Investigation of the anti-cancer Effects of the Tyrosine Kinase Inhibitor-Pexidartinib on the Lung Cancer Cell Line

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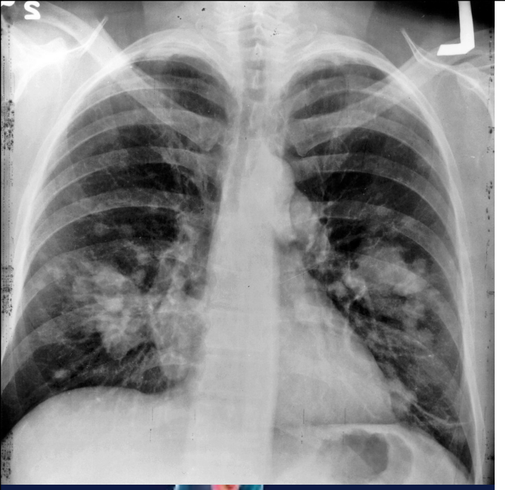
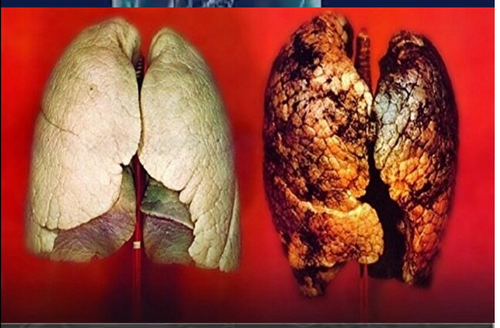
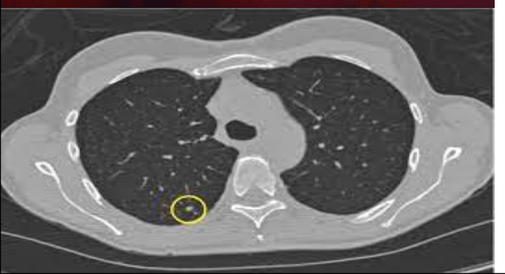
Outline

- Lung Cancer
- Tyrosine Kinase Inhibitors
- Pexidartinib
- Aim
- Material, Methods and Results
  - Cell Culture and Treatment
  - 1. MTT Assay
  - 2. Flow Cytometry
  - 3. SDS Page / Western Blot
  - 4. Cell Migration Analyses
- Discussion
- Summary
- References

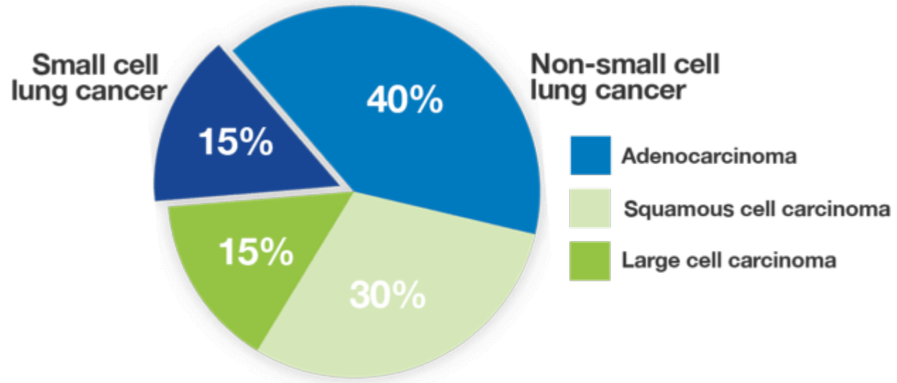




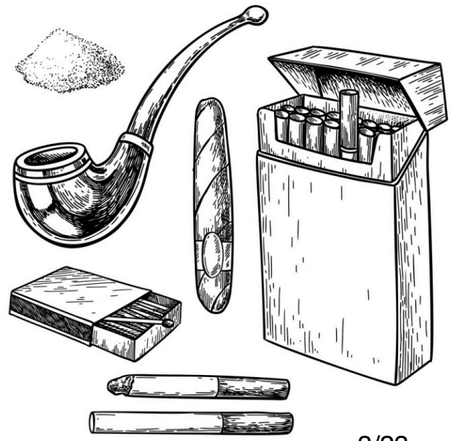


Lung Cancer

- Second most common type of cancer
- Most deaths in both sexes in all cancers
- Etiology is not fully explained
- Most important risk factor is tobacco use
- However, lung cancer in non-smokers has also increased in recent years.

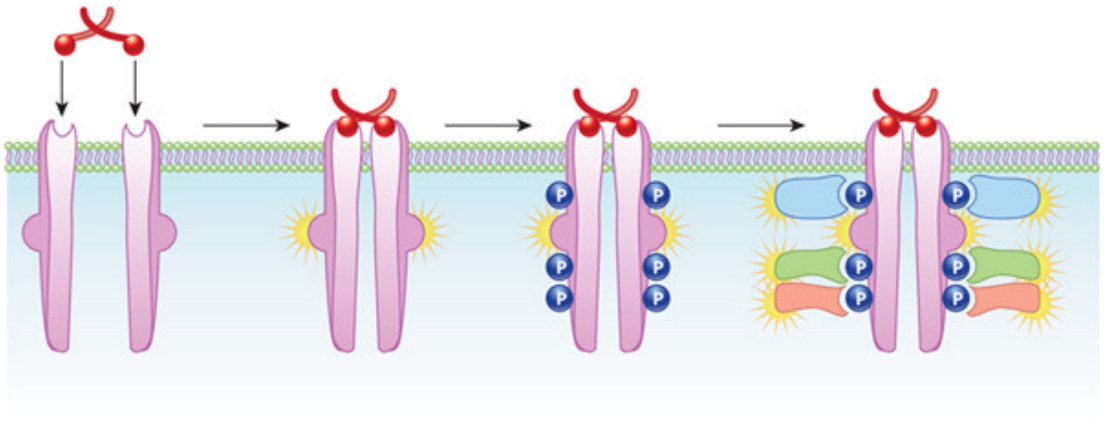


- The most common lung cancer is adenocarcinoma. Tyrosine kinase inhibitors targeting driver genes encoding various tyrosine kinase families such as EGFR, ALK and ROS1 are effectively used in the treatment of adenocarcinoma.



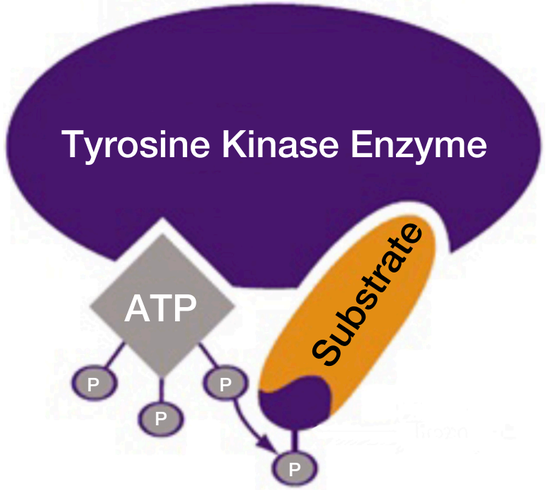
Tyrosine Kinase Inhibitors (TKIs)

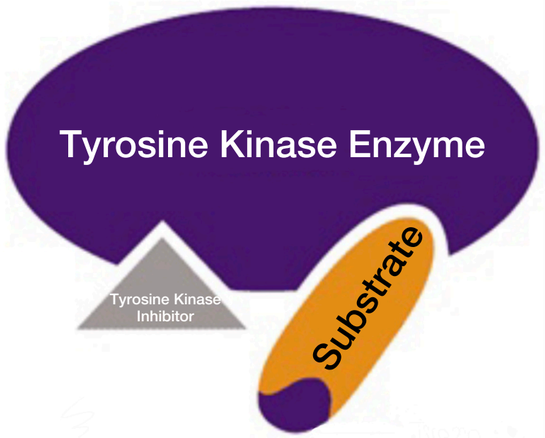
Tyrosine Kinase Inhibitors



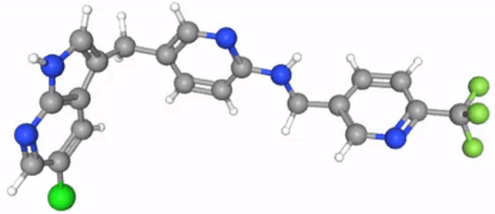
- Gain of function mutations
- Excessive protein kinase synthesis
- Genomic rearrangments

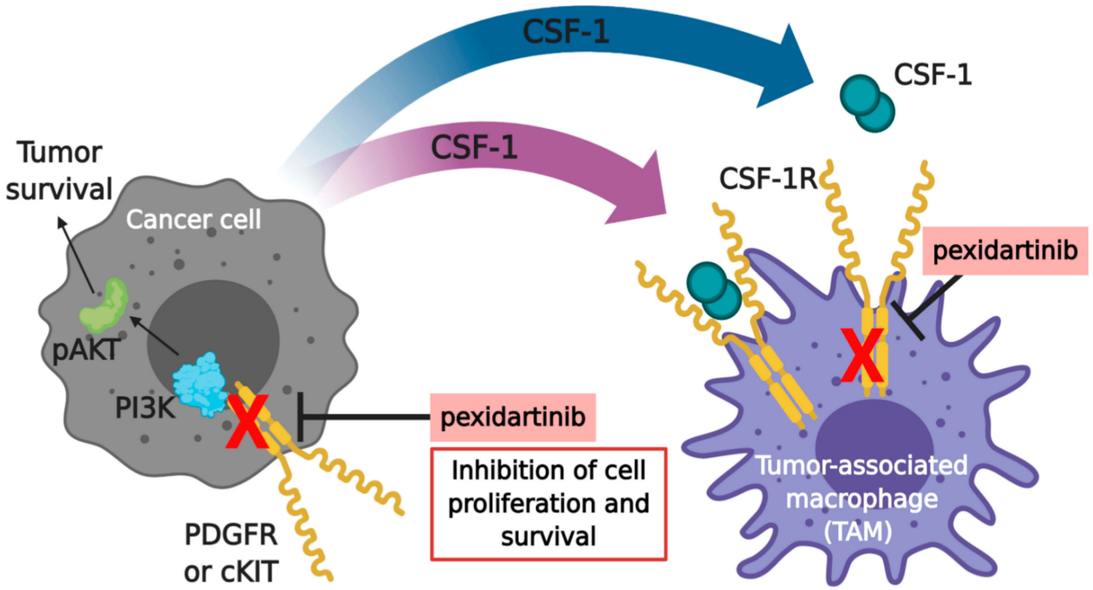
- Intracellular signal transduction
- Proliferation
- Differentiation
- Movement of cells





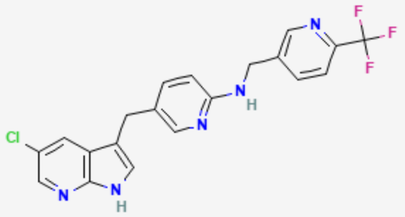
Pexidartinib





http://doi.org/10.2147/DDDT.S253232

- Approved by the FDA in 2019
- First systemic agent for tenosynovial giant cell tumors
- Inhibits CSF-1R
- Also can inhibit C-KIT and FMS-like tyrosine kinase 3

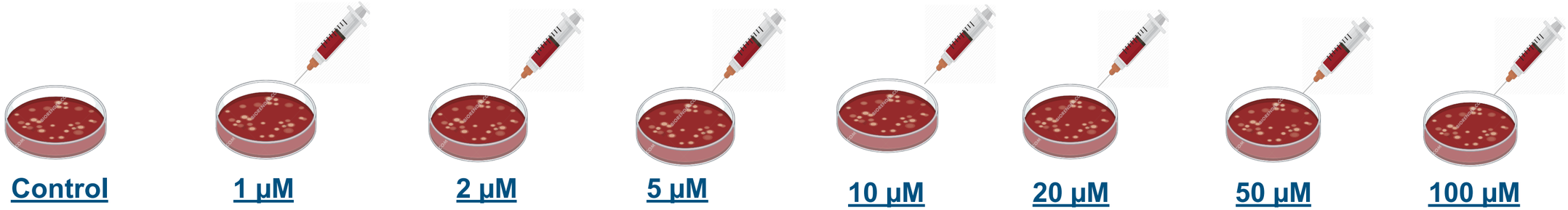


- In addition to various in vitro and in vivo studies, clinical studies on metastatic KIT-mutated melanoma, metastatic pancreatic and colorectal cancers, newly diagnosed glioblastoma, advanced GIST, breast cancer, advanced solid tumors and many other cancers are ongoing.

Aim

In this study, we aimed to investigate the anti-proliferative and anti-metastatic effects of a tyrosine kinase inhibitor named Pexidartinib on BEAS-2B lung epithelium cell and A549 lung adenocarcinoma cell line.

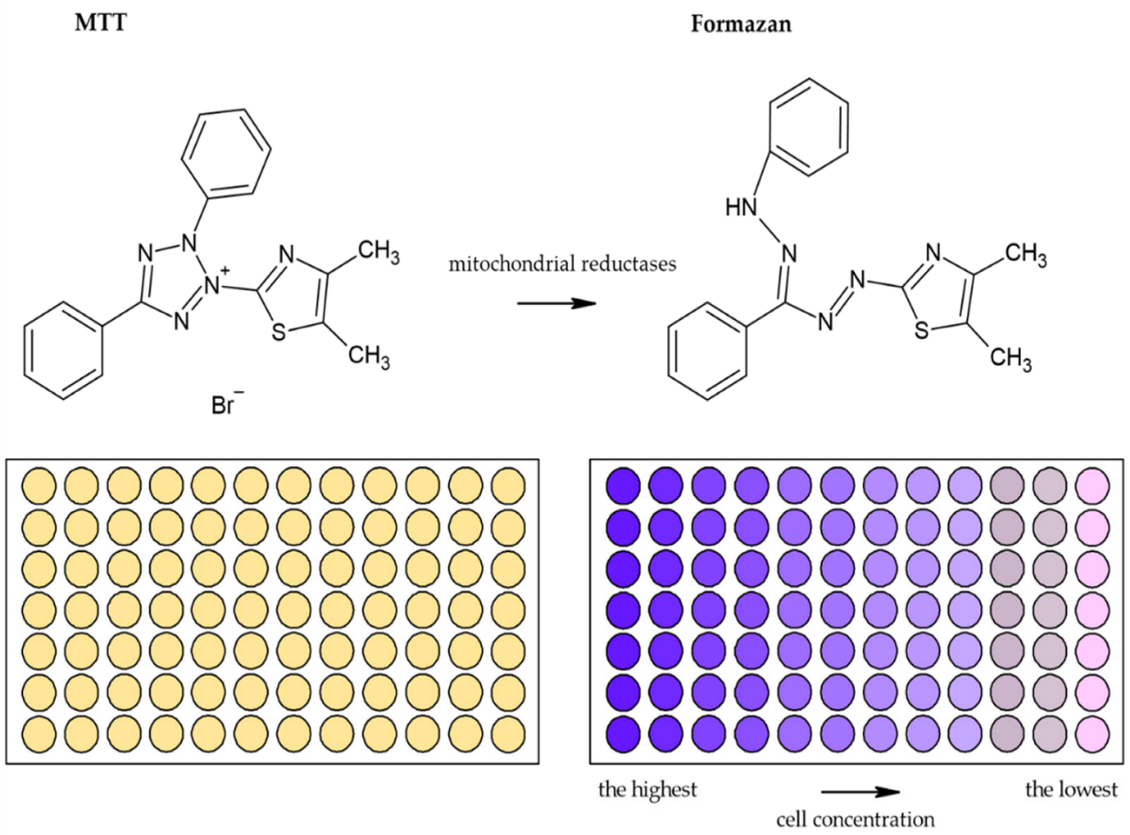
Material & Methods
Cell Culture and Treatment



24 h incubation

BEAS-2B cell line as a control cell and A549 lung cancer cell line were cultured in standard conditions using DMEM-F12 medium with 10%FBS and %1 penicillin/streptomycin. Cells were seeded in 96-well plate and treated with pexidartinib in increasing concentrations (1, 2, 5, 10, 20, 50 and 100 µM) for 24h and 48 h.

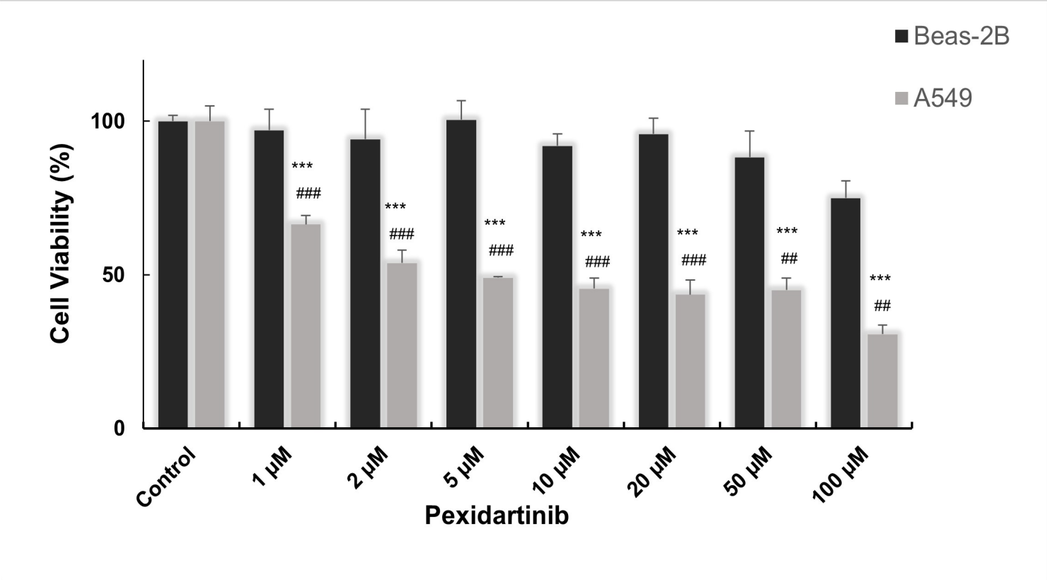
1. Material & Methods: MTT Assay



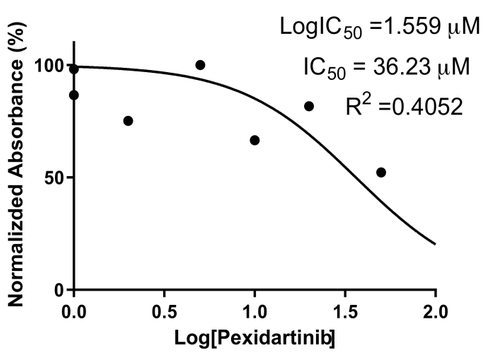
- The viability of cells was tested using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
- Cells were incubated at 37 °C with 0.1 mg/mL MTT for two hours. The supernatants were decanted without being dispensed to the cells.
- Then, 100 µL DMSO was added to the cells, which were then kept in the dark.
- After 30 minutes, optical densities were measured using a microplate reader) at 570 nm. The cell viability assay for each group was performed in triplicate.

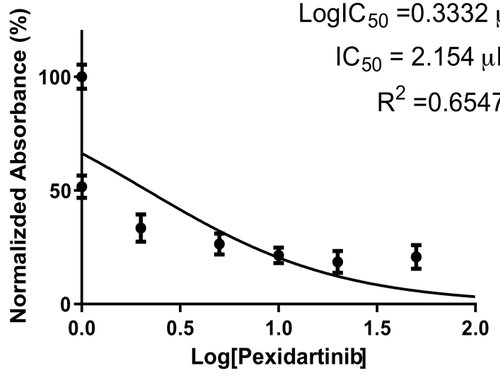
1. Results: MTT Assay

Cell Viability - 24 h

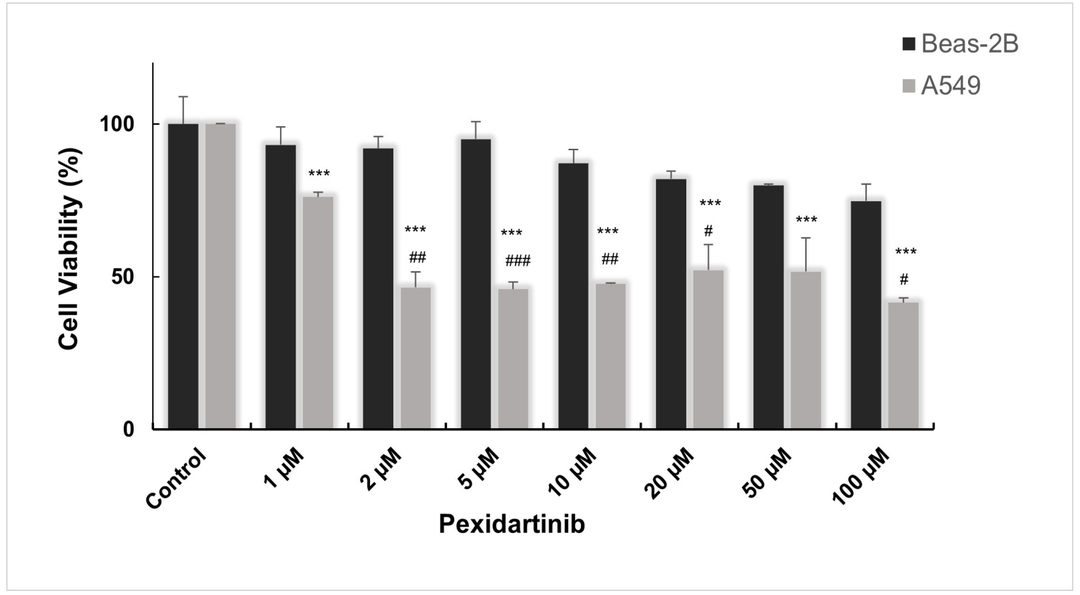


Significant differences compared to control of A549 cells = *
Significant differences compared between Beas-2B and A549 cells = # *, #=p<0.05 , **,##=p<0.01, ***,###=p<0.001

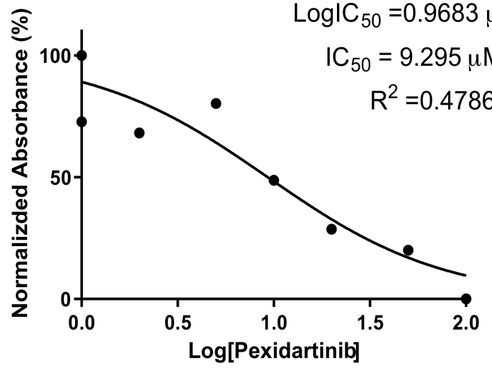


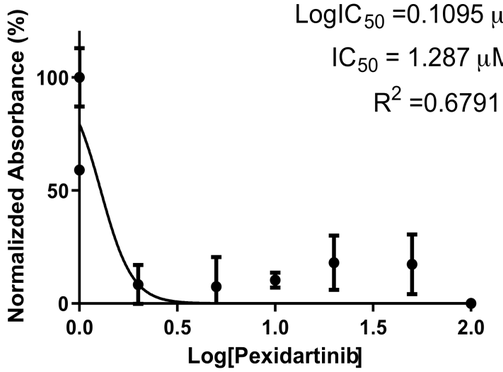


Cell Viability - 48 h



Significant differences compared to control of A549 cells = *
Significant differences compared between Beas-2B and A549 cells = # *, #=p<0.05 , **,##=p<0.01, ***,###=p<0.001





1. Results: MTT Assay

Selectivity Index

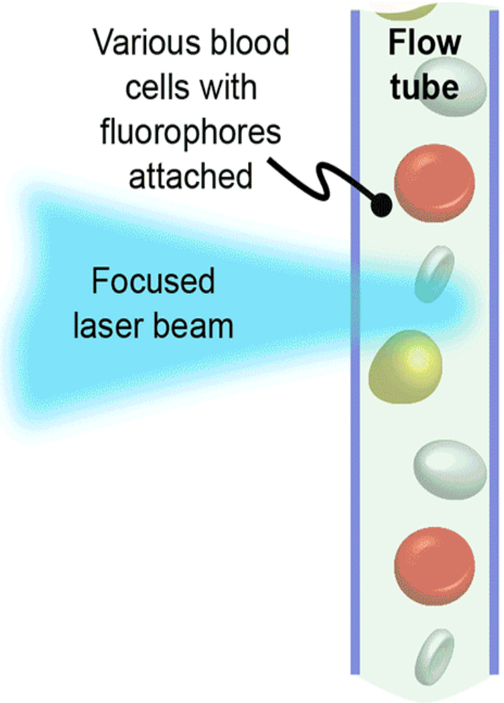
$$\text{Selectivity index (SI)} = \frac{\text{IC50 normal cells}}{\text{IC50 cancer cells}}.$$

Selectivity Index (SI) > 3.

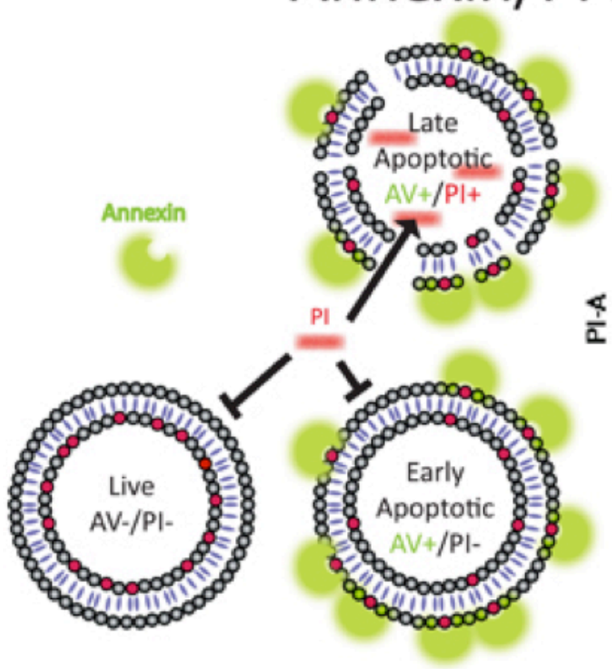
	Time Point	IC_{50} (µM) ± SEM A549 cells	IC_{50} (µM) ± SEM Beas-2B cells	SI
Pexidartinib	24	2.15±1.12	36.2±0.96	16.84
	48	1.3±0.164	9.3±1.41	7.15

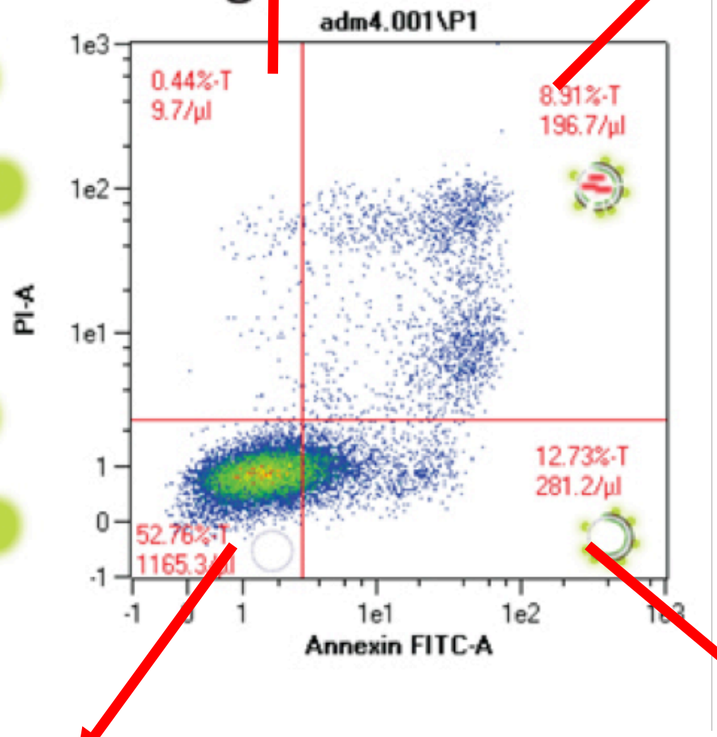
"Cytotoxicity and Genotoxicity of Biogenic Silver Nanoparticles in A549 and BEAS-2B Cell Lines", Bioinorganic Chemistry and Applications, vol. 2022, Article ID 8546079, 22 pages, 2022. https://doi.org/10.1155/2022/8546079

2. Material & Methods
Annexin V/PI Flow Cytometry Analysis



Light detection system





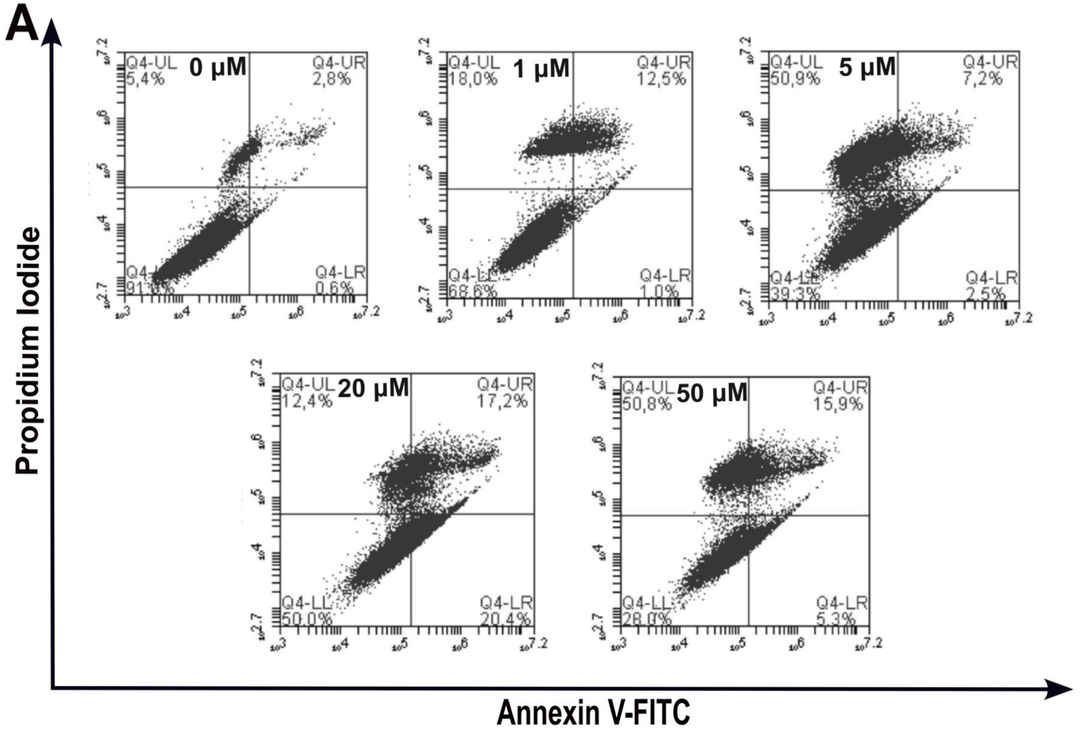
Necrotic Cells

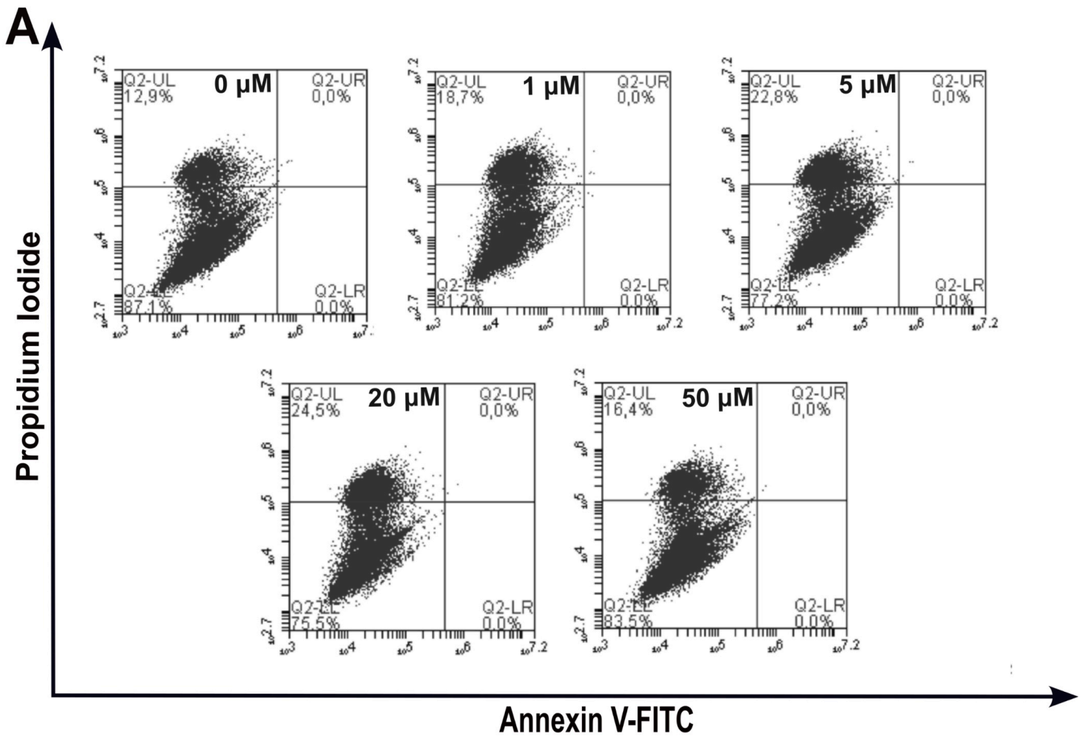
Late Apoptotic Cells

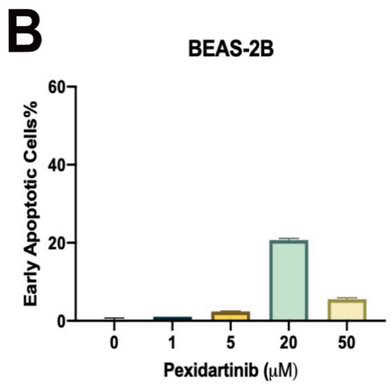
Live Cells

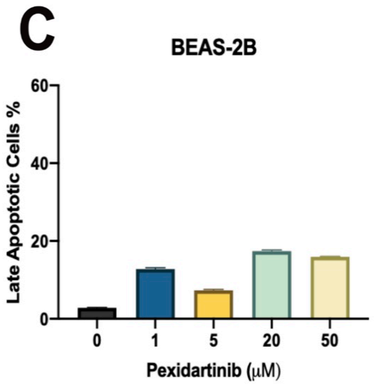
Early Apoptotic Cells

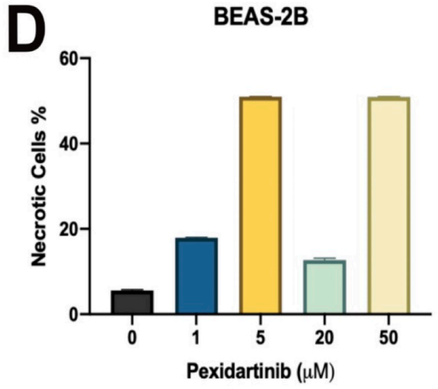
2. Results: Annexin V/PI Flow Cytometry Analysis

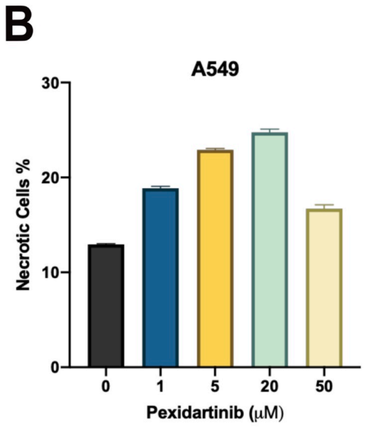




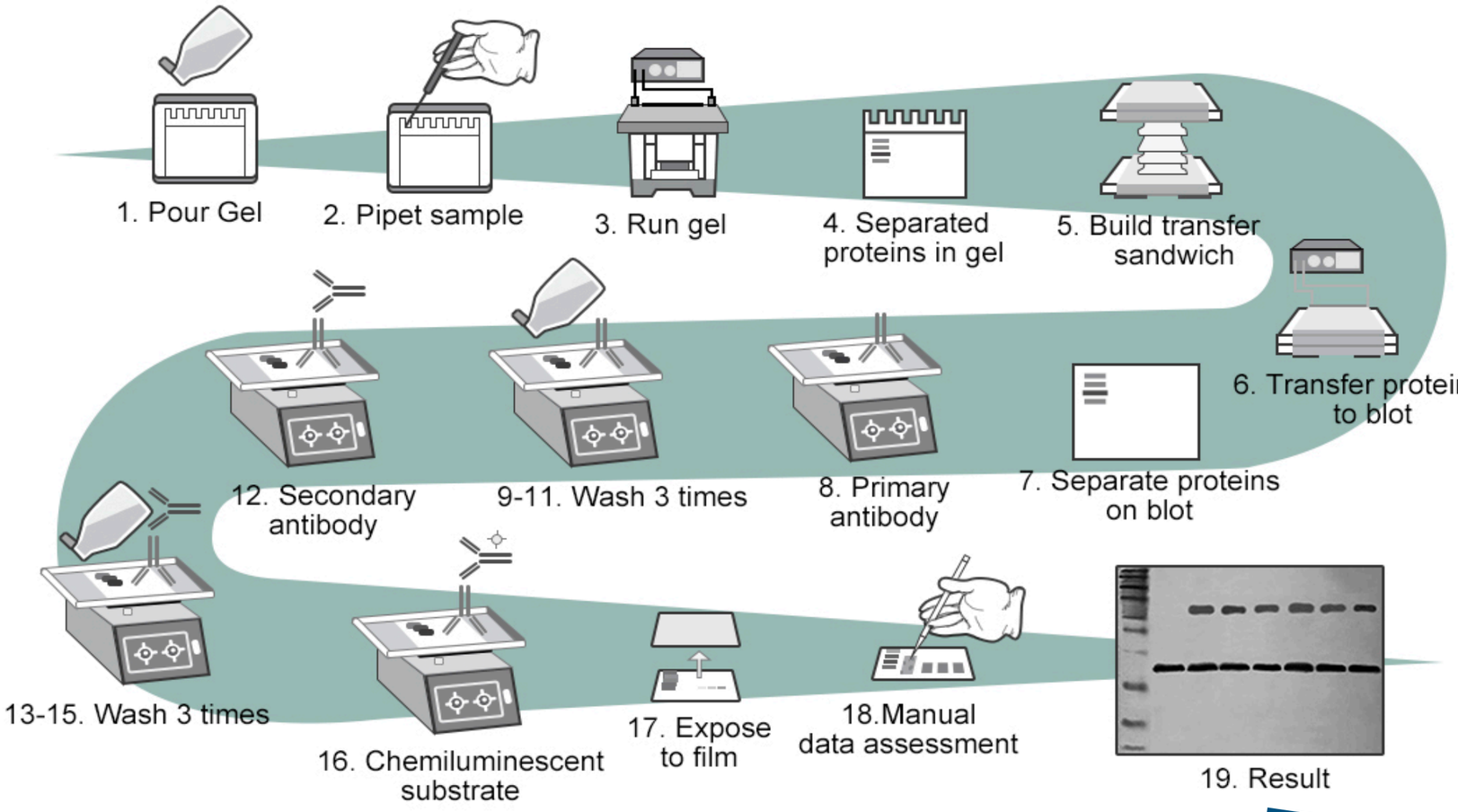




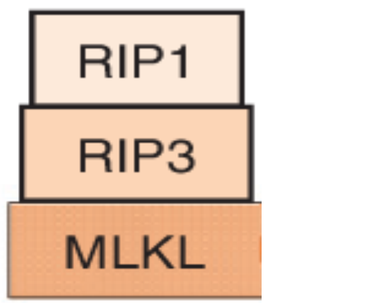




3. Material & Methods: SDS-PAGE/Western blot



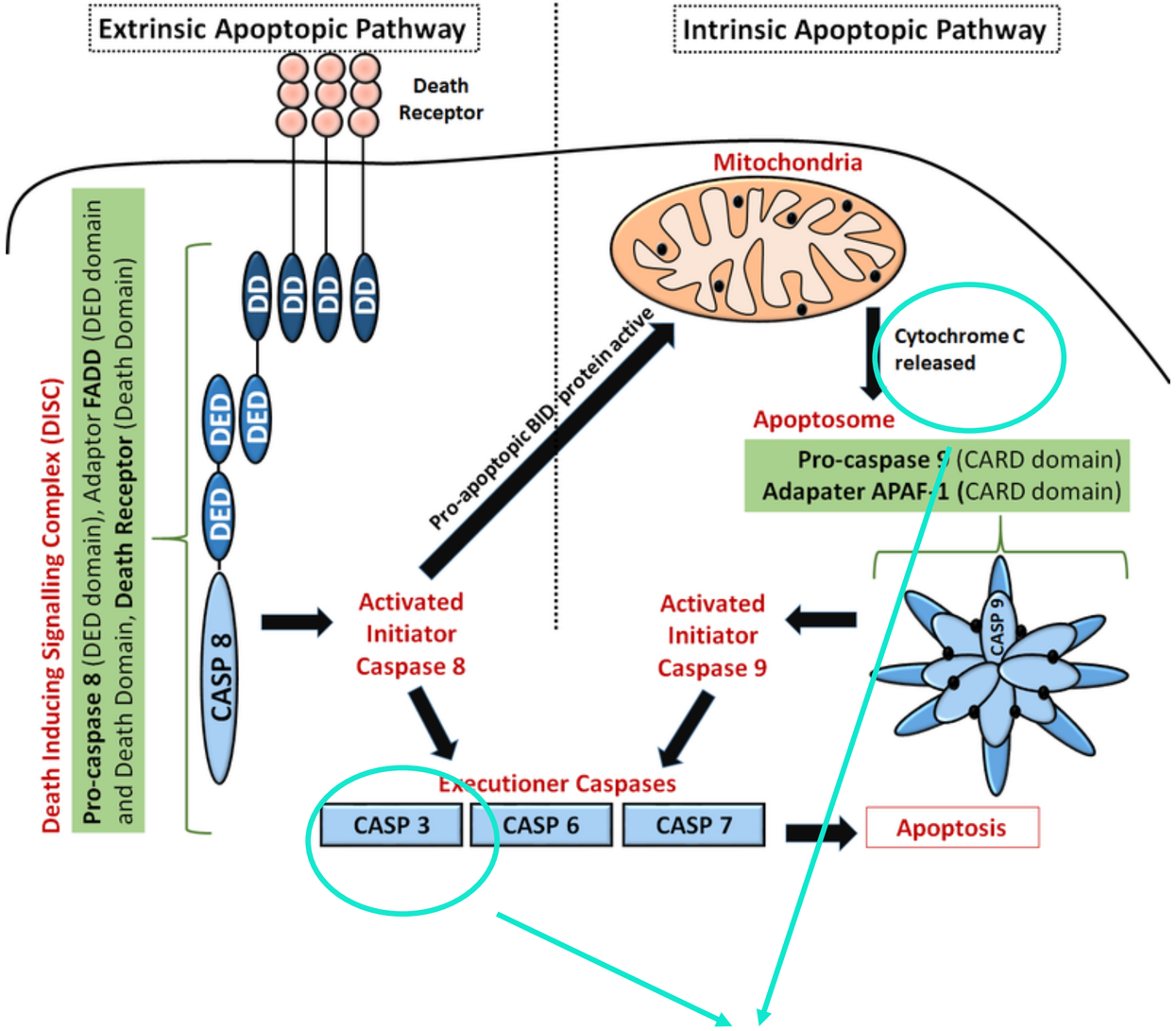
RIP3
MLKL
pMLKL
Caspase-3
Cytochrome c



The complex of RIP1, RIP3 and MLKL is major compenents of necrosis

Necroptosis

Necroptosis is the caspase-independent “cellular suicide” or “regulated” necrosis. It is an alternative mode of regulated cell death mimicking features of both apoptosis and necrosis.

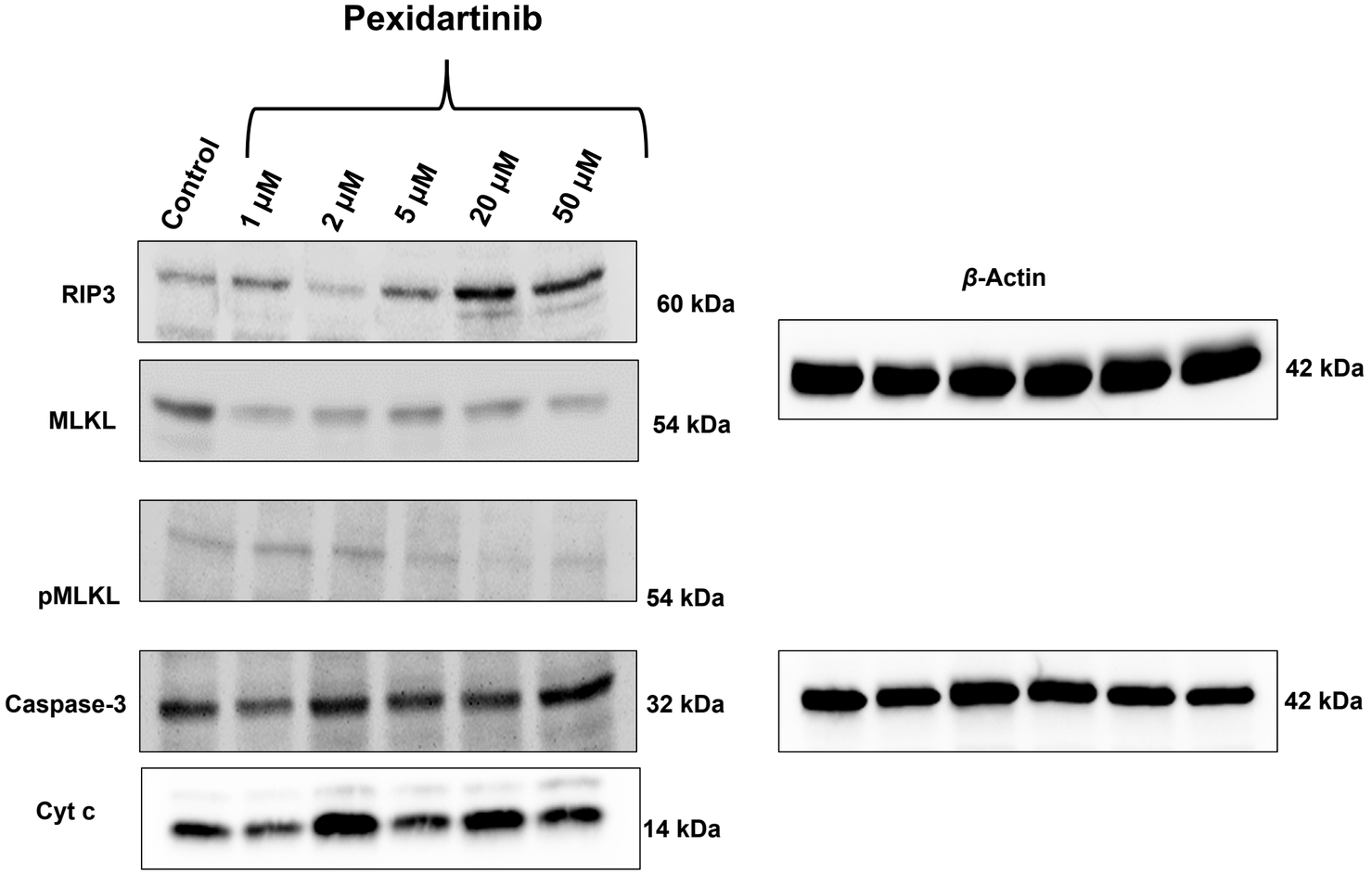


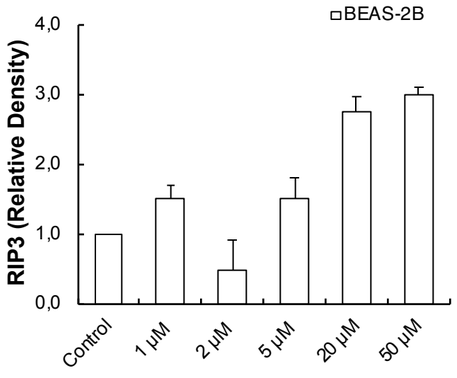
Caspase-3 playing a central role, the extrinsic activation initiates the caspase cascade specific to the apoptotic pathway Cyt c release are regarded as key upstream molecular events of apoptosis

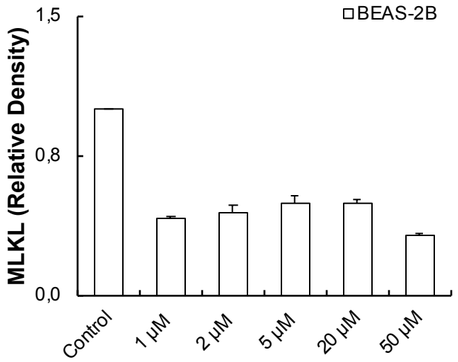
Regulation of necroptosis signaling and cell death by reactive oxygen species,
The coming decade of Cell Death Research: Five Riddles

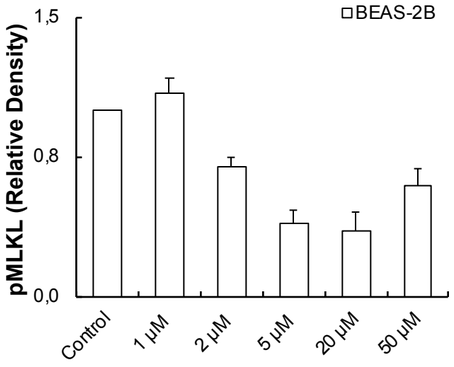
Apoptosis Regulators Bcl-2 and Caspase-3

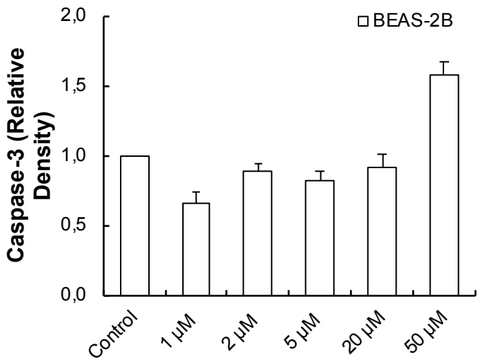
BEAS-2B

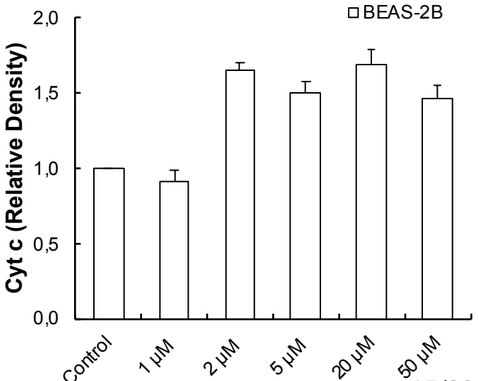

Pexidartinib
Control
1 μM
2 μM
5 μM
20 μM
50 μM
RIP3
60 kDa
MLKL
54 kDa
pMLKL
54 kDa
Caspase-3
32 kDa
Cyt c
14 kDa
β-Actin
42 kDa
42 kDa


BEAS-2B
RIP3 (Relative Density)
4,0
3,0
2,0
1,0
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM

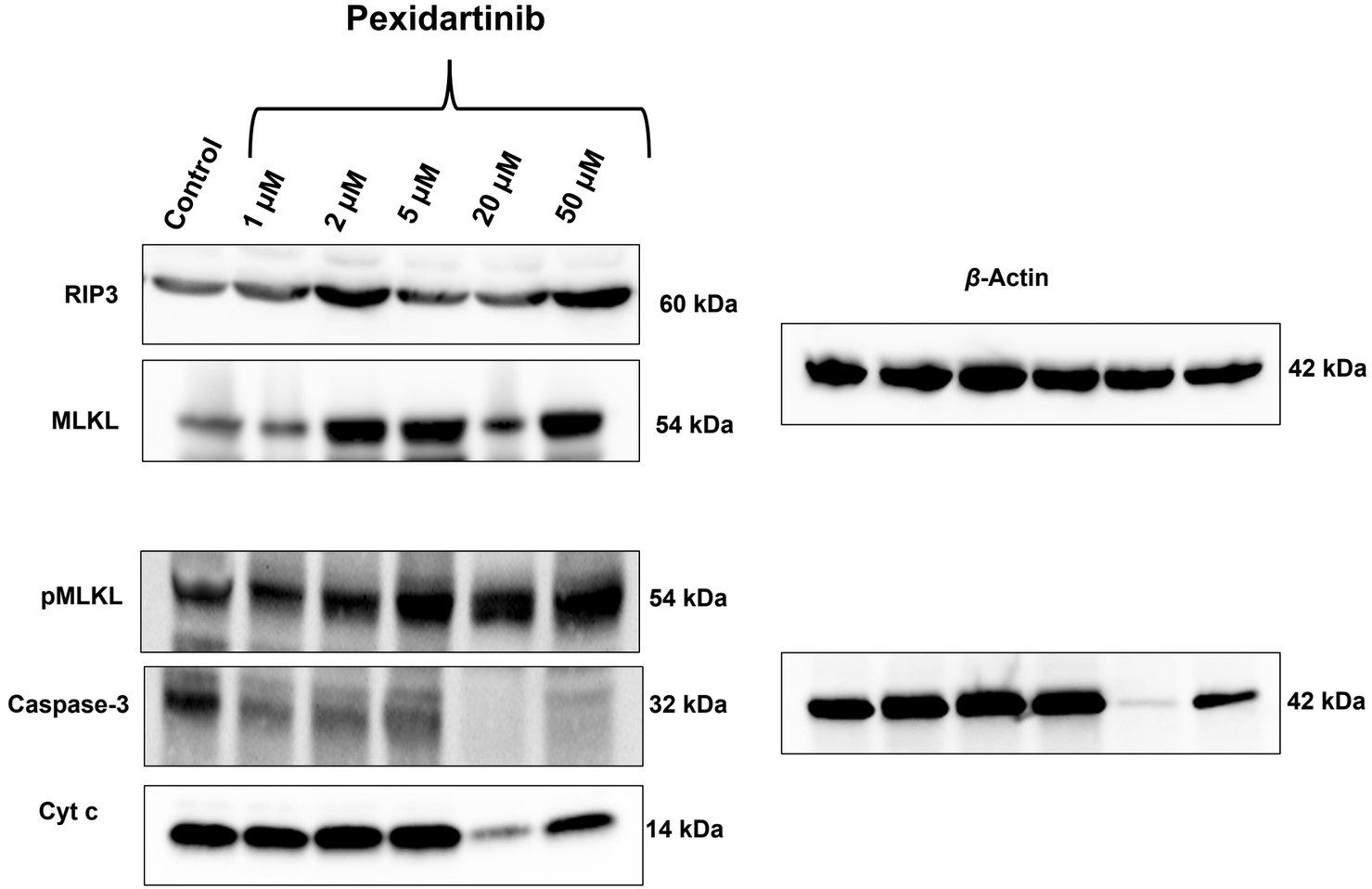

BEAS-2B
MLKL (Relative Density)
1,5
0,8
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM

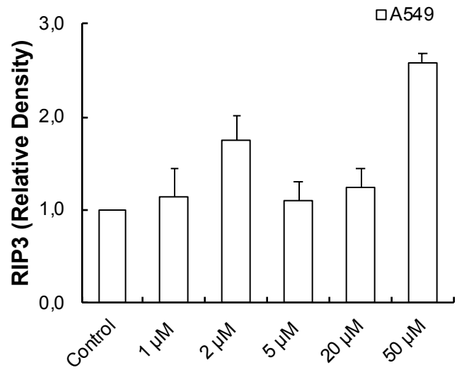

BEAS-2B
pMLKL (Relative Density)
1,5
0,8
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM

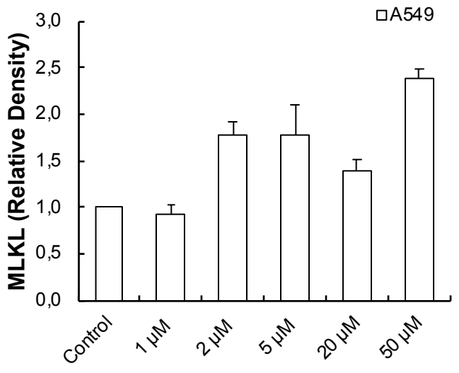

BEAS-2B
Caspase-3 (Relative Density)
2,0
1,5
1,0
0,5
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM

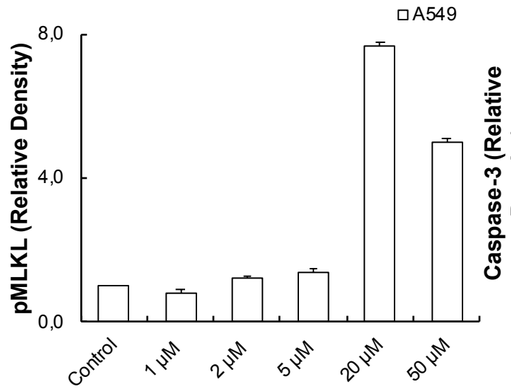

BEAS-2B
Cyt c (Relative Density)
2,0
1,5
1,0
0,5
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM

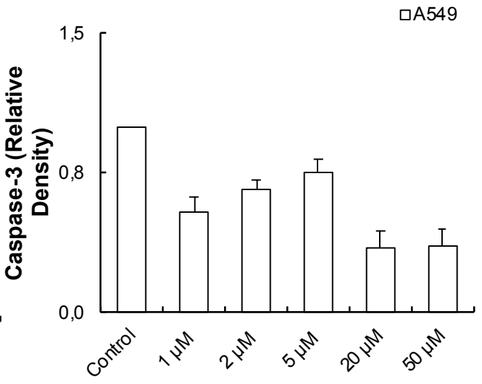
A549

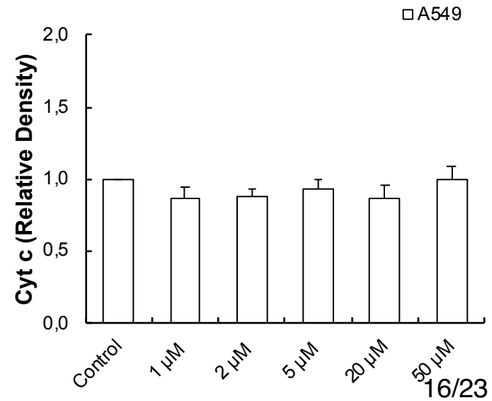

Pexidartinib
Control
1 μM
2 μM
5 μM
20 μM
50 μM
RIP3
60 kDa
MLKL
54 kDa
β-Actin
42 kDa
pMLKL
54 kDa
Caspase-3
32 kDa
42 kDa
Cyt c
14 kDa


A549
RIP3 (Relative Density)
3,0
2,0
1,0
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM

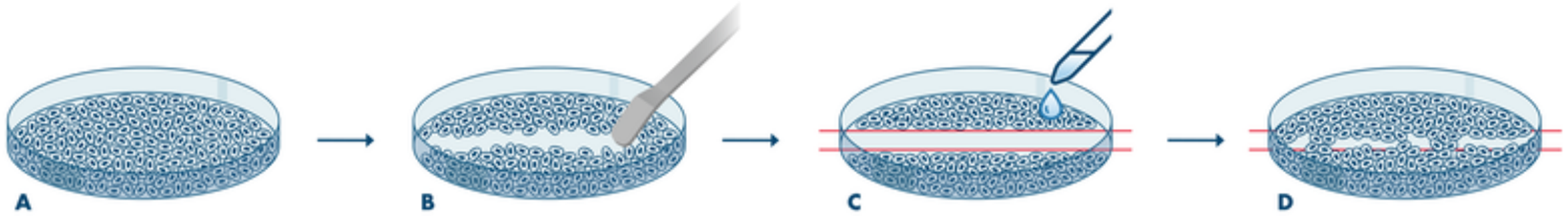

A549
MLKL (Relative Density)
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM


A549
pMLKL (Relative Density)
8,0
4,0
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM


A549
Caspase-3 (Relative Density)
1,5
0,8
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM


A549
Cyt c (Relative Density)
2,0
1,5
1,0
0,5
0,0
Control
1 μM
2 μM
5 μM
20 μM
50 μM

4. Material & Methods: In vitro Cell Migration Assay



Create a physical gap within a cell monolayer.

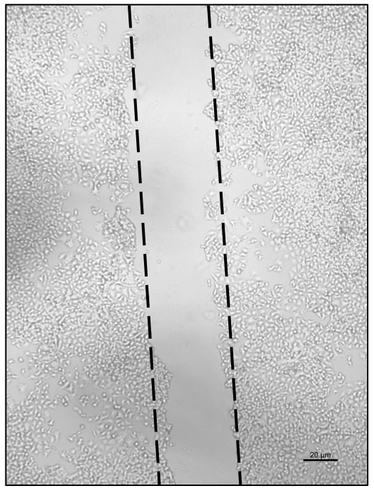
↓

Monitor the process of cell migration into the gap with live cell imaging or by taking photos at different time points.

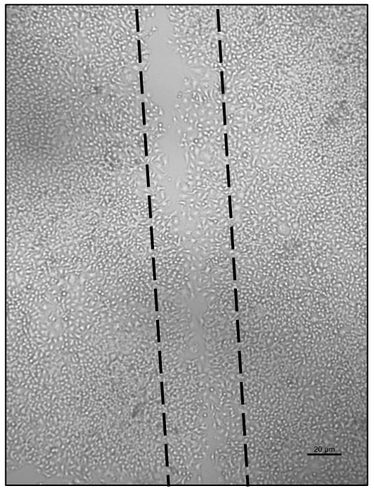
↓

Analyze the gap closure rate, which is a typical experimental readout, manually or by using automated software.

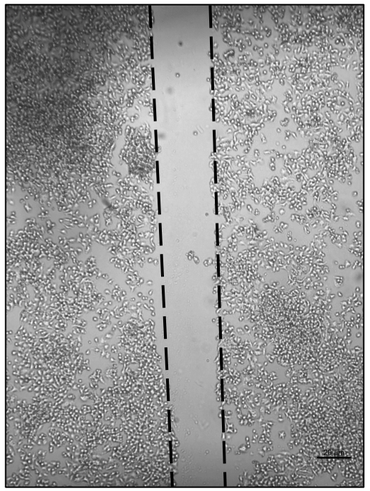
4. Results: *In vitro* Cell Migration Assay



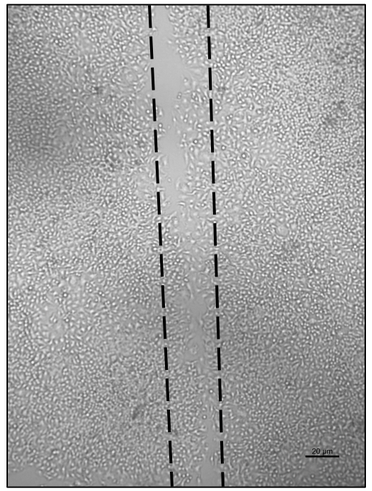
Control (0.h)



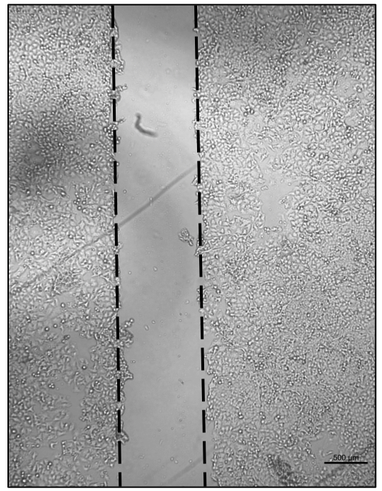
Control (24.h)



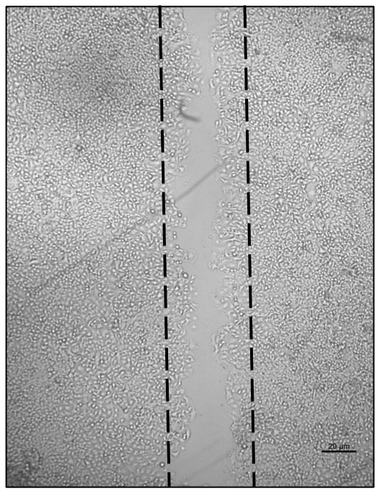
1 µM (0.h)



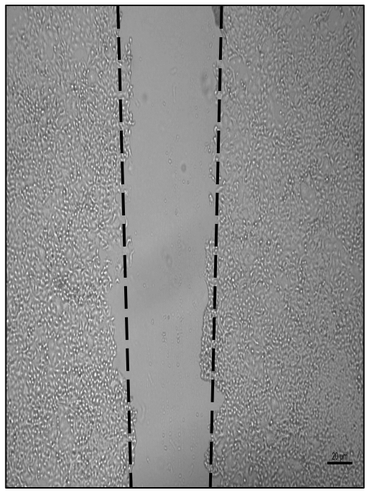
1 µM (24.h)



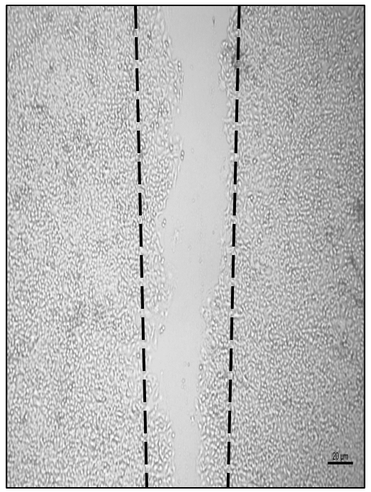
5 µM (0.h)



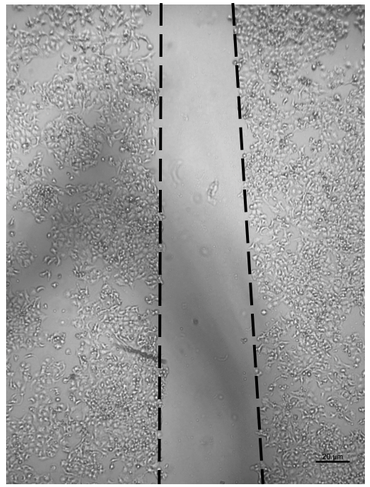
5 µM (24.h)



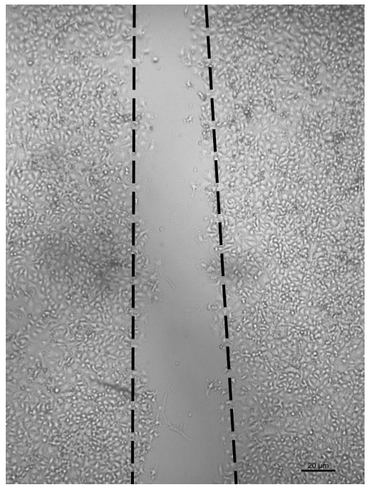
20 µM (0.h)



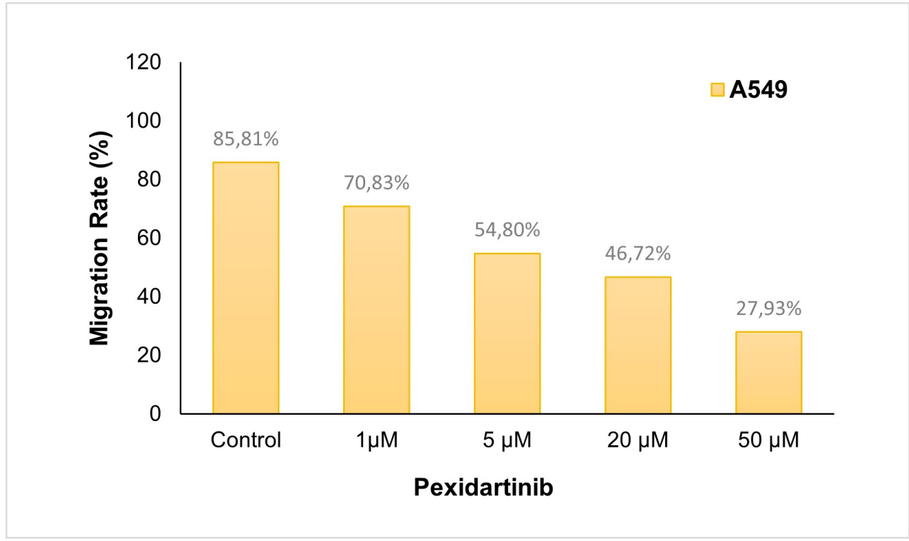
20 µM (24.h)



50 µM (0.h)



50 µM (24.h)



A549

Discussion

- There is no significant effect of treatment of pexidartinib on Beas-2B cells was observed on cell viability. However, cell viability of lung cancer cells, A549, was decreased with treatment of pexidartinib that is even 1 μM ($p<0.005$).

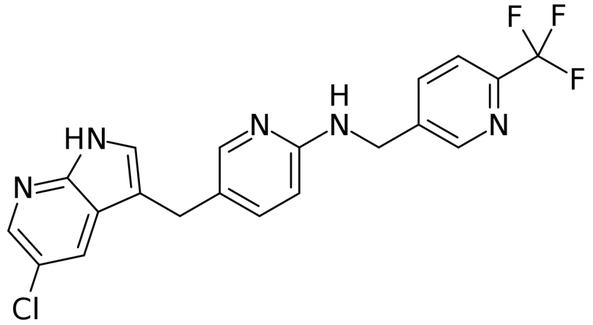
- Necrotic cells stained with PI were increased pexidartinib treatment at A549 cells respectively. There was no apoptosis induced at any concentration of pexidartinib at A549 cells.

- These results suggest that Effect of Pexidartinib on lung adenocarcinoma should need further investigations.

Limitations

- Limited financial support ☺️
- Limited time of study

Summary



- Lung cancer is the most common cause of death among all cancers.
- Tyrosine kinase inhibitors are used effectively in many cancer treatments.
- Pexidartinib significantly kills lung adenocarcinoma cells compared to healthy cells with necroptosis.
- Pexidartinib significantly decreases cell migration rate on A549 lung adenocarcinoma cells.

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Thank you

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