ENRICHMENT OF DENDRITIC CELLS FOR ESTABLISHMENT OF IMMORTALIZED CELL LINE

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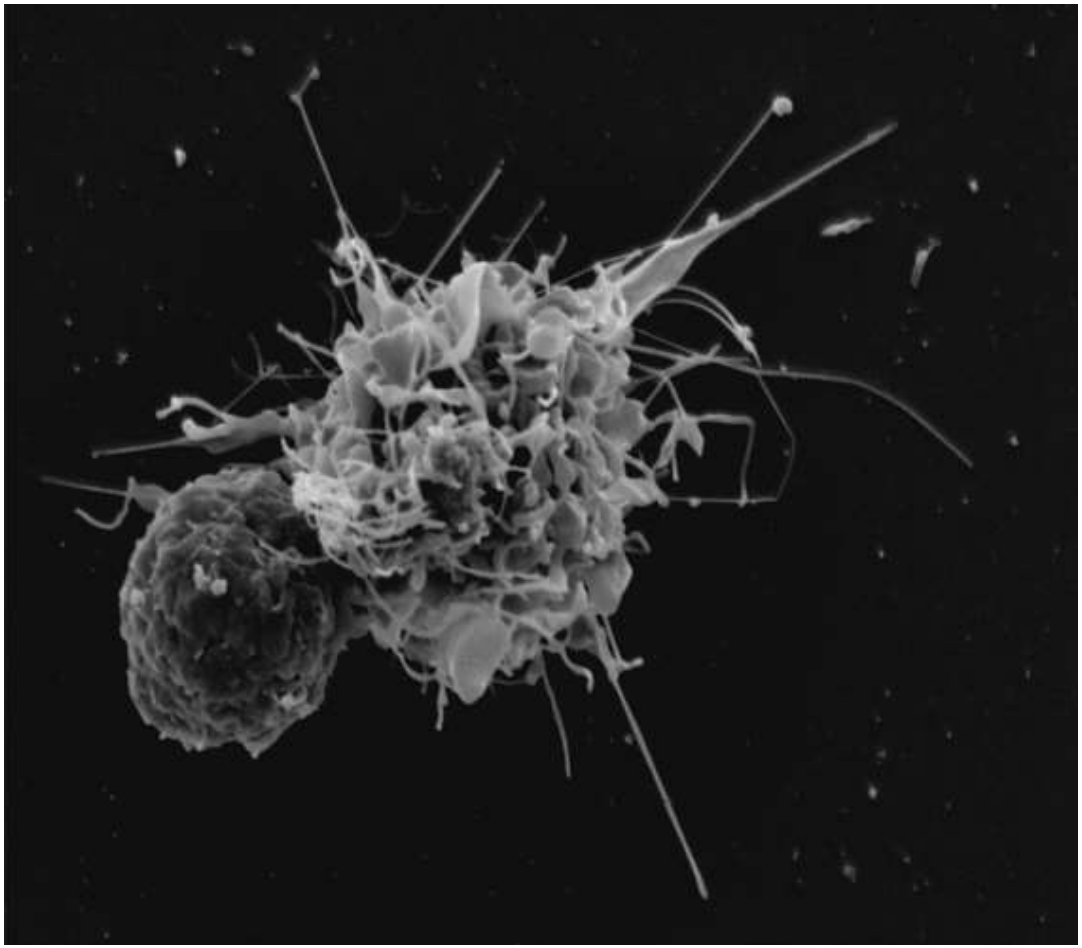
OVERVIEW

- **Introduction to Dendritic Cells (DC) and *Brucella abortus***
- **Specific Goals of the Project**
- **Methods Developed and Used**
- **Results**
- **Conclusions**
- **Future Work**

INTRODUCTION TO DENDRITIC CELLS AND *BRUCELLA ABORTUS*

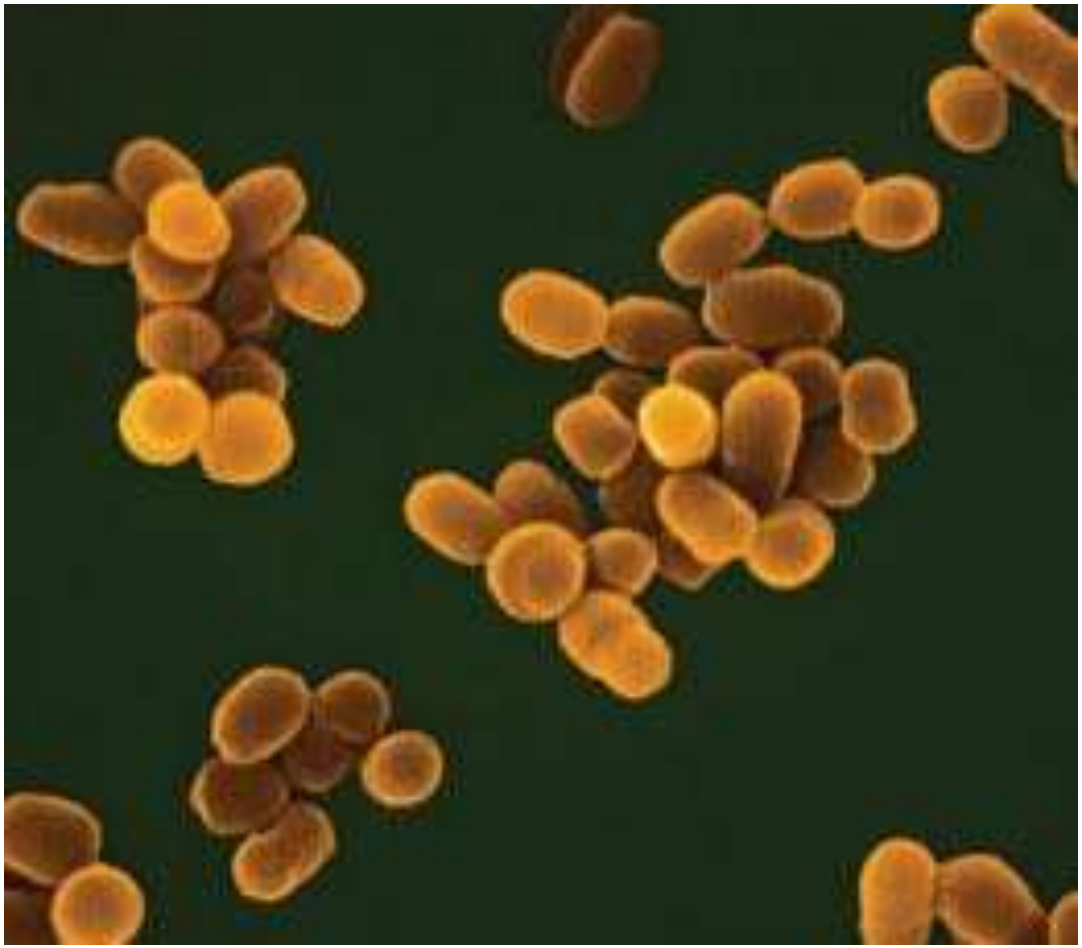
WHAT ARE DENDRITIC CELLS?

- Antigen presenting cells of the immune system
- Monocyte derivatives
- Adherent during immature state
- Migrate to lymph tissue once activated



WHAT ABOUT *BRUCELLA ABORTUS*?

- **Gram negative, coccobacilli**
- **Establish chronic infection of dendritic cells**
- **Can cause spontaneous abortion**
- **Zoonotic pathogenesis**
- **Can result in large economic losses**



SPECIFIC GOALS OF THE PROJECT

BACK GROUND TO THE PROJECT

Began working with Jake Berg (masters candidate in Veterinary Science) under the guidance of Dr. Adamovicz to establish an immortalized dendritic cell line for *Brucella abortus* studies.

SPECIFIC AIMS OF THE PREVIOUS SEMESTER

- **Modify and develop protocols for the following:**
  - Differentiation of DC from Peripheral Blood Mononuclear Cells (PBMC)
  - Immortalization of DC using a recombinant viral vector
  - Sub-culturing of immortalized DC

METHODS

DIFFERENTIATION OF DC FROM PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

Isolate monocytes from PBMC using negative magnetic bead isolation

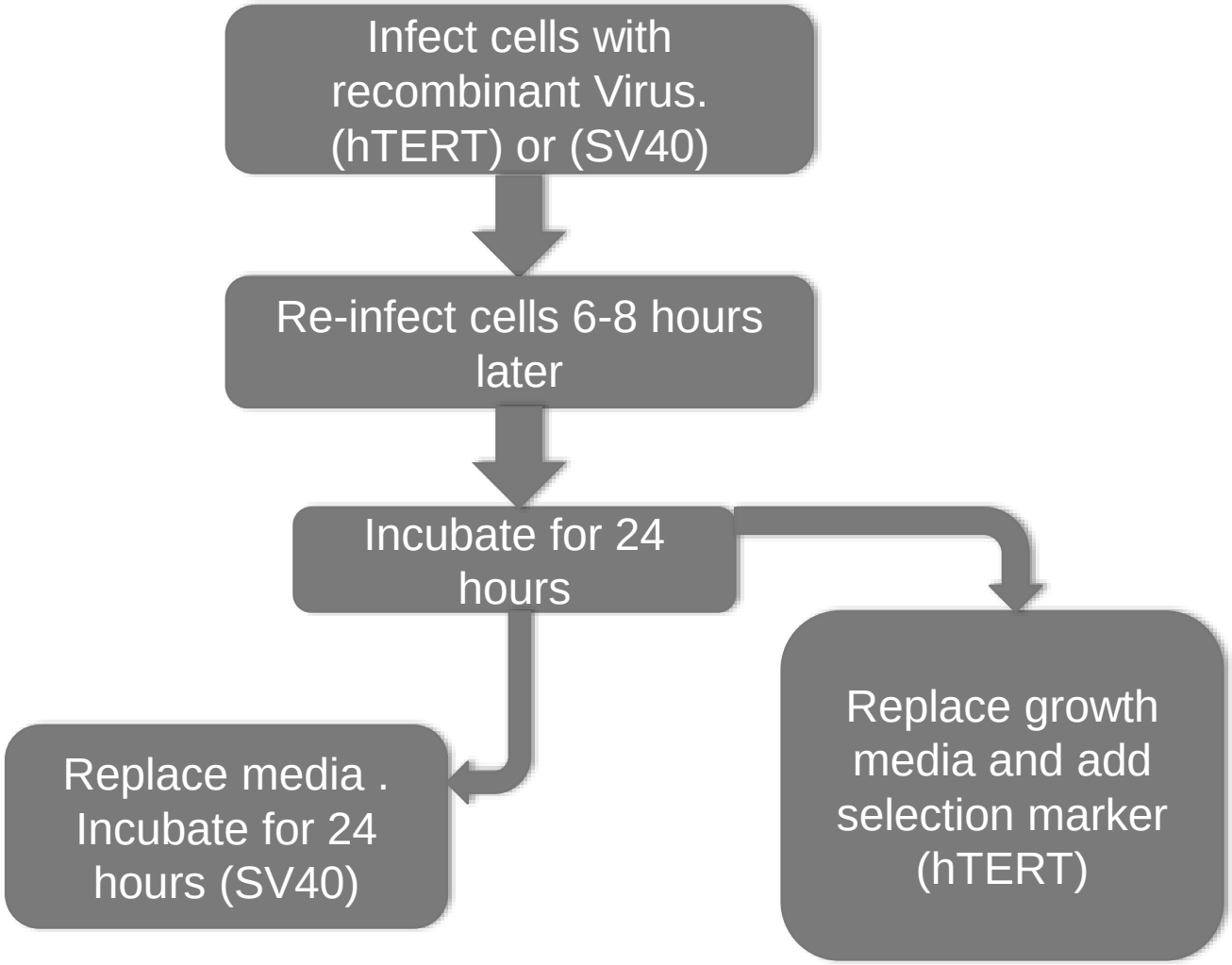
Plate viable cells in growth media

Stimulate monocytes to differentiate into DC

Wait

MAGNETIC BEAD ISOLATION

IMMORTALIZATION OF DC USING A RECOMBINANT VIRAL VECTOR



RETRO – E1 HTERT VIRUS VS. SV40 VIRUS

hTERT

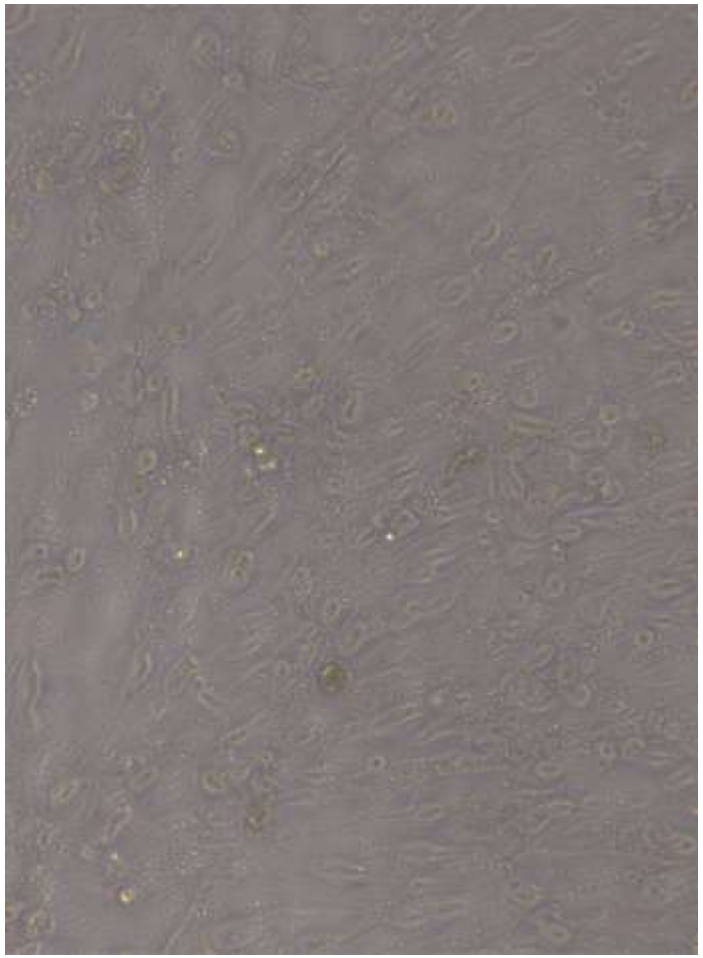
- Human Telomerase Reverse Transcriptase
- Inactive in most somatic cells
- One of the newest immortalization techniques developed
- Puromycin used as selection marker

SV40

- Simian virus 40
- Polyomavirus
- Inhibits apoptotic pathway via p53 inhibition
- common technique for immortalization

SUB-CULTURING DENDRITIC CELLS

- 4 days post infection, pass cells to new growth flask
- Trypsinize cells
- Centrifuge
- Re-suspend and transfer to new growth flask



RESULTS

TRIAL RUN

- **Started semester by doing a trial run on the protocols that had been modified and developed**
- **4 days post monocyte isolation, growth flasks appeared to be contaminated with bacteria**
- **Gram stains were performed to confirm bacterial contamination**
- **Bacteria were also observed in the growth flasks using the EVOS inverted microscope in Dr. Millers lab**

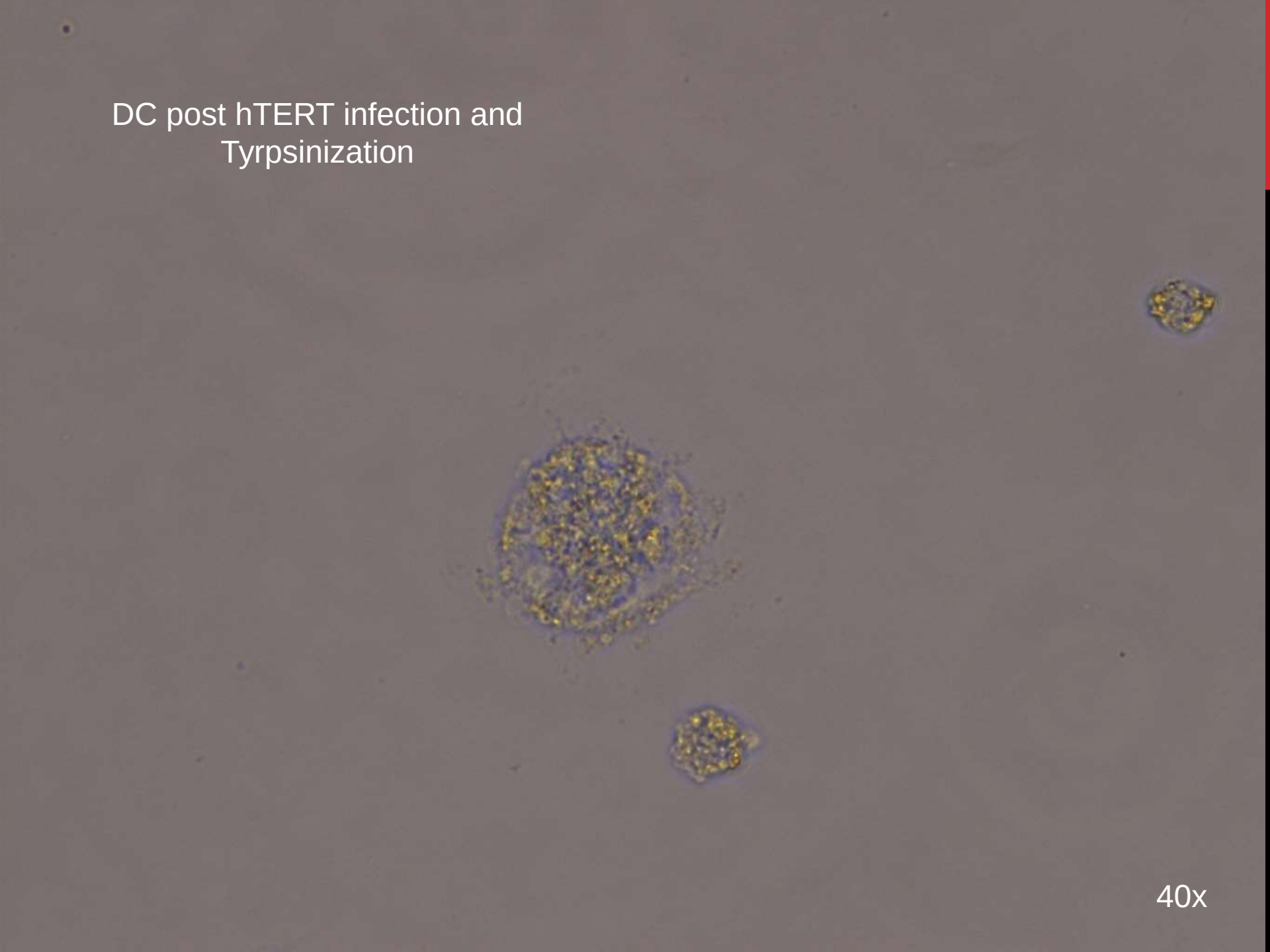
ROUND 2

- **Monocytes were isolated**
- **Differentiated into immature DC using il-4 and GM-CSF**
- **Then moved onto hTERT infection….**

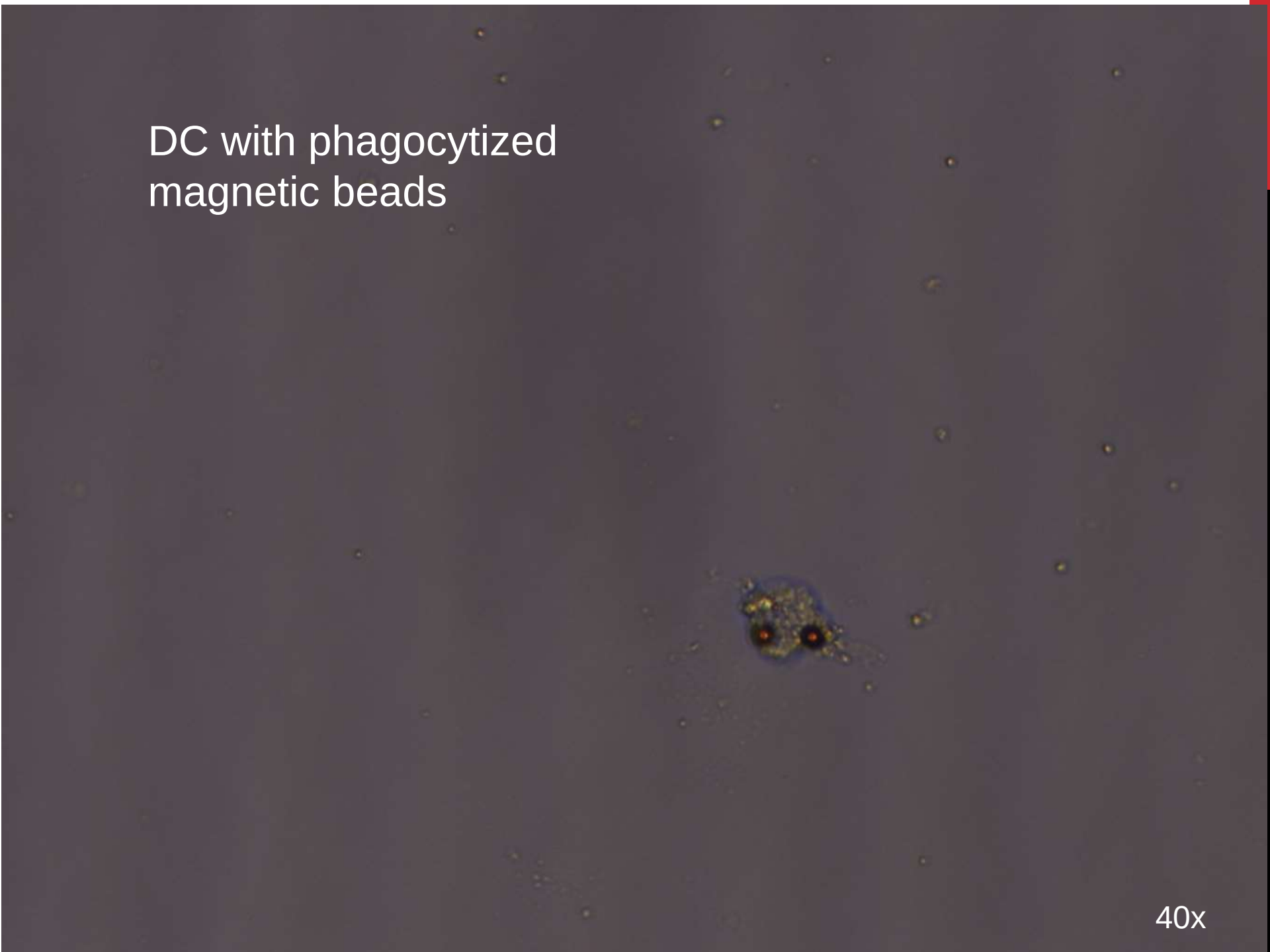
HTERT INFECTION

- **2 wells of a 6 well plate were infected with 2ml of hTERT**
  - PBMC from cows 798 and 309.
- **6 hours after first infection the same 2 wells were re-infected with 2ml of hTERT**
- **15µL of polybrene concentrated at .8mg/ml was added to each well per infection.**
- **4 days post infection DCs were trypsinized and transferred to T12.5 growth flasks**

DC post hTERT infection and Tyrpsinization



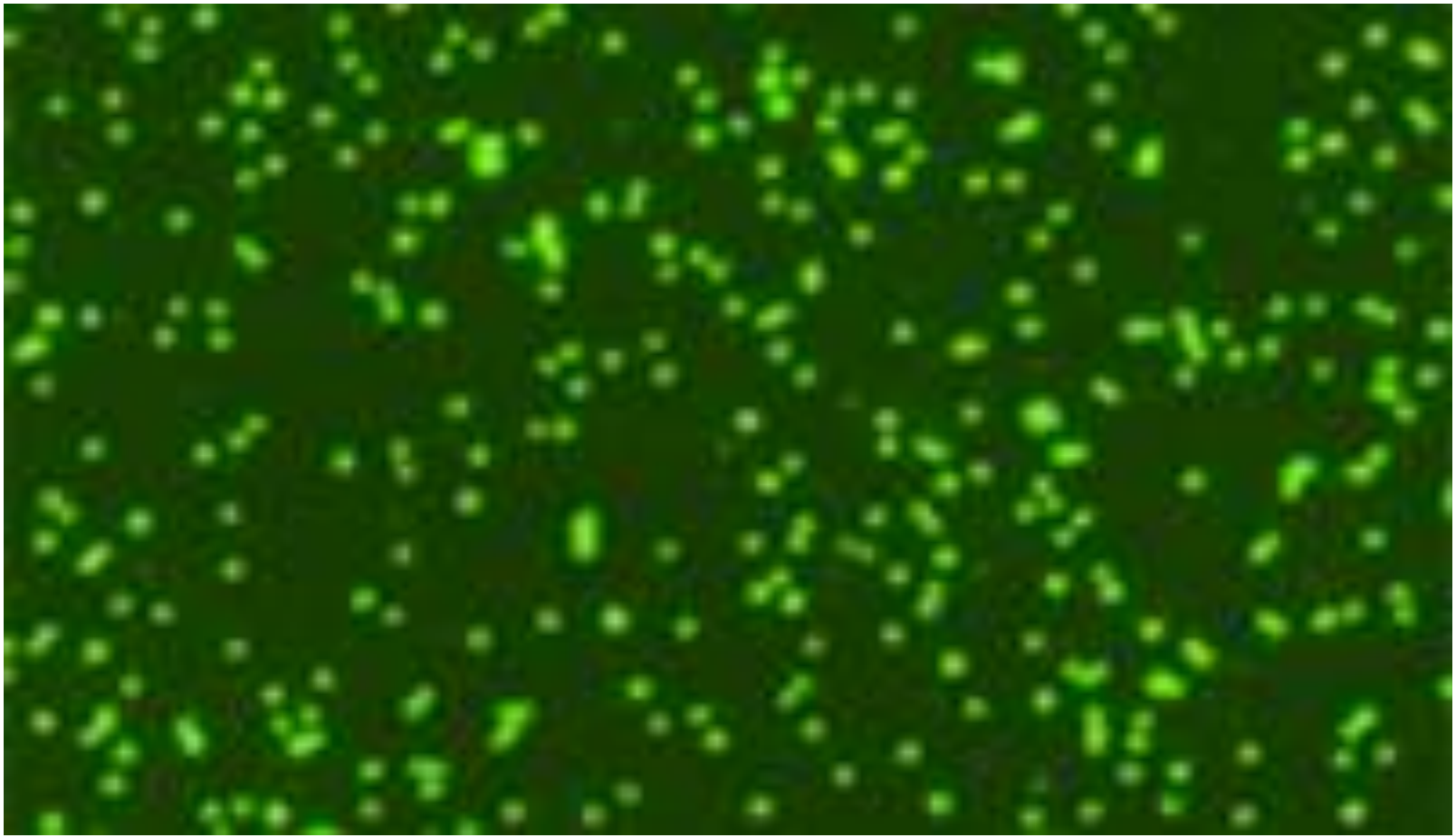
40x


DC with phagocytized
magnetic beads
40x

DEATH AND ALL HIS FRIENDS

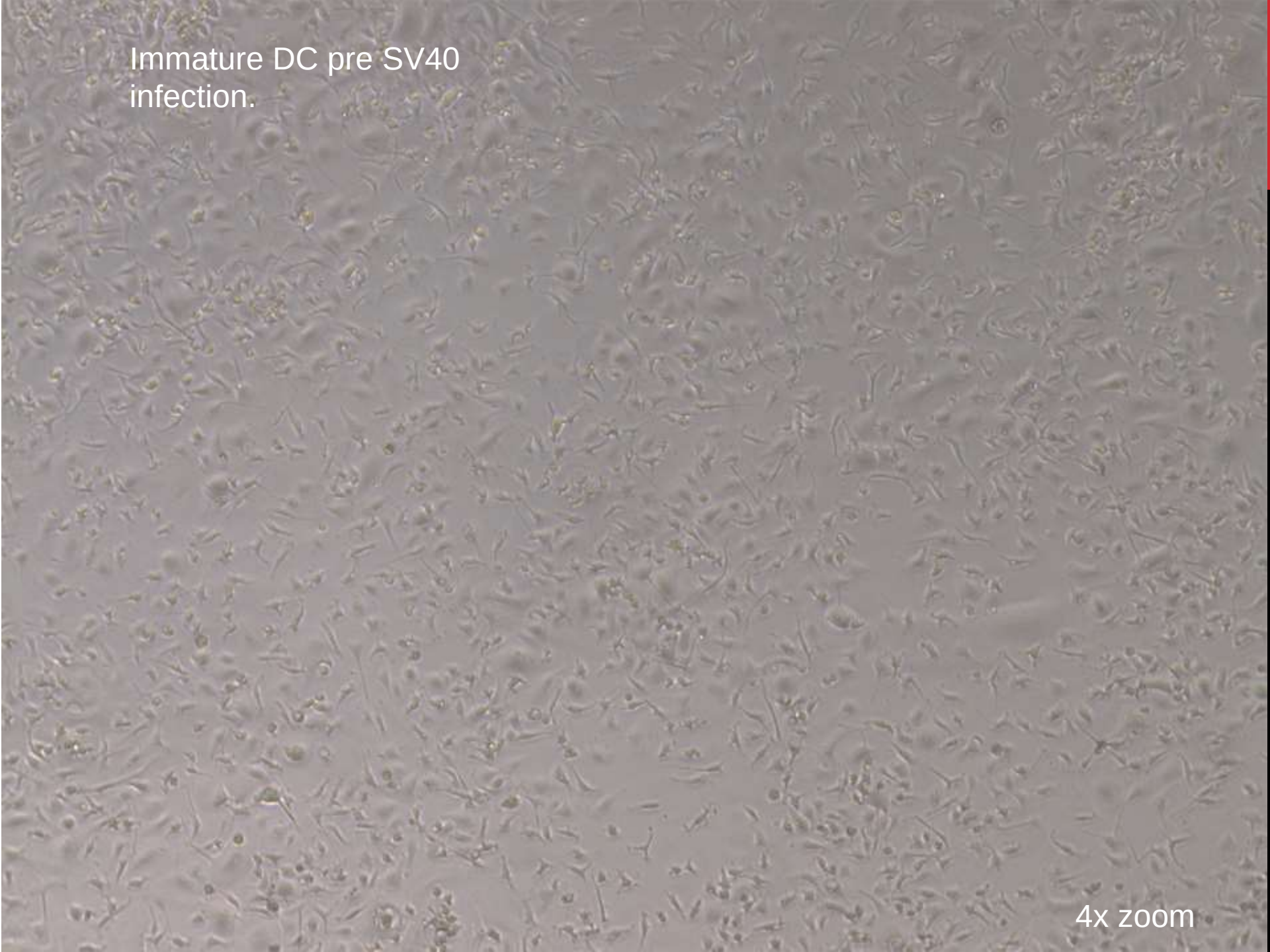
- **Replacement of growth media occurred for the next 3 weeks**
- **24 days post infection DCs infected with hTERT were determined to be dead.**
- **DC were determined to be dead after performing a cell count using acridine orange stain and a fluorescent microscope**

ACRIDINE ORANGE/ ETHIDIUM BROMIDE STAINING



BACK TO THE DRAWING BOARD

- **We began again but made a few changes in the protocol**
  - Magnetic bead isolation was not used
  - SV40 polyomavirus was used for infection
    - Better established immortalization protocol
    - Does not require a selection marker
  - Cells were allowed to grow in flask before being trypsonized
  - Varying volumes of SV40 were used to infect 6 PBMC growth flasks
  - Stimulating factors IL-4 and GM-CSF replaced with every change of media


Immature DC pre SV40 infection.
4x zoom

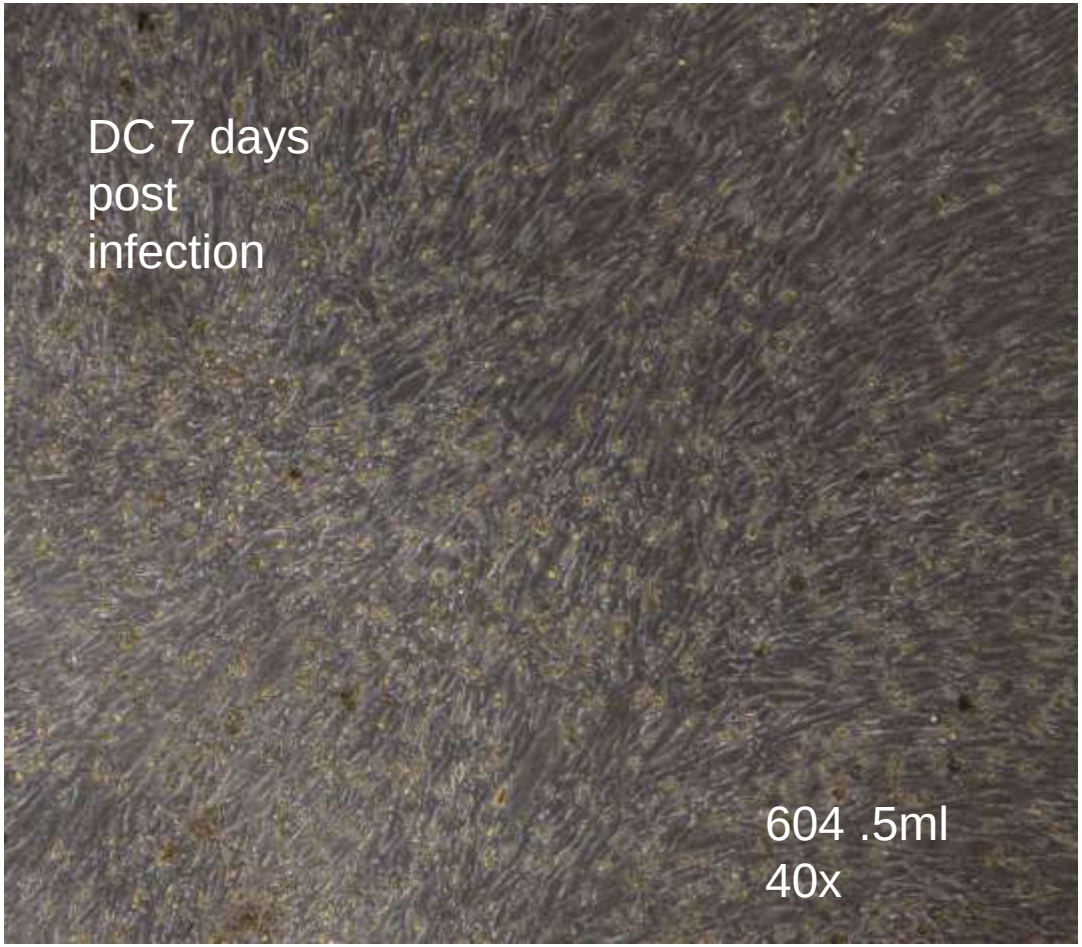
SV40 INFECTION

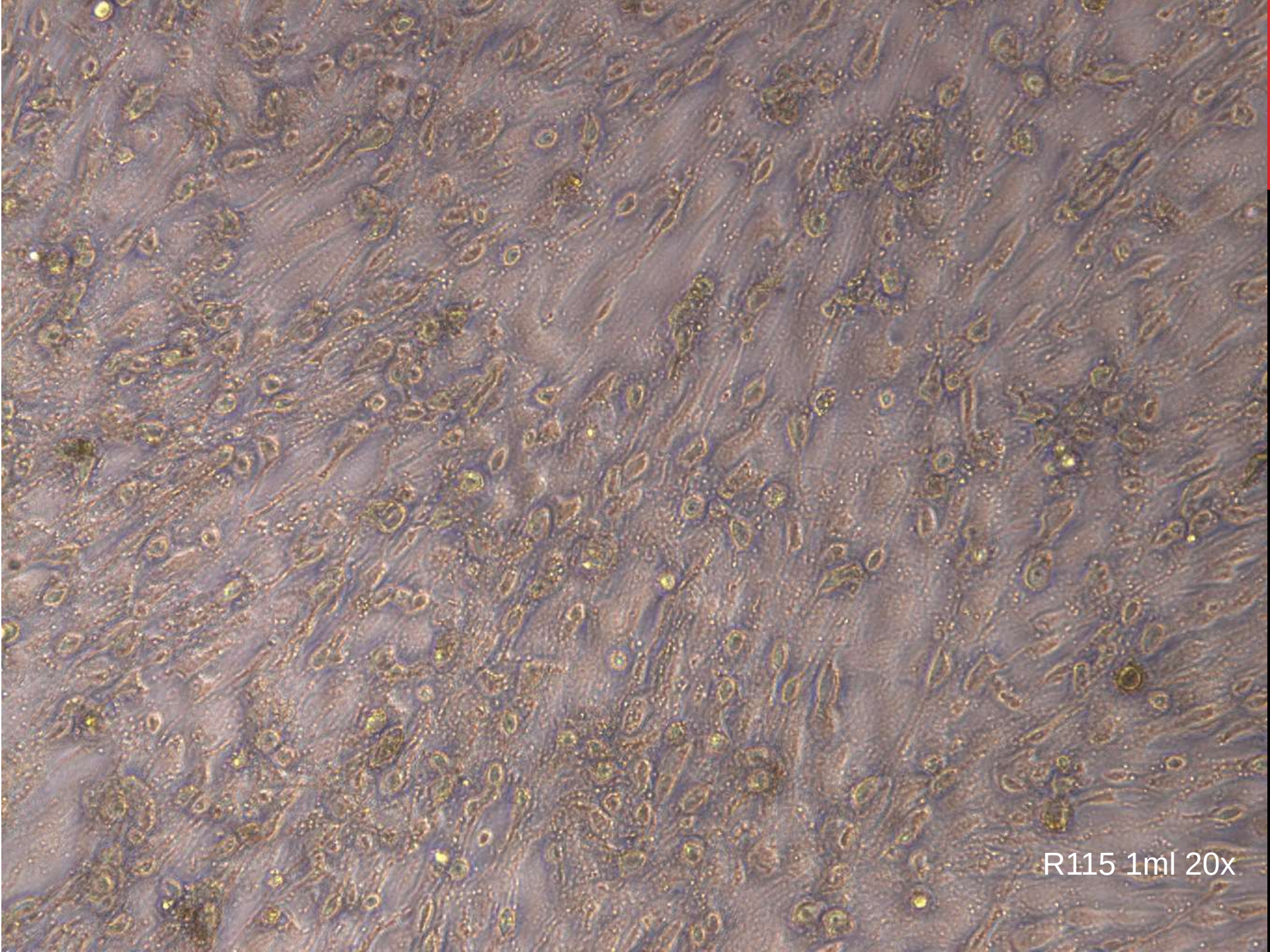
- **PBMC's were from 3 different cows; 604,R115, N238.**
- **PBMC's from each cow were divided into 2 growth flasks a piece.**
  - Each 604 flask respectively received .5ml or 1ml of SV40
  - Each R115 flask respectively received 1ml or 2ml of SV40
  - Each N238 flask respectively received .5ml or 2ml of SV40

IMMORTALIZATION?

- **7 days post infection cell proliferation was observed in flasks 604 (.5ml), 604 (1ml), R115 (1ml)and R115 (2ml).**

- **Cell proliferation was not observed in flask N238 (1ml) and N238 (2ml)**




R115 1ml 20x

TROUBLE IN PARADISE

- After 2 passages, the morphology of the cells changed
- Larger, fibrous vacuolated cells observed
- Cells were believed to be in senescence

CONCLUSIONS

WHERE THE PROJECT WENT WRONG THE FIRST TIME

The source of the contaminant was believed to be a result of our technique, Gram (+) bacteria thought to be commensals.

WHERE THE PROJECT WENT WRONG THE SECOND TIME

Cell death attributed too….

- Trypsinizing cells in weakened state
- To great of a concentration of puromycin used as a selection marker
- Lack of stimulating factors

- **Lead to the changes in protocol stated previously**

WHERE THE PROJECT WENT WRONG THE THIRD TIME

- **Cells may not be transformed**
- **Could be in senescence:**
  - Lack of outside stimulation
  - Denatured IL-4
    - Resulting from continuous freezing and thawing
- **R115 1ml was challenged with Lipopolysaccharide (LPS)**

FUTURE WORK

ITS ALL UP HILL FROM HERE

- **We plan to sub-culture cells until the end of the semester**
  - Challenge cells with different antigens
- **At the end of the semester the cells will be frozen down for the summer.**
- **In the fall we will begin categorizing sub-populations within the population of immortalized cells**

ACKNOWLEDGEMENT

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- **Masters Candidate : Jake Berg**
- **WSVL staff: Dr. Myrna Miller , Jenifer McKenna**
- **Classmate: Mat Rorke**
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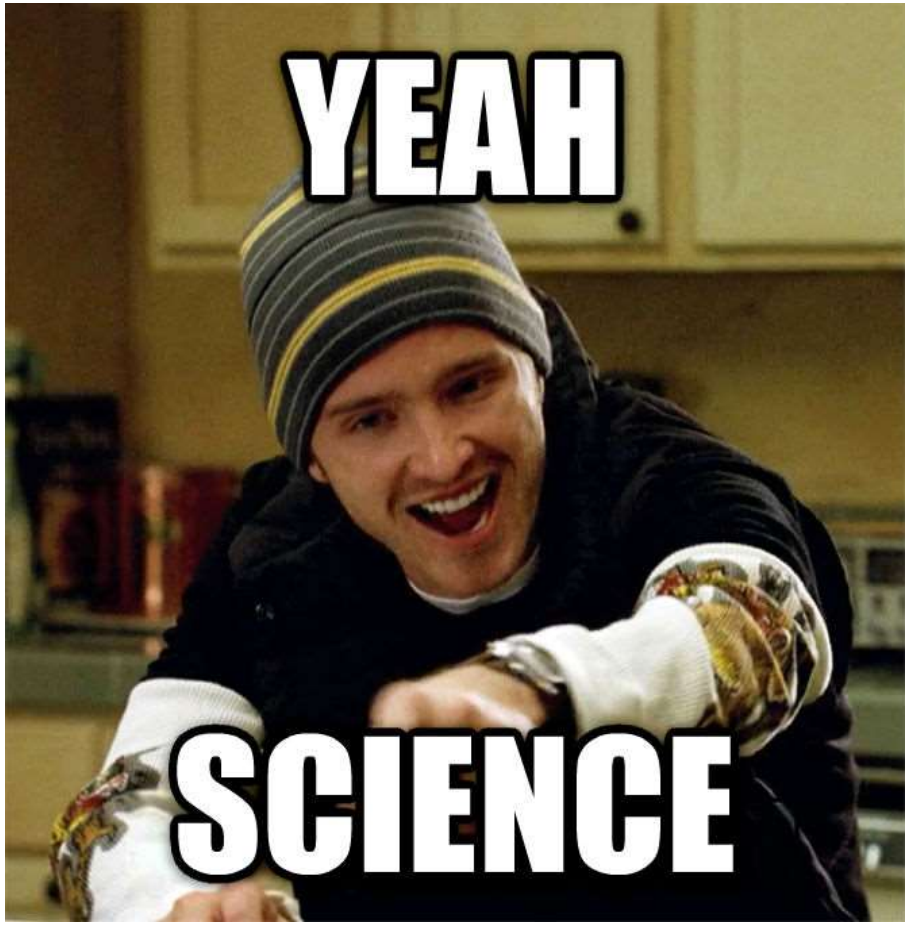
QUESTIONS?



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