Targeting Ataxia-Telangiectasia Mutated to Enhance the Radiation Response of Glioblastoma

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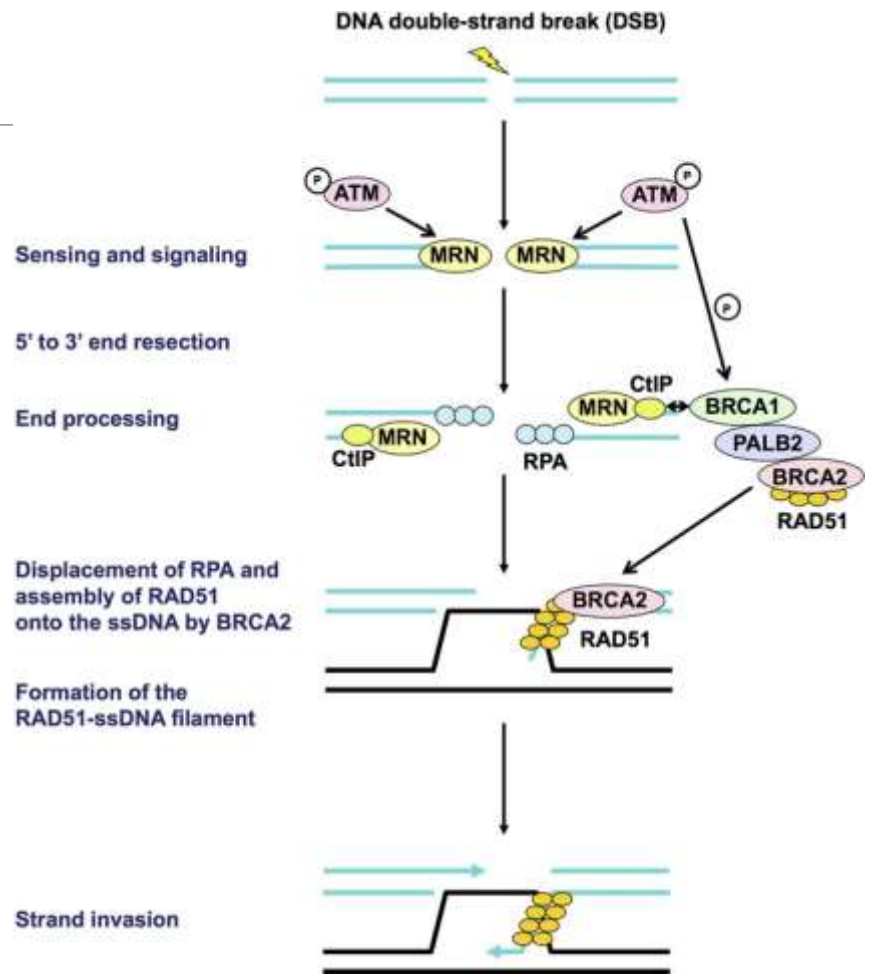
Glioblastoma

- Glioblastoma (GBM) is the most prevalent type of brain cancer
  - 12,390 cases estimated to occur in 2017
- Standard of care
  - Surgical resection
  - Radiation therapy
  - Chemotherapy with temozolomide (TMZ)
- Even with this treatment, the median patient survival remains between 12 and 15 months
- By sensitizing tumor cells to radiotherapy, we can increase the effectiveness of treatment and the patient survival of the disease

DNA Damage Response

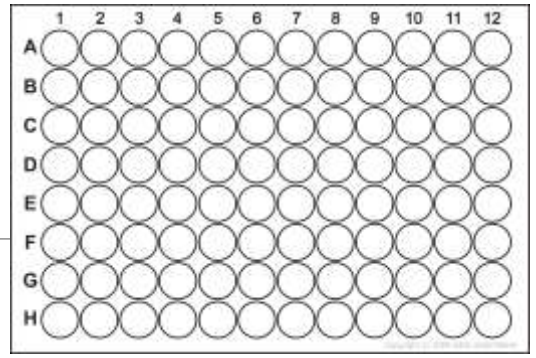
- Radiation therapy produces DNA damage in different forms
  - The most lethal form of damage, double-strand breaks (DSBs), is mainly repaired by two pathways
    - Non-homologous end-joining (NHEJ) – involves the protein DNA dependent protein kinase (DNApk)
      - Faster, more error-prone
    - Homologous recombination (HR) – involves the protein ataxia telangiectasia mutated (ATM)
      - Slower, error-free
- My Project: Evaluate the effect of ATM inhibitor KU60019 on radiation response of U251 tumor cells and normal astrocytes



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4317796/figure/fig02/

Methods

- **Cytotoxicity** – Amount of cell kill the drug produces at different concentrations over time
  - 96 well plate
  - 0.5, 1, 3, 5, 10 uM KU60019 for u251s
  - 0.1, 1, 3, 5, 10, 50, 100 uM KU60019 for NA
  - 24, 48 and 72 hours
- **Western Blotting –** Changes in protein levels in response to KU60019 and IR
  - Tested the effect of drug with irradiation on U251 tumor cells
  - Concentrations of 1, 3, and 5 uM
  - Collected samples 30m and 24h after IR
- **yH2AX Immunofluorescence assay –** Measured how successful the cells were in repairing double strand breaks (DSBs) after IR
  - 4-well chamber slides
  - U251s and normal astrocytes, 3 uM KU60019



http://www.cellsignet.com/media/templ.html

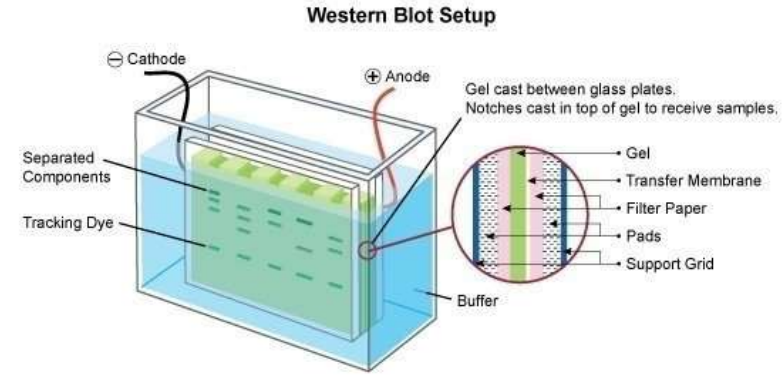
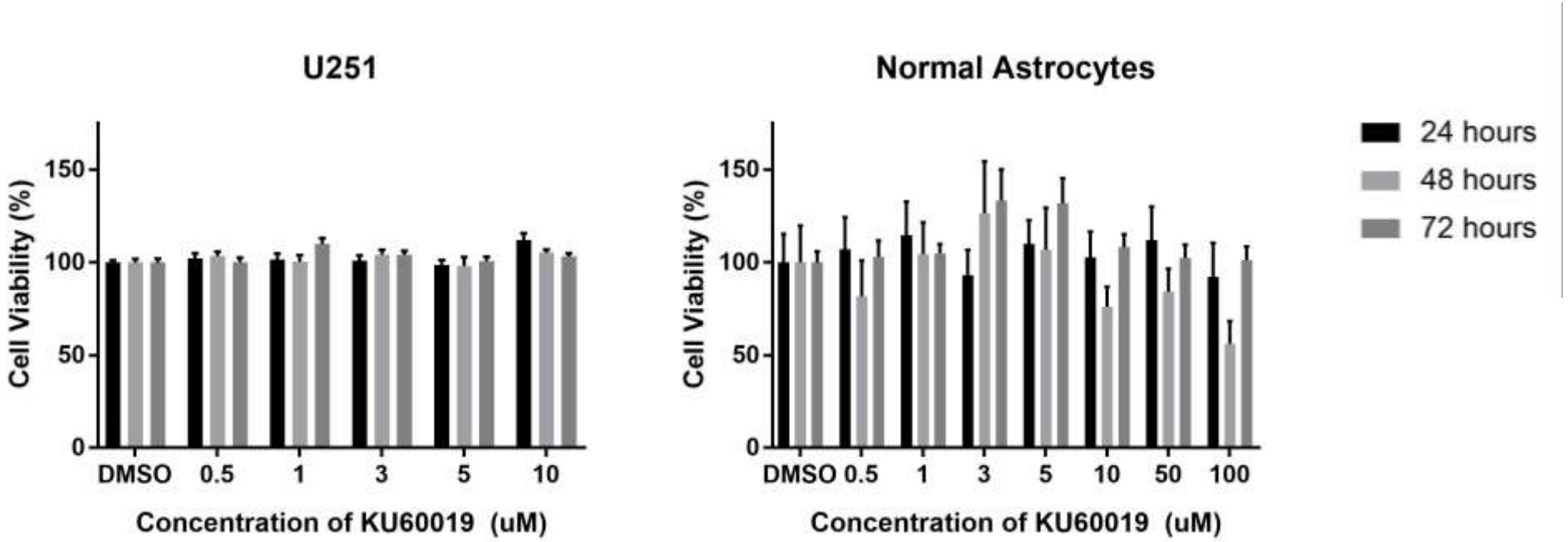


Diagram 1: *Illustration of Western Blot Setup.*

http://www.antibodies-online.com/resources/17/1224/western-blotting-immunoblot-gel-electrophoresis-for-proteins/

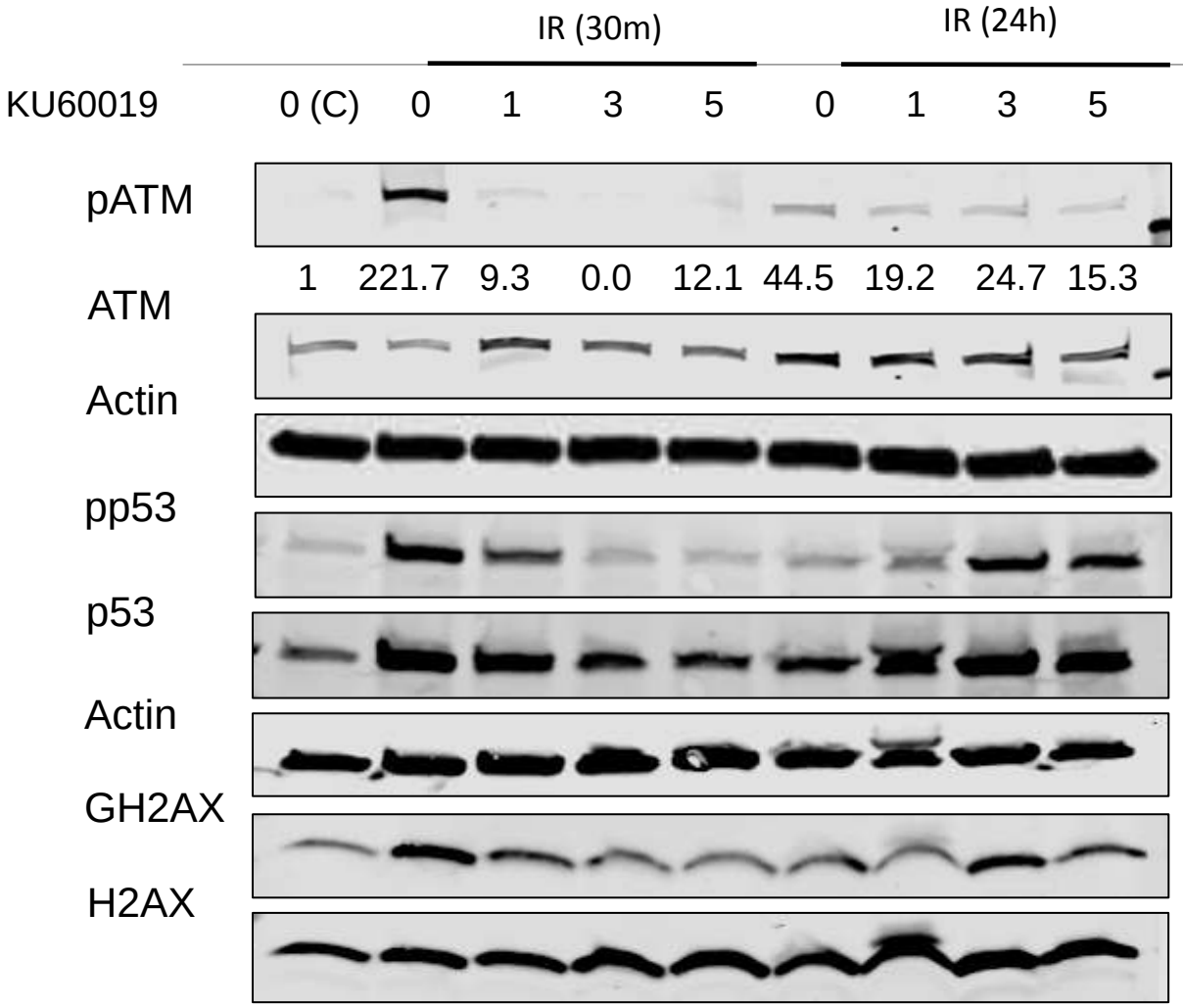
https://ibidi.com/open-slides-dishes/38--slide-4-well-ph.html

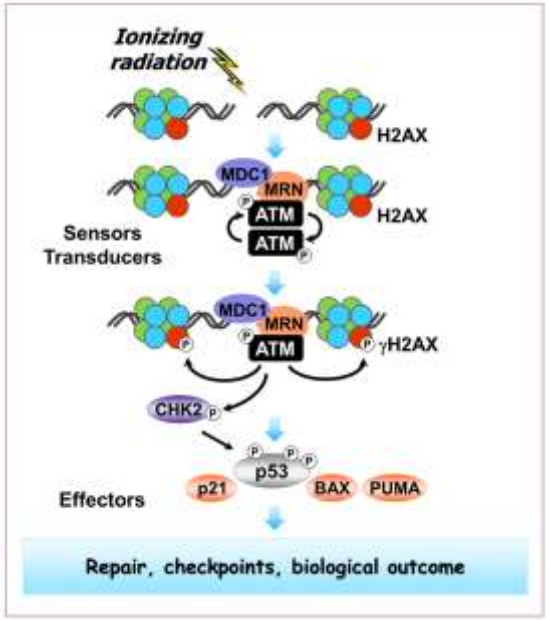
Results: Cytotoxicity



- KU60019 showed no toxicity to the U251s
- Normal Astrocytes were more sensitive to KU60019, with cellular viability decreasing at higher drug concentrations – this helped us choose a drug dose for our experiments
  - Most cell kill with normal astrocytes occurred at 48 hours at 10, 50 and 100uM – we don't want this

Results: Western Blots

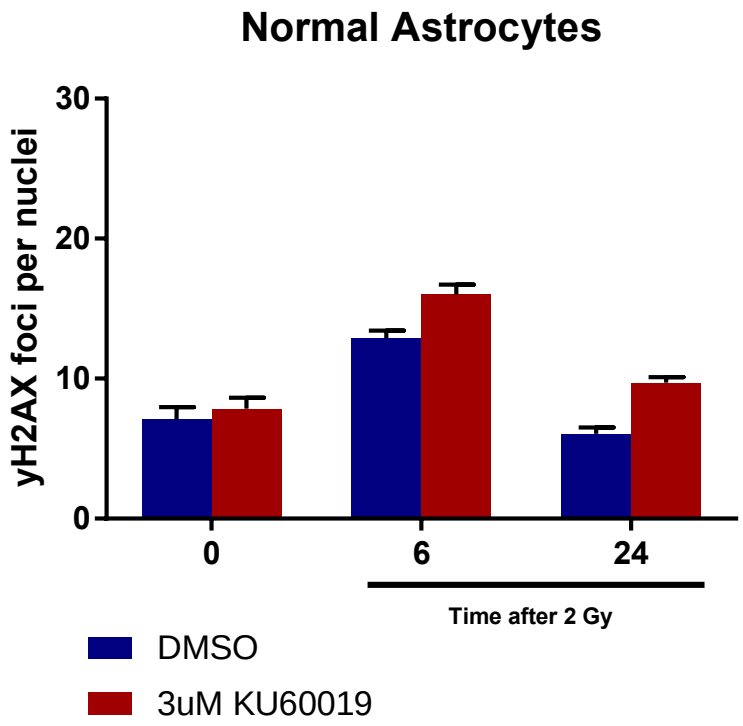


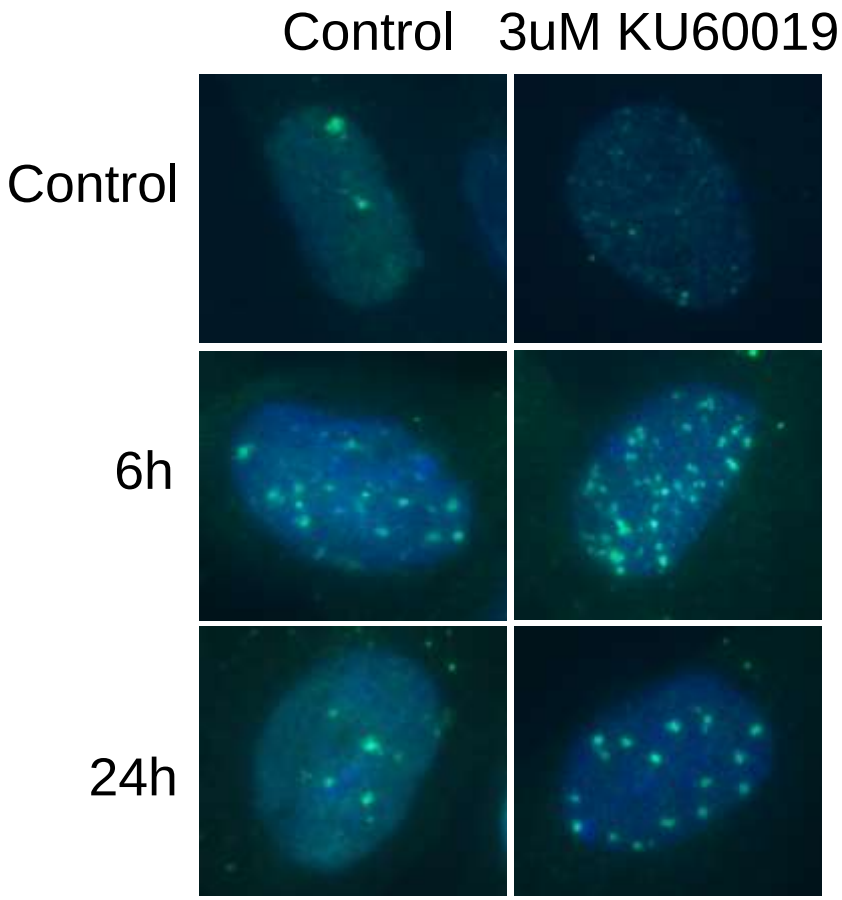


http://www.mdpi.com/1422-0067/14/11/22409/htm

- Gene silencing of pATM achieved completely at 30m with KU60019 concentration of 3uM
- pATM levels began increasing at 1 and 3uM KU60019 at 24 hours
- Inhibits pp53 and most effectively at 1, 3 and 5 uM KU60019 at 30m
- GH2AX and p53 followed similar patterns

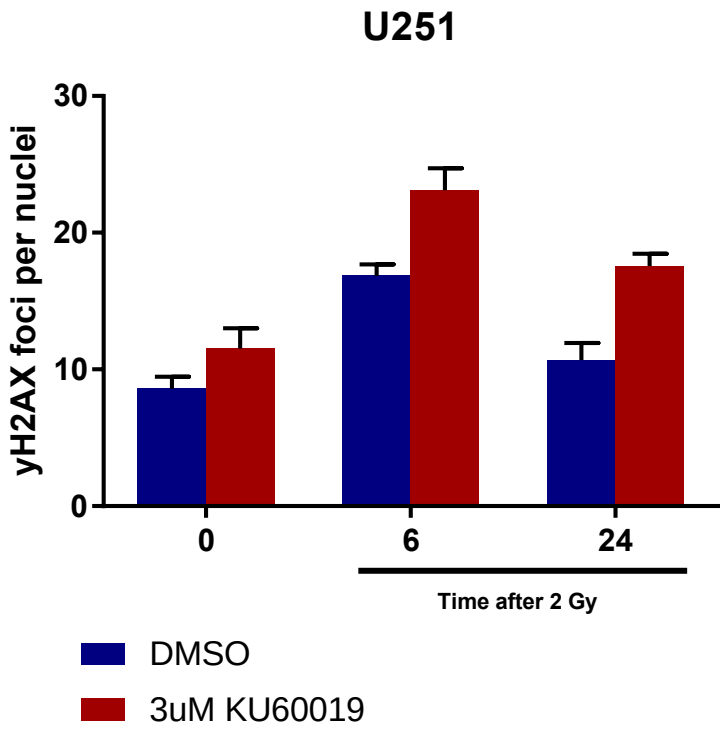
Results: yH2AX assay

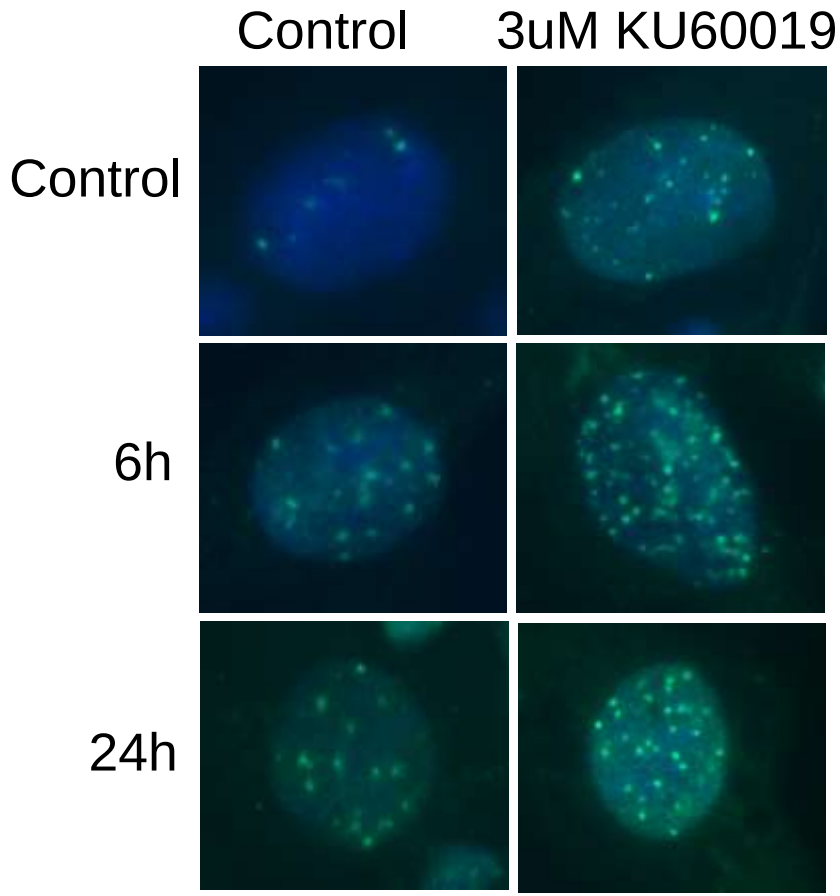




- General increase in foci within time points from control to treated samples
  - Not statistically significant
  - KU60019 does not create significant amounts of DNA damage in NA at this concentration

Results: yH2AX assay





- Difference in the number of foci at 24 hours compared to non-irradiated cells
  - Statistically significant
- Tells us the drug at 3uM is able to keep U251s from repairing DSBs

Summary

- KU60019 showed no toxicity to U251 cells at the concentrations tested and therefore would not be sufficient by itself – it needs to be used with radiation to be effective
- From the concentrations we tested, KU60019 is an effective inhibitor. It works best at 3uM but also shows activity at lower concentrations when U251 cells are irradiated at 2Gy
- As shown in the yH2AX assay, KU60019 prevents U251 cells from successfully repairing DSBs, which could make it an effective radiosensitizer

What I learned

- Research is tiring
- You have to stay REALLY organized
  - It's impossible to keep track of everything in your head when you're doing multiple experiments at once!
- You need to be super patient
- I'm awful at calculating dilutions
- It's really easy to mess something up every time
  - You have to accept mistakes!!
- The people aren't intimidating!!
  - Everyone's so friendly and the people are a huge reason for why I had such a good experience

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