YM115, A PHARMACOLOGICAL SUPPRESSANT OF SURVIVIN/XIAP, EXHIBITS ANTINEOPLASTIC ACTIVITY IN JAK2V617F CELLS

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Objective: The central role of the control of apoptosis in the pathophysiology of Philadelphia chromosome-negative myeloproliferative neoplasms (MPN) has been recently reinforced in genetic and pharmacological studies. The inhibitor of apoptosis protein (IAP) family has eight members and plays an important role in apoptosis, the most studied ones being survivin (BIRC5) andX-linked inhibitor of apoptosis (XIAP). YM155 is a small molecule with antineoplastic potential that has been described as a suppressant of survivin and XIAP. **Material and methods:** *BIRC5* and *XIAP* mRNA expression data from blood samples of healthy donors and MPN patients were obtained from GEOR2. HEL and SET2 $JAK2^{V617F}$ cells were used. YM155 with potential antineoplastic activity was used. Ruxolitinib was used as a reference drug. Cell viability was assessed by MTT assay, apoptosis by annexin V/propidium iodide labeling and flow cytometry (FC), clonogenicity by autonomous colony formation, cell cycle by propidium iodide and FC, protein expression by Western Blot analysis and gene expression by quantitative PCR. **Results:** *BIRC5* expression was significantly increased in primary myelofibrosis patients compared to healthy donors ($p < 0.05$). On the other hand, XIAP expression was reduced in MPN patients (all $p < 0.05$). In cellular models with $JAK2^{V617F}$ mutation, *BIRC5* expression was higher in HEL and SET2 cell lines when compared to normal hematopoietic cells. In a time-concentration manner, HEL cells showed great sensitivity compared to SET2 cells when exposed to the drug. IC_{50} values for HEL cells were 48.4, 1, and 0.6 μM, and for SET2 cells were 31.4, 3.8, and 3.3 μM for 24, 48, and 72h, respectively ($p < 0.05$). In both cell lines, synergic, additive, or antagonistic effects were not observed in the combination of YM155 and ruxolitinib. In HEL and SET2 cells, YM155 induced apoptosis in a dose-dependent manner being the less concentration responsible for inducing apoptosis (0.32 μM) in both cells line ($p < 0.05$). As observed in the apoptotic effect after a low exposure of YM155, both cells had a decrease in colony formation and an increase in cell death after being exposed to a higher concentration of YM155 as seen in the cell cycle where increased cells in the $subG_1$ population were observed ($p < 0.05$). Genes involved in cell cycle progression, autophagy, DNA damage, and apoptosis were modulated in HEL and SET2 cells (all $p < 0.05$). YM155 induced modulation in apoptosis, autophagy, and DNA damage proteins. In both cell lines, ULK1, SQSTM1/p62, and LC3BII (markers of autophagy) cleaved PARP1 (marker of apoptosis) and yH2AX (marker of DNA damage) were modulated. **Discussion and conclusion:** Our results indicate that survivin/BIRC5 and XIAP are differently expressed in MPN and YM155 has multiple antineoplastic effects in MPN cell models, suggesting that IAP proteins may be a point of pharmacological intervention for the treatment of these diseases. **Funding:** Supported by FAPESP, CNPq, and CAPES.

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IDOSO COM LMA MANTENDO TRATAMENTO E REMISSÃO HÁ 50 MESES: RELATO DE CASO

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Objetivos: Relatar o caso de um paciente idoso mantendo sobrevida livre de progressão de doença superior ao estudo e ao esperado segundo o estudo pivotal do protocolo utilizado. **Material e métodos:** Relato de caso retrospectivo por revisão de prontuário associado à revisão de literatura. **Resultados:** Paciente masculino de 69 anos, encaminhado após internação hospitalar para transfusão de hemácias devido a anemia severa. Na internação, apresentava anemia macrocítica severa e plaquetopenia moderada, sem alteração da série leucocitária. O paciente foi submetido a exame de medula óssea sendo diagnosticado como Leucemia Mieloide Aguda (LMA) M4-M5. Foi proposto protocolo com Azacitidina e Venetoclax, conforme estudo Viale-A, pois o paciente foi considerado inelegível para quimioterapia intensiva. Paciente tornou-se politransfundido às custas principalmente de transfusão de Hemácias, entretanto alcançou a independência transfusional ao final do terceiro ciclo do protocolo. A partir do sexto ciclo desenvolveu episódios de neutropenia, sendo reduzido a dose de Azacitidina. Uma vez que continuou a apresentar episódios de neutropenia optou-se por iniciar Peg-filgrastima, mas paciente desenvolveu como para efeito dores ósseas prejudicando sobremaneira sua qualidade de vida. Uma vez que o paciente já havia atingido a sobrevida mediana esperada para o tratamento, optou-se por suspender o Neulastim e reduzir a dose de Venetoclax, ficando com Azacitidina 50 mg/m^2 por 5 dias e Venetoclax por 21 dias, em ciclos de 28 dias, permanecendo sem mais episódios de neutropenia e recuperando sua qualidade de vida. Neste momento o paciente permanece em remissão hematológica com ótima qualidade de vida, ECOG 0, em 50 meses de tratamento. **Discussão:** A LMA compreende um grupo heterogêneo de neoplasias de células hematopoiéticas de linhagem mieloide, que interferem na diferenciação celular, levando à síndrome de falência medular. É a forma mais comum de leucemia aguda entre, sendo mais frequente quanto mais elevada a faixa etária. Pacientes com LMA geralmente apresentam sintomas relacionados a complicações de citopenias, cursando com a sintomas como fadiga, palidez, fraqueza, sangramentos e infecções. O diagnóstico de LMA requer infiltração da medula óssea, com ao menos 20% de blastos ao Mielograma ou anomalias genéticas específicas, independentemente da contagem de blastos. O

prognostico dos pacientes idosos em geral é prejudicado pelo fato de não suportarem quimioterapia intensiva e não poderem ser submetidos a Transplante de Medula Óssea, sendo atualmente as melhores alternativas as associações de Venetoclax com Azacitidina ou Citarabina. Conforme publicação suplementar da revista Blood referente as apresentações orais do ASH de 2022 o acompanhamento de longo prazo do ensaio clínico Viale-a, que avaliou a associação de Venetoclax e Azacitidina para pacientes com LMA, em um acompanhamento médio de 43,2 meses, a sobrevida global mediana foi de 14,7 meses, mesmo em pacientes com doença residual mínima indetectável a sobrevida global mediana foi de 34,2 meses. Aos 50 meses de acompanhamento menos de 20% dos pacientes que participaram do estudo estavam vivos. **Conclusão:** O caso descrito chama atenção pois o paciente em questão permanece com ótima qualidade de vida após 50 meses de seu diagnóstico, extrapolando os dados de sobrevida global do estudo pivotal de aprovação do protocolo empregado.

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AD80 HAS HIGHER POTENCY COMPARED TO CLINICALLY USED FLT3 INHIBITORS IN FLT3-ITD AML CELLS

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Objectives: Acute myeloid leukemia (AML) with *fms-like tyrosine kinase 3* (FLT3) mutations account for around 30% of all AML cases. FLT3 is a transmembrane receptor with important roles for hematological cells. The mutations will cause overactivation of signaling pathways such as PI3K, RAS, and STAT5. AD80 has been described as a multikinase inhibitor and previous data from our group has shown that treatments with AD80 decreased cell proliferation and clonogenicity, induced apoptosis, and inhibited the activation of different proteins. The present study aims to further investigate the effects of the multikinase inhibitor. **Materials and methods:** For the study, AML FLT3-ITD cell lines MV4-11 and MOLM-13 were utilized. Flow cytometry was used to analyze apoptosis and cell cycle progression. Gene expression was performed by qPCR analysis and molecular docking was performed using the softwares Autodock Vina and Gold, and viability comparison between AD80, quizartinib, and midostaurin was performed by MTT assay. **Results:** AD80 treatments resulted in an increased subG1 cell population, confirming previous findings about it inducing cell death. The apoptosis assay using chloroquine was performed to investigate the autophagy found in early results, and it showed that the inhibition of autophagy causes apoptosis. AD80 decreased the expression of genes related to cell survival such as cyclins and increased the expression of genes related to cell cycle arrest, apoptosis, and autophagy. Lastly, the molecular docking showed that AD80 binds to the FLT3 protein on the same site as the drug quizartinib. Treatments with AD80 were also more effective in reducing cell viability than quizartinib and midostaurin treatments (all $p < 0.05$). **Discussion:** Treatments as low as 0.64 nM were able to induce cell death and increase subG1 populations, evidenced by cell cycle analysis. Previous results confirmed that AD80 induced autophagy in FLT3-ITD cell lines, so chloroquine was used as an autophagy inhibitor to further investigate the process. Similarly to other FLT3 inhibitors, results showed that autophagy happens as a cell protector, hence when it is inhibited the apoptosis levels increase. qPCR confirmed what was found in the protein expression analysis from previous data, in which pathways related to cell survival and cell proliferation were downregulated and pathways related to cell cycle arrest, cell death, and autophagy were overexpressed. When it comes to the protein binding analysis, AD80 was able to form bonds with more residues in the site of action when compared to quizartinib, which could justify that AD80 was nearly twice as potent as quizartinib in both cell lines. **Conclusions:** AD80 exhibits antineoplastic effects affecting different cell processes, highlighting its potential as a treatment for FLT3-ITD AML patients. **Funding:** Supported by FAPESP, CNPq, and CAPES.

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DOENÇA RESIDUAL MENSURÁVEL POR CITOMETRIA DE FLUXO MULTIPARAMÉTRICA É VIÁVEL E DETERMINA RISCO PROGNÓSTICO DINÂMICO EM PACIENTES DE UMA COORTE BRASILEIRA DE LEUCEMIA MIELOIDE AGUDA

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