9. CONCLUSIONS

The major findings of this work can be summarised as follows:

- After treatment with UCN-01, the presence of the hMLH1 protein is correlated with a functional G1-phase checkpoint, mediated through inhibition of the cdk2 kinase activity by $p27^{KIP1}$. $hMLH1^-$ cells have a not-functional G1-phase checkpoint; therefore, after UCN-01 treatment, $hMLH1^-$ cells enter the cell cycle and are driven to apoptosis. The long-term result expressed by clonogenic assay may be indicating that, after treatment with UCN-01, cell cycle arrest is likely to inhibit more effectively cell growth than apoptosis.
- In the response to CPT-11, the p53 protein seems to be a major genetic determinant. $p53^{+/+}$ cells maintain for long time a G2/M-phase arrest that is only transient in the $p53^{-/-}$ cell line. Maintenance of G2/M-phase arrest is preventing $p53^{+/+}$ cells to undergo apoptosis, while in $p53^{-/-}$ cells mitotic catastrophe and apoptosis are induced. Notwithstanding these differences in cellular reaction, both cells undergoing massive apoptosis and those making a long-term arrest showed a similar result in clonogenic survival assay. This observation was recently refined by experiments performed in our research group. Indeed, it was shown that $p53^{wt}$ cell lines are generally more sensitive to SN-38 than $p53^{mut}$ cells in long-term clonogenic assay.

Cell cycle arrest and apoptosis are two processes affecting the sensitivity and/or cytotoxicity to the chemotherapeutic agents UCN-01 and CPT-11. It is shown in this work that cell cycle arrest may be a more effective response and lead to inhibition of cell growth more successfully than apoptosis. The present work indicates, that when isogenic cell lines with or without intact cell-cycle checkpoints are compared, the more rational means to obtain information about the effect of anticancer agents is the employment of assays that distinguish between cell cycle arrest and cell death.

The present data clarify the effects of p53 or of hMLH1 on arrest and apoptosis after treatment with UCN-01 or with CPT-11 in isogenic cell systems and suggest possibilities of rational manipulation of the cellular responses of tumors with a known set of lesions.