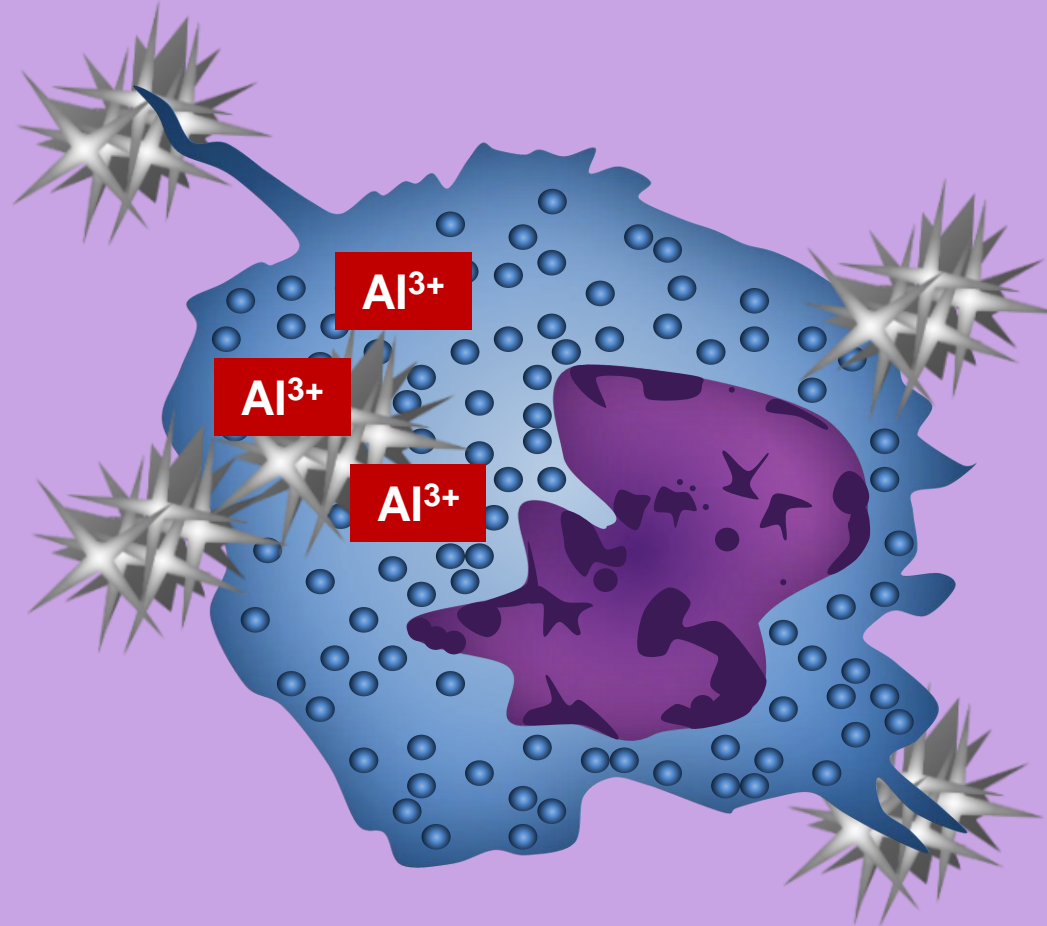
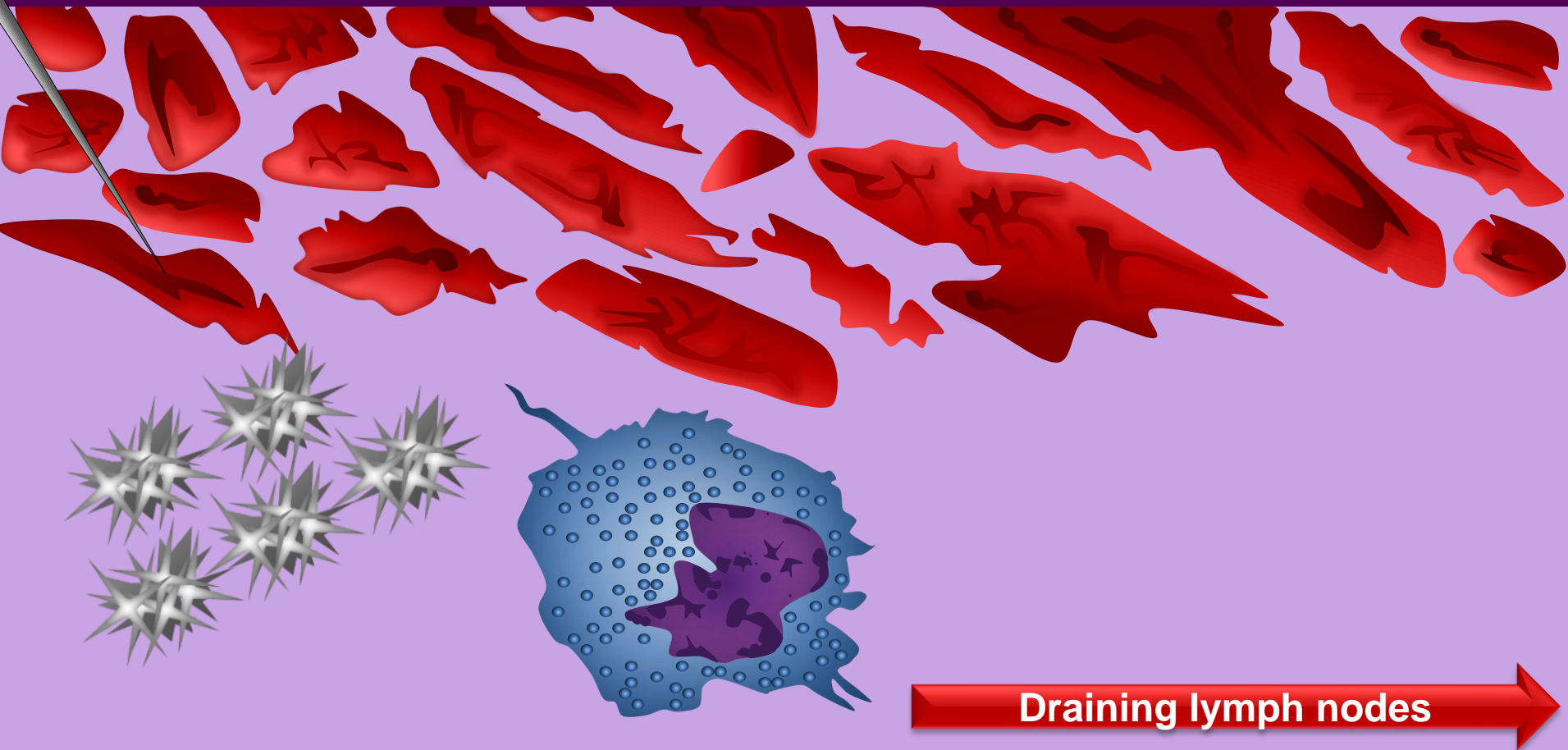


A matter of survival: Assessing the long term viability of ABA loaded macrophages



Introduction - site of intramuscular injection



- Vaccine depot – Antigen-adjuvant complexes.
- APCs recruited to injection site ca 24hrs post vaccination.

- Monocytes phagocytose ABAs (Mold et al., 2016).
- ABAs have different cytotoxicity profiles.

- How long do cells survival once loaded with ABA?

Exp 1: THP-1 macrophages – Viability vs. time

Aim

- To monitor survival of THP-1 macrophages over 8 days.

Methods – differentiation protocol

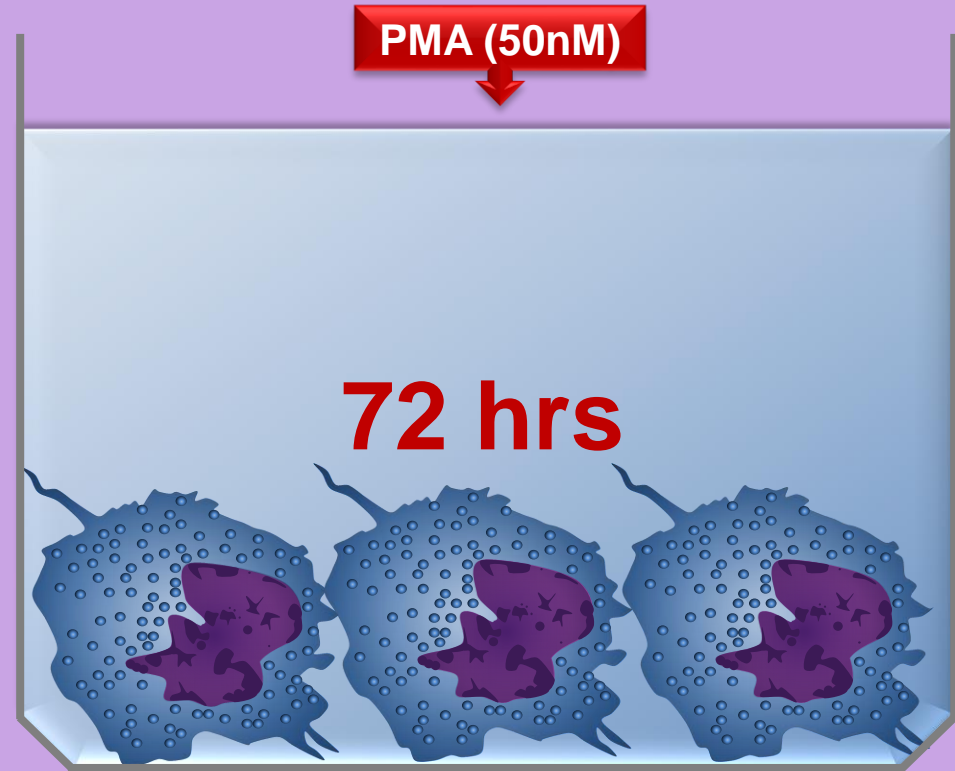
- PMA (50nM) for 48hrs.
- Rest period in fresh medium – 24hrs.
- No medium change thereafter.

Methods – Viability

- LDH assay – 490nm abs.

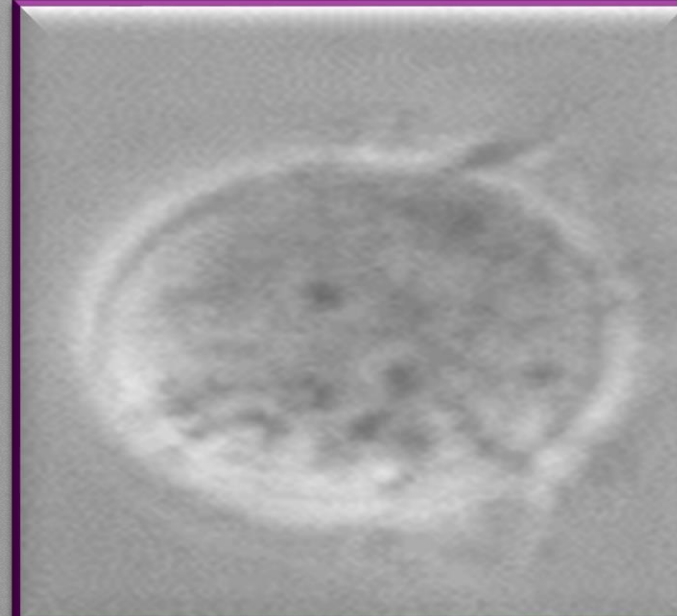
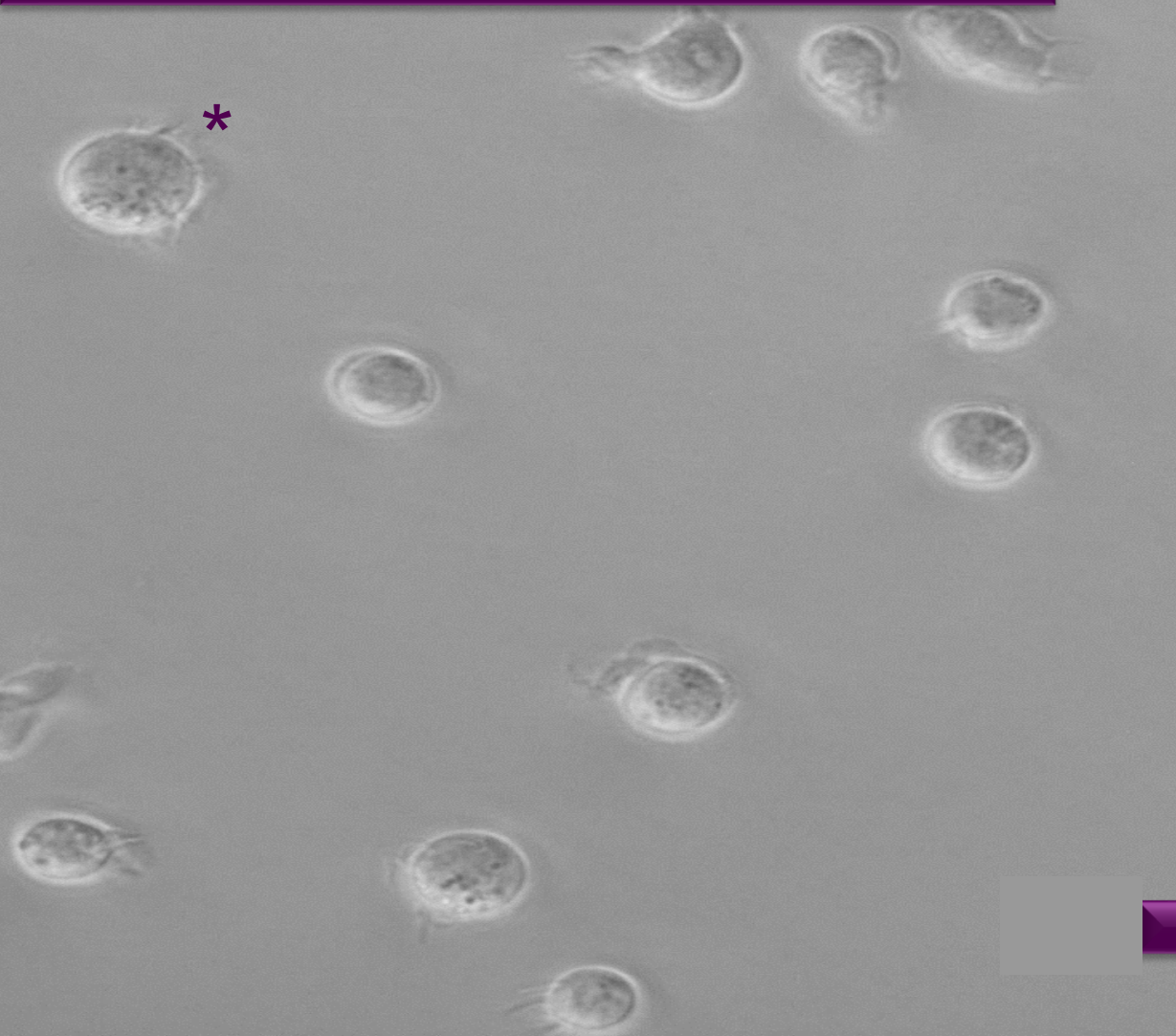
Methods – morphology

- Light microscopy.



THP-1 cells – 15 mins post PMA exposure

50nM PMA – BF and PC overlay, x 40obj

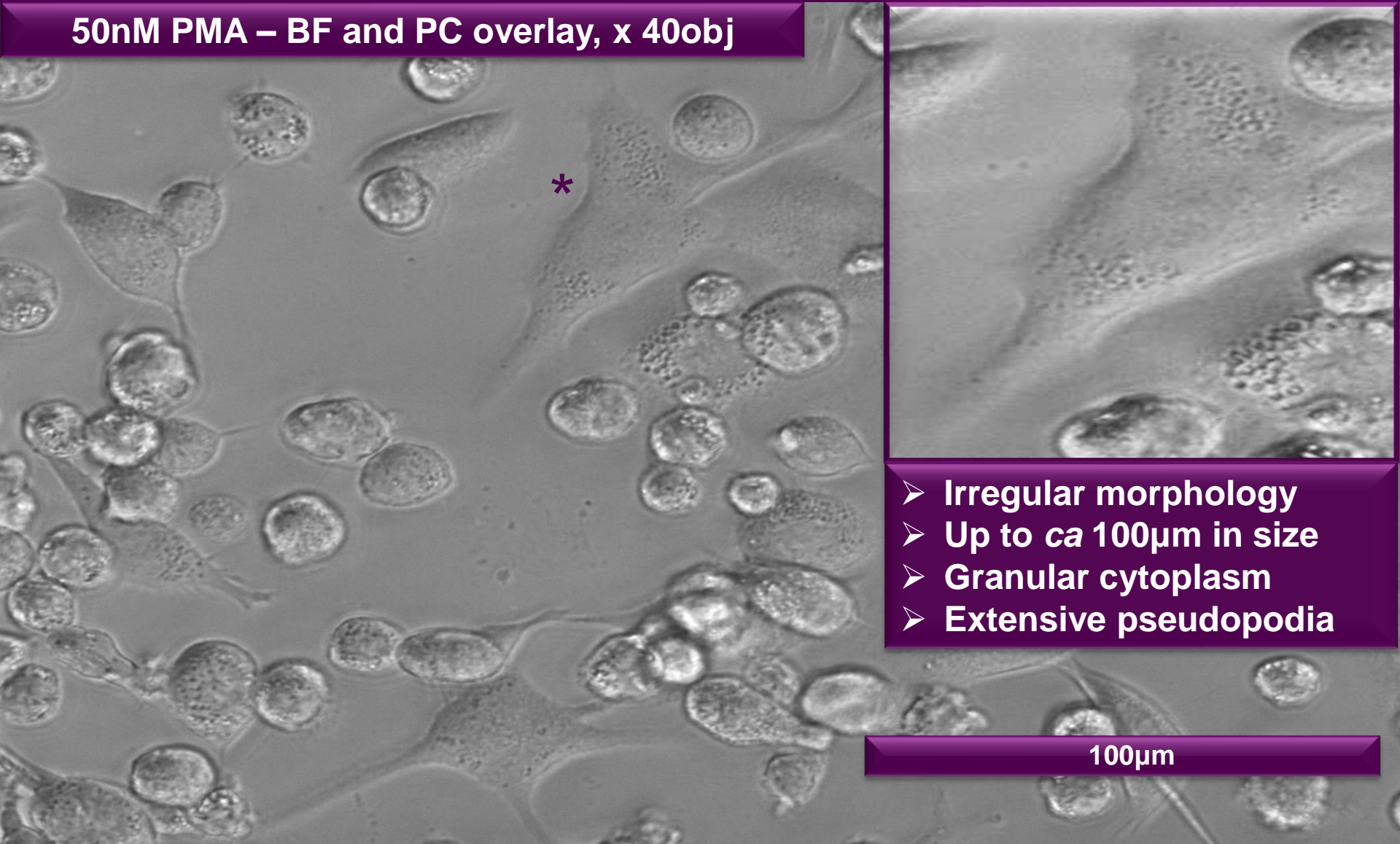


- Spherical morphology
- ca 10-20 μ m in size
- Few granules in cytoplasm
- Limited pseudopodia

100 μ m

THP-1 cells – 72 hrs post PMA exposure

50nM PMA – BF and PC overlay, x 40obj



- Irregular morphology
- Up to ca 100µm in size
- Granular cytoplasm
- Extensive pseudopodia

100µm

THP-1 macrophages – Viability over time

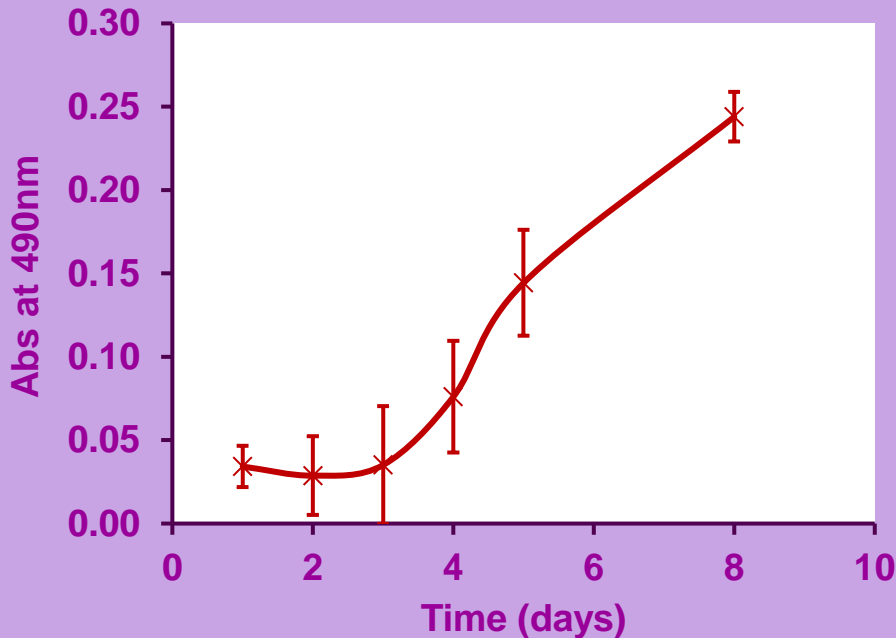


Fig 1: Viability of THP-1 macrophages over 8 days post exposure to PMA (50nM) as measured via an LDH assay. Error bars represent \pm SD of the measurement where $n=2$.

Time (days)	% mortality
1	5
2	2
3	7
4	12
5	25
8	41

Table 1: Percentage mortality of THP-1 macrophages over 8 days post exposure to PMA (50nM) as measured via an LDH assay.

- LDH release was consistently low over first three days (0.034 ± 0.012 – 0.035 ± 0.035)
- LDH release increased steadily from day three onwards (0.035 ± 0.035 – 0.244 ± 0.015)
- Cell viability is ca 60% 8 days following PMA exposure

Exp 2: THP-1 macrophages – uptake of ABA vs. time

Aim

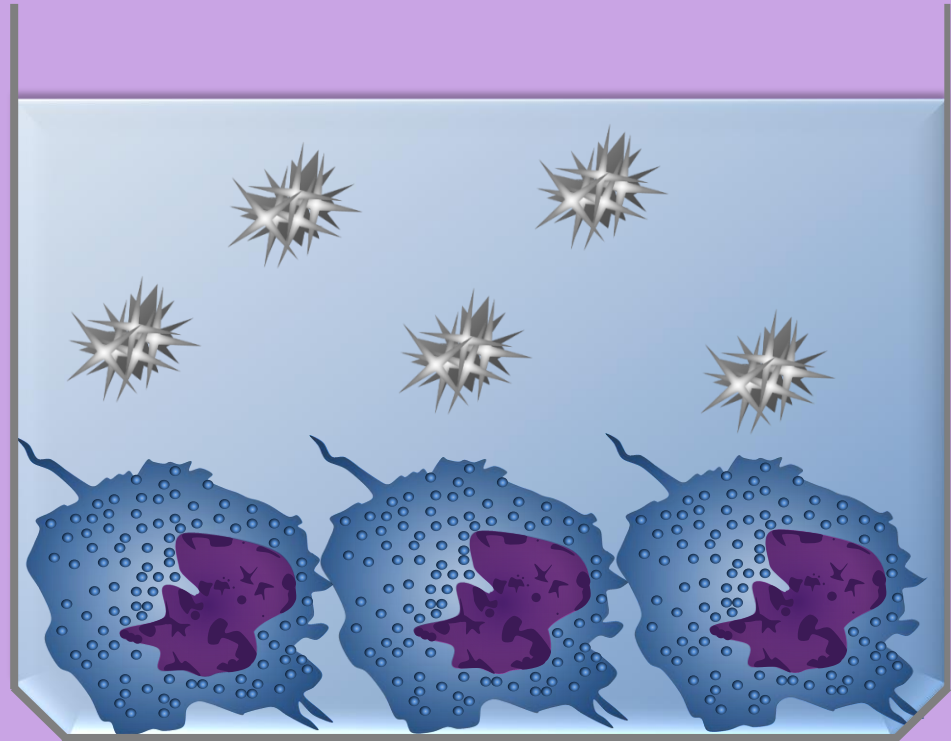
- To monitor uptake of ABA by THP-1 macrophages – cytoplasmic loading.

Methods – treatment regime

- Alh 1.7 μ g/mL – 3 and 24hrs.
- Stained with lumogallion (100 μ M) 3 hrs prior to processing.

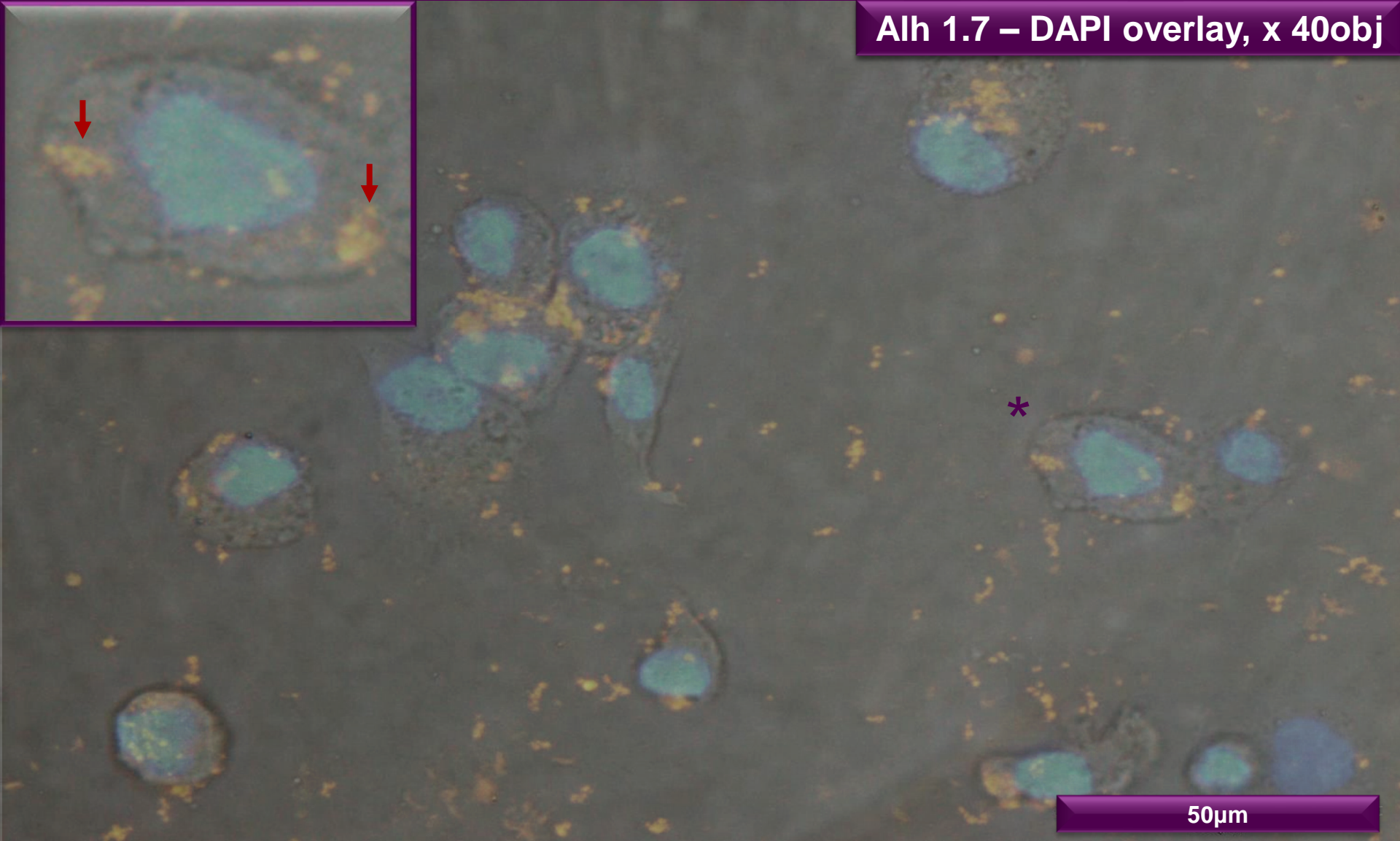
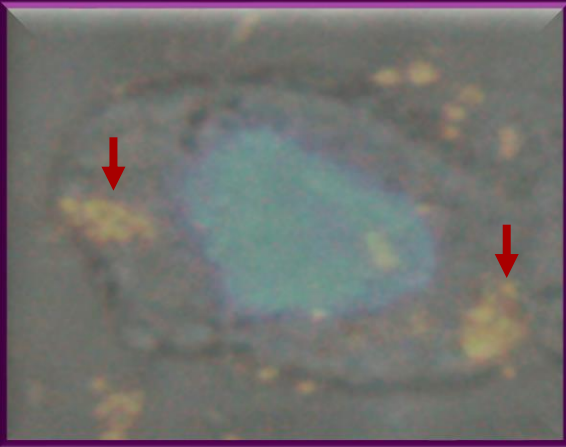
Methods – visualising Al uptake

- Cells fixed with PFA (4% w/v)
- Cytoplasmic loading observed using fluorescence microscopy.
 - ❖ Lumogallion – Al
 - ❖ DAPI – nuclear stain



THP-1 macrophages (3hrs post exposure)

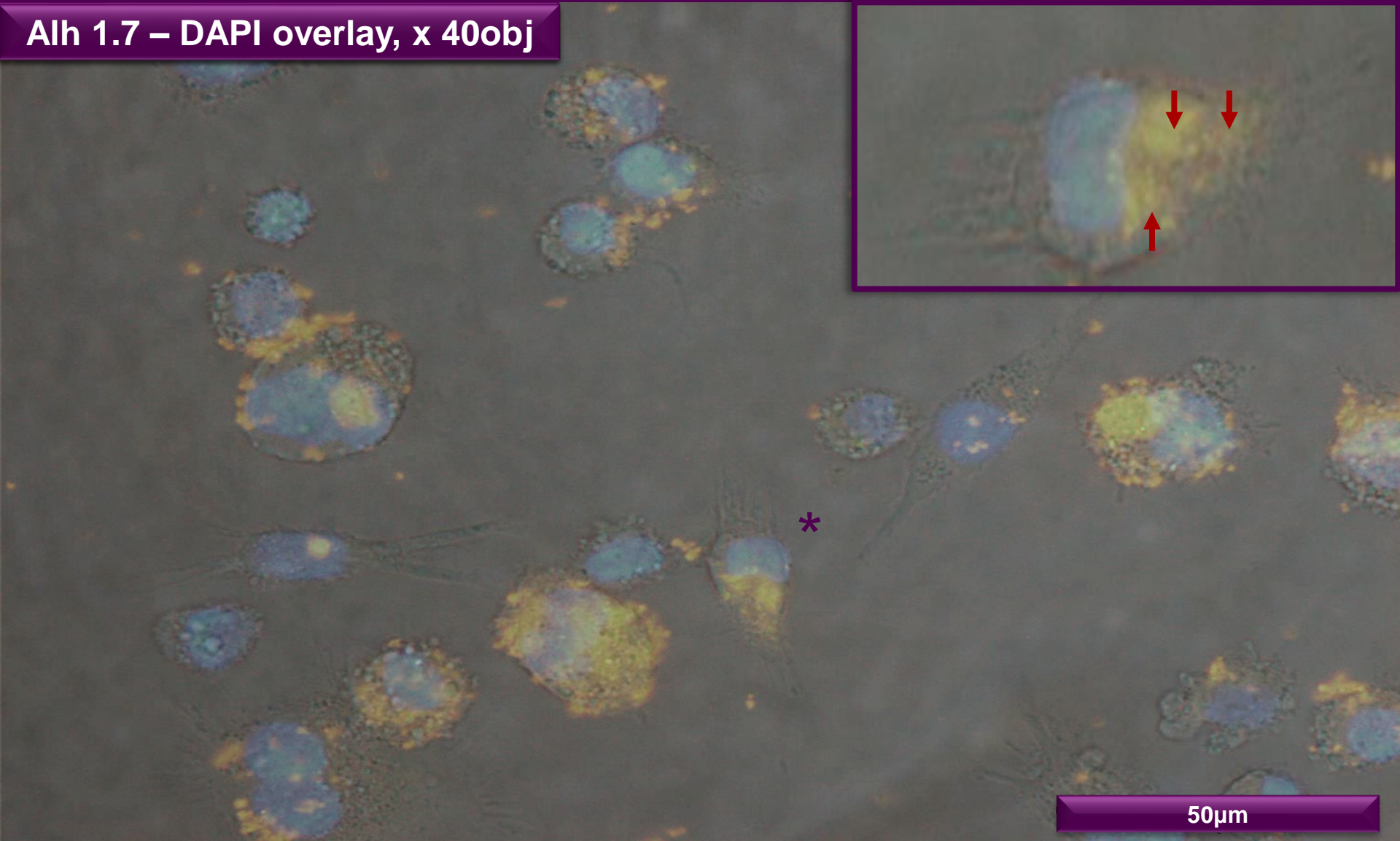
Alh 1.7 – DAPI overlay, x 40obj



50µm

THP-1 macrophages (24hrs post exposure)

Alh 1.7 – DAPI overlay, x 40obj



Exp 3: THP-1 macrophages – survival in the presence of ABAs (7 day study)

Aim

- To monitor viability of cells post exposure to ABA.

Methods – treatment regime

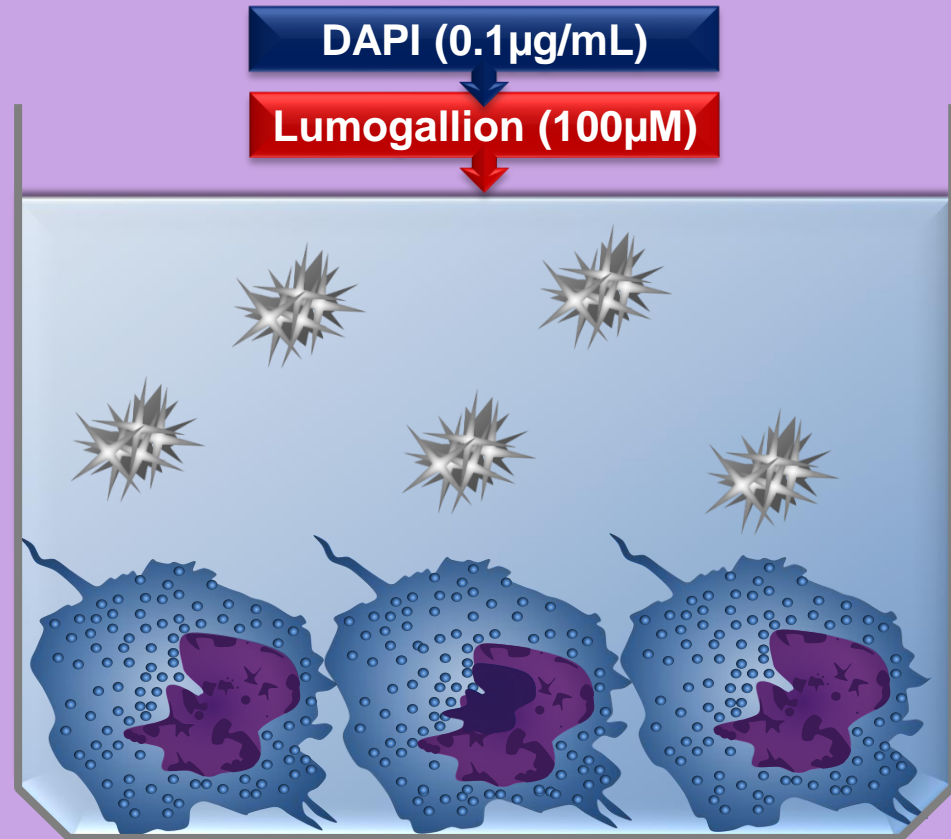
- Ah and Adj (1.7 μ g/mL) - 24hrs.

Methods – visualising AI uptake

- Cytoplasmic loading - fluorescence microscopy.
 - ❖ Lumogallion (100 μ M) – AI

Methods – viability

- Fluorescence microscopy
 - ❖ DAPI (0.1 μ g/mL) – dead cell stain
- LDH assay



Day 1 – Fluorescence microscopy

R10 control – DAPI overlay, x 40obj



100μm

Day 1 – Fluorescence microscopy

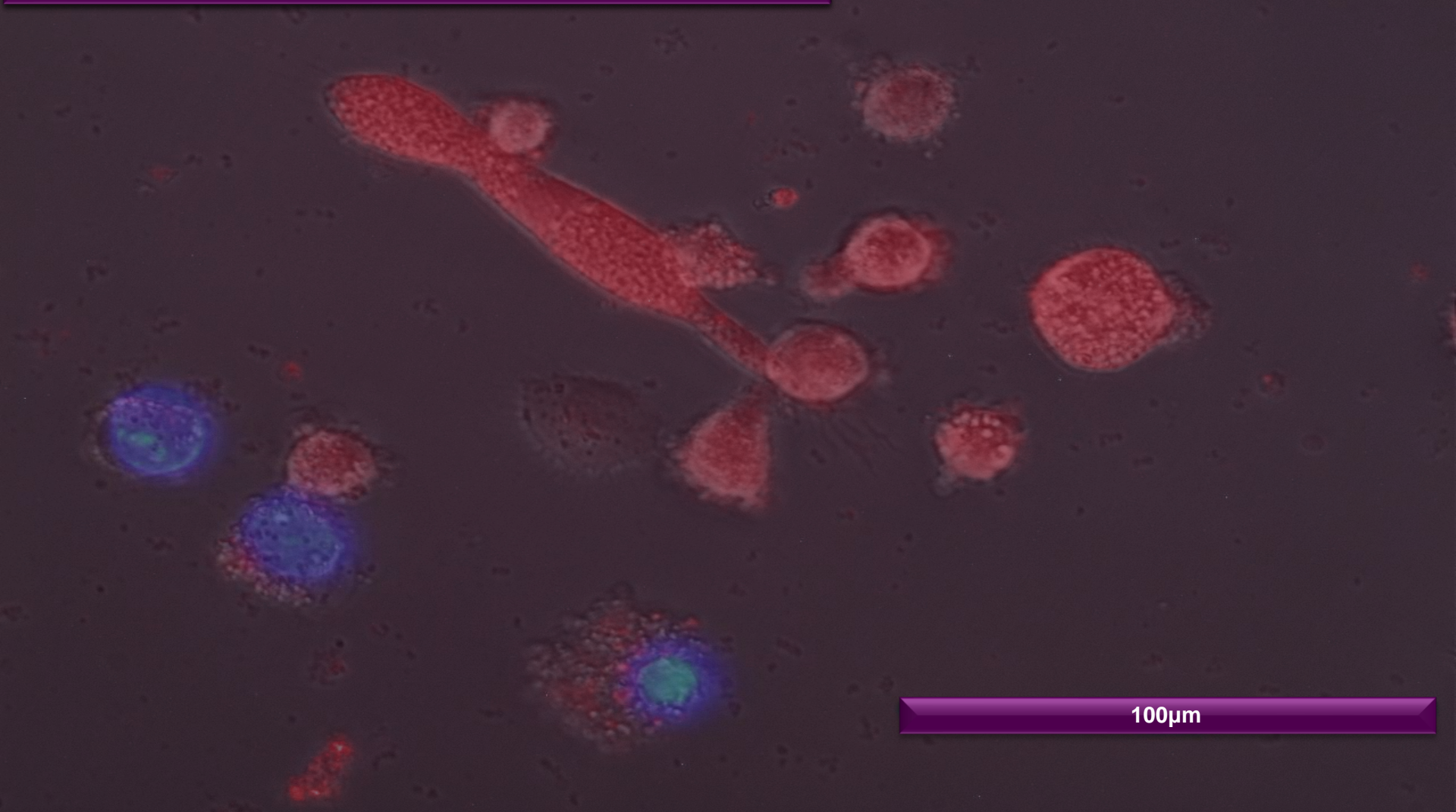
Alh 1.7 – RFP + DAPI overlay, x 40obj



100μm

Day 1 – Fluorescence microscopy

Adj 1.7 – RFP + DAPI overlay, x 40obj



Day 1 – Viability (LDH)

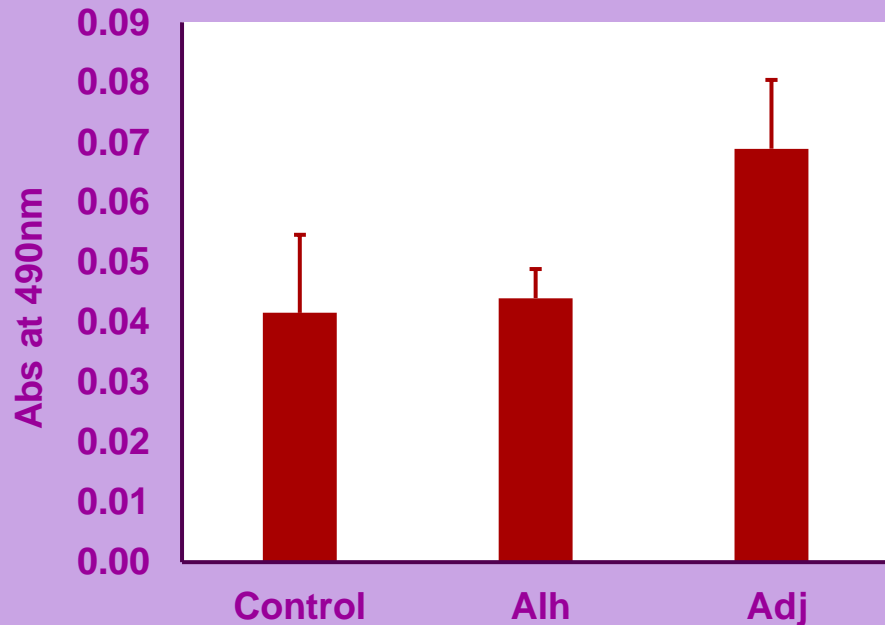


Fig 2: Viability of THP-1 macrophages 24hrs post exposure to Alh and Adj (1.7µg/mL) as measured via an LDH assay. Error bars represent ±SD of the measurement where n=5.

Treatment	% mortality
Alh	9
Adj	13

Table 2: Percentage mortality of THP-1 macrophages 24hrs post exposure to Alh and Adj (1.7µg/mL) as measured via an LDH assay.

- Greatest LDH release– Adj treatment (0.069±0.011, P=0.004 vs. control)
- Alh vs. control – similar LDH release (0.044±0.005 vs. 0.042±0.013, P=0.932)
- Alh vs. Adj (P=0.008).

Day 3 – Fluorescence microscopy

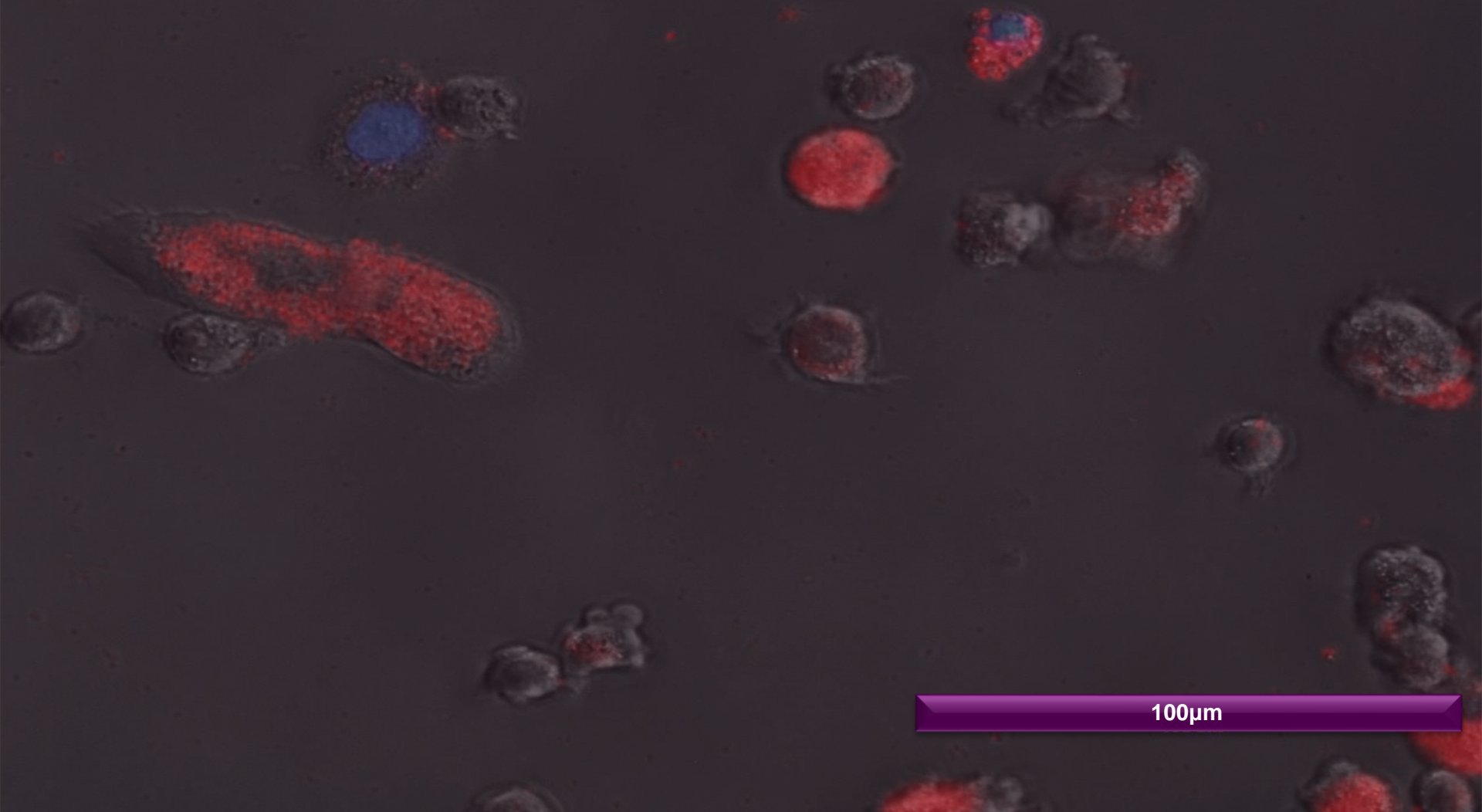
R10 control – DAPI overlay, x 40obj



100μm

Day 3 – Fluorescence microscopy

Alh 1.7 – RFP + DAPI overlay, x 40obj



100μm

Day 3 – Fluorescence microscopy

Adj 1.7 – RFP + DAPI overlay, x 40obj



100μm

Day 3 – Viability (LDH)

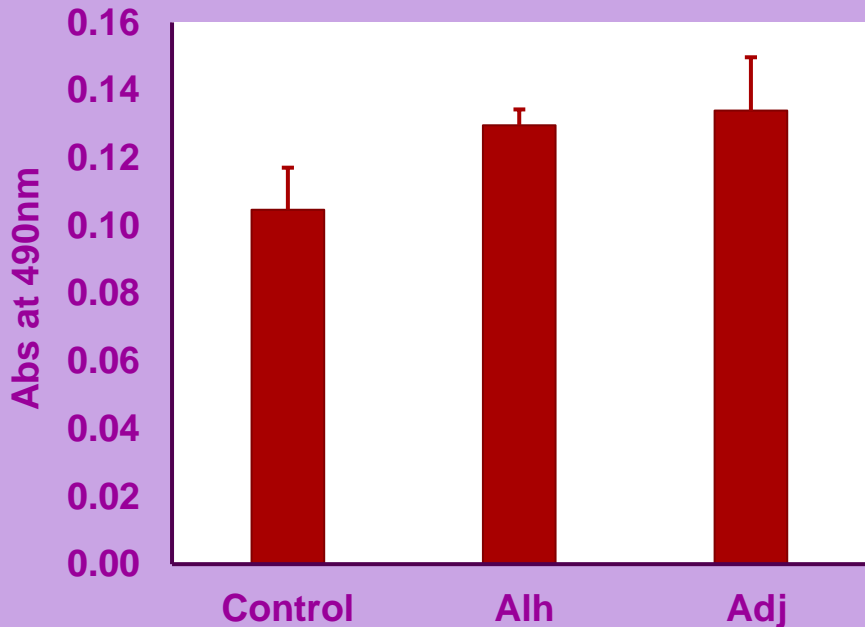


Fig 3: Viability of THP-1 macrophages 3 days post exposure to Alh and Adj (1.7µg/mL) as measured via an LDH assay. Error bars represent ±SD of the measurement where n=5.

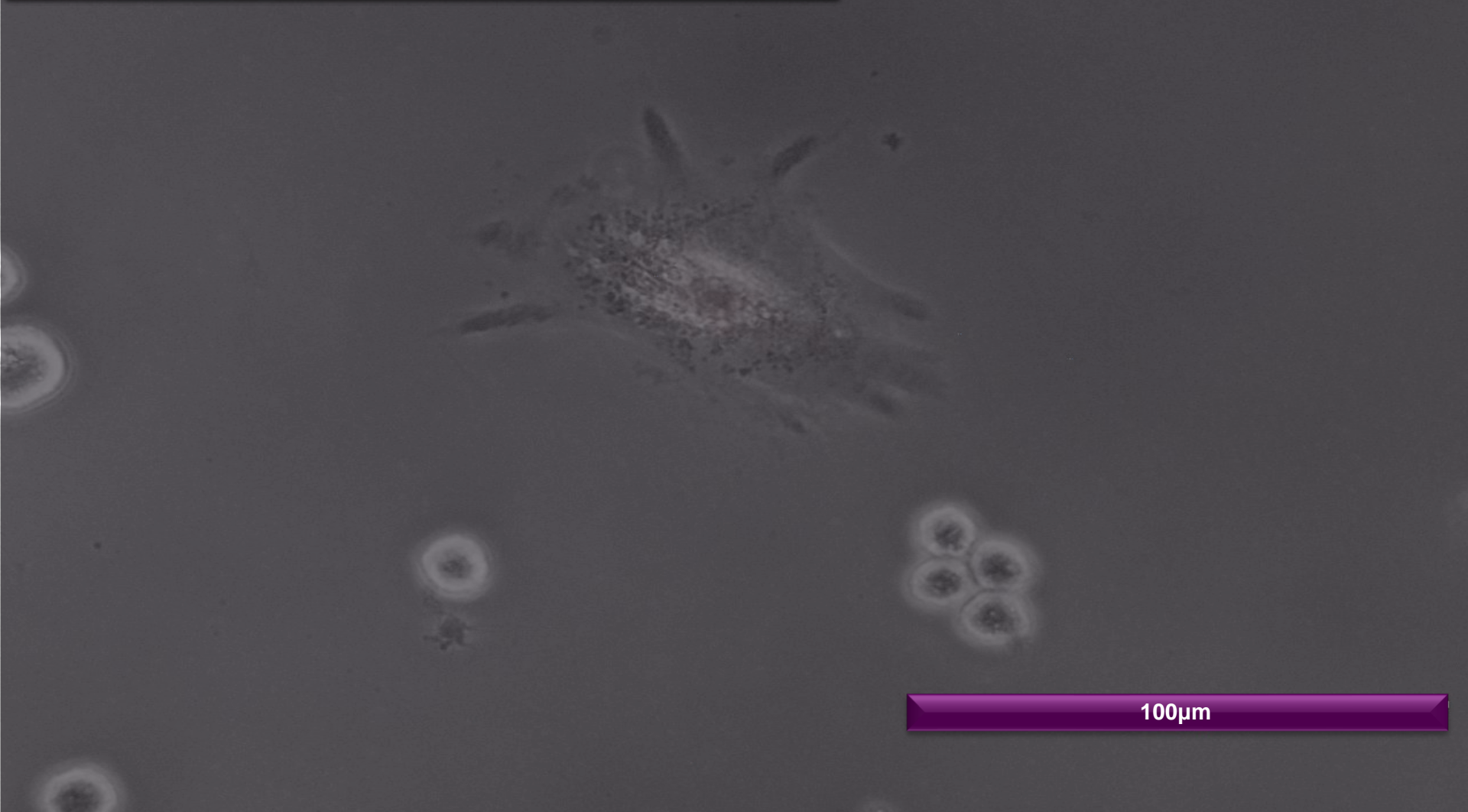
Treatment	% mortality
Alh	20
Adj	26

Table 3: Percentage mortality of THP-1 macrophages 3 days post exposure to Alh and Adj (1.7µg/mL) as measured via an LDH assay.

- Greatest LDH release– Adj treatment (0.134±0.016, P=0.005 vs. control)
- LDH release in Alh treatments > control (0.130±0.005 vs. 0.105±0.012, P=0.015)
 - Alh vs. Adj (P=0.813).

Day 7 – Fluorescence microscopy

R10 control – DAPI overlay, x 40obj



100µm

Day 7 – Fluorescence microscopy

Alh 1.7 – RFP + DAPI overlay, x 40obj



100μm

Day 7 – Fluorescence microscopy

Adj 1.7 – RFP + DAPI overlay, x 40obj



Day 7 – Viability (LDH)

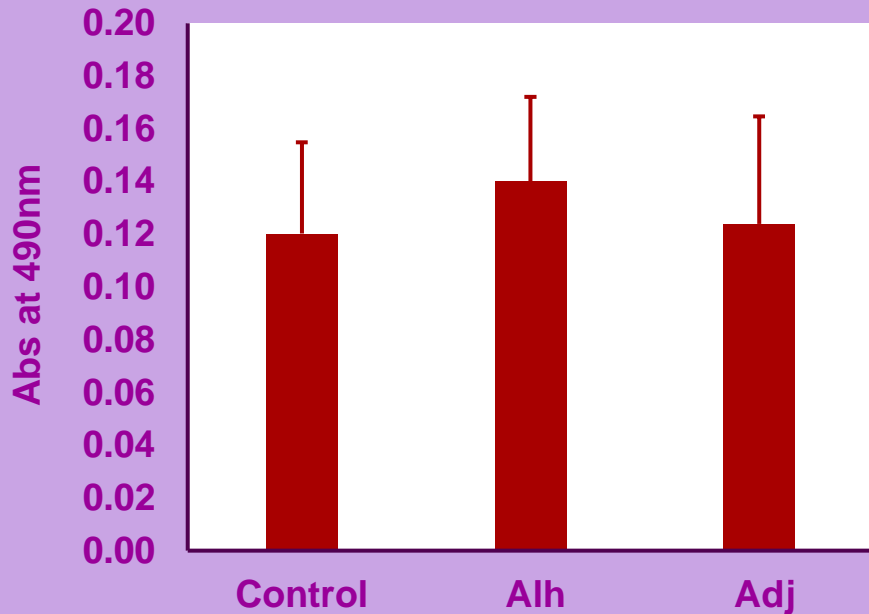


Fig 4: Viability of THP-1 macrophages 7 days post exposure to Alh and Adj (1.7µg/mL) as measured via an LDH assay. Error bars represent ±SD of the measurement where n=5.

Treatment	% mortality
Alh	19
Adj	25

Table 4: Percentage mortality of THP-1 macrophages 7 days post exposure to Alh and Adj (1.7µg/mL) as measured via an LDH assay.

- Greatest LDH release - Alh treatments (0.140±0.032, P=0.967 vs. control)
- LDH release in Adj treatments similar to control (0.124±0.041, P>0.999 vs. control)
- Alh vs. Adj (P>0.999).

Viability of adjuvant treated cells vs. time

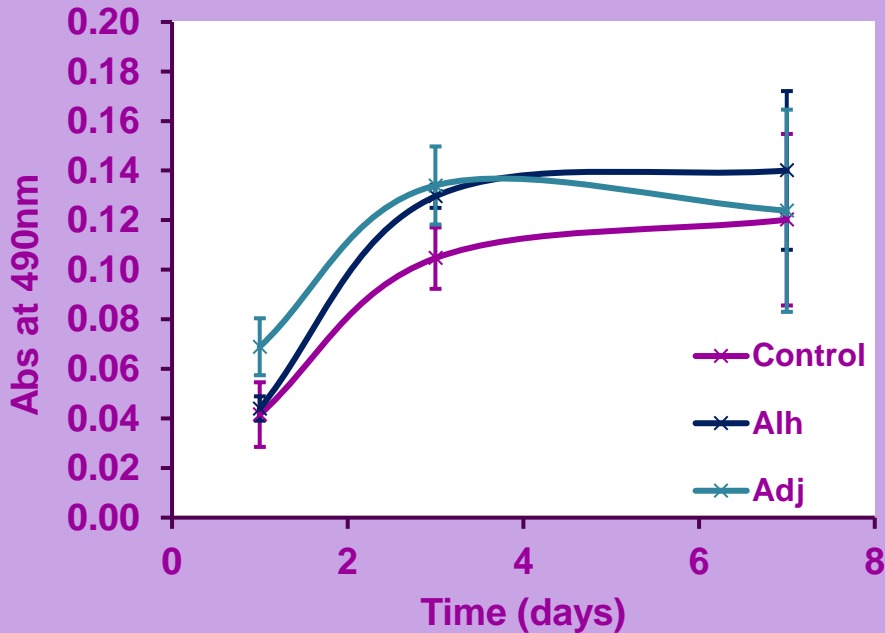


Fig 5: Viability of THP-1 macrophages over 7 days post exposure to Alh and Adj (1.7µg/mL) as measured via an LDH assay. Error bars represent \pm SD of the measurement where $n=5$.

Days	1	3	7
Treatment	% mortality		
Control	7	16	13
Alh	9	20	19
Adj	13	26	25

Table 5: Percentage mortality of THP-1 macrophages over 7 days post exposure to Alh and Adj (1.7µg/mL) as measured via an LDH assay.

- Cellular mortality gradually increases under all conditions plateauing after day 3.
- Increase in cell mortality is initially more pronounced in Adj treatments (day 1).
- No difference in LDH release observed between treatments by day 7.

Conclusions

Survival *in vitro*

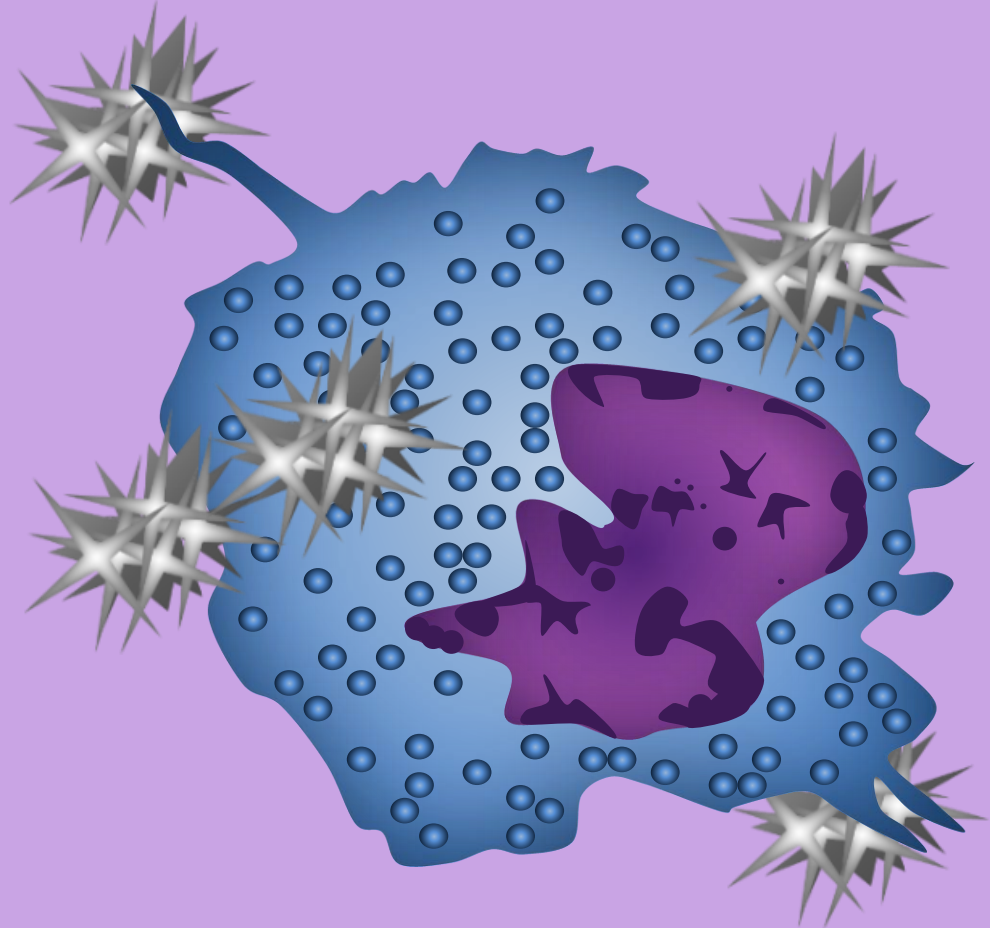
- Majority of cells viable after 8 days
- De-differentiation occurs after *ca* 7 days.

Phagocytosis of ABA

- Extensive uptake of both ABAs observed.

Viability of AI loaded cells over time

- Majority of cells loaded with ABA remain viable after 7 days.
- Systemic cellular translocation of ABA is likely.



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