# MDM4 POLYMORPHISMS ASSOCIATED WITH AN INCREASED RISK OF ACUTE LYMPHOBLASTIC LEUKAEKMIA IN CAUCASIAN POPULATIONS

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## Abstract

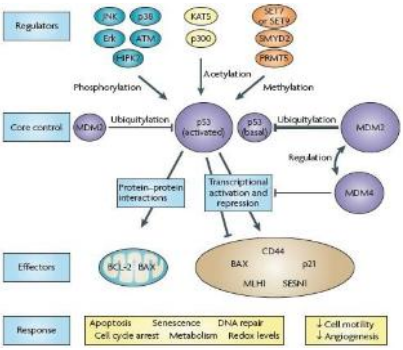
Acute lymphoblastic leukemia (ALL) is a well-known malignant disorder affecting both children and adults. Although the therapeutic limits have been maximized and optimized greatly, a better understanding of ALL is required for further improvements as prognostic value and cancer risk, to name a few. The genetic background has been revealed as critical to further improve the prognosis and diagnosis of ALL and other multiple malignant disorders. Hence this project proposes to study the individual genetic polymorphisms in Mdm4, a downstream gene in the P53 pathway in order to determine the susceptibility to this affection in the Caucasian population in Barcelona, Spain.

## Introduction

**Multiple studies** of the p53 pathway elements - being the most relevant the ones regarding p53 and its negative regulator MDM2 - have proven useful to assign **targets for ALL therapy** and to broaden the understanding of this cancer. Interestingly, the role of **polymorphic variants** in the p53 pathway genes has been significantly **related with cancer susceptibility.**

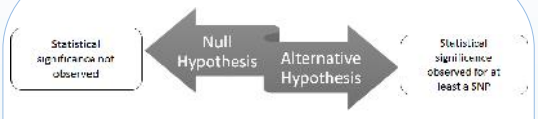
The **p53 pathway** is essential to mediate **cellular stress responses** such as DNA damage, hypoxia and aberrant proliferation. **P53**, a well-characterized transcription factor, is the **core regulator** to initiate cell-cycle arrest, apoptosis, senescence and DNA repair. Under no cellular stress conditions, p53 is usually **repressed** by its direct negative regulator: **MDM2**, which **binds** to p53 and **targets it for degradation** via ubiquitination. **MDM4** represses p53 by binding too, inhibiting p53 transcriptional activity while inhibiting MDM2 degradation. MDM4 has been reported to have an astonishing **structural similarity** with MDM2. Likewise, it seemed fairly reasonable to use MDM2 as an **antecedent** to further compare with MDM4.

Further studies have shown the **correlation** between specific **genetic variants and ALL susceptibility** in *Mdm2* and *p53* but the correlation between *Mdm4* genetic variants and ALL has **yet to be reported**



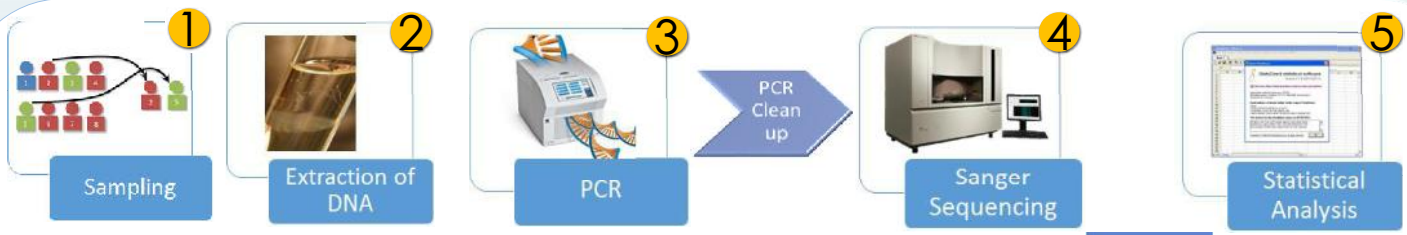

**The p53 pathway.** Reference: *Nat Rev Cancer © 2009 Nature Publishing Group.*

## Hypotheses and Objectives




- To determine which, if any, ***Mdm4*** **SNPs** are the ones responsible for an **increased ALL risk** in population according to **statistical significance** and thus to provide, if possible, **new targets** for further cancer **susceptibility studies**.
- To provide more clarity regarding future polymorphism association studies, using a **non-neutral haplotype procedure** to test the possibility of **selection pressure**, providing more valuable information and taking into account **SNPs clusters linkage disequilibrium** as it might have **concealed the real SNPs association**.
- To broaden the knowledge regarding *Mdm4* as one of the most relevant **negative regulators in the p53 pathway** and its role regarding **tumor susceptibility**, with multiple applications in modern **pharmacogenomics**.

## Material and Methods




Sources of the mouthwash samples:
(i) the Caucasian (CEU) population of the **International Hap Map project** and (ii) Barcelona´s **anonymous donors** and (iii) **ALL cancer patients** from Barcelona´s Hospital Vall D'Hebron.

It is mandatory to hand them an **Informed Consent Statement** according to the **Committee on Bioethics**

It has been determined that **60** samples from each group are necessary for this project, including **20 trios** from the CEU population of the Hap Map project.

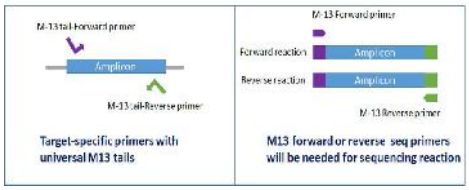
DNA extraction is required using the **phenol-chloroform protocol**.

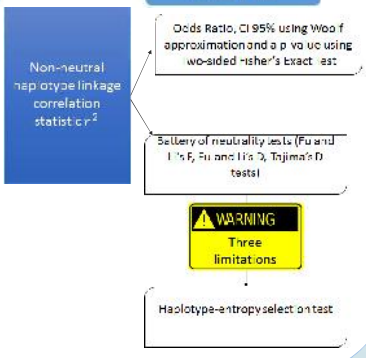
The DNA samples will be amplified in **GeneAmp® PCR System 9600** thermo cycler. To estimate the annealing temperature, **Tm Calculator** web tool is required.

The product will be genotyped by Sanger's method using **the Big Dye® Terminator v3.1 DNA Cycle Sequencing Kit** on a **3730XL DNA Analyzer.**

The primers used were selected using **Primer Designer™ Tool** including an **M-13F 5' TGTAAAACGACGGCCAGT / M-13R 5' CAGGAAACAGCTATGACC** tail to **validate** the sequence by having **double coverage** from either side.

Moreover, it may be instrumental to delineate the break points of **heterozygous insertion / deleti**ons or strong **stops induced** by difficult sequences such as an extensive **homopolymer**.



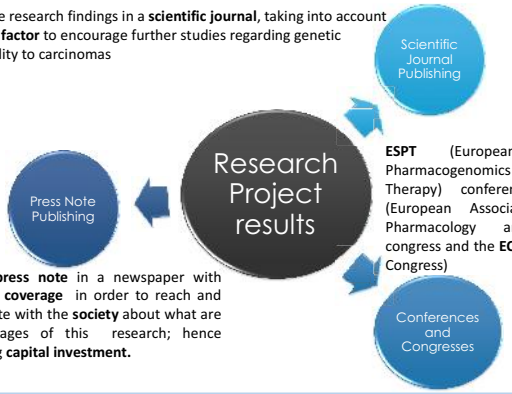

## Expected results

- The most likely result is **accepting the alternative hypothesis** and refute the null one (**rs4245739 A>C polymorphism**).
- A detailed amount of information regarding non neutral haplotypes and alleles and whether they are or they aren't **under selection pressure**.
- The study may identify **novel associated SNPs**, which can be translated into **novel susceptibility markers** for ALL and possible **targets** for further studies.

| SNP | Variants | Associated risk with | Described Function |
|---|---|---|---|
| **rs4245739** | A>C | Ovarian and Breast cancer, squamous cell carcinoma, Retinoblastoma | Creates a miR-191 target site |
| **rs1380576** | C>G | Prostate | Yet to be reported |
| **rs116197192** | C>T | Retinoblastoma | Yet to be reported |

## Diffusion plan

Publish the research findings in a **scientific journal**, taking into account its **impact factor** to encourage further studies regarding genetic susceptibility to carcinomas




Publish a **press note** in a newspaper with **nationwide coverage** in order to reach and communicate with the **society** about what are the advantages of this research; hence encouraging **capital investment.**

**ESPT** (European Society of Pharmacogenomics and Personalised Therapy) conferences, the **EACPT** (European Association for Clinical Pharmacology and Therapeutics) congress and the **ECC** (European Cancer Congress)

## References

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