Effect of inhibition of cellular *non-sense mediated decay (NMD)* on p53 tumor suppressor gene expression in hepatocarcinoma cell lines

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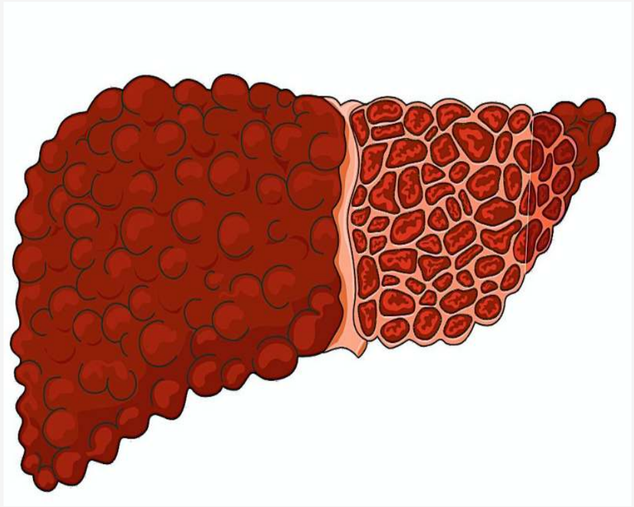
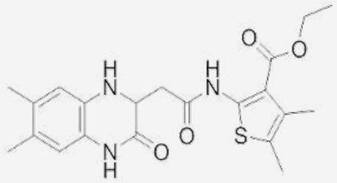
Índex

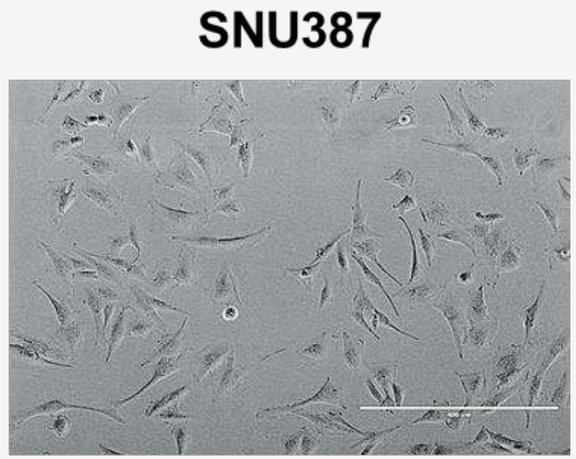
1.- INTRODUCTION

NMDI14





Hepatocarcinoma

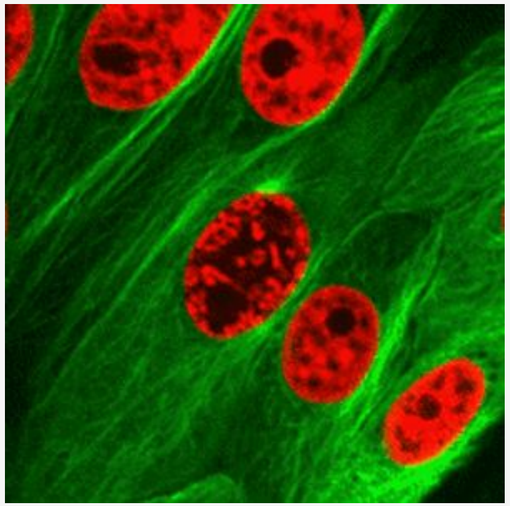


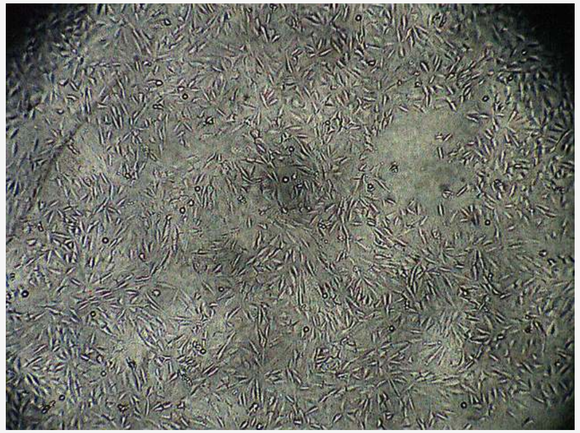
Affected tissue
(cirrhosis, fibrosis)

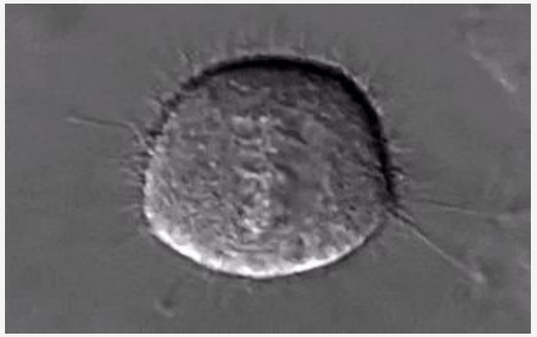
NMDI14 (drug)
or control

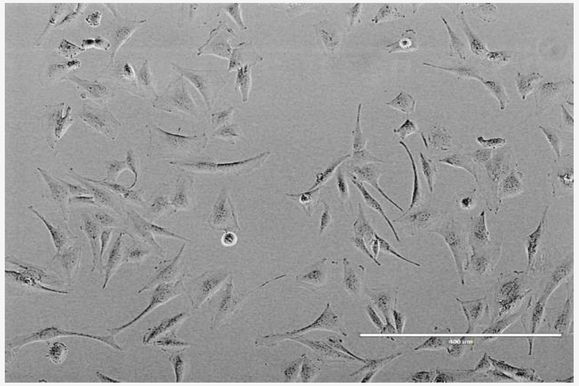
2.- OBJECTIVE

To analyze the expression (mRNA) of the p53 gene in a hepatocarcinoma cell line (SNU387) treated with an NMD inhibitor (NMDI14) and compare it with the negative control (untreated 387 cells).





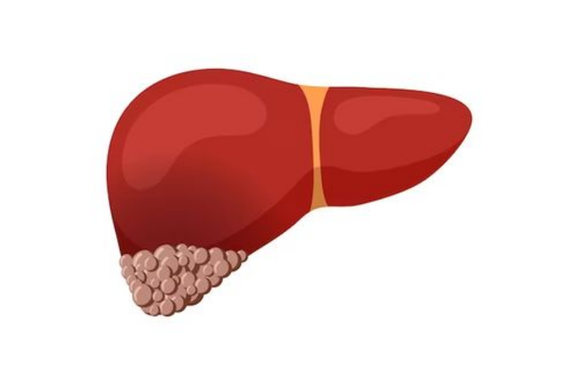




TUMOR CELLS

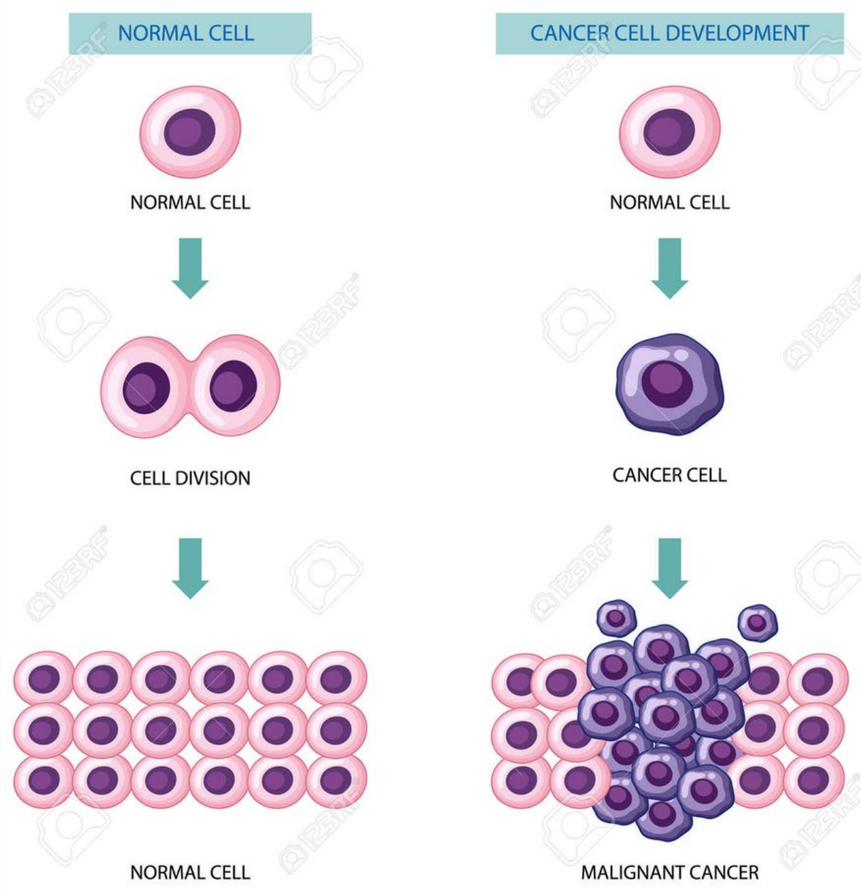
3.-THEORETICAL FRAMEWORK

CELLULAR HEPATOCARCINOMA



Causes

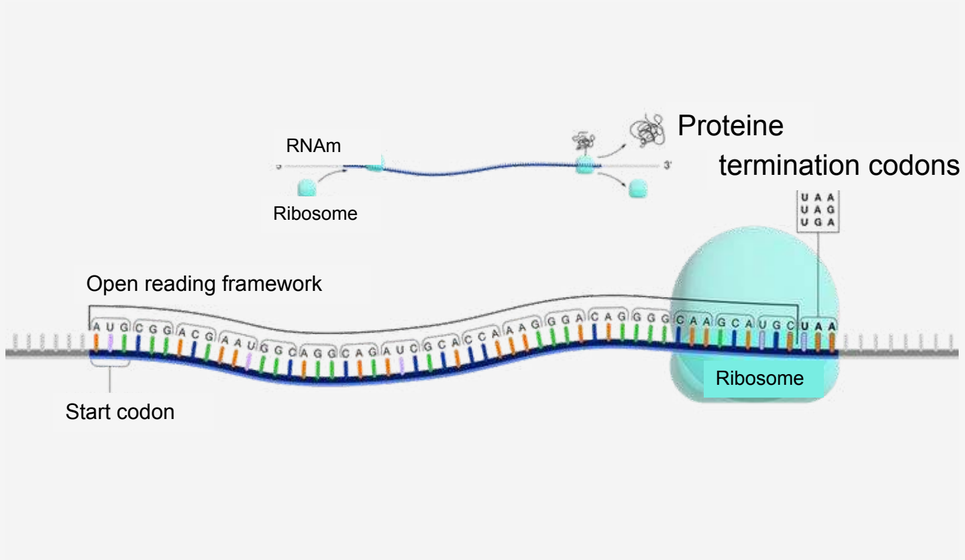
It can be caused in people with cirrhosis or fatty liver disease. It affects mostly older people, especially men rather than women

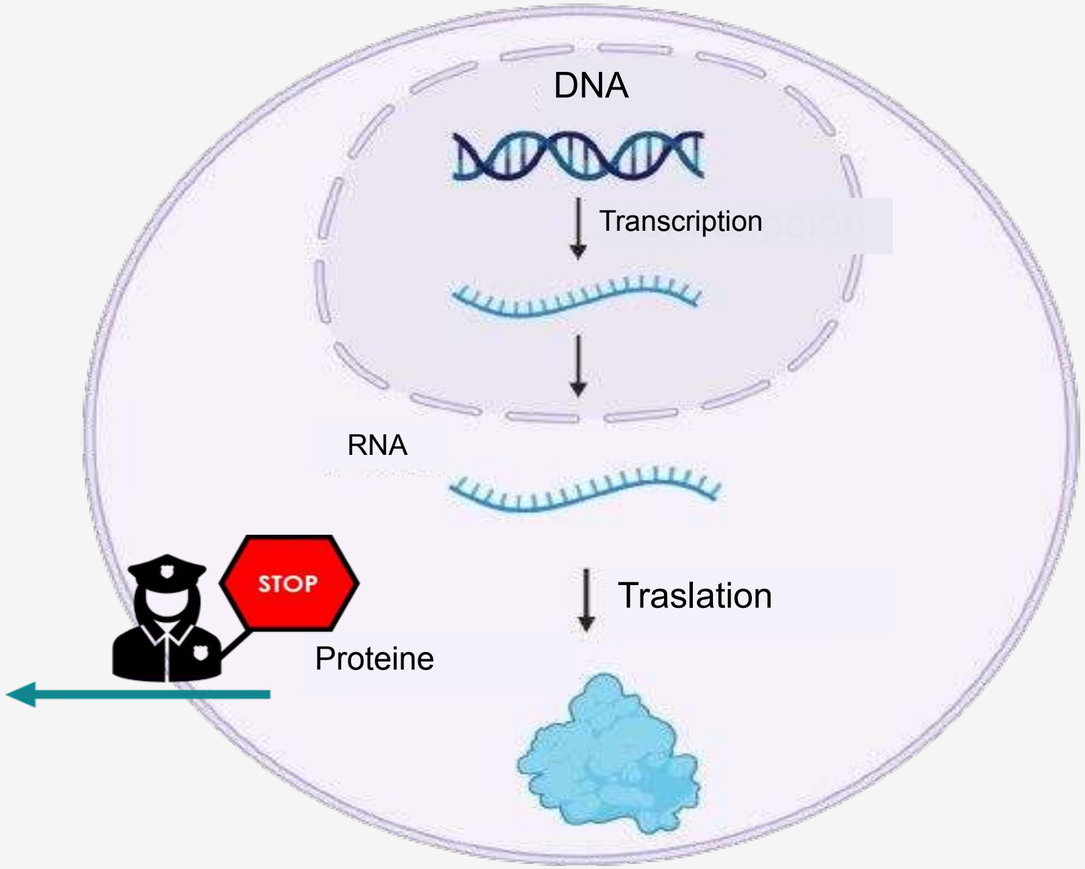


How cancer forms?

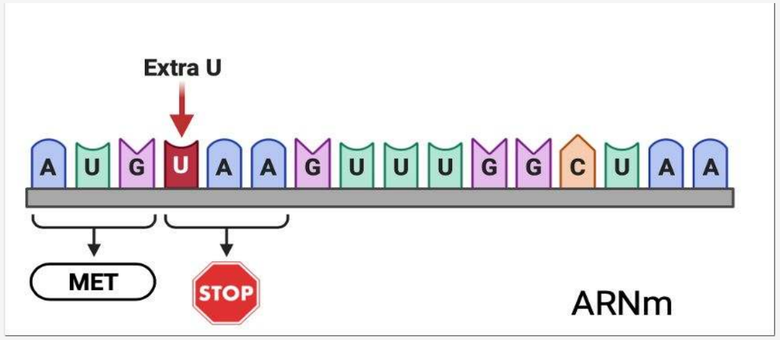
Cancer occurs when normal cells begin to divide uncontrollably and transform into cancer cells. To prevent this from happening, the NMD is responsible for regulating the transcription process in the cells.

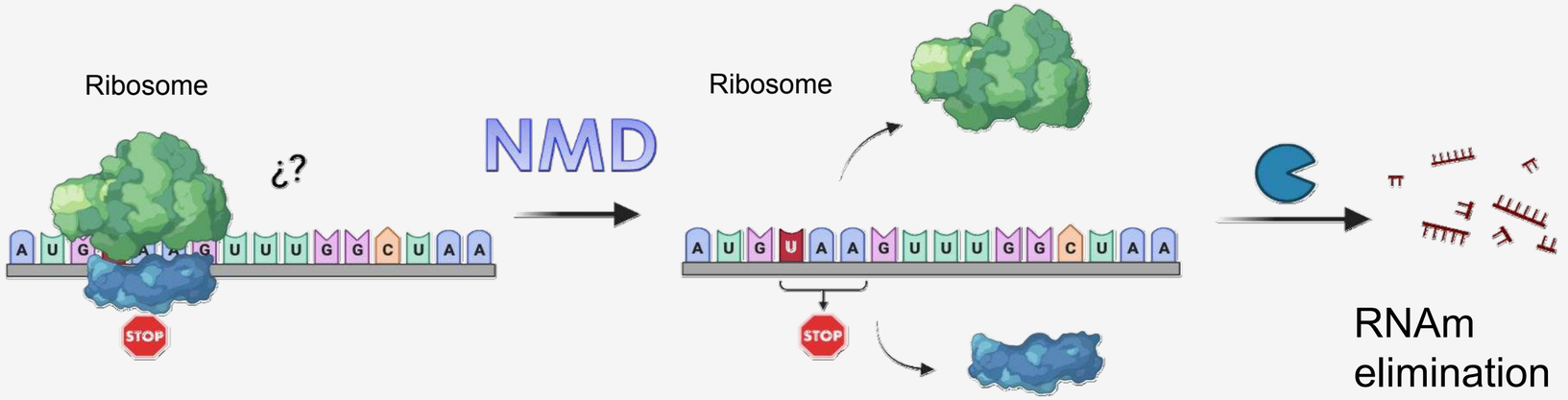
Non-sense mediated decay (NMD) mechanism





Non-sense mediated decay (NMD) mechanism

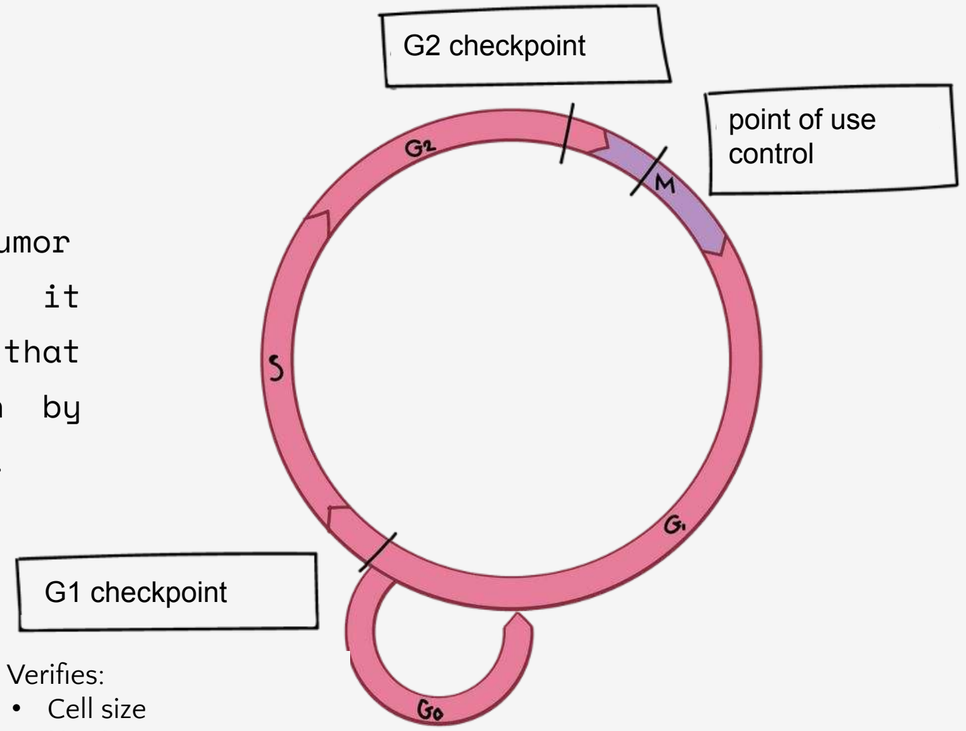




P53 GEN

¿What is?

The *p53* gene is a tumor suppressor gene as it encodes a protein that regulates cell division by keeping it under control.

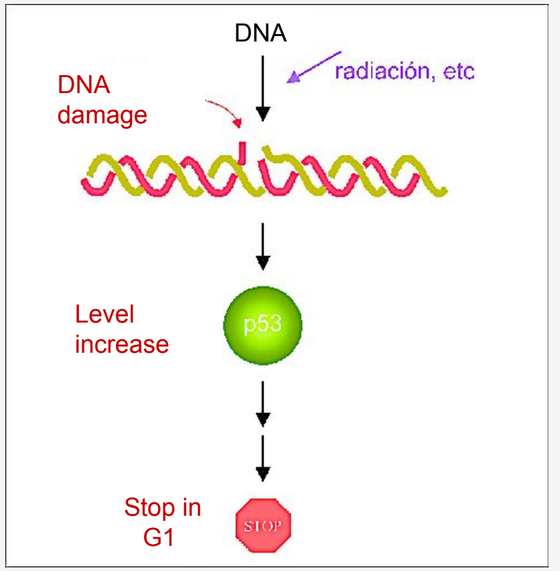


Verifies:

- Cell size
- Nutrients
- Growth factors
- DNA damage

How it works?

This gene is activated when a cell suffers DNA damage or is subjected to cellular stress. If the *p53* gene is inactivated, cells can proliferate with unrepaired genetic damage, which would occur at the G1 checkpoint.



RESEARCH PLANNING

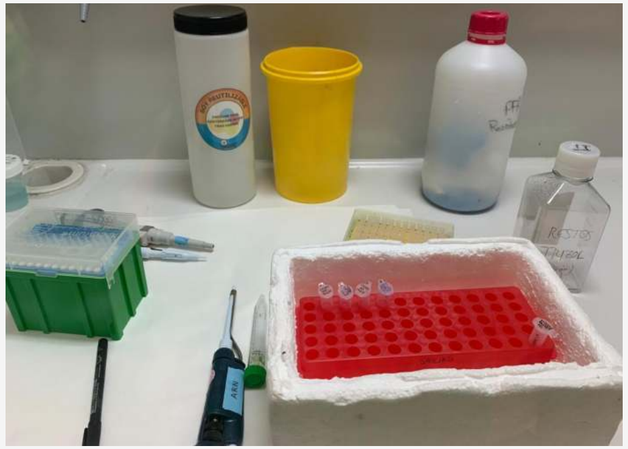
IMIBIC (Córdoba)

1- First session

30 october 2023

2- Second session

29 november 2023



3- Third session

31 January 2024

4- Fourd session

21 February2024

5- online sessions

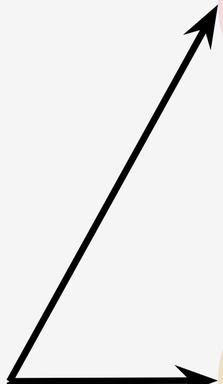
Shared files on Google-Drive.

4.-MATERIALS AND METHODS

Reagents

- Trizol (600µl/sample)
- Chloroform (120µl/sample)
- Isopropanol (300µl/sample)
- Ethanol 75% (600µl/sample)
- Glycogen (1µl/sample)
- H_2O RNAsa free (8µl/sample)
- Retrotranscriptase (1 µl/sample)

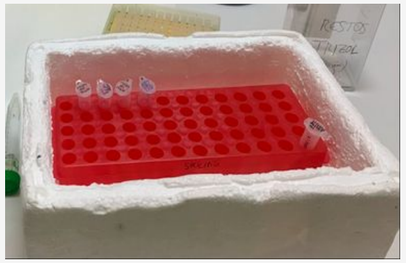
Kit DNAsa



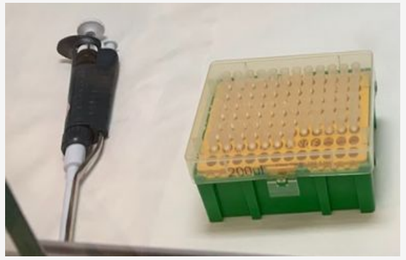
- Enzime DNAsa (1µl/sample)
- Buffer 10x DNAsa (1µl/sample)
- STOP Solution DNAsa (1µl/sample)

Equipment used

Box with ice



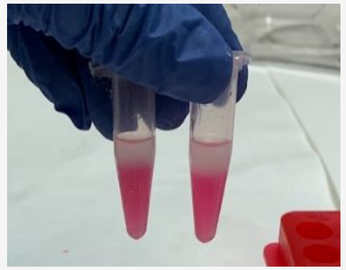
Pipettes and tips

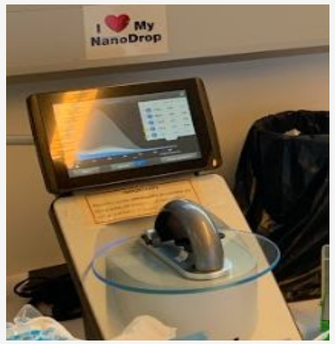


Termoblock

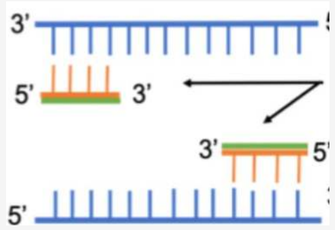
Centrifuge

Kit of retrotranscription





Primers



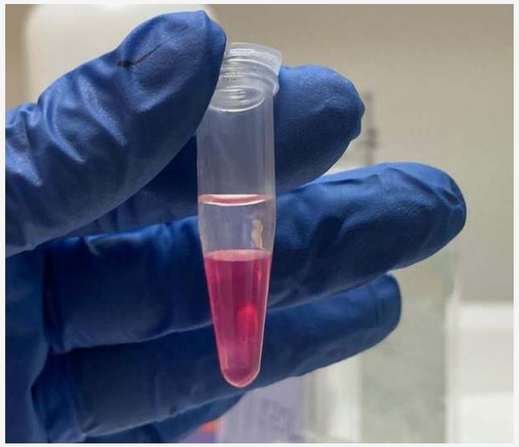
Equipment of qPCR

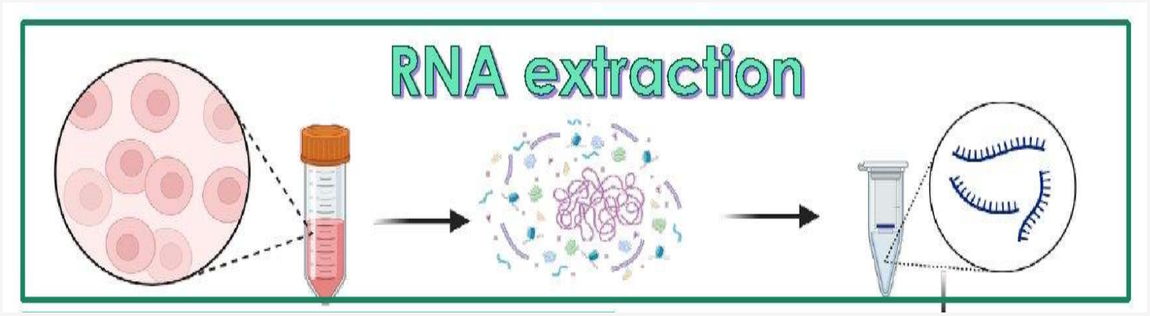
Plaque qPCR

SYBR mixture for PCR

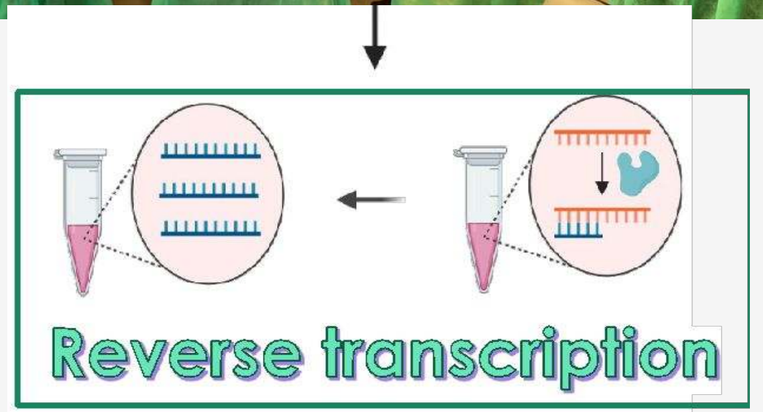
DESIGN OF LABORATORY WORK

1 RNA is extracted from hepatocarcinoma cells.



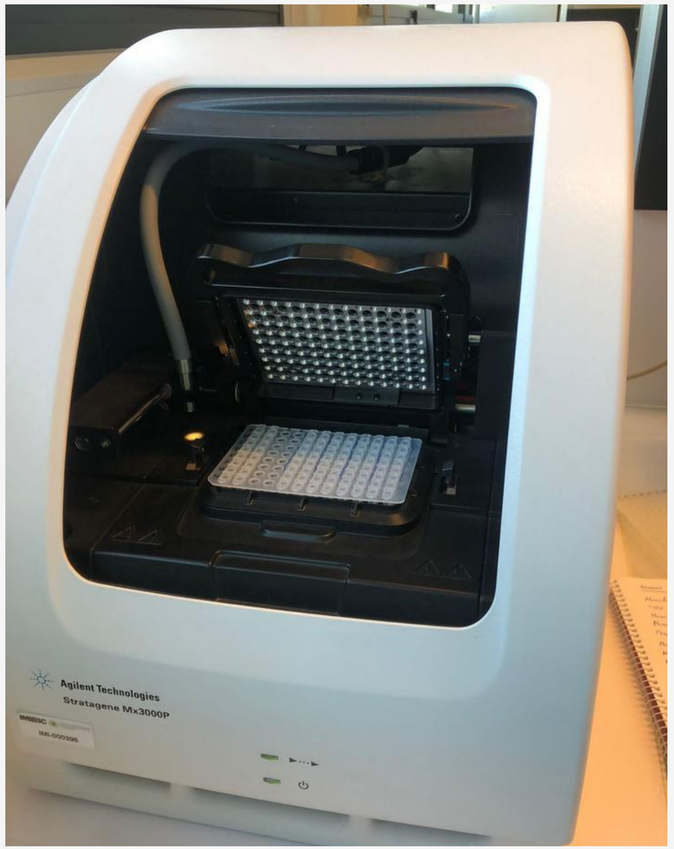


2 Retrotranscription

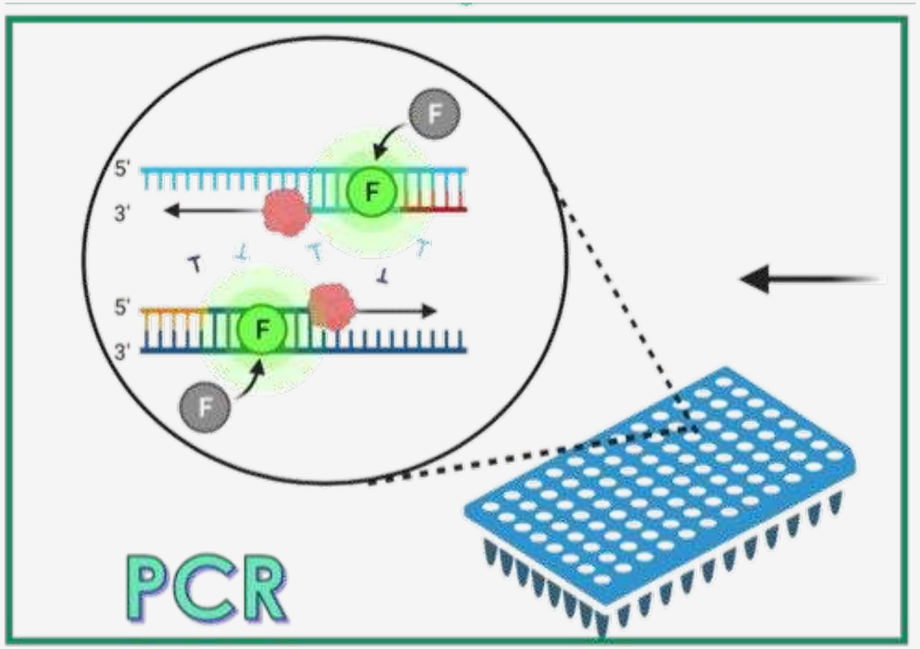


DESIGN OF LABORATORY WORK

3 Cuantitative PCR



4 Data standardization



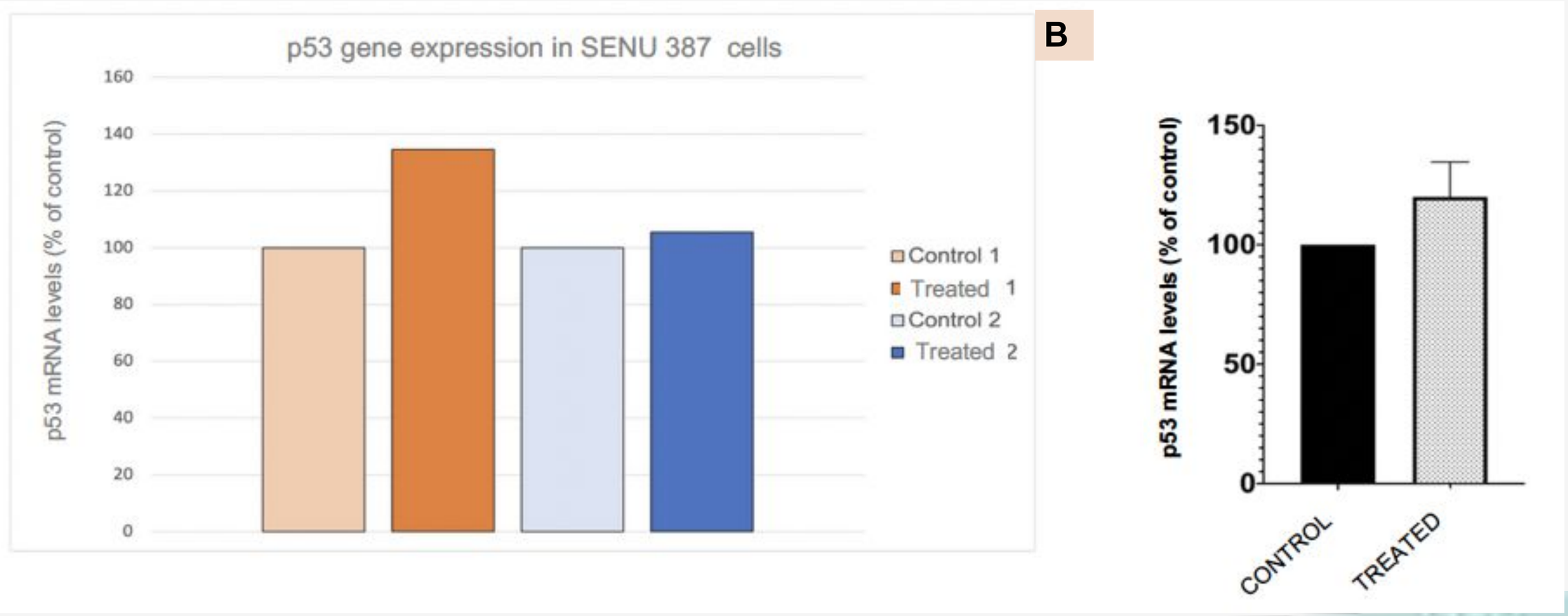
5.-RESULTS

Table A. RNA concentration in NMD 14-treated and untreated SNU 387 SNU 387 cells

SAMPLES	RNA CONCENTRATION
Control 1 (C1)	4.184,8 ng/µl
Treated 1 (T1)	4.601,3 ng/µl
Control 2 (C2)	1.882 ng/µl
Treated 2 (T2)	1.806,9 ng/µl

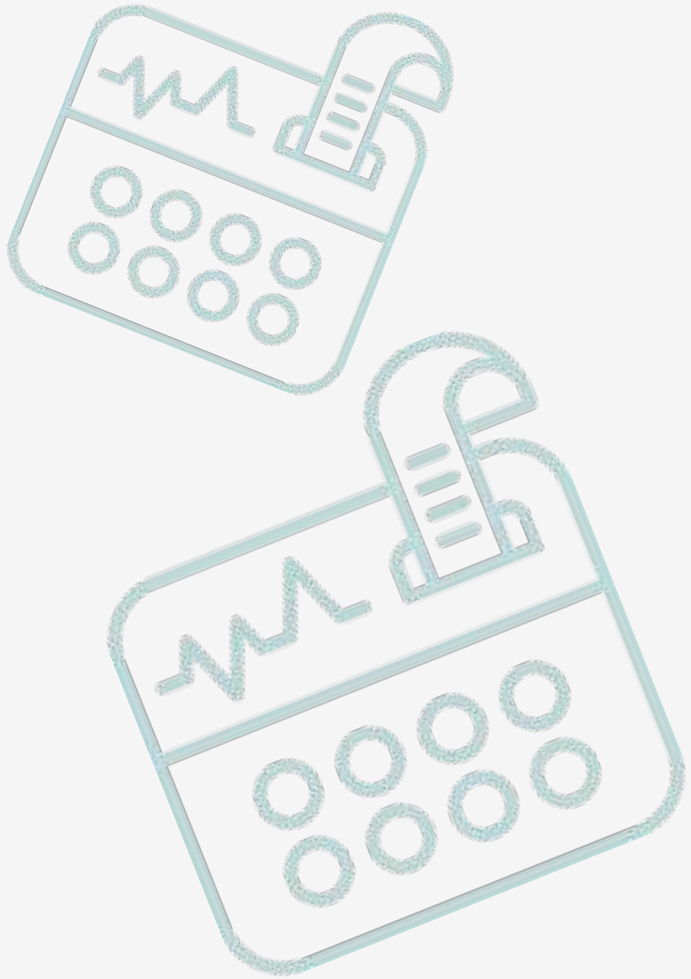
Table (A) shows the concentration of RNA obtained from NMDI14-treated SNU387 cells (T1 and T2) and their respective controls (C1 and C2)

5.- RESULTS



Graph 1. (A) Shows the *p53* mRNA levels in each of the samples, represented by the percentage in the samples of treated cells with respect to their respective control. (B) **shows the mean (of the replicates) of *p53* mRNA** levels in both treated cells (T1 and T2) and controls (C1 and C2).

6.-CONCLUSION



We can conclude that NMD inhibition has the potential to increase the expression of the p53 gene whose role in controlling cell proliferation could help reduce the growth of a hepatocarcinoma cell line.

7.- Acknowledgments

Acknowledgments

Finally, we would like to thank all the people and institutions that have made this project possible.:

- To researcher Betsaida Ojeda Pérez
- To the coordinating teacher Elena León Rodríguez
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- To the group GC-30 Molecular Hepatology and to IMIBIC
- To the ISCIII project (ERDF/ESF, "Investing in your future"; PI20/01301, FI21/00141, DTS22/00057, PI23/00652) co-funded by the European Union, JdA (PEMP-0036-2020, BIO-0139).

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