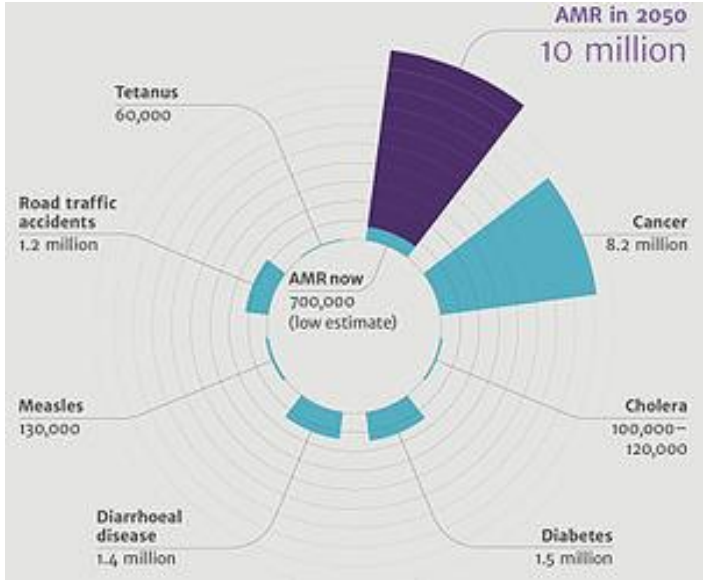
What should be on your slides?

- Depends on your project, but you will need to cover

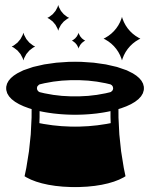
- Title
- Here's the big picture
- Here's what *I* am going to do
- Here's how *I* am going to do it
- Here's what I found and what it means
- Here's why you should care and what it means for the future
- (Acknowledgements and Questions)

Setting the Scene

- Your introduction should give people an overview of the field enough that they can be introduced to what you will talk about
- Think about what will grab their attention – what is the big picture?



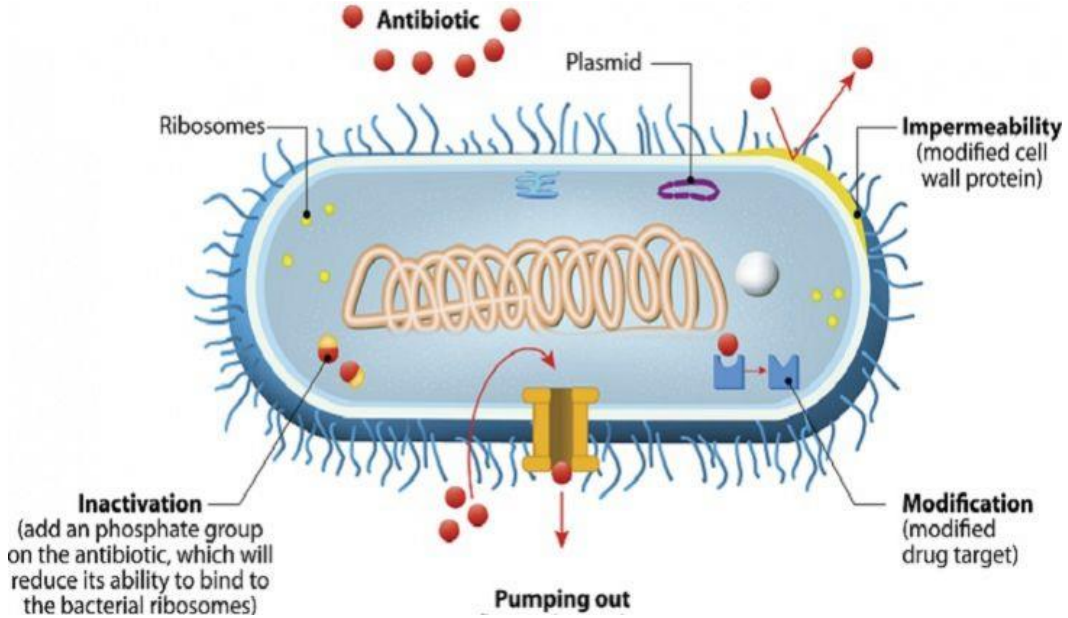
- Antimicrobial Resistance (AMR) is a global health concern
- If left untreated, by 2050 the number of deaths due to AMR is expected to rise to 10 million



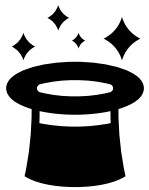
Posing a question to the audience can be a good way of getting them engaged from the start

What is your project or pitch about?

- You then need to put **your work / idea** into the bigger picture context
- What has been done previously – what are the unanswered questions? What will you do that is novel? Why should I care?



- There are a number of methods to kill bacteria
- However bacteria have evolved strategies to overcome these
- In this work we take a different approach



Try to make sure you can summarise your idea in ONE SENTENCE.

Outline what you've done or what your idea is

- Here's where you get to talk about your idea in more detail
- What techniques did you use, what question were you trying to answer?
- Remember this is not a chronological description– it's a story about your work or ideas and stories don't have to be told in the exact order that you did things

Start with key images / data – what are the minimum number of pictures you need to show to get your point across?

Show me the numbers!

- Never put up a huge table of numbers and apologise for it not being readable. If it’s not readable, don’t show it
- A couple of clear graphs and images say far more than text and tables can ever manage
- Try to use the highest resolution, simplest graphics you can
- Think about how you group images together
- Think about using schematics

If someone in your group has a flair for graphic design LET THEM DO THE SLIDES
If you are not confident with graphics keep them simple

What does it all mean?

- Remember to refer back to your research question or your pitch idea (don't assume anyone has remembered what it is you said you were doing)
- Be explicit in your discussion – no one in the audience will know the significance of your findings as well as you do
- Remember to say what you have found is important – how does it link to the broader context
- Remember your narrative

Don't be afraid to use questions, things like "So what does it all mean?" "And what are the implications of this?"

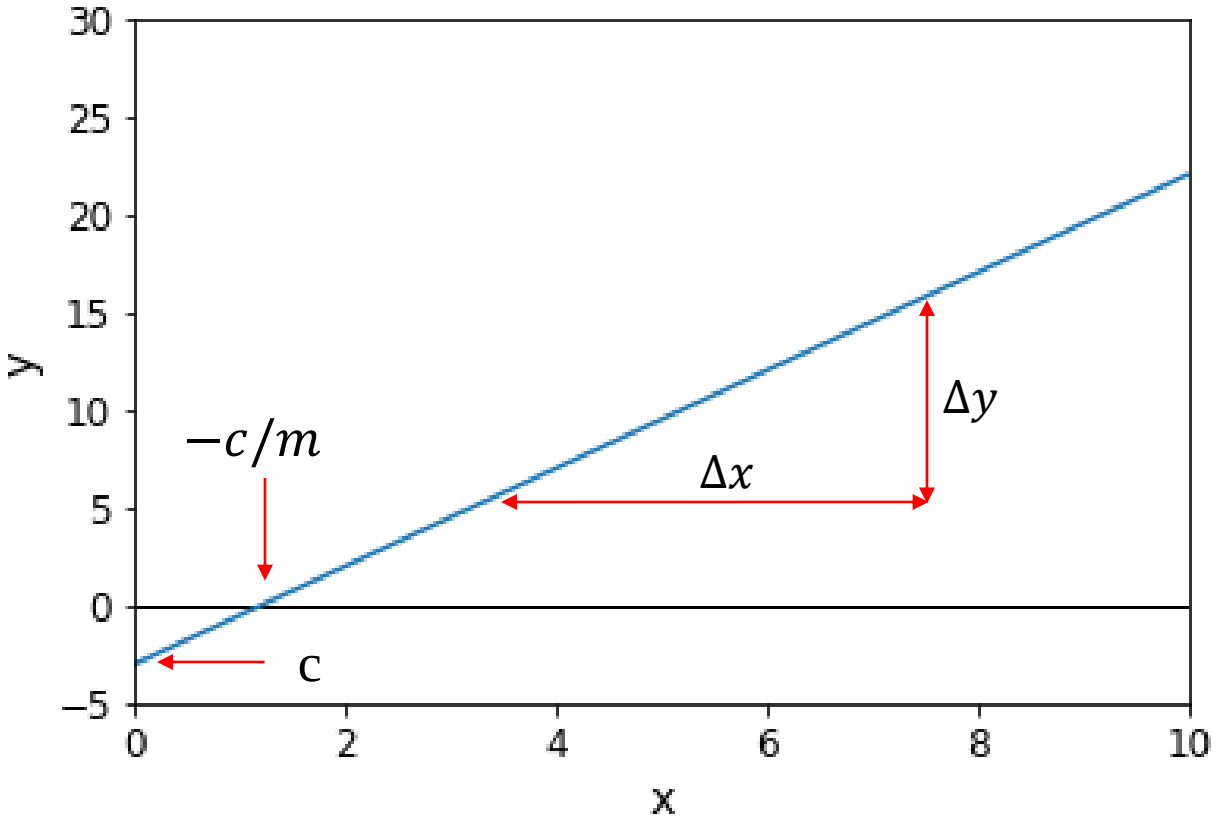
What will I do next?

- A conclusion should summarise your findings
- But it should also point to what's next
- Leave the audience thinking about what you've said
- Hopefully leave them wanting you to win!

Maybe try restating the question you began with, or reminding the audience of a key fact.

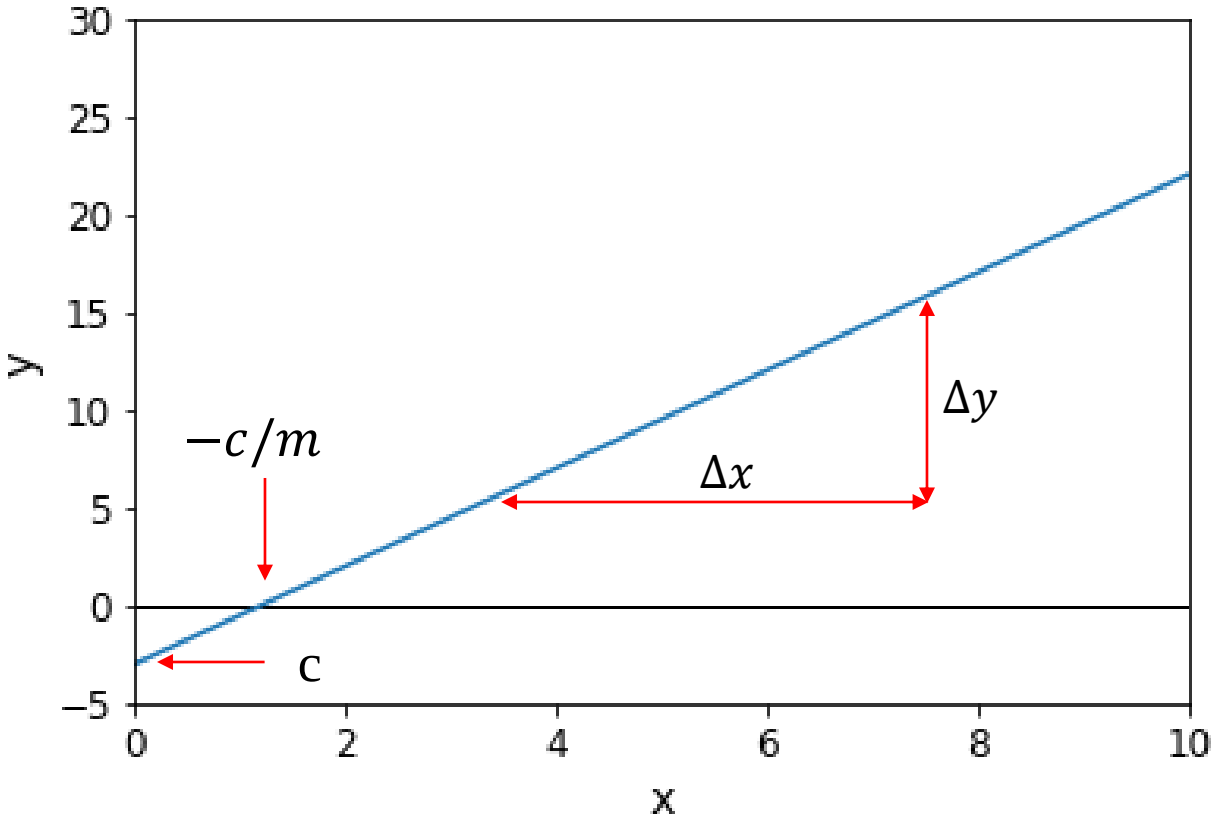
Your slide is not your script

- A straight line can be described by the equation: $y = mx + c$
- m represents the gradient of the line and c represents the intercept with the y axis
- the gradient can be determined from the ratio of the change in y, Δy for a corresponding change in x, Δx
- the x intercept can be determined as $-c/m$

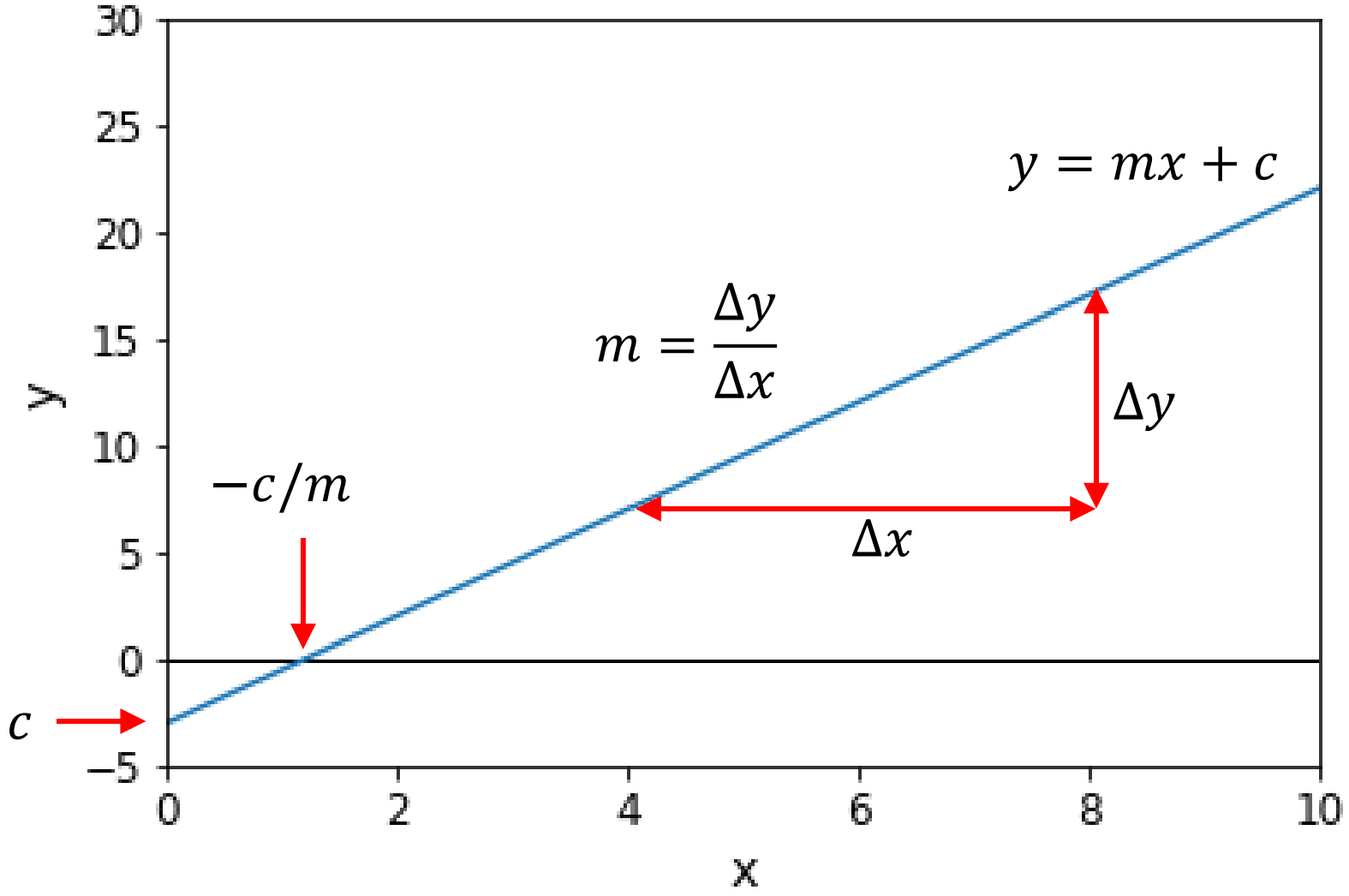


Your slide isn't even your summary notes

- Straight line: $y = mx + c$
- Gradient: $m = \frac{\Delta y}{\Delta x}$
- y axis intercept: c
- x axis $-c/m$

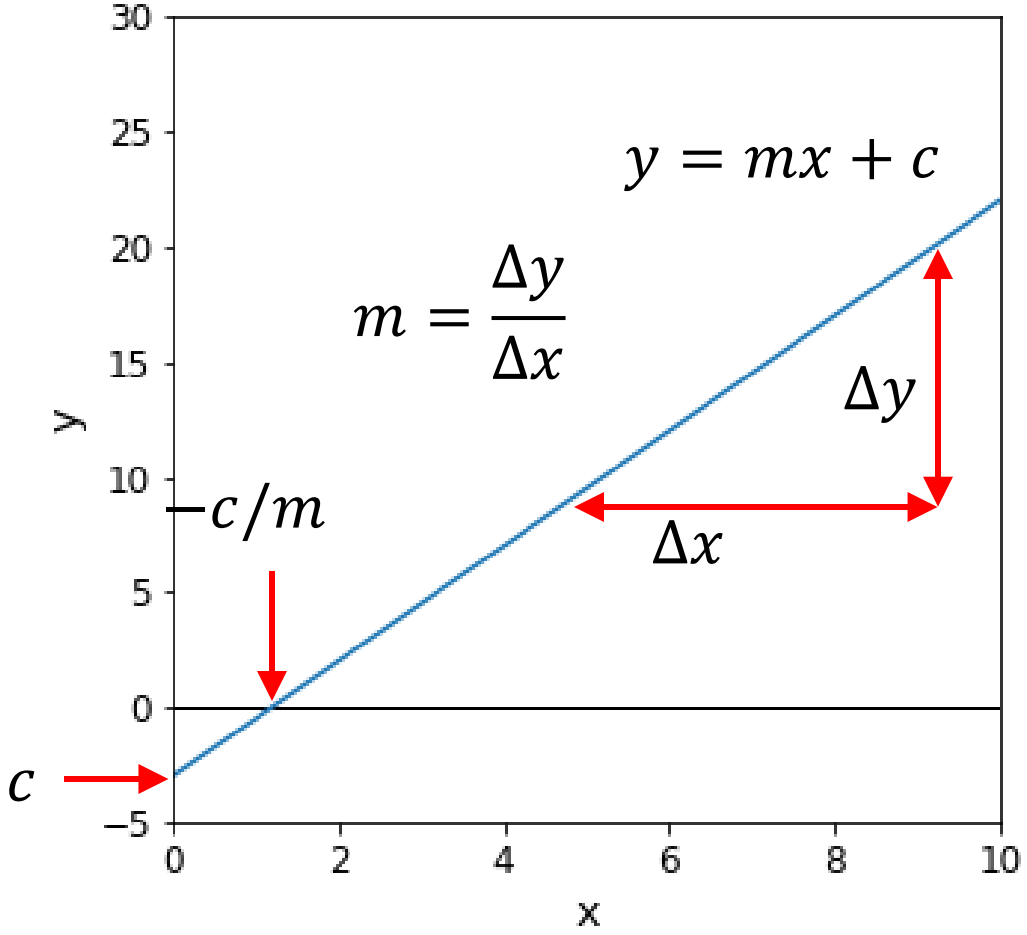


The audience listen and look, not listen and read



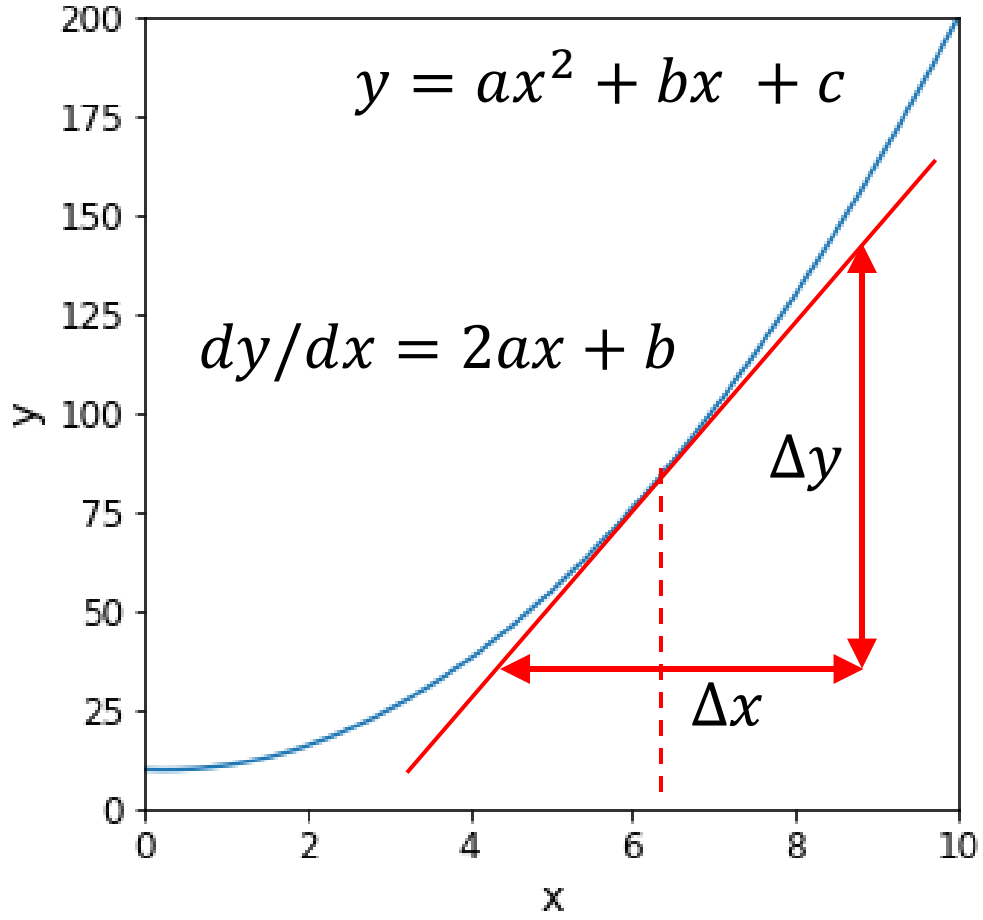
Don’t try to cram too much into one slide

Linear



Constant gradient

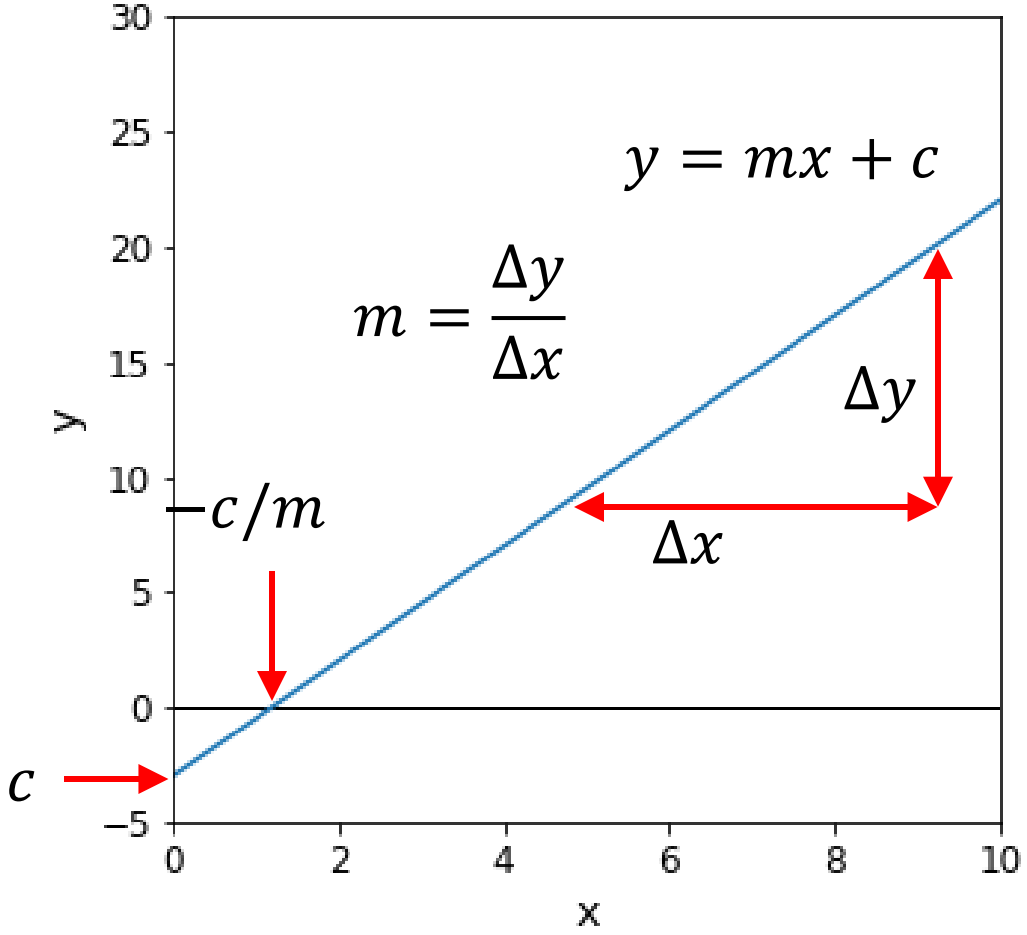
Quadratic



Continuous variation of gradient

Don’t try to cram too much into one slide

Linear



Constant gradient

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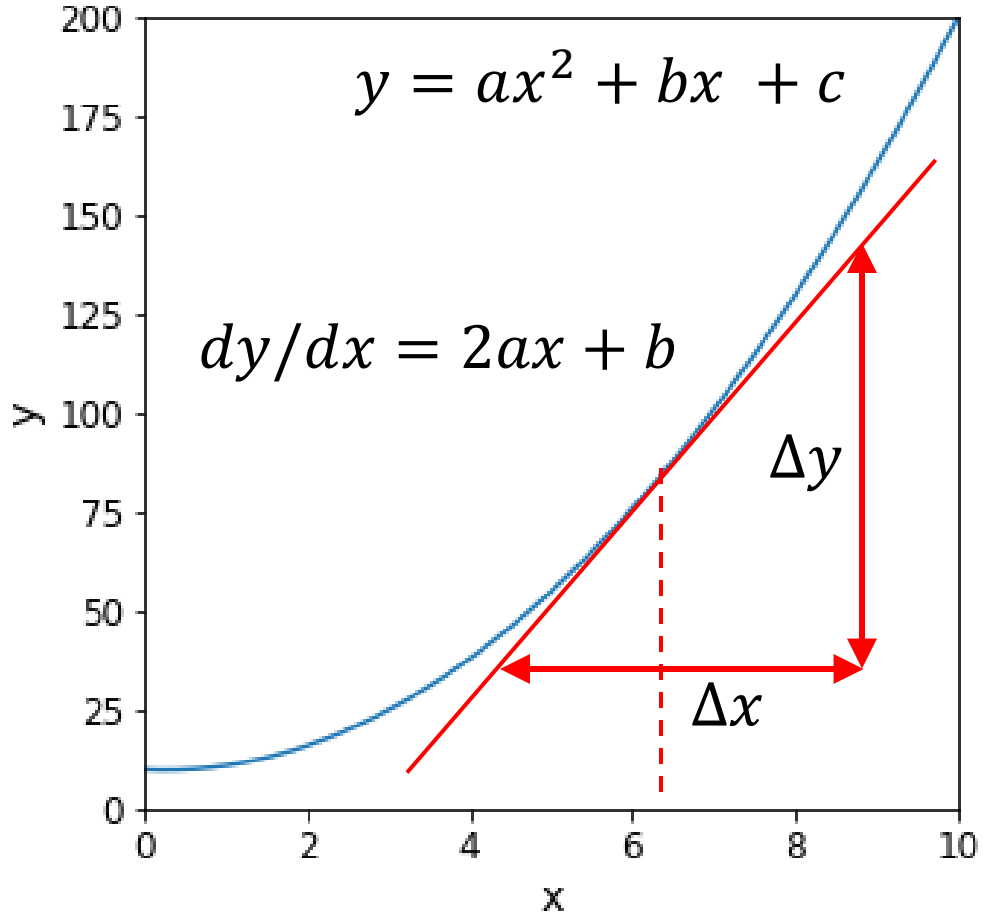


Continuous variation of gradient

Lots of prose-style text is not a good look:

A straight line plotted on a horizontal axis x and vertical axis y can be described by the equation: $y = mx + c$, where m represents the gradient of the line and c represents the intercept with the y axis. The gradient can be determined from the ratio of the change in y, Δy for a corresponding change in x, Δx. In this case the x intercept can be determined as $-c/m$.

Visual clarity

This is 32 point

This is 28 point

This is 24 point

This is 20 point

This is 18 point

This is 16 point. This is 16 point

This is 14 point. This is 14 point

This is 12 point. This is 12 point

This is 10 point. This is 10 point. This is 10 point

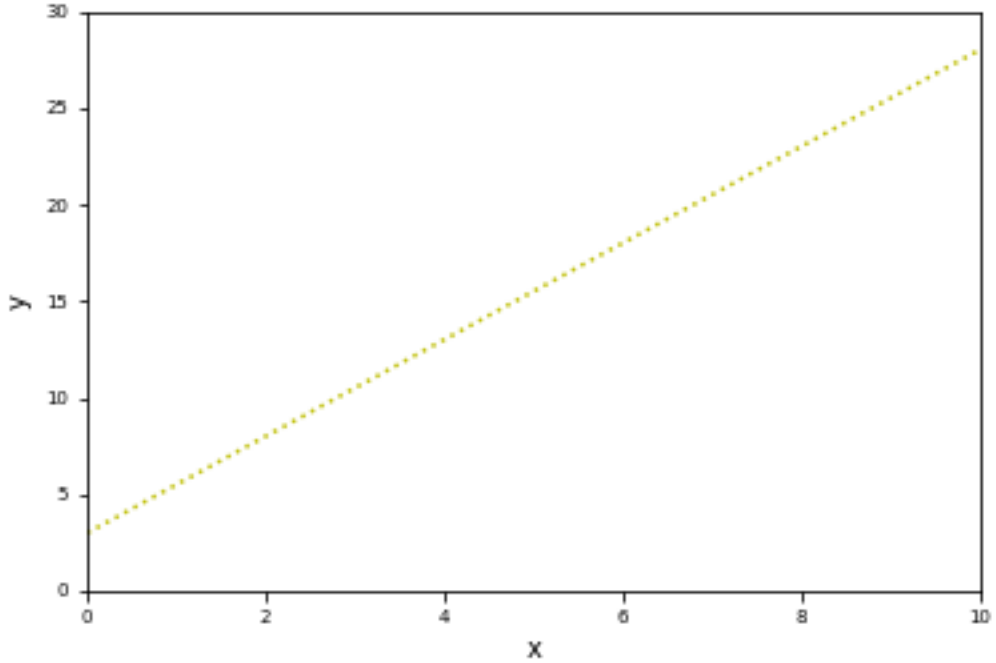
Simple, sans-serif fonts are best

Serifed fonts are more distracting

"Amusing" fonts look terrible

ALL-CAPITALS ONLY INFREQUENTLY TO MAKE A POINT

Choose sensible font / colours in diagrams



Use colour sparingly, for **emphasis**

Some colours like yellow / green can be very hard to see

As can low-contrast combinations

Bad things Powerpoint encourages

- Multi-level bullet point lists:
  - Are quite distracting:
    - If you need them you probably have too much text
    - Yet people seem to love using them
      - ✓ I've really no idea why!
  - They can sometimes be useful
    - But avoid more than one indent level
    - Don't use wild mixtures of bullets

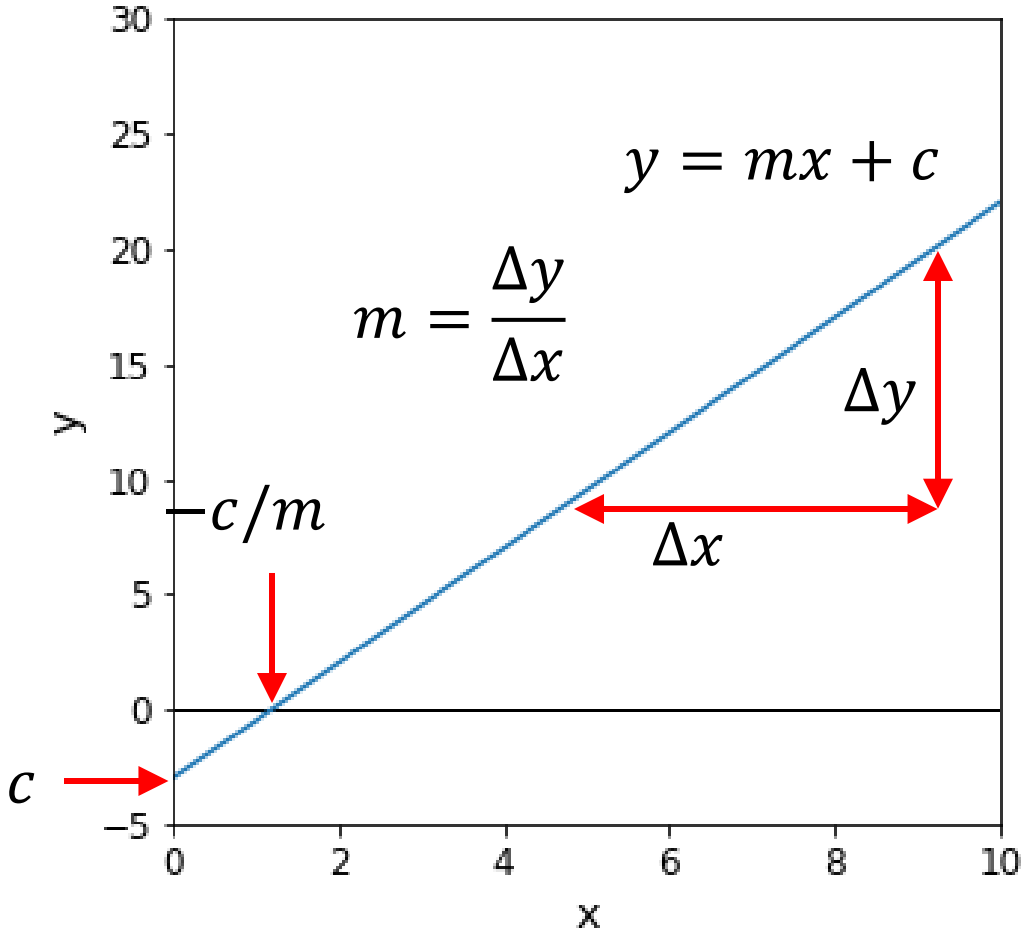
MORE BAD THINGS POWERPOINT ENCOURAGES

Avoid dodgy clipart and cheesy stock images!

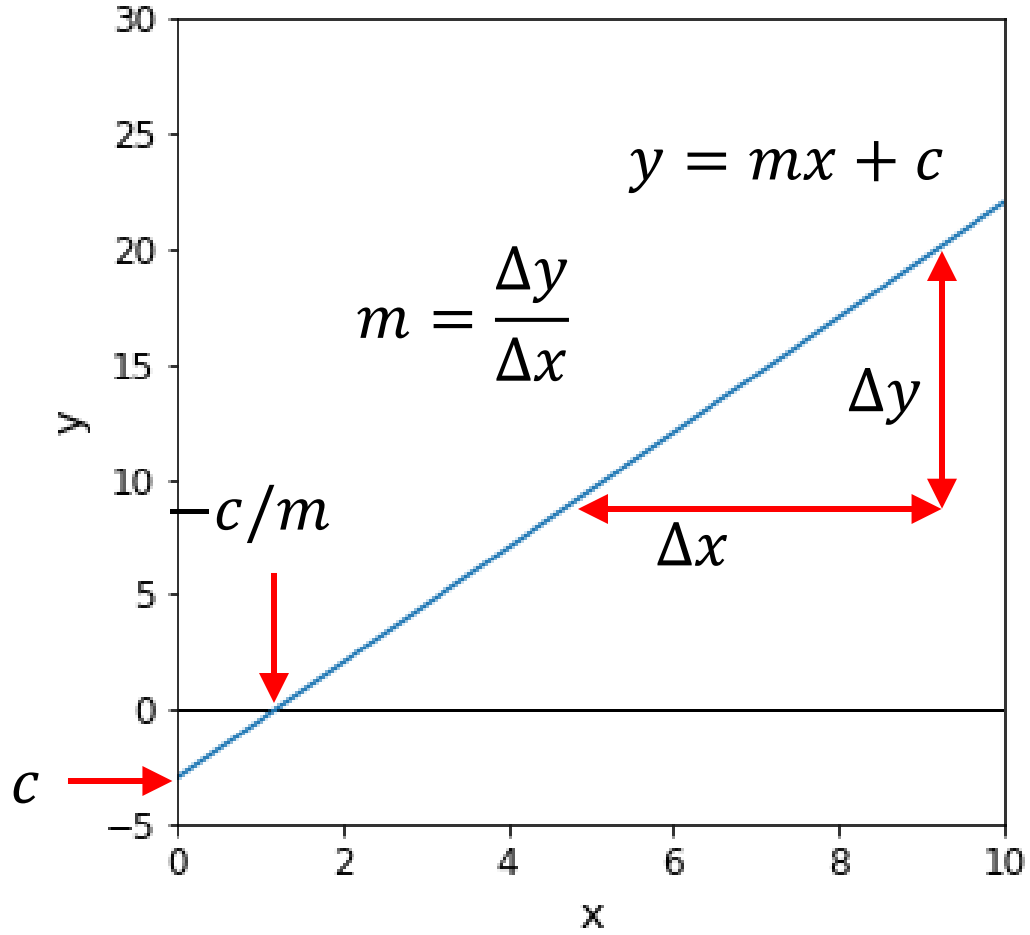
Most of the built-in themes are pretty terrible – keep it clean, simple and relevant!

Yet more Powerpoint horrors…

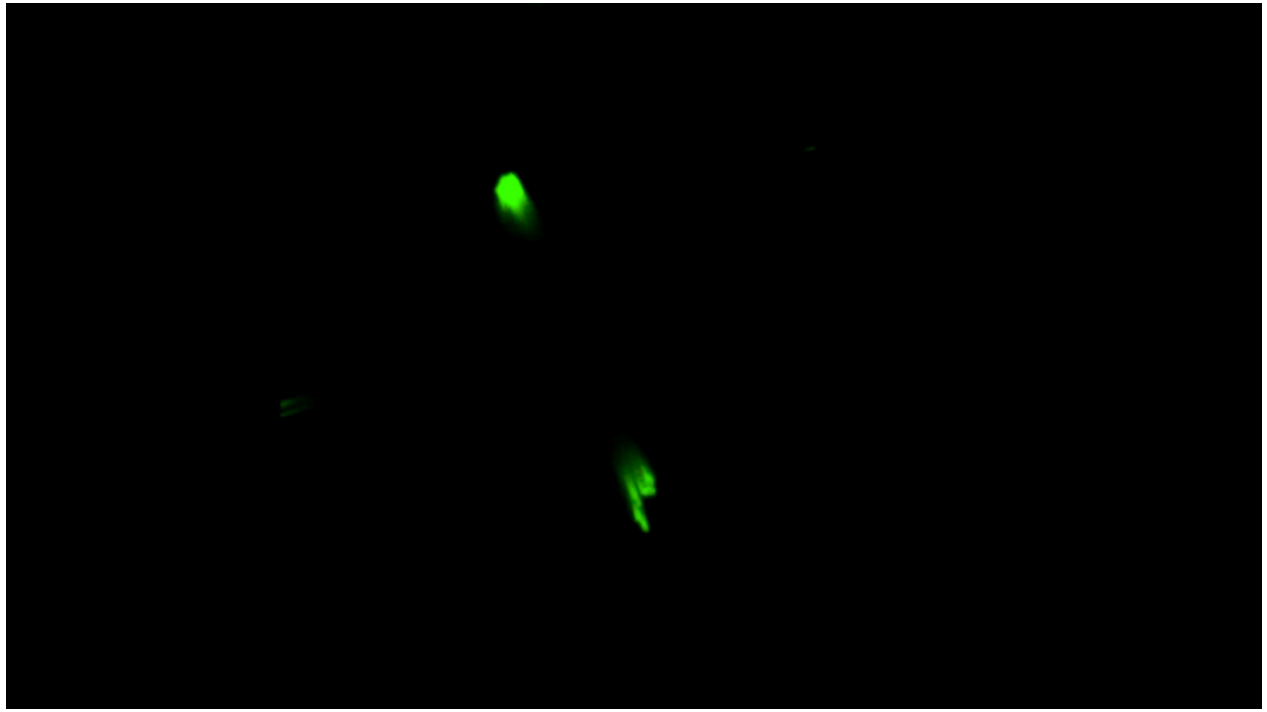
Building a slide in steps can be useful:



"Appear" is the only animation effect you should be using:



Video / animation can be useful if used correctly



Do include video / animation if it is relevant and illustrative

(Protein crystallisation, Rui Cheng, BCFN THETA project presentation, April 2020).

Questions

- When you're preparing your presentation, think about what people might ask you
- If you don't know the answer DON'T MAKE SOMETHING UP – instead say "that's really interesting, I hadn't considered that", or "that's something we'll be looking at in the future"
- Don't get defensive – 99% of the people in the audience want you to do well and are asking things because they are interested. Ignore the 1% who are fond of the sound of their own voice

Presenting - Try to speak naturally

- Word-for-word scripting doesn't sound natural
- Use bullet-point notes as prompts for the points you want to make
- Memorise your opening sentence and know how you're going to get from one slide to the next
- Slides with too much text will encourage you to read the slide out – this is very dull for your audience
- Ideally, don't use notes
- Practice – you will remember what you want to say
- Getting "lost" in your notes confuses you and the audience

The only tip here is practice, practice, practice

Presenting

- Stand up straight, face the audience, smile, breathe…
- Try not to move around too much – don't be rigid but moving a lot can be distracting, try to be natural
- Think about how fast you speak, how loud you speak
- If you're sharing the talk, think about the handover
- Bring energy to your talk, but don't go overboard (you're not a kids TV presenter…)
- Jokes are *very* hard to land well unless you are extremely confident (and you run the risk of offending people)
- Do NOT, under any circumstances, swear

Consider asking someone to video you doing a practice – do you have any obvious verbal or physical things you do that you can work on eliminating?

Body language

- In person:
  - Look at your audience, not down at your notes / computer
  - Be animated – point, make gestures, use laser pointer (don’t overdo it)
  - Address the screen the audience can see
  - Watch out for physical tics / fidgeting!

- Online:
  - Loose a lot of audience engagement
  - Speak / look to the camera
  - Use virtual laser pointer / mouse pointer

Other things to think about

- Visualising yourself giving a great presentation can be useful to relax you if this is something you find stressful
- Make sure you have multiple copies of your presentation in different formats and always try to set up the AV or load your talk and check it in plenty of time – particularly if you have videos or animations
- It gets easier with practice – take every opportunity you can to give talks
- Even really seasoned academics get stage fright…

Summary

- Plan your talk with a clear narrative
- Give the audience context
- Watch your pace
- Pitch at the right level
- Clear, simple slides reliant on images
- Stand up straight, face the audience, use the microphone, speak clearly
- Try to avoid scripts and learn to make minimal use of notes
- Prepare for questions, and don’t ever be afraid to say you don’t know!
- Practice, practice and practice again!

Tips for Good Posters

Posters vs talks: similarities

- Narrative (a “story”) is important – how do sections link together?
- Sections: introduction / motivation, procedures, results, summary / conclusions. Can often be more creative in a poster.
- Avoid large blocks of text. More opportunity in a poster than a talk but you are not “sticking a paper on the wall”
- Data are almost always best presented graphically if possible. Tables, if used, should be small and simple.
- Readability is vital: font sizes, quality of graphics, etc. are important
- Audience: Who are you presenting to? What level should you pitch the material at?

Posters vs talks: differences

- Always present for your talk but not necessarily for your poster – readers should be able to navigate it themselves.
- Narrative order is obvious in a talk (sequential in time). Needs more thought in a poster.
- More opportunity for in-depth Q+A. The poster isn't a script – it usually initiates further discussion. Be prepared!
- Material can be examined more closely and all at once. Quality and consistency of presentation and layout become very obvious.
- Your audience probably want to be there (chose to come to your poster) but can also choose to leave!

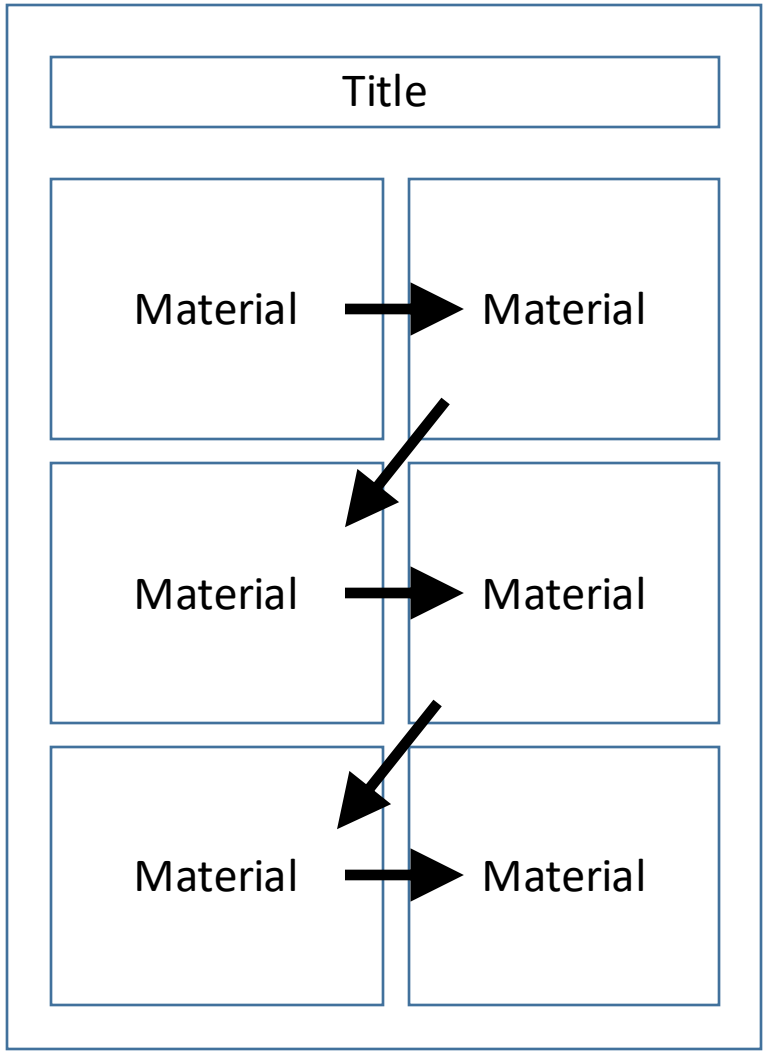
What should I put on my poster?

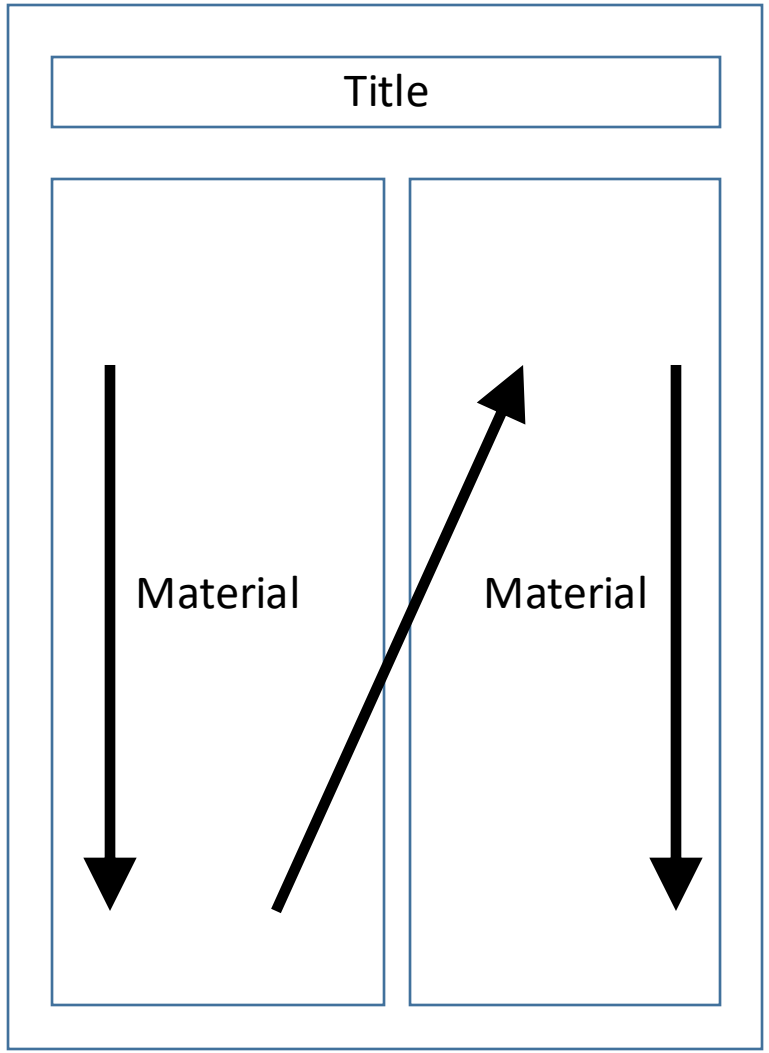
- Think about the following questions…
- How will I introduce why I am doing what I am doing?
- What is the most important or interesting part?
- How can I visually share my research? Should I use charts, graphs, photos, images?
- What information might I want to convey when talking to people that complements my poster?

What makes a good poster

- Important information should be readable from about 2-3m away
- Title is short and draws interest
- Clear introduction / motivation and “pathway” through the poster
- Word count about 300 to 800 words with text clear and to the point
- Good contrast between text and background
- Sectioning: boxes, headlines bullets, numbering make it easy to read
- Effective use of graphics, colour and fonts
- Consistent and clean layout

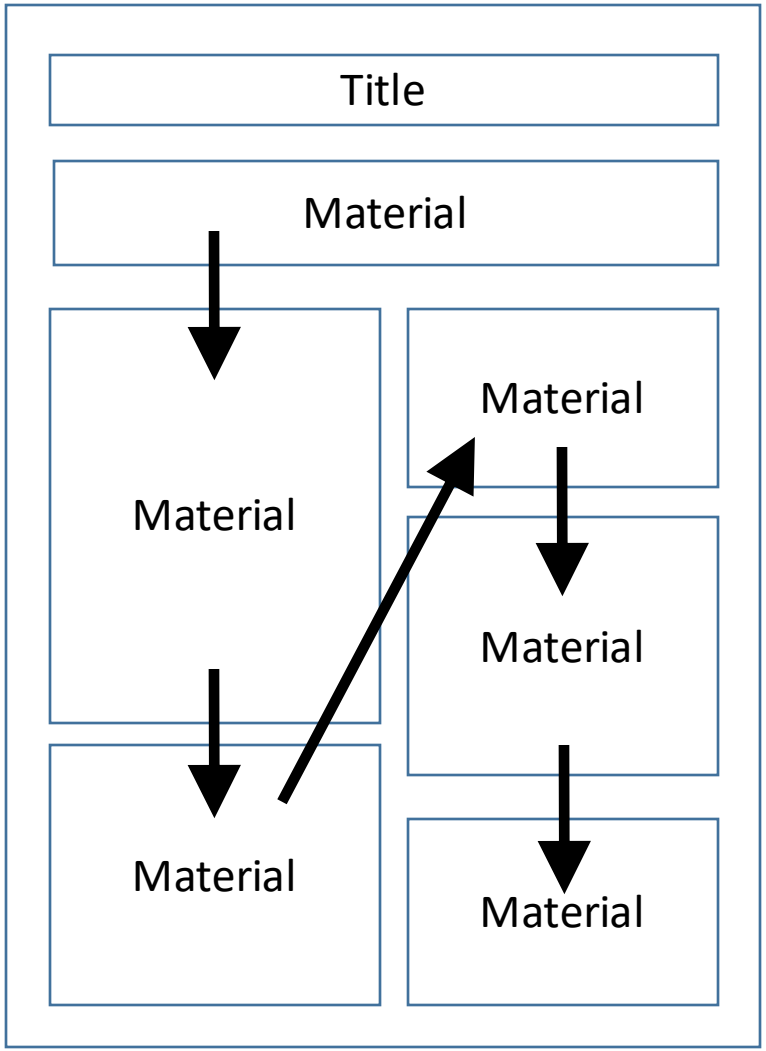
Narrative “pathway”

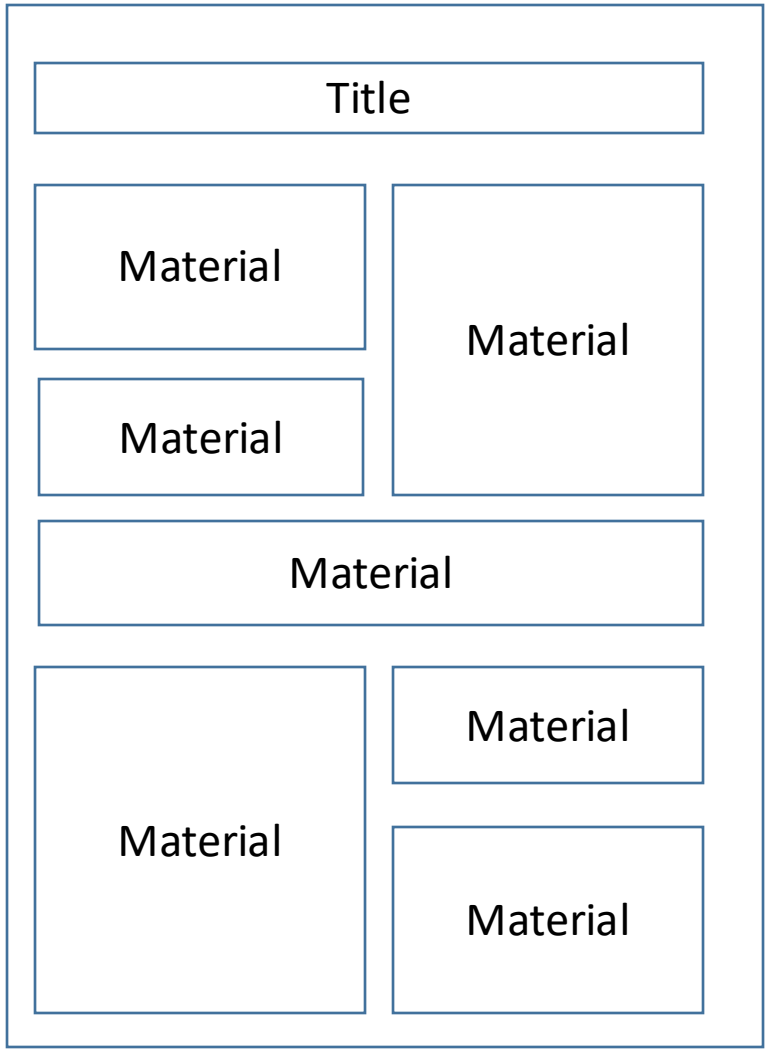




- Think about order in which the reader will process the material.
- What information needs to come earlier for later material to make sense?
- Layout is very important for this.

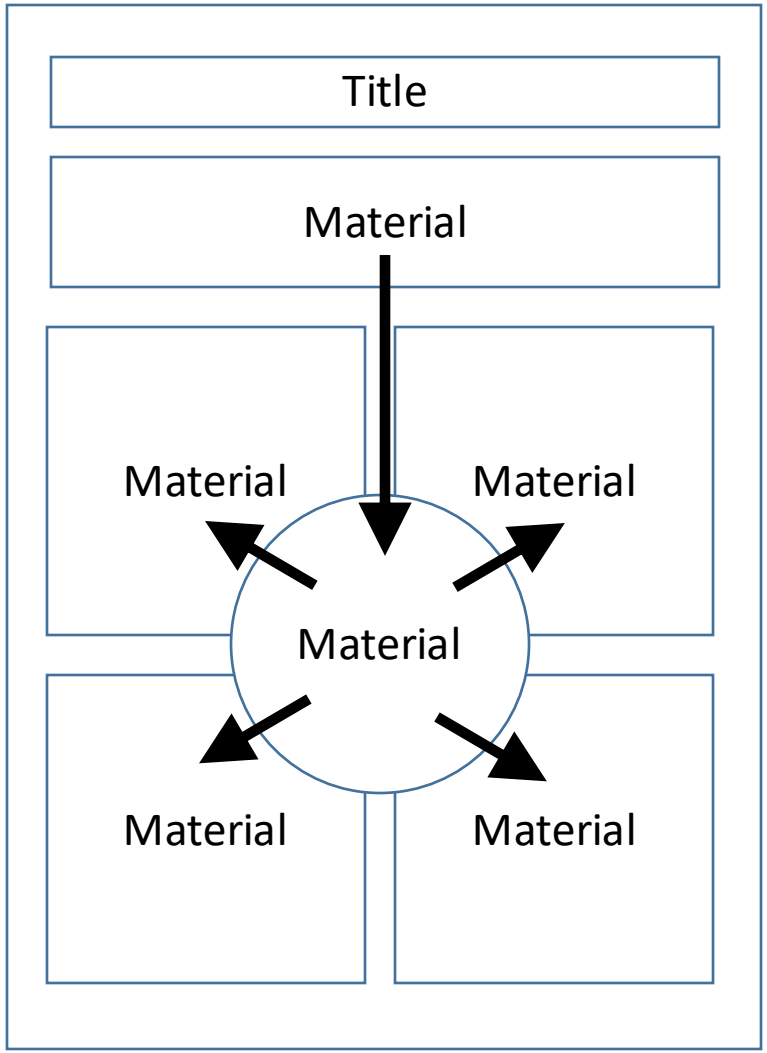
Narrative “pathway”





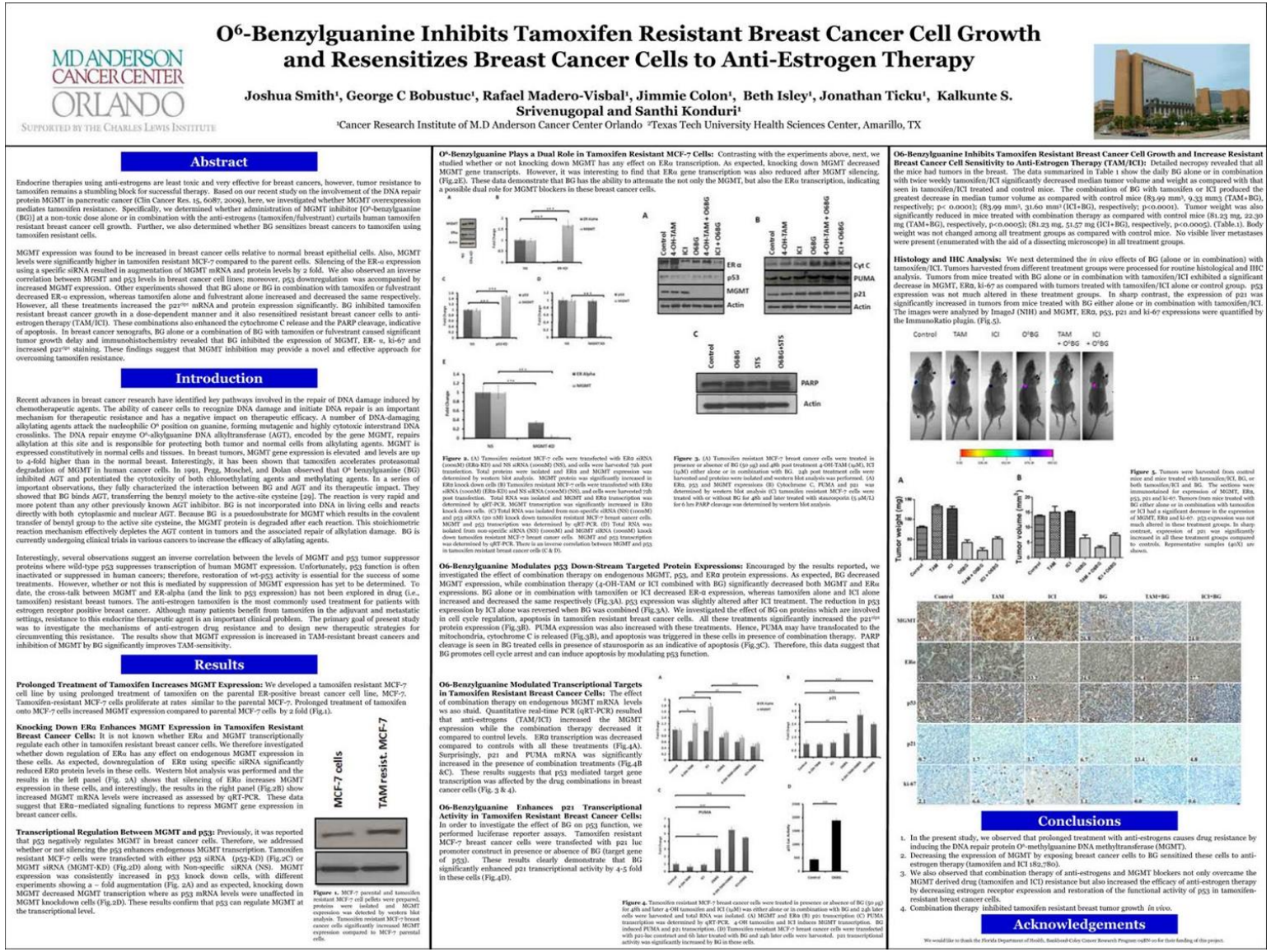
- More complex layouts can make this process confusing

Narrative “pathway”



- Be creative, but think about how your audience will navigate your poster.

What isn't a good poster?



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

MD ANDERSON CANCER CENTER ORLANDO
SUPPORTED BY THE CHARLES LEWIS INSTITUTE

Joshua Smith[1], George C Bobustuc[1], Rafael Madero-Visbal[1], Jimmie Colon[1], Beth Isley[1], Jonathan Ticku[1], Kalkunte S. Srivenugopal and Santhi Konduri[1]
[1]Cancer Research Institute of M.D Anderson Cancer Center Orlando [2]Texas Tech University Health Sciences Center, Amarillo, TX

Abstract

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

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

Introduction

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

MCF-7 cells | TAMresist. MCF-7

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

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

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

Figure 3. (A) Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μg) and 48h post treatment 4-OH-TAM (1μM), ICI (1μM) either alone or in combination with BG. 24h post treatment cells were harvested and proteins were isolated and western blot analysis was performed. (A) ERα, p53 and MGMT expressions (B) Cytochrome C, 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^{WAF1} protein expression (Fig.3B). PUMA expression was also increased with these treatments. Hence, PUMA may have translocated to the mitochondria, cytochrome C is released (Fig.3B), and apoptosis was triggered in these cells in presence of combination therapy. PARP cleavage is seen in BG treated cells in presence of staurosporin as an indicative of apoptosis (Fig.3C). Therefore, this data suggest that BG promotes cell cycle arrest and can induce apoptosis by modulating p53 function.

O6-Benzylguanine Modulated Transcriptional Targets in Tamoxifen Resistant Breast Cancer Cells: The effect of combination therapy on endogenous MGMT mRNA levels ws aso stuid. 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 activity was significantly increased by BG in these cells.

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

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

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

Conclusions

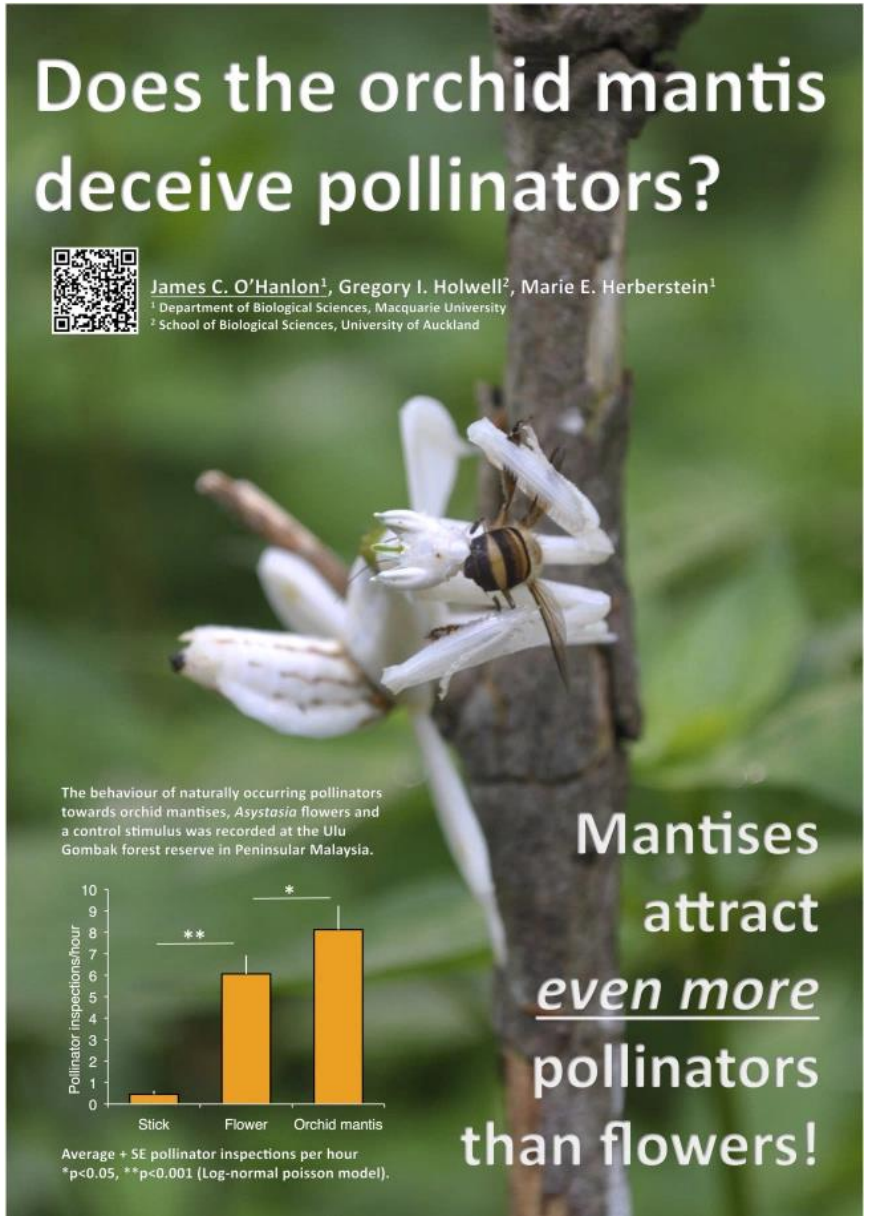
1. In the present study, we observed that prolonged treatment with anti-estrogens causes drug resistance by inducing the DNA repair protein O⁶-methylguanine DNA methyltransferase (MGMT).
2. Decreasing the expression of MGMT by exposing breast cancer cells to BG sensitized these cells to anti-estrogen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

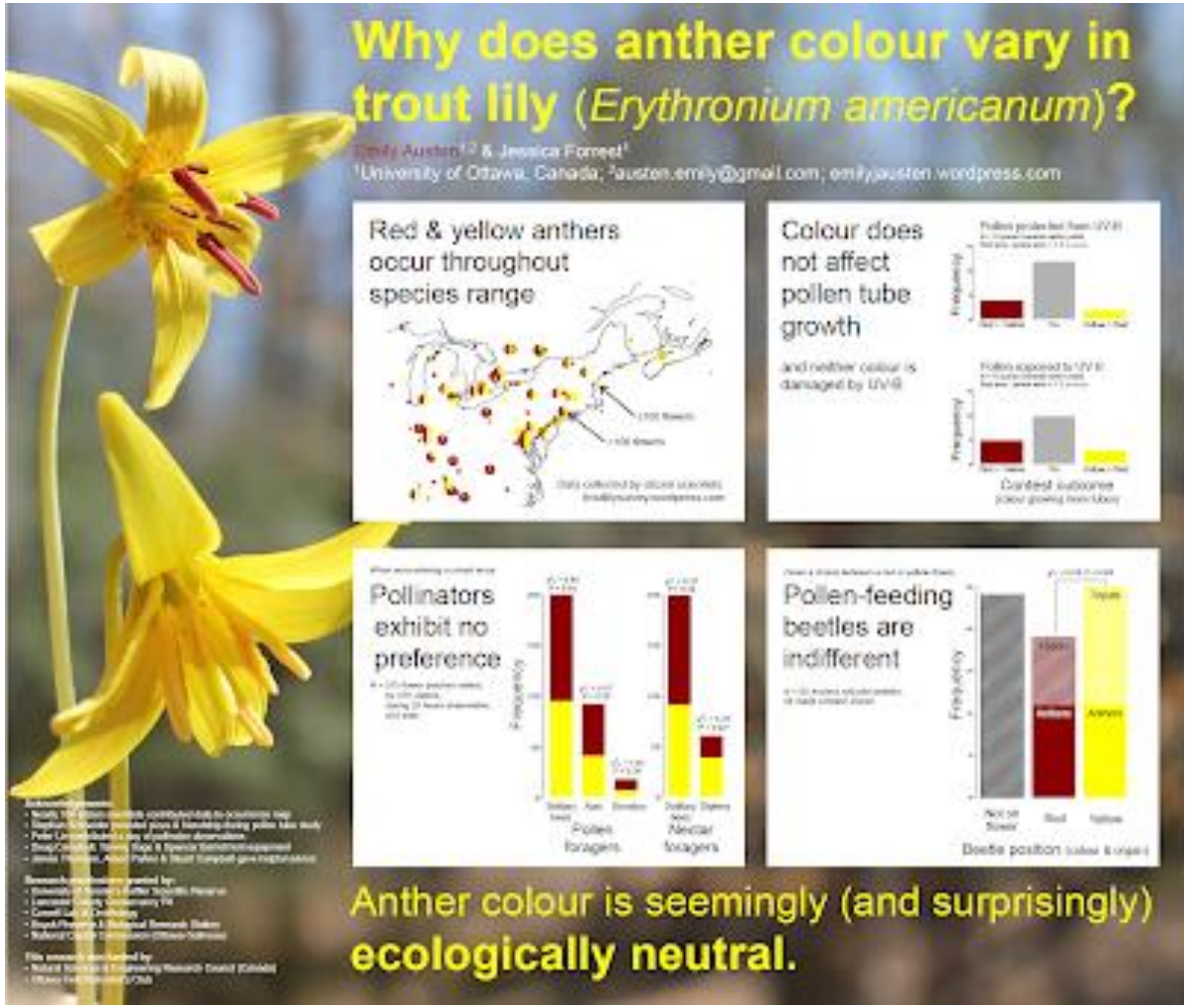
Acknowledgements

We would like to thank the Florida Department of Health, Bankhead-Coley Cancer Research Program 09BN-10 for their funding of this project.

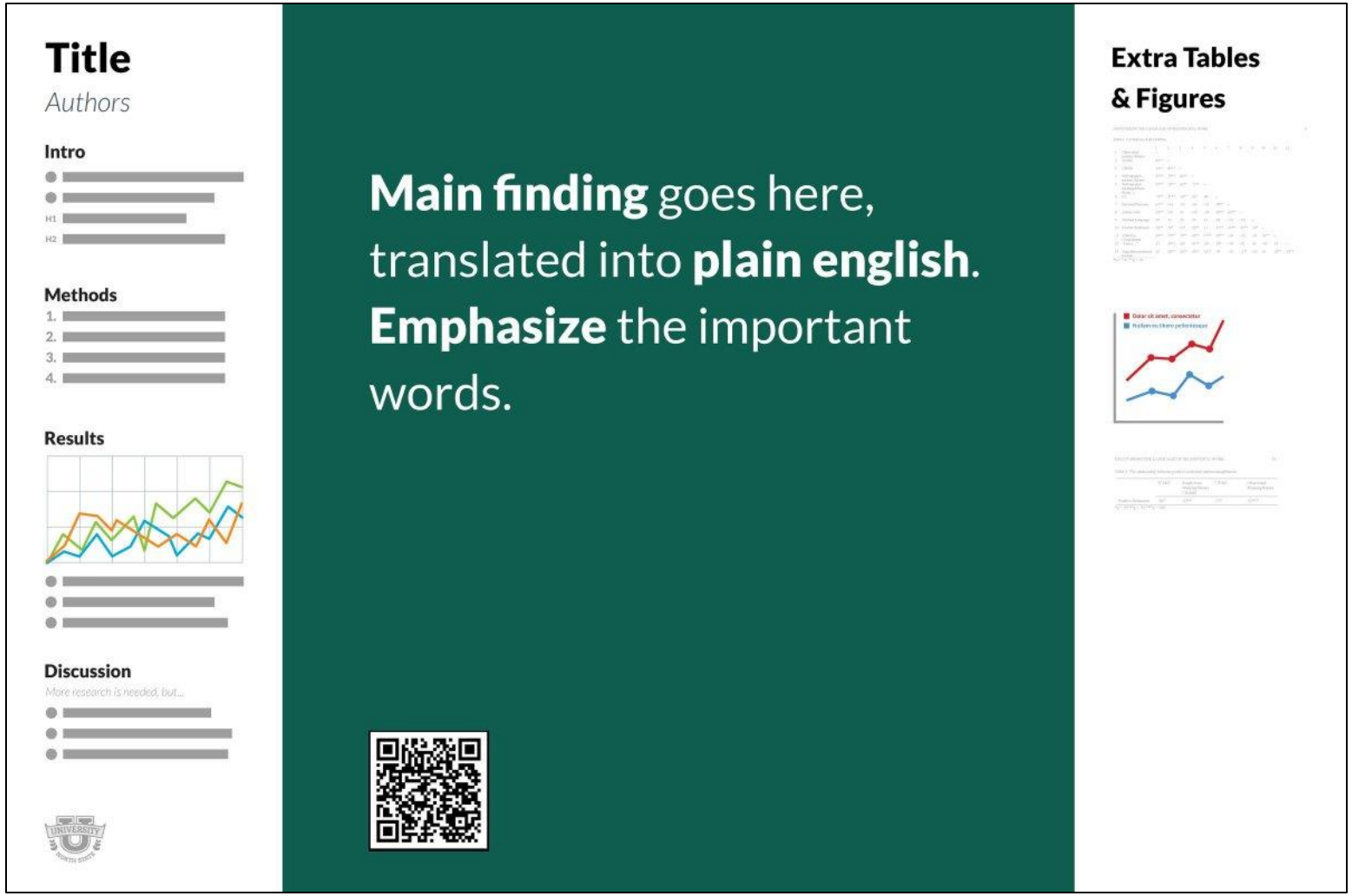
- Grey, grey, grey
- Text too small and too much
- Cramped
- Looks like someone has tried to take a paper and make it into a poster

Sometimes less is more…





Context and personal taste do play a big part…



"This video brilliantly and eloquently captures the inefficacy of the poster presentation. It's 2019 and the world changes at lightening speed. And yet, cardboard posters and awkward conversation are the BEST methods for sharing ideas and insight?!?" (comment: Paula Kay)

"This whole Instagram-a-fication of scientific posters movement is my least favorite science trend right now. It misses the entire point of scientific posters, which is to facilitate networking and scientific discussion." @CousinAmygdala

https://www.youtube.com/watch?v=1RwJbhkCA58

http://betterposters.blogspot.com/2019/04/critique-morrison-billboard-poster.html

Practicalities – print

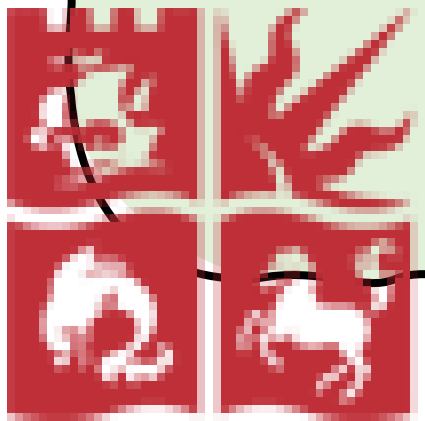
- Title: approximately the entire width of the poster
- Main text broken into multiple columns or panels, but think about narrative order. Use section headings within these if necessary.
- Sans-serif fonts (rather than serifed) work best for posters, particularly for titles, subtitles, and headers. Arial is a good default, Garamond is also nice. Don't ever, EVER use Comic Sans.
- Adjust the font size depending on amount of text on your poster but think about readability.
- Use consistent formatting for headings, sub-headings, body text.
- Use a grid to keep all text aligned with each other and with figures

Practicalities – font sizes

- For guidance:
  - Title: 72-120 pt.
  - Subtitle: 48-80 pt.
  - Section headers: 36-72 pt.
  - Body text: 24-48 pt
  - No text should be smaller than 18pt.

Practicalities - graphics

- Web images are low resolution (72 dpi) and are not proper quality for a printed poster. Limit image resolution to 150 dpi minimum to ensure printability.
- Images should be inserted directly into the layout, not linked from another program. Jpeg is preferred for photographs, tif, gif or (mainly) png for geometric graphics or if you require transparency.
- Do not use the default Excel chart settings on posters (or indeed ever). Consider using a decent graph package – e.g. Origin, or MatPlotLib if you're a Python user



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Practicalities – preparation

- DTP or page layout software is ideal (InDesign, Quark Express) but may not be available.
- Powerpoint adequate in most cases (most people use this). Graphical tools are painful in newer versions.
- Word processors are not really suitable.
- LaTeX can be used (beamer and beamerposter classes) but hard work unless you are an expert.
- Adobe Illustrator, Photoshop allow preparation of high-quality graphics.
- FOSS alternatives: LibreOffice Impress (for PowerPoint), Scribus (for InDesign), Inkscape and Gimp (for graphics).

Practicalities – PowerPoint posters

- Create your poster on ONE slide. The page size MUST be the print size – set this before you start or you'll end up with a really low-quality print.
- To prevent cropping when printing, be sure you have a 1-inch margin around the edges of the poster.
- To set the page size go to Design > Slide Size > Custom Slide Size
- For A0 Portrait the dimensions are 84.1cm x 114.9cm
- Always check whether you are required to prepare portrait or landscape!

Practicalities – Printing

- Convert your poster to a pdf and print an A4 copy
- This will let you look at layout, colours, whether characters have been printed properly.
- You can also hand out A4 copies of your poster to people who are interested (if there's nothing on your poster that you consider to be information you wouldn't want to share in hard copy…)
- When printing, think about finish – Gloss? Matt? Canvas?
- You'll want to think about a poster tube to store and transport it also
- Allow time for printing (and re-printing, just in case).
- www.bristol.ac.uk/print-services/posters-and-banners/

Summary

- Clarity of narrative
- Guide the reader through the poster in a way that they can do on their own
- Simple, clean, high resolution graphics, with minimal text
- Make sure text is readable from ~2m away
- You don’t have to tell them *everything* - start the discussion

Best of luck to all of you, enjoy it, have fun, and contact me if you have questions or concerns!