Introduction: Acute Lymphoblastic Leukaemia (ALL) is a malignant disease characterized by an accumulation of early lymphoid precursors in bone marrow, and can affect both linages, B and T cells (B-ALL and T-ALL). NOTCH signalling plays a significant role in cell fate decision during development, stem cell self-renewal and differentiation in haematopoiesis, and activating NOTCH1 mutations are present in more than half patients with T-ALL. Therefore, modulation of NOTCH signalling pathway, for example with gamma-secretase inhibitors, might provide a new therapeutic approach in ALL. In this context, the aim of this study was to evaluate the therapeutic potential of a γ-secretase inhibitor, GSI-XXI, in two in vitro models of ALL.
Materials and methods: For this purpose, we used two different cell lines, a T-ALL (CEM) and a B-ALL (KOPN-8). These cells were incubated in the absence and presence of GSI-XXI. Cell viability and proliferation were assessed by trypan blue exclusion assay. Cell death was evaluated by optical microscopy and flow cytometry (FC) using annexin V/propidium iodide double staining and JC-1 probe. Apoptotic protein levels (BAX and BCL-2) and cell cycle distribution were also evaluated by FC. The expression levels of *CCND1*, *CCNB1*, *CCNE1* and *NF-κB* genes were determined by RT-PCR. Results were considered statistically significant when $p < 0,05$.
Results: Our results suggest that GSI-XXI reduced cell proliferation and viability in a dose- and cell type dependent manner with an IC50 at 24h of approximately 40 μM for CEM and 30 μM for KOPN-8 cells. This compound induced cell death mainly by apoptosis in both cell lines, mediated by an increase in BAX/BCL-2 ratio and a decrease in mitochondrial membrane potential. The analysis of cell cycle progression also revealed a significant arrest in G0/G1 phase in CEM cells. This analysis also showed a sub-G1 peak in KOPN-8 treated cells which correspond to DNA fragmentation a typical feature of apoptosis. Finally, GSI-XXI did not induce significant changes in the expression levels of *CCND1*, *CCNB1*, *CCNE1* and *NF-κB* genes.
Conclusions: In conclusion, if these results can be translated to clinical practice, they suggest that γ-secretase inhibitors, like GSI-XXI, might be a good therapeutic approach in acute lymphoblastic leukaemia patients.

P43. THE INFLUENCE OF FIXATION TEMPERATURE IN IN VITRO DNA ANALYSIS

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Introduction: Formalin-fixed and paraffin-embedded (FFPE) are important sources for molecular studies, namely to identify cancer-related biomarkers. Nevertheless, the quality of FFPE-obtained DNA is lower than that of fresh/frozen tissues, since fixation induces several chemical modifications. The impact of tissue fixation duration, fixative type and pH on the integrity of FFPE-extracted DNA has been the subject of several studies. It's well established that fixation using formalin for less than 72 hours allows extraction of high quality DNA. Although it is known that DNA stability is highly dependent on temperature, the influence of the fixation temperature on the quality of FFPE-extracted DNA is not understood.
Objective: Evaluate the influence of the fixation temperature in the quality of FFPE-extracted DNA through a systematic literature review.
Materials and methods: The search was performed in PubMed for studies published in English, up to December 2017. The included studies compared two or more fixation temperatures using formalin, to perform DNA analysis.
Results: 7 studies met the defined criteria. All compared room temperature (RT) with 4 °C (5 studies), 0-4 °C (1 study) or 37 °C (1 study). DNA integrity was evaluated by agarose gel electrophoresis, PCR, multiplex ligation-dependent probe amplification (MLPA) and whole gene amplification (WGA). In 5 studies the best results were obtained for fixation at 4 °C (electrophoresis, PCR and WGA); whereas RT allowed the best results in 2 cases (PCR and MLPA), one of which demonstrated that fixation at 37 °C preserved DNA similarly to RT.
Conclusions: DNA stability is highly temperature-dependent as DNAses are inhibited at low temperatures. Accordingly, it is not surprising that fixation at 4 °C allows the extraction of less degraded DNA in most studies. Nevertheless, RT seems to be also an acceptable temperature, as it allowed successful MLPA and PCR when using small DNA fragments. From these observations one may conclude that tissue fixation must be performed at 4 °C if the goal is to analyse high-molecular-weight DNA. As RT is the standard fixation temperature in diagnostic lab units, these conclusions may imply the adequate adjustments in order to prevent false conclusions from molecular analysis. Nevertheless, additional studies that analyse not only DNA integrity, but also DNA purity, yield and concentration should be done to determine the optimal fixation temperature to perform molecular analysis.

P44. THE ROLE OF RADIATION THERAPY IN PEDIATRIC POPULATION: A 5 YEAR EXPERIENCE IN AN ONCOLOGY CENTER

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Introduction: The incidence of childhood cancer has been increasing over the last years. It is estimated that approximately 43 children are diagnosed every day, and 1 out of 8 patients will not survive the disease. Depending on the diagnosis and the staging degree, treatment may include surgery, chemotherapy and radiotherapy, or an association of these. The treatment approach by radiotherapy is responsible for the improvement of loco-regional tumoral control and overall survival. Childhood cancer represents a very heterogeneous group of diseases, which suffered major treatment advances in the last years. The impact of those advances in the quality of life and survival in this population is still not completely understood. The aim of this study is to evaluate the impact of the treatment in the tumoral response, toxicity profile and overall survival of the patients.
Materials and methods: Retrospective study that included all the children treated with radiotherapy in the IPOC, between August 2012 and March 2017. Statistical assessment was calculated by IBM SPSS.
Results: This study included a total of 64 children with a median age of 9 years (range 0-17). The most common diagnosis were lymphoblastic leukemia (16%), rhabdomyosarcoma (14.1%) and Hodgkin lymphoma (10.9%). At the time of the diagnosis, only 14% of the patients presented metastatic disease, more frequently observed in the lung. The majority were treated with curative intent (88%), including adjuvant radiotherapy and chemo/radiotherapy, with 3D-conformal therapy (77%) and intensity modulated radiation therapy (14%). The early toxicities most commonly seen were radiodermatitis (34%), headaches (14%) and alopecia (12%). In the clinical and imaging assessment following the conclusion of the treatment, a partial tumoral response was the most common outcome. The median follow-up was 30 months and the overall survival was 54.3 months.
Conclusions: Overall, pediatric cancer is relatively rare; however it has shown a progressive increasing incidence in the last few decades. With the improvements in cancer treatment there has been a major growth in the population of survivors. This