**Introduction**

- Lung cancer (LC) is the most common cause of cancer death in the world and Portugal are considered the leading cause of death.1
- It is divided into two histological types: small cell lung cancer and non-small cell lung cancer.1
- TP53 gene mutation is present in over 50% of existing malignant tumors;
- These mutations may change P53 functions promoting defects in the checkpoints of the cell cycle, cell immortalization, genomic instability and inappropriate survival.2
- Radiotherapy is a major therapeutic modality for the treatment of cancer, with the aim to destroy tumor cells and prevent them from proliferating.3

**Aim**

To determine what the influence X-radiation on P53 expression in cell lines of lung cancer.

**Material and Methods:**

**Samples**
- Small Cell Lung Cancer Cell Line NCI-H69 (ATCC ® HTB-119 ™)
- Non-Small Cell Lung Cancer Cell Line NCI-H1299 (ATCC ® CRL-5803 ™)
- Lung Cancer Cell Line A549 (ATCC ®CCL-185 ™)

**Trypan Blue Dye:** Determines the number of viable cells in a cell suspension through examination under a microscope.4 Based on the principle that the living cells have intact cell membranes that exclude certain dyes, while dead cells do not.4

**Alamar Blue Reagent:** Non-fluorescent component that produces a fluorescent product upon reduction by living cells, without leading to cell death thereof.5 In contact with the cells, the dye undergoes reduction and turns red. The reduced form is highly fluorescent and the extent of the conversion, which is of cell viability can be measured by reflective optical density or fluorescence.5

**Western Blot Assay:** Transference of the proteins by electrophoresis from the polyacrylamide gel to support a nitrocellulose membrane.6 Proteins are fixed by providing a band which is detected by a specific protein, using primary antibody (Ab) directed against the P53 protein are then added secondary Ab that recognize the primary Ab and that are conjugated to the enzyme, alkaline phosphatase, which will produce a colored precipitate.6

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<tr>
<th>Advantages</th>
<th>Limitations</th>
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<tr>
<td>Trypan Blue Dye</td>
<td>Gives a direct measure of cell viability.6</td>
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<tr>
<td>Alamar Blue Reagent</td>
<td>Simple and versatile way to assess cell proliferation.8</td>
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<td>Western Blot Assay</td>
<td>Sensitive, and does not require radioactive probe.9</td>
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<td>It’s toxic and can cause cancer in operator.7</td>
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<td>The lack of sharp contrast often causes difficulty in differentiating between stained and unstained cells.6</td>
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<td>The accumulation of fluorescent product in the middle. The large reduction of dye by metabolically active cells leads to a non-fluorescent end product.8</td>
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<td>Are manually intensive and time-consuming.10</td>
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<td>The method has not been miniaturized, which wastes materials and reduces sensitivity.10</td>
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**References:**

9. David R. Krey CID. Visualization of antioxidant proteins on Western blots. Elsevier Inc [Internet]. Wisconsin; 1988;180–4