FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68469
Scriptwriter Name: Pallavi Sharma
Project Page Link: https://review.jove.com/account/file-uploader?src=20883093

Title: Comet Assay to Quantify DNA Damage in FLT3 Mutant-Expressing 32D Cells after Exposure to Type I and Type II FLT3 Inhibitors

Authors and Affiliations:

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? No

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? No

3. **Filming location:** Will the filming need to take place in multiple locations? NO

Current Protocol Length
Number of Steps: 06
Number of Shots: 20

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Jiani Ge:** We employed comet assays to systematically evaluate the genotoxicity of Type I and Type II FLT3 inhibitors on 32D-FLT3 mutant cells, providing critical data for clinical optimisation in AML.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.4*

What are the current experimental challenges?

1.2. **Dandan Tang:** During the procedures, improper handling may cause the gel to fall out of the slide, resulting in sample loss.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1, 2.3.2*

What research gap are you addressing with your protocol?

1.3. **Jiani Ge:** Research indicates that AC220, as a type II inhibitor, inflicts more severe DNA damage upon FLT3-mutant cells, providing crucial insights for developing combination therapies by exploiting DNA damage vulnerability to overcome therapeutic resistance.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Figure 3*

What research questions will your laboratory focus on in the future?

1.4. **Songbai Liu:** Investigate the mechanism of action of FLT3 inhibitors in the treatment of acute myeloid leukaemia, and develop dual-drug or multi-drug combination therapy strategies based on FLT3 inhibitors.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.5. Jani Ge:

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. Jiani **Ge:**

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Comet Assay Procedure

Demonstrator: Jiani Ge

2.1. To begin, prepare a 10 to 1 mixture of low-melting-point agarose and cell suspension in 1.5-milliliter tubes using a pipette and mix gently **[1]**. Dispense 55 microliter aliquots of the agarose-cell mixture onto comet slides using a pipette held at a 45-degree angle, **[2-TXT]**. Then, place the slides coated with the mixture at 4 degrees Celsius for 30 minutes in a dark environment **[3]**.

2.1.1. WIDE: Talent pipetting a 10 to 1 mixture of low-melting-point agarose and cell suspension into a 1.5 milliliter tube and mixing gently.

2.1.2. Talent carefully dispensing 55 microliters of the mixture onto the comet slide at a 45-degree angle. **TXT: Maintain a 2 mm distance from the slide surface**

2.1.3. Talent transferring the coated slides into a light-protected box at 4 degrees Celsius.

2.2. Submerge the solidified slides in precooled lysis solution **[1]** and incubate for at least 1 hour at 4 degrees Celsius, protecting the slides from light **[2]**.

2.2.1. Talent placing the solidified comet assay slides into a container filled with precooled lysis solution.

2.2.2. Talent covering the container and placing it in a 4 degrees Celsius storage area protected from light.

2.3. After lysis, remove the slides from the lysis solution **[1]**. Using a pipette gun, carefully suck up as much residual liquid as possible from around the paddle **[2]**. Then, place the slides in alkaline electrophoresis buffer **[3]** and allow them to pre-equilibrate for 1 hour at 4 degrees Celsius in the dark **[4]**.

2.3.1. Talent lifting a slide from the lysis solution.

2.3.2. Talent using a pipette gun to remove the liquid surrounding the slide paddle.

2.3.3. Talent placing the slide into a container filled with alkaline electrophoresis buffer.

2.3.4. Talent places the covered container in the refrigerator.

2.4. Now, place the slides into the electrophoresis chamber **[1]**. To fully immerse the slides, add enough precooled alkaline electrophoresis solution **[2]**. Run electrophoresis at 24 volts, with a constant current for 30 minutes using a buffer pre-equilibrated to reduce

DNA damage **[3]**.

2.4.1. Talent positions the comet assay slides into the electrophoresis chamber.

2.4.2. Talent pouring alkaline electrophoresis buffer into the chamber until the slides are completely submerged.

2.4.3. Talent setting the electrophoresis unit to 24 volts and 300 milliamperes and starting the run.

2.5. After electrophoresis, use a pipette gun to aspirate the alkaline electrophoresis buffer from around the slide **[1]**. Submerge the slides in double-distilled water for 5 minutes, **[2-TXT]**, then submerge the slides in 70 percent ethanol for 5 minutes **[3]**. Place the slides in an incubator set at 37 degrees Celsius for 15 minutes to dry **[4]**.

2.5.1. Talent using a pipette gun to remove the electrophoresis solution from the slide container.

2.5.2. Talent placing the slides into a container with double-distilled water. **TXT: Repeat 1x**

2.5.3. Talent transferring the slides into 70 percent ethanol for 5 minutes.

2.5.4. Talent placing the slides into a 37 degrees Celsius incubator to dry.

2.6. Next, apply a 100-microliter aliquot of YeaRed *(Yea-Red)* nucleic acid stain to each well of the slide **[1]**. Incubate the slides for 30 minutes at 28 degrees Celsius in a light-protected environment **[2]**. Rinse the slides gently with water to remove excess stain **[3]** and dry them in a 37 degrees Celsius incubator **[4]**.

2.6.1. Talent pipetting 100 microliters of YeaRed nucleic acid stain into each well of the comet slide.

2.6.2. Talent places the covered slides in the incubator

2.6.3. Talent gently rinsing the slides with water.

2.6.4. Talent placing the slide in a 37 degrees Celsius incubator to dry.

Results

3. Results

3.1. The comet assay was systematically employed to quantify differential DNA damage profiles induced by gilteritinib and AC220 *(A-C-Two-Two-Zero)* in FLT3- *(F-L-T-Three)* mutant cell lines **[1].**

3.1.1. LAB MEDIA: Figure 1 and Figure 2.

3.2. Quantitative analysis of Tail DNA percentage confirmed that both compounds significantly increased DNA damage after 2, 4, and 6 hours **[1],** with AC220 producing higher damage levels than gilteritinib at each time point **[2].**

3.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the bars for both treatments at 2, 4, and 6 hours, showing increasing Tail DNA percentages.*

3.2.2. LAB MEDIA: Figure 3. *Video editor: Emphasize that the black bars are consistently taller than the white bars at each time point*.

3.3. Olive Moment Tail measurements mirrored the Tail DNA trend, with statistically significant increases for both treatments from 2 to 6 hours **[1],** and higher OTM values observed in the AC220 group throughout **[2].**

3.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight the bars for 2, 4, and 6 hours for both treatments, showing rising OTM values.*

3.3.2. LAB MEDIA: Figure 4. *Video editor: Emphasize that the black bars (AC220) are consistently higher than the white bars (gilteritinib), especially at 6 hours.*

- **Agarose**

Pronunciation link:
https://www.merriam-webster.com/dictionary/agarose
IPA: /əˈgæroʊs/
Phonetic Spelling: uh-GAIR-ohs

- **Aliquot**

Pronunciation link:
https://www.merriam-webster.com/dictionary/aliquot

IPA: /ˈælɪkwət/
Phonetic Spelling: AL-i-kwut

- **Electrophoresis**

Pronunciation link:
https://www.merriam-webster.com/dictionary/electrophoresis
IPA: /ɪˌlɛktroʊfərˈiːsɪs/
Phonetic Spelling: ee-lek-tro-for-EE-sis

- **Incubator**

Pronunciation link:
https://www.merriam-webster.com/dictionary/incubator
IPA: /ˈɪŋkjubeɪtər/
Phonetic Spelling: ING-kyoo-bay-ter

- **Gilteritinib**

Pronunciation link:
No confirmed link found
IPA: /gɪlˌtɛrətɪˈnɪb/
Phonetic Spelling: gil-ter-uh-TIN-ib

- **AC220**

Pronunciation link:
No confirmed link found (alphanumeric code)
IPA: /ˌeɪˈsiː ˈtuːˌtuːˈzɪəroʊ/
Phonetic Spelling: A-C-two-two-zero

- **FLT3**

Pronunciation link:
No confirmed link found (abbreviation)
IPA: /ˌɛfˌɛlˈtiː θri/
Phonetic Spelling: F-L-T-three

- **Olive Moment**

Pronunciation link:
No confirmed link found (as a technical term)
IPA: /ˈɑlɪv ˈmoʊmənt/
Phonetic Spelling: OL-iv MOH-ment