Self-presentation

1. Name and surname.

Ewa Augustin

2. Diplomas and academic degree.

Master's degree in Biology: 1986, the Faculty of Biology and Earth Science, University of Gdańsk; the title of the thesis: "Esterase's polymorphism of several national population of *Cyprinotus (Heterocypris) incongruens"*. Supervisor: Prof. dr hab. Tadeusz Sywula

Doctoral degree in Biology: 1998, the Faculty of Biology, Geography and Oceanology, University of Gdańsk, on the basis of the thesis: "The influence of biological active compounds on the cell cycle progression and programmed cell death (apoptosis) of tumor cells". Supervisor: Prof. dr hab. inż. Jerzy Konopa (Gdańsk University of Technology), reviewers: Prof. dr hab. med. Jacek M. Witkowski (Medical University of Gdańsk), Prof. dr hab. Grzegorz Węgrzyn (University of Gdańsk).

3. Professional Development.

1986 – 1998 - Chemist, Specialist at the Department of Pharmaceutical Technology and Biochemistry, Chemical Faculty, Gdańsk University of Technology.

1999 – 2000 – Assistant at Department of Pharmaceutical Technology and Biochemistry, Chemical Faculty, Gdańsk University of Technology.

2000 – 2011 – Assistant Professor at Department of Pharmaceutical Technology and Biochemistry, Chemical Faculty, Gdańsk University of Technology.

2011 – until now – Senior Lecturer at Department of Pharmaceutical Technology and Biochemistry, Chemical Faculty, Gdańsk University of Technology.

4. Scientific achievements as indicated in the Article 16, Act No. 2 of 14th March 2003 regarding Law on Academic Degrees and Title and Degrees and Title in Arts (Official Journal of Laws, No. 65, item 595, as amended):

Scientific achievement which is underlies my application for habilitation procedure is a series of publications concerning the determination of new biological and biochemical effects induced by antitumor acridine derivatives in tumor cells, in terms of their potential use in antitumor therapy.

A) Title of scientific project:

Biological and biochemical effects induced by antitumor acridine derivatives in tumor cells.

B) Authors, the title of the publication, publisher, year of publishing.

Impact factor (IF) on the base of Journal Citation Reports according to the year of publishing, with the exception of the manuscript no 7 (monograph), where IF is from years 2013/2014; MNiSW points according to the List of scientific journals, (list A) published by Ministry of Science and Higher Education (MNiSW) on 31 of December 2014.

Original papers constituting the basis of the scientific achievement:

1. **E. Augustin***, A. Moś-Rompa, A. Skwarska, J.M. Witkowski, J. Konopa. Induction of G2/M phase arrest and apoptosis of human leukemia cells by potent antitumor triazoloacridinone C-1305. *Biochemical Pharmacology,* 72 (12), 1668-1679, 2006.
   IF 3,581; 5-years IF 4,772; points MNiSW 40

2. **E. Augustin*,** B. Borowa-Mazgaj, A. Kikulska, M. Kordalewska, M. Pawłowska. CYP3A4 overexpression enhances the cytotoxicity of the antitumor triazoloacridinone derivative C-1305 in CHO cells. *Acta Pharmacologica Sinica,* 34 (1), 146-156, 2013.
   IF 2,496; 5-years IF 2,673; points MNiSW 30

3. **E. Augustin***, M. Pawłowska, J. Polewska, A. Potęga, Z. Mazerska. Modulation of CYP3A4 activity and induction of apoptosis, necrosis and senescence by the anti-tumour imidazoacridinone C-1311 in human hepatoma cells. *Cell Biology International,* 37(2), 109-120, 2013.
   IF 1,635; 5-years IF 1,543; points MNiSW 15

4. **E. Augustin***, M. Niemira, A. Hołownia, Z. Mazerska. CYP3A4-dependent cellular response does not relate to CYP3A4-catalysed metabolites of C-1748 and C-1305 acridine antitumor agents in HepG2 cells. *Cell Biology International,* 38 (11), 1291 - 1303, 2014.
   IF 1,635; 5-years IF 1,543; points MNiSW 15

5. A. Skwarska, **E. Augustin**, M. Beffinger, A. Wojtczyk, S. Konicz, K. Laskowska, J. Polewska. Targeting of FLT3-ITD kinase contributes to high selectivity of imidazoacridinone C-1311 against FLT3-activated leukemia cells. *Biochemical Pharmacology,* 95(4), 238 - 252, 2015.
   IF 4,650; 5-years IF 4,772; points MNiSW 40

6. **E. Augustin***, A. Skwarska, A. Weryszko, I. Pelikant, E. Sankowska, B. Borowa-Mazgaj. Antitumor triazoloacridinone C-1305 inhibits FLT3 kinase activity and potentiates apoptosis in mutant FLT3-ITD leukemia cells. *Acta Pharmacologica Sinica*, 36 (3), 385 - 399, 2015.
   IF 2,496; 5-years IF 2,673; points MNiSW 30

Monograph:

7. Z. Mazerska, **E. Augustin*,** A. Składanowski, M. Bibby, J. A. Double, J. Konopa. C-1311. Antineoplastic. *Drugs of the Future,* 23 (7), 702-706, 1998.
   IF 0,253; 5-years IF 0,46; points MNiSW 15

   * corresponding author.

Total Impact factor: 16,746

Total 5-years Impact Factor: 18,436

Total number of points awarded by the Ministry of Science and Higher Education (MNiSW): 185

Individual contribution of the author to each of the articles written by more than one person is described in the Appendix No. 3 (The list of achievements in scientific and research work, scientific collaboration, teaching and popularization of science). Declarations of all the co-authors, stating their individual contribution, can be found in the Appendix No. 5.

C) Description of the scientific objectives and results presented in the papers forming the basis of the scientific achievements.

In the papers being the background of my scientific achievement I made an attempt to explain the biological and biochemical mechanism of action of antitumor acridine derivatives, synthesized at Department of Pharmaceutical Technology and Biochemistry at Gdańsk University of Technology, in tumor cells.

Introduction.

Malignant tumors in Poland are the second, after cardiovascular diseases, cause of death, and treatment of cancer is still one of the most difficult problems of modern medicine and its greatest challenge. Failures in the treatment of cancers arise, among others, from not fully known causes of this disease, no significant difference between cancer and normal cell, and the relatively low effectiveness of chemotherapeutic agents. Therefore, it is still searching for compounds with high antitumor activity, while reduced toxicity, which could make a significant contribution to improving the efficacy of these extremely complicated and insidious in their nature diseases. According to R. Weinberg and D. Hanahan, the main features of tumor cells are: sustaining proliferative signaling, evading growth suppressors, avoiding apoptosis, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis [1]. 10 years later, the same authors added 4 features of tumor cells more: genome instability, avoiding immune destruction, deregulation cellular energeting, and as enabling characteristics, tumor-promoting inflammation [2]. In the case of developing cancer, order of appearance of these changes is different, in addition, not all of which must appeared in order to developed the disease. Furthermore, due to the polymorphism and different expression of enzymes metabolizing xenobiotics and proteins controlling the cell cycle, patients respond differently to an applied chemotherapeutic. Therefore, still did not manage to get a drug that would be effective against most cancers. Thus, the goal of modern tumor therapy is to design the drug not only with a high therapeutic potential, but also with increased efficiency and pointed directly at the tumor cells, which included differences between individual patients. Targeted therapy uses the newly known mechanisms of proliferating cancer cells and works by selective inhibition of tumor growth without affecting normal cells.

In the Department of Pharmaceutical Technology and Biochemistry, the studies for the search for potential anticancer drugs among acridine derivatives are conducted from many years. Due to the central role of DNA in the control of structure and function of normal and cancer cells, particularly valuable group of drugs are compounds disrupting its proper functioning. These compounds may interact with DNA by intercalation, by non-covalent binding into grooves or by

formation of covalent bonds, mono- or bifunctional [3, 4]. These studies led to receive the first and so far the only one original Polish anticancer drug called Ledakrin (Nitracrine[R], C-283) [5, 6], which was introduced to the clinic in 1976 for patients with ovarian cancer, peritoneal and certain types of skin cancer. However, due to the high toxicity, mutagenic potential and side effects [7, 8], it was relatively quickly withdrawn from clinic. Further studies conducted at the Department of Pharmaceutical Technology and Biochemistry led to receive the next groups of acridine derivatives: imidazoacridinones, triazoloacridinones and 4-methyl-1-nitroacridines.

The development of a new, high active anticancer agents raises the questions about their mechanism of action, what is very important for the rational design of individual therapy, currently under of great interest. Furthermore, this kind of the research allows to point out for the possibility of modifying the structure of these compounds in order to obtain new derivatives with improved antitumor properties. Therefore, selected derivatives from imidazoacridinones (C-1311), triazoloacridinones (C-1305) and 4-methyl-1-nitroacridines (C-1748) are the subject of my studies whose goal is to explore and explain the biological and biochemical mechanism of action of these compounds against tumor cells.

The short description of studied compounds which are the subject of presented studies.

The derivative C-1311 (5-diethylaminoethylamino-8-hydroxyimidazoacridinone, Figure 1) is the most active compound among imidazoacridinones. It was selected on the base on extended preclinical studies and as Symadex[TM] reached phase II clinical trials and showed antitumor activity against malignant breast cancer in patients previously treated with anthracyclines and taxanes [9, 10]. It was show, that C-1311 binds to DNA non-covalently by intercalation and following metabolic activation covalently crosslinks DNA strands [11]. Moreover, C-1311 is topoisomerase II inhibitor and is able to stabilize cleavable complexes of this enzyme [12]. In 1998 we wrote a monograph on biological activity and biochemical mechanism of action of C-1311 derivative, discussed below, which I am a co-author.

Z. Mazerska, E. Augustin*, A. Składanowski, M. Bibby, J. A. Double, J. Konopa. C-1311. Antineoplastic. *Drugs of the Future,* 23 (7), 702-706, 1998.

The most important pharmacological properties of C-1311 described in this work were: 1) preferential cytotoxic and antitumor activity against solid tumors, including xenograft of human colon carcinoma HT29 in nude mice; 2) inability of induction of oxygen free radicals (ROS) suggesting lack of cardiotoxic properties; 3) rapid accumulation in tumor cells, especially in the nucleus; 4)

induction of apoptosis in tumor cells (L1210, HeLa) without irreversible inhibition of DNA synthesis; 5) high activity against MDR expressing cell lines; 6) low mutagenic potential.

High antitumor activity exhibited also structural analogs of imidazaocridinones – triazoloacridinones. The derivative C-1305 (5-dimethylaminopropylamino-8-hydroxytriazoloacridinone, Figure 1) is the most active compounds among triazoloacridinones and was selected for the extended preclinical trials. Triazoloacridinones similarly to imidazoacridinones, possess pharmacophoric 5-diaminoalkyl group in the side chain and the hydroxyl group at the position 8 of the heterocyclic ring whose presence is essential for high biological activity of these derivatives [13, 14]. Triazoloacridinones exhibit high cytotoxic activity towards many tumor cell lines *in vitro* (NCI screening system, Bethesda, USA), as well as high antitumor activity against several experimental tumors in mice, especially leukemias or colon [14]. Triazoloacridinones, like imidazoacridinones, intercalate to DNA and covalently crosslink DNA strands [15]. C-1305 stabilizes cleavable complexes of topoisomerase II with DNA, but in contrast to other topoisomerase II poisons, C-1305 has a strong anti-proliferative effect against cells lacking functional poly(ADP-ribose) polymerase1 (PARP1), involved in DNA repair [16, 17]. Moreover, binding to DNA, C-1305 induces unique structural changes within a DNA sequence comprising guanine triplets [16, 17], what results in diminished affinity of the proteins TRF1 and TRF2 to telomeric DNA [18].

The third group of antitumor agents developed in the Department of Pharmaceutical Technology and Biochemistry are 4-methyl-1-nitroacridine derivatives [19]. The derivative C-1748 (9-[(2'-hydroxyethylamino)-4-methyl-1-nitroacridine] (Figure 1) exhibits high antitumor activity against prostate, colon, kidney, melanoma xenografts in nude mice [20, 21] and lower toxicity compare to other 1-nitroacridines derivatives [22, 23, 24]. C-1748 covalently binds to DNA [25] and after metabolic activation induces DNA crosslinking in human prostate cancer, ovarian, colon and leukemias, which is important for its biological activity [26]. Currently, this compounds is prepared for the first phase of clinical trials and undergoing extensive preclinical studies conducted in the Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, as well as in other laboratories.

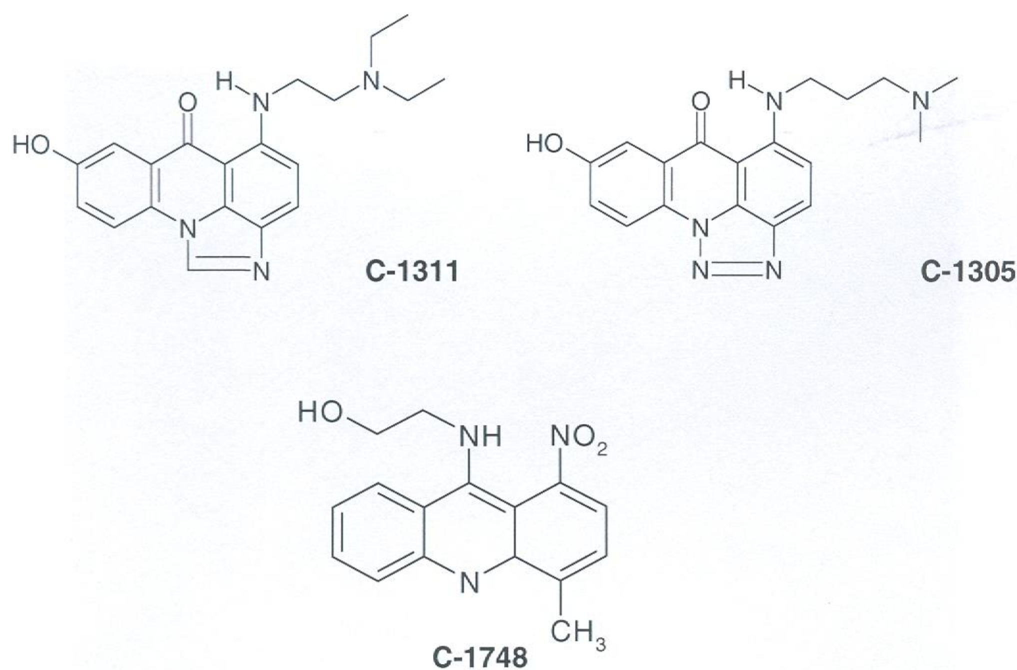

Figure 1 Chemical structures of studied derivatives: imidazoacridinone, C-1311 [27], triazoloacridinone, C-1305 [28] and 4-methyl-1-nitroarcidine, C-1748 [19].

The aim of my research was to determine the biological and biochemical mechanism of action of antitumor acridine derivatives against tumor cells. To achieve this goal I conducted research in three directions leading to:

1. Understanding the impact of selected acridine derivatives on the cell cycle progression of tumor cells and determination of the ability of studied drugs to induce apoptosis taking into account the interrelationships between cell cycle inhibition and apoptosis.
2. Examination whether expression of isoenzyme cytochrome P450 genes (*CYP3A4*) affects the cell cycle progression and final cellular response (apoptosis, necrosis, mitotic catastrophe, cellular senescence) induced by the selected acridine derivatives in tumor cells.
3. Elucidation whether the studied acridine derivatives are able to inhibit receptor tyrosine kinase FLT3 in acute leukemia cells and whether it is important for biological activity of these drugs.

In this work I present the results of my research concerning the above-mentioned research tasks, in order to clarify the biological and biochemical mechanism of action of acridine derivatives with high antitumor activity against tumor cells.

The first of the proposed research directions concerned the impact of acridine derivatives, represented by triazoloacridinone C-1305 on the cell cycle progression of human leukemia cells and its ability to induce apoptosis in these cells.

It has been previously shown that the biological activity of the synthesized acridine derivatives is related to their damaging interaction with DNA. DNA damage may be the first step leading to cell death, which largely depends on the type of chemotherapeutic agent, the dose of the drug, duration of action or cell type [11, 15, 25, 26]. It should also be take into account that the final results of chemotherapeutic treatment will depend not only on whether in cells treated with a chemotherapeutic agent cell death occurs or not. Extremely important they are also time relations or progression of cells in the cell cycle. Considering the above facts, I raised the question whether and what changes in the cell cycle progression are induced by triazoloacridinone C-1305 and what will be the consequences for the final biological effect caused by this drug. These studied were performed in the framework of KBN Project in which I was a leader (Appendix No. 3), and the results are presented in the below publication.

E. Augustin, A. Moś-Rompa, A. Skwarska, J.M. Witkowski, J. Konopa. Induction of G2/M phase arrest and apoptosis of human leukemia cells by potent antitumor triazoloacridinone C-1305. ***Biochemical Pharmacology,*** **72 (12), 1668-1679, 2006.**

We showed that C-1305 derivative in the biological active concentrations corresponding to its EC_{90} (concentration of the drug in which proliferation of the cells are inhibited in 90%) induces transient accumulation of human leukemia cells MOLT4 and HL60 in the G2/M phase, and these cell population started to decrease after longer incubation time and fraction of hypodiploid cells are appeared (sub-G1 fraction), what indicated for DNA degradation because of apoptosis (Figure 2). It is worth to point out that sub-G1 population was significant bigger in MOLT4 cells (52% after 72 h of C-1305 incubation) than in HL60 cells (15% after 72 h of C-1305 incubation).

Induction of apoptosis by C-1305 derivative we confirmed by other tests. Obtained results showed for the first time that both human leukemia cells underwent apoptosis following C-1305 treatment at EC_{90} concentration and apoptosis run according to classical mitochondrial pathway with active caspase 3 (Figure 3). Moreover, lymphoblastic leukemia MOLT4 was more sensitive to drug treatment and apoptosis process was induced in a greater extent than in mieloblastic leukemia HL60.

Summing up, I showed for the first time that studied triazoloacridinone C-1305 induced G2/M arrest followed by apoptosis in human leukemia MOLT4 and HL60 cells what gives argument to consider this compound as a novel, potent antitumor therapeutic agent. The results have made reasonable further, wider examination of the mechanisms of action of these compounds.

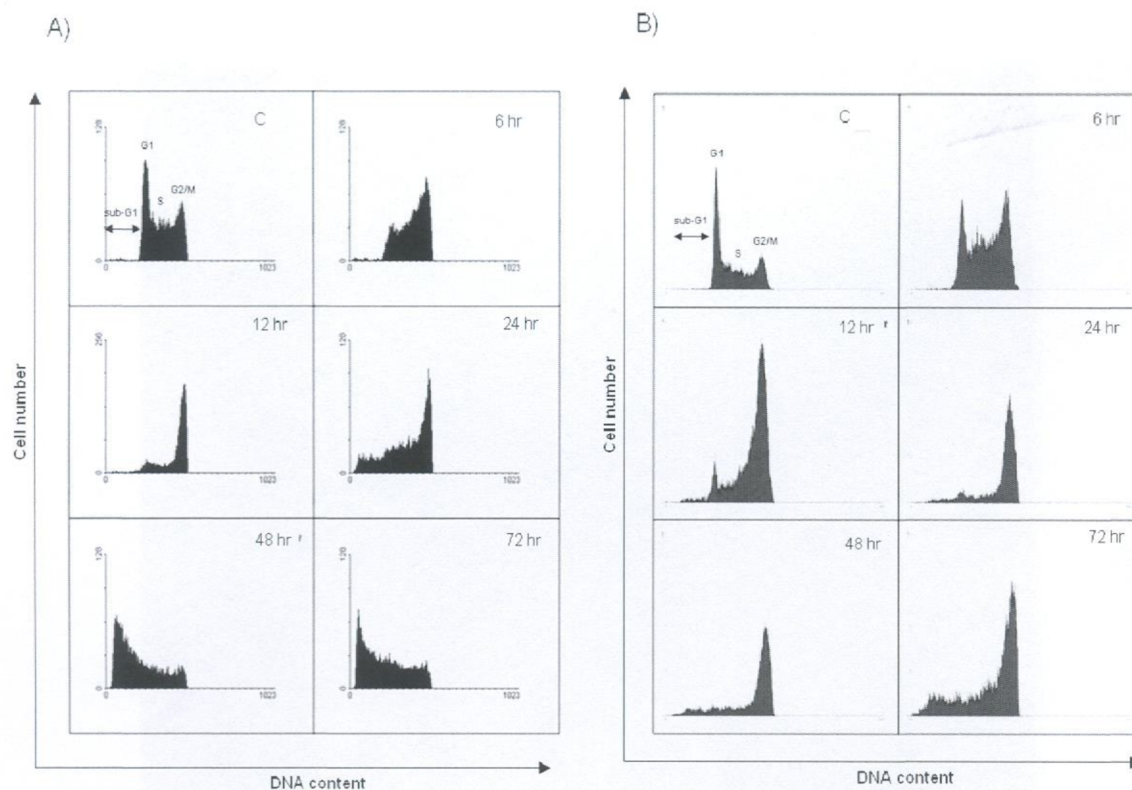

Figure 2 The changes in the cell cycle distribution of MOLT4 (A) and HL60 (B) cells treated with C-1305 at EC_{90} concentration for the time indicated [Augustin et al., *Biochemical Pharmacology,* 72 (12), 1668-1679, 2006].

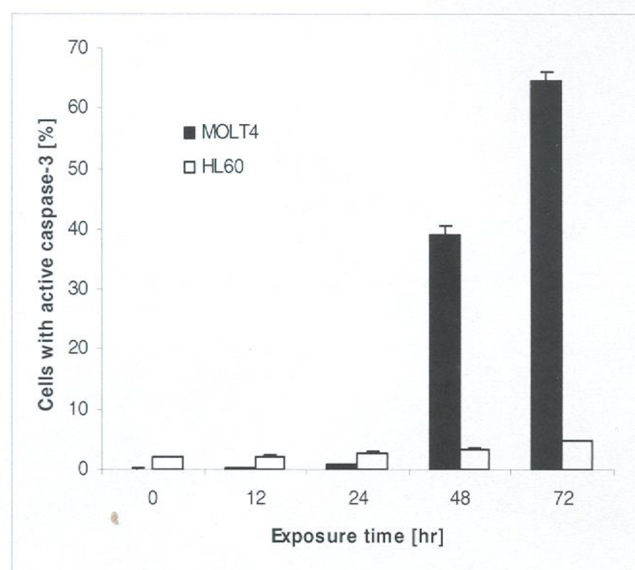

Figure 3 The number of MOLT4 and HL60 cells with active caspase 3 following C-1305 treatment at EC_{90} concentration for the time indicated [Augustin et al., *Biochemical Pharmacology,* 72 (12), 1668-1679, 2006].

An important aspect of "target therapy" is the considering of individual protein expressions which are important for physiology of tumor cells. From the point of view of antitumor therapy each patient has a different ability to metabolic transformation of drugs used in therapy.

Therefore, **the second research direction was to investigate the influence of the expression of CYP3A4 isoenzyme on the cell cycle progression and final cellular response induced by studied acridine derivatives in tumor cells.**

The majority of cancer therapeutics are metabolised in host organizm and the metabolism leads to their activation or detoxification. Metabolism activation can be responsible for the transformation of the pro-drug into its active form or for the induction or increasing the toxic effects. In turn, detoxification changes may be responsible for the reduction of toxic effects, but also for the weakening of the therapeutic effect. Therefore, the studies on drug metabolism and the activity and gene expression of drug metabolizing enzymes can play a crucial role in its final therapeutic effects. The major enzymes involved in drug metabolism, both in the activation and detoxification are the cytochrome P450 isoenzymes. [29, 30, 31]. Gene expressions of cytochrome P450 are different in normal and tumor tissue of the same organism. These facts have consequences in the effects of antitumor drug therapy, which is characterized by narrow "therapeutic window". Therefore, a priority direction of chemotherapy is currently *targeted therapy*, which takes into account metabolic inter-individual differences and differences in gene expression of metabolizing enzymes between normal and cancer tissue. There is increasing evidence that changes in the expression of metabolic enzymes of cytochrome P450 might affect the cellular response induced in tumor cells by xenobiotics, including carcinogenic and antitumor compounds [32, 33]. The obtained results concerning the influence of the changes in the expression level of cytochrome P450 proteins on apoptosis. Interestingly, expression of these enzymes may both inhibit and enhance the effect of apoptosis depending on the cytostatic compound [34, 35, 36]. In view of these facts, I undertook the attempts to investigate whether the increase level of cytochrome P450 isoenzymes can affect the type of cellular response induced by acridine derivatives in tumor cells. Results of these studies resulted in three papers, which I discussed below. I am the first author in all of them.

The aim of the first from three publications was to investigate the influence of CYP3A4 overexpression on cytotoxicity and cellular response induced by triazoloacridinone C-1305 in the model system of Chinese hamster ovary CHO cells.

<u>E. Augustin</u>, **B. Borowa-Mazgaj, A. Kikulska, M. Kordalewska, M. Pawłowska. CYP3A4 overexpression enhances the cytotoxicity of the antitumor triazoloacridinone derivative C-1305 in CHO cells.** ***Acta Pharmacologica Sinica,*** **34 (1), 146-156, 2013.**

There is a little cell lines overexpressing isoenzyme of cytochrome P450 so far. Therefore, I chose immortal Chinese hamster ovary CHO cell lines for my These cell are very easy to culture and are sensitive to triazoloacridinone C-1305. We performed our studies with the cells: wild-type CHO cells, CHO-WT, and with two recombinant cell lines: CHO-HR cells with overexpression of human cytochrome P450 reductase (CPR) and CHO-HR-3A4 cells with coexpression of human CYP3A4 and CPR. These cell lines were developed and obtained from Dr Thomas Friedberg (Biomedical Research Centre, Dundee, UK) [37]. The studies performed with the graduates revealed that CHO-HR cells overexpressing human P450 reductase exhibited the highest sensitivity to studied compound, whereas CHO-WT were the least sensitive to this drug (Table 1).

Cell line	EC_{50} [μM]	EC_{80} [μM]
CHO	**0.032** ± 0.006	**0.087** ± 0.005
CHO-HR	**0.012** ± 0.0008	**0.032** ± 0.0001
CHO-HR-3A4	**0.0099** ± 0.0041	**0.064** ± 0.0095

Table 1 Cytotoxic activity of C-1305 derivative against CHO cell lines after 72 h continuous drug incubation [Augustin et al., *Acta Pharmacologica Sinica,* 34 (1), 146-156, 2013].

Moreover, overexpression of P450 isoenzymes affected the cellular response induced by C-1305 derivative in CHO cells model system. CHO wild-type cells presented morphological features typical for mitotic catastrophe and necrosis. CHO-HR cells overexpressing human reductase P450 underwent also mitotic catastrophe and apoptosis. In turn, CHO-HR-3A4 cells coexpressing CYP3A4 and CPR underwent only apoptosis. (Figure 4).

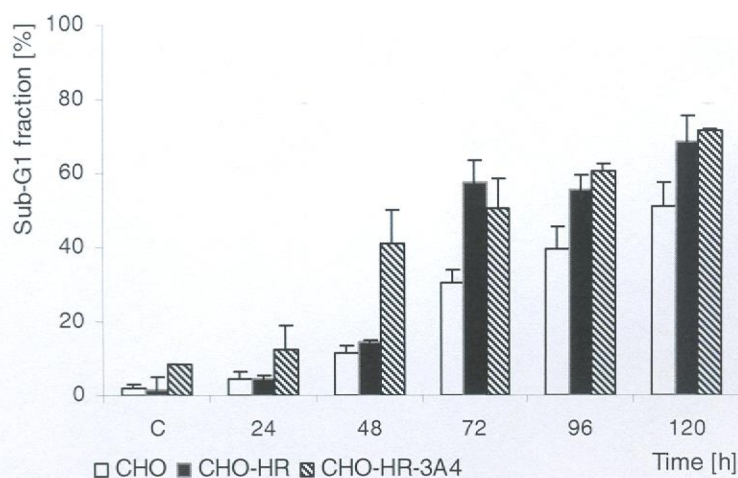

Figure 4 The percentage of wild-type and recombinant CHO cells in the sub-G1 fraction, considered as apoptotic, treated with C-1305 at IC_{80} for the time indicated [Augustin et al., *Acta Pharmacologica Sinica,* 34 (1), 146-156, 2013].

Moreover, we showed that CHO and CHO-HR cells which did not die by apoptosis, necrosis or senescence, underwent cellular senescence. CHO-HR-3A4 cells with coexpression of CPR and CYP3A4 did not undergo cellular senescence. These cells died by apoptosis following C-1305 treatment.

Summing up, we showed that different level of metabolic cytochrome P450 enzymes has a significant impact on the nature and kinetics of biological response induced by triazoloacridinone derivative C-1305 in CHO cells model system.

The aim of the next publication was to determine whether high expression of CYP3A4 isoenzyme will have an impact on cytotoxicity and the type of cellular response induced by antitumor imidazoacridinone C-1311 in human hepatoma HepG2 cells.

E. Augustin, M. Pawłowska, J. Polewska, A. Potęga, Z. Mazerska. Modulation of CYP3A4 activity and induction of apoptosis, necrosis and senescence by the anti-tumour imidazoacridinone C-1311 in human hepatoma cells. *Cell Biology International,* 37(2), 109-120, 2013.

HepG2 cell line is very often used for investigation of drug metabolism, particularly for the regulation of drug metabolising enzymes [38, 39]. However, these cells express CYP3A4 isoenzyme at low level. Therefore I used a HepG2 cell line that constitutively expresses CYP3A4 – the Hep3A4 cell line. We showed that C-1311 inhibits proliferation of both hepatoma cell lines in a dose-dependent manner, however HepG2 cells were more sensitive to C-1311 than cells overexpressing CYP3A4.

Cell cycle analysis showed that C-1311 induced transient G2/M arrest in HepG2 and Hep3A4 cells, however the population of the cells in this compartment was higher in HepG2 cells then in Hep3A4 cells. This cell cycle effect was accompanied by appearance of population with DNA lower than 2N, fraction sub-G1, which was slightly higher in Hep3A4 cells, what indicated for the cells with degradated DNA, mainly apoptotic. Induction of apoptosis by C-1311 in hepatoma cells was confirmed by other tests (Figure 5). Because apoptosis was induced only in part of hepatoma cells and on the low level and some of the cells underwent also necrosis or mitotic catastrophe, I put the hypothesis, like in CHO cells, that the cells which survived can undergo cellular senescence following C-1311 exposure. Light microscopy let to investigate the characteristic blue color in cytoplasm of senescent cells. The senescence process was induced by C-1311 in HepG2 and Hep3A4 cells, but in the latter much earlier and in larger population than in HepG2 cells.

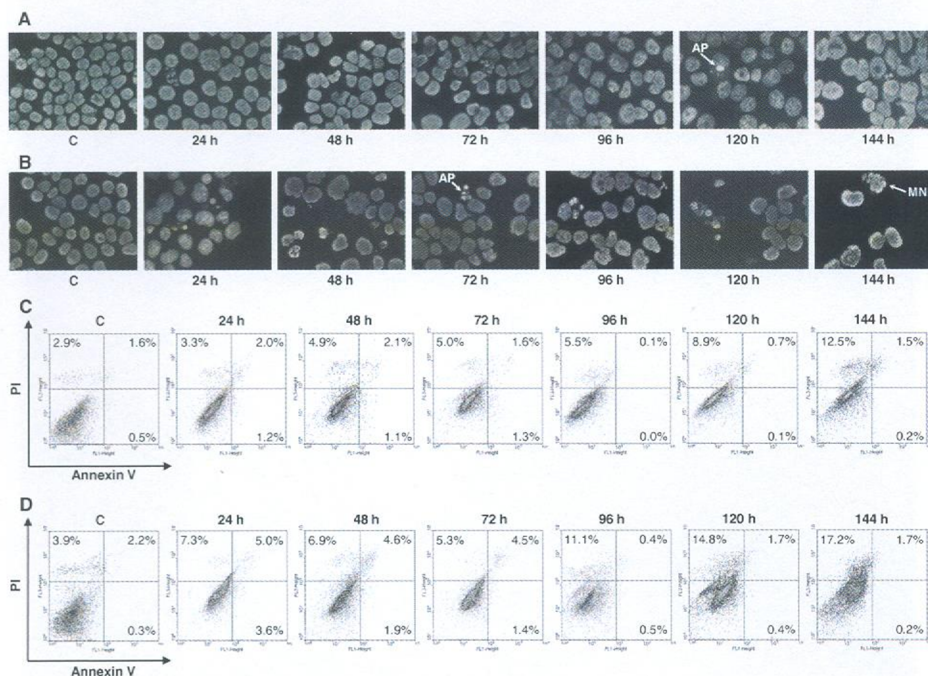

Figure 5 Analysis of nuclear morphology of HepG2 (A) and Hep3A4 (B) cells treated by C-1311 derivative for the time indicated. AP – apoptotic cells; MN – multinucleated cells characteristic for mitotic catastrophe. (C) HepG2 cells, (D) Hep3A4 cells – analysis of membrane alterations, AnnexinV/PI staining. Left bottom quadrant– viable cells (A-/PI-); right bottom quadrant – early apoptotic cells (A+/PI-); right upper quadrant – late apoptotic and necrotic cells (A+/PI+); left upper quadrant – necrotic cells (A-/PI+) [Augustin et al., *Cell Biology International*, 37(2), 109-120, 2013].

Summing up, we showed for the first time, that CYP3A4 overexpression in hepatoma cells affects, although in this cellular system to a small extent, the type of cellular response induced by imidazoacridinone derivative C-1311. HepG2 cells with normal and higher expression of CYP3A4 underwent apoptosis and necrosis, but these precesses only include a small population of cells in

both cell lines. After longer incubation time with studied compound, the cells of both cell lines underwent accelerate senescence.

In the next paper, using the same cellular system, we extended our research concerning the influence of CYP3A4 isoenzyme on the cellular response of tumor cells for another acridine derivatives: C-1748 and C-1305.

E. Augustin, M. Niemira, A. Hołownia, Z. Mazerska. CYP3A4-dependent cellular response does not relate to CYP3A4-catalysed metabolites of C-1748 and C-1305 acridine antitumor agents in HepG2 cells. *Cell Biology International,* 38 (11), 1291 - 1303, 2014.

These compounds were selected considering their different chemical structures and different metabolic pathways seen earlier in rat liver [40, 41]. In order to study the cellular effects depending on CYP3A4 overexpression, I chose HepG2 cells stably transfected with CYP3A4 isoenzyme (Hep3A4 cell line) and HepG2 cells stably transfected with empty vector (HepC34 cell line). The latter cell line is control line for Hep3A4 cells, allowing eliminate the effect of transfection on the cell growth and on the biological response after exposure for studied compounds. Cell cycle analysis revealed that C-1748 induces transient accumulation of HepC34 cells in the G2/M phase, but only after short incubation time (up to 24 h) and started from 48 h the population of cells in this phase significantly decrease. This cell cycle effect was slightly weaker for CYP3A4 overexpressing cells. After longer incubation time with C-1748 derivative, the sub-G1 population increase what indicate for induction of apoptosis in these cells (Figure 6A). Triazoloacridinone derivative C-1305 induced transient accumulation of the cells of both cell lines in the G2/M phase already after 12 h of incubation and following longer exposition (up to 144 h) the population with degradated DNA increase (sub-G1), reached 90% only in CYP3A4 overexpressing cells (Figure 7A). In control HepC34 cells this population reached hardly 18%. Cytometric analysis of membrane alteration and fluorescence microscopy confirmed the presence of late apoptotic and necrotic population following treatment with C-1748 and C-1305 (Figure 6 and 7B and C).

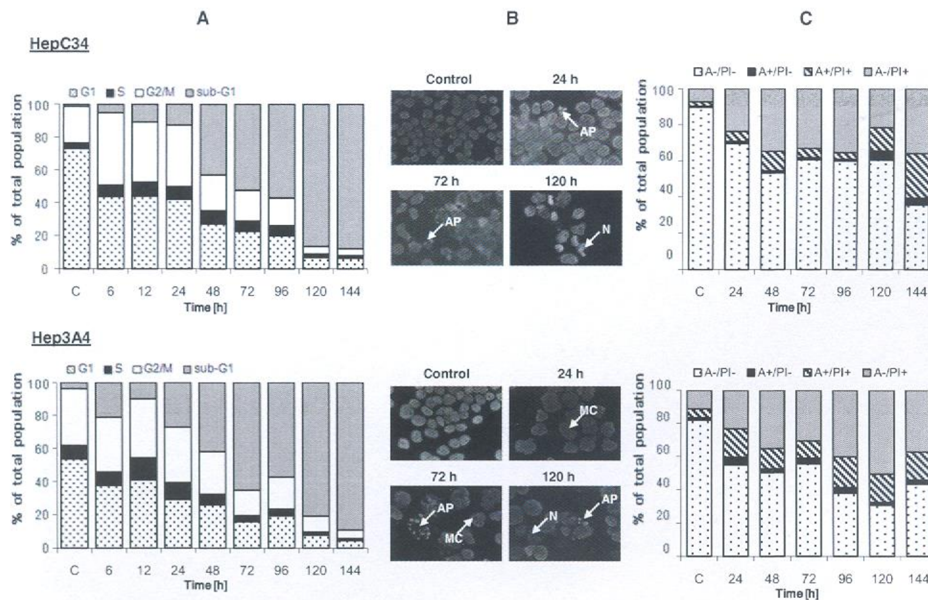

Figure 6 Cellular effects induced by C-1748 derivative in hepatoma cells. A) Distribution of HepC34 and Hep3A4 cells in the cell cycle. B) Analysis of nuclear morphology of cells stained with DAPI. C) Analysis of membrane alteration (Annexin V/PI dual staining) [Augustin et al., *Cell Biology International,* 38 (11), 1291 - 1303, 2014].

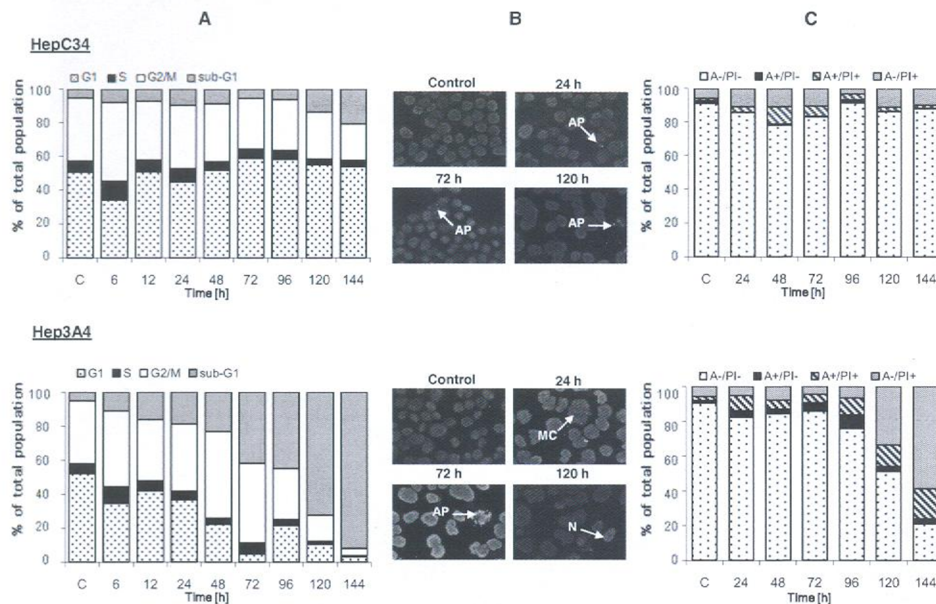

Figure 7 Cellular effects induced by C-1305 derivative in hepatoma cells. A) Distribution of HepC34 and Hep3A4 cells in the cell cycle. B) Analysis of nuclear morphology of cells stained with DAPI. C) Analysis of membrane alteration (Annexin V/PI dual staining) [Augustin et al., *Cell Biology International,* 38 (11), 1291 - 1303, 2014].

Summing up, I showed that CYP3A4 overexpression modulates cellular response induced by triazoloacridinone derivative C-1305 in hepatoma cells. Hep3A4 cells underwent apoptosis and necrosis in a greater extent than control HepC34 cells. These cells which did not die following C-1305

treatment, underwent cellular senescence. In turn, C-1748 derivative induced apoptosis and necrosis in CYP3A4 overexpressing cells as well as in control HepC34 cells at the same level. So, I investigated that changes in CYP3A4 expression modulate C-1305-induced final cellular response in human hepatoma cells, despite the fact that CYP3A4 did not influence C-1305 metabolism. In contrast, C-1748 metabolism was influenced by CYP3A4, but has no significant impact on the cellular response induced by this drug in HepG2 cells. This result indicates that CYP3A4 overexpression affects cellular response in a way other than directly through metabolism.

In the third stage of the study I returned to human leukemia cells in order to determine the ability of acridine derivatives to inhibit activity of receptor tyrosine kinase FLT3. Recent studies in cell-free kinase assays revealed that imidazoacridinone C-1311 demonstrated low nanomolar inhibition (8-15 nM) of recombinant FLT3 kinase [45]. Therefore, I put the hypothesis that FLT3 kinase can be a new molecular target for studied acridine derivatives.

Thus, **the third line of research was to determine the ability of compounds C-1311 and C-1305 to inhibit the tyrosine kinase FLT3 activity in acute leukemia cells and possible role of this process for biological activity of these compounds.**

Receptor tyrosine kinases (RTK) play a central role in signal transduction by running multi-enzymes signaling pathways. The regulation of activity of these kinases is essentials for the proper functioning of all cells. Oncogenic activation of RTKs caused by mutation, deletion or translocation in the genes of tyrosine kinases may lead to the constitutive activation of these enzymes, resulting in uncontrolled proliferation and cell growth. It is estimated that one third of patients with acute myeloid leukemia (acute myeloid leukemia) has a constitutively active mutant form of FLT3 receptor tyrosine kinase (FMS-like tyrosine kinase-3), which is associated with low survival predictions in the treatment of this type of pathologies [42]. FLT3 kinase is a member of the class III tyrosine kinase. Expression of *FLT3* gene is detected in normal, progenitor of hematopoietic cells and in lymphopoietic cells of thymus and lymph nodes [43, 44]. Internal tandem duplications (ITD) in the juxtamembrane domain of FLT3 are the most frequent mutation in *FLT3* gene and what I mentioned above, this mutation is detected within 20-30% of patients with AML [42]. The second type of mutation occurs relatively rarely and there are point mutations within the activation loop of the FLT3 tyrosine kinase domain, which led to change aspartate for tyrosine (D835Y) and was detected in 5-10% of patients with AML [43]. In the light of mentioned above informations, receptor tyrosine kinase FLT3 has become a promising molecular target, especially in view of the observed resistance of AML leukemias for FLT3 inhibitors in clinical trials. Therefore, the aim of the studies which I conducted as part of the Project KBN in which I was a Leader (Appendix No. 3) included answer on

the question whether imidazoacridinone C-1311 and triazoloacridinone C-1305 have the ability to inhibit activity of receptor tyrosine kinase FLT3 in selected human acute leukemia cell lines and how this inhibition affects the proliferation, signal transduction and induction of cell death of acute leukemia cells with normal and constitutively active kinase FLT3. Obtained results are the subject of two publication in which I am the first or the second author, and I will present below.

A. Skwarska, E. Augustin, M. Beffinger, A. Wojtczyk, S. Konicz, K. Laskowska, J. Polewska. Targeting of FLT3-ITD kinase contributes to high selectivity of imidazoacridinone C-1311 against FLT3-activated leukemia cells. *Biochemical Pharmacology,* 95(4), 238 – 252, 2015.

We used a panel of human leukemic cell lines: MV-4-11 and MOLM13 (FLT3-ITD) cells with constitutively active mutated FLT3, RS-4-11 and HL60 (wt-FLT3) with wild-type FLT3 in order to determine the influence of C-1311 to inhibit activity of FLT3. We showed that C-1311 inhibited phosphorylation of both wild-type and ITD-mutated FLT3 kinase during 3h of drug treatment. However, after 24 h of C-1311 exposure, its inhibitory potential was much more pronounced in FLT3-ITD cells, especially in homozygous MV-4-11 cells (IC_{50} ~0.6 μM) compared to heterozygous MOLM13 cells (IC_{50} 1 μM). Moreover we investigated that C-1311-mediated inhibition of FLT3-ITD activity resulted from inhibition of FLT3 autophoshorylation, but does not result either from decrease *FLT3* transcription or from reduced expression of FLT3 receptor on cellular surface (Figure 8).

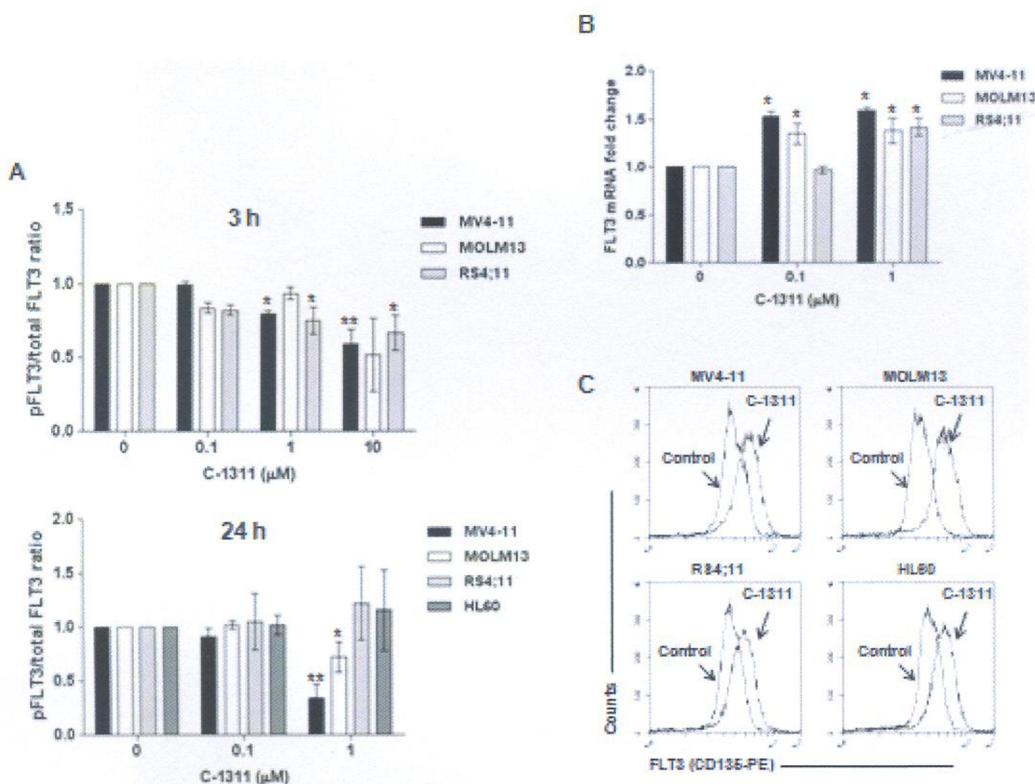

Figure 8 Inhibition of FLT3 autophosphorylation by C-1311 in human acute leukemia AML cells. A) Phospho-FLT3 level (pTyr591) was determined using ELISA and normalized to total level of FLT3. The level of FLT3 phosphorylation was expressed as a fold change relative to untreated controls (equal to 1). B) Expression level of *FLT3* mRNA in MV-4-11, MOLM13 i RS-4-11 cells following 24 h drug exposure. RT-PCR, normalized to *GDPH* mRNA levels. C) Representative flow cytometry histograms of cell surface FLT3 receptor expression. [Skwarska et al., *Biochemical Pharmacology,* 95(4), 238 – 252, 2015].

Activation of FLT3 kinase results in phosphorylation of downstream effector kinases in signaling pathways such as MAPK (*ang. mitogen-activated protein kinase),* that subsequently regulate cell growth of hematopoietic cells. In addition, FLT3 is involved in Akt pathway (*ang. protein kinase B)*, which is a key protein in phosphorylation of trancription factor FOXO, resulted in inhibition of apoptosis. Further, we investigated whether observed FLT3 inhibition in aculte leukemia cells AML were correlated with inhibition of mentioned above signaling pathways. Additionally, we used human leukemia cells U937 and CEM that do not express FLT3 (FLT3-negative). We showed that, independly of FLT3 status, C-1311 inhibited phosphorylation of MAPK having no influence on protein expression. In turn, C-1311 treatment decreased Akt phosphorylation and overall expression of this protein especially in FLT3-ITD mutated leukemia cells. What is very important, C-1311 inhbited MAPK and Akt signaling pathways in concentrations caused inhibition of FLT3 autophosphorylation.

Next, we studied the infuence of C-1311 on the cell cycle progression of AML cells, at concentrations responsible for FLT3 inhibition. MV-4-11 cells accumulated in G0/G1 phase for the

first 24 h of C-1311 treatment, whereas MOLM13 cells accumulated in G2/M phase. Transient accumualtion in G2/M phase underwent also RS-4-11 cells (FLT3-wild type). On the other hand, we did not observe any significant changes in the cell cylce progression of HL60 (FLT3 wild-type) and U937 (null FLT3) cells after 24 h of C-1311 incubation. Further we showed, that C-1311 induced typical for apoptosis changes in nuclear morphology of MV-4-11 and MOLM13 FLT3-ITD cells, and in a limited extent in RS-4-11 and HL60 cells (FLT3 wild- type) cells and U937 and CEM cells (null-FLT3). Moreover, HL60 cells exhibited features typical for mitotic catastrophe. Moreover, C-1311-induced apoptosis involved caspase 3 activation and mitochondria. We investigated that C-1311 down-regulates expression of *bcl-2* and *survivin* in leukemia cells carrying FLT3-ITD mutation (MV-4-11 and MOLM13), having no impact on transcription of these genes in wild-type FLT3 cells and with cells without FLT3. Moreover, silencing of *FLT3-ITD* decreases activity of C-1311 and enhances leukemia cell survival (Figure 9). Obtained results confirms that FLT3 inhibition by C-1311 is crucial for high antitumor activity of this drug against leukemia cells carrying FLT3-ITD mutation.

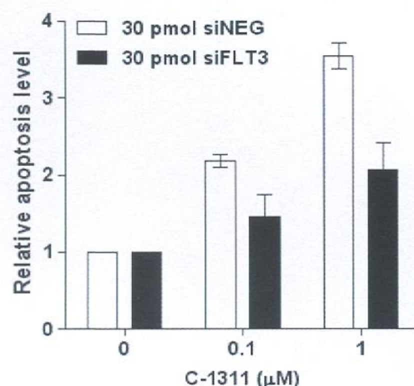

Figure 9 The infuence of silencing FLT3 by siRNA on apoptosis induced by C-1311 in MV-4-11 cells (FLT3-ITD). [Skwarska et al., *Biochemical Pharmacology,* 95(4), 238 – 252, 2015].

It is well documented, that prolonged exposition for FLT3 inhibitors can lead to resistance. Hence, targeting of FLT3 in combination with cytotoxic therapy may significantly improve clinical outcome [46]. Thus, we dermined the effect of C-1311 on leukemia cells proliferation in combination with cytarabine or doxorubicin. We used MV-4-11 and RS-4-11 cells and also, U937 cells without FLT3. Combination of C-1311 with cytarabine or doxorubicin showed distinct synergistic activity in FLT3-ITD positive cells.

Summing up, imidazoacridinone C-1311 is very effective inhibitor of receptor tyrosine kinase FLT3 in human acute leukemia AML cells. While brief C-1311 exposure blocked wild-type and FLT3-

ITD activity, profound and sustained inhibition was achieved only for FLT3-ITD mutants. These results indicate that mutated FLT3-ITD kinase is a new, potential molecular target for C-1311.

The next paper concerning the ability of triazoloacridinone derivative C-1305, close analog of C-1311, to inhibit activity of receptor tyrosine kinase FLT3 and the resuls are presented below.

E. Augustin, A. Skwarska, A. Weryszko, I. Pelikant, E. Sankowska, B. Borowa-Mazgaj. Antitumor triazoloacridinone C-1305 inhibits FLT3 kinase activity and potentiates apoptosis in mutant FLT3-ITD leukemia cells. *Acta Pharmacologica Sinica*, 36 (3), 385 - 399, 2015.

The studies performed with human acute leukemia cells with FLT3-ITD – MV-4-11 cells, with wild-type FLT3 – RS-4-11 cells and with the cells without FLT3 – U937 cells revealed that FLT3-ITD mutated cells were the most sensitive to C-1305 (Table 2).

Cell line	Incubation time	24 h	48 h	72 h
MV-4-11	IC_{50} [μM] ±SD*	1.2 ± 0.17^{c}	$0.19 \pm 0.03^{b,e}$	$0.07 \pm 0.011^{c,e}$
RS-4-11	IC_{50} [μM] ±SD*	2.0 ± 0.9^{c}	0.38 ± 0.06^{b}	0.30 ± 0.074^{c}
U937	IC_{50} [μM] ±SD*	7.6 ± 1.6	1.90 ± 0.29	0.35 ± 0.11

Table 2 Cytotoxic activity of C-1305 against AML cells [Augustin et al., *Acta Pharmacologica Sinica*, 36 (3), 385 - 399, 2015].

ELISA test shows, that C-1305 (1-10 μM) dose-dependly inhibited the kinase activity of FLT3, which was more pronounced in the mutant FLT3-ITD cells than in the wild-type FLT3 cells. Importantly, if C-1311 inhibited FLT3 in the concentration range of 0.1 μM – 1 μM, in the case of C-1305 comparable effect was observed at concentrations ten times higher. We also showed that C-1305 dose-dependly dereased the phosphorylation of STAT5 and MAPK and the inhibitory phosphorylation of Bad,which is one of the pro-apoptotic protein [47]. This effect was observed in MV-4-11 and RS-4-11 cells, but not in the cells FLT3 negative. Then we asked the question whether the inhibition of FLT3 and its down-stream targets: MAPK, AKt, Bad by C-1305 had an impact on cell cycle progression and apoptosis of AML cells. After C-1305 treatment the population of MV-4-11 cells dereased concomitant with appearance of hypodiploid cells (sub-G1). Simultaneously, inhibition of FLT3 by C-1305 decreased level of MAPK, STAT5 or Bad and we observed activation of caspase 3, decrease in $\Delta\Psi_m$ and translocation of phosphatydilserine to outer membrane, which indicates the induction of apoptosis in these cells. Moreover, silencing of *FLT3-ITD* significantly increased survival of MV-4-11 cells after C-1305 treatment. In turn, RS-4-11 cells (wild-type FLT3) underwent a transient G2/M

arrest and sub-G1 population were relatively lower compare to MV-4-11 cells. We also observed a weaker inhibition of signaling pathways via MAPK and Akt by C-1305 in these cells than in MV-4-11. In the U937 cells (null-FLT3) C-1305 treatment resulted in transient G2/M accumulation concomitant with low level of sub-G1 population. Moreover, the levels of Akt and Bad remained unchanged and the level of MAPK dereased.

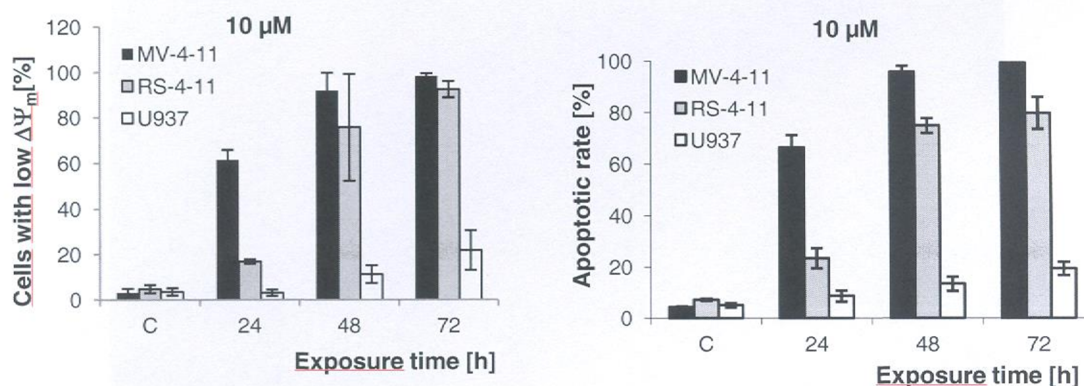

Figure 10 Induction of apoptosis by C-1305 derivative in leukemia AML cells. Left panel: reduction of mitochondrial membrane potential; right panel: the number of early- and late apoptotic cells, test Annexin V/PI [Augustin et al., *Acta Pharmacologica Sinica*, 36 (3), 385 - 399, 2015].

In conclusions, we extended the previous conclusion that receptor tyrosine kinase FLT3 can be molecular target not only for C-1311, but also for triazoloacridinone C-1305. This compound inhibited autophosphorylation of both mutated and wild-type FLT3, however more effective in mutant FLT3-ITD cells. Inhibition of FLT3-ITD resulted in induction of apoptosis and wild-type FLT3 cells being less susceptible to C-1305-induced apoptosis. Regardless of the status of the *FLT3* gene, induction of apoptosis and inhibition of signaling pathways were observed at concentrations of C-1305 responsible for its cytotoxic activity.

A summary of the results in the 3 proposed research directions.

Obtained results allow me to draw the following conclusions:

1. Triazoloacridinone derivative C-1305 induced G2/M arrest of human leukemias MOLT4 and HL60 followed by apoptosis, which involved mitochondria and caspases. The capacity of HL60 to execute apoptosis after C-1305 treatment was significantly weaker compared to MOT4 cells.

2. The level of cytochrome P450 isoenzyme (CYP3A4) had significant impact on the type of cellular response induced by triazoloacridinone derivative C-1305 in CHO cell model system. Wild-type CHO cells underwent mitotic catastrophe, necrosis and cellular senescence. CHO-HR cell with overexpression of human cytochrome P450 reductase underwent mitotic catastrophe, but also apoptosis and cellular senescence. CHO-HR-3A4 cells with coexpression of human CYP3A4 and reductase underwent apoptosis, but they did not show the features of cellular senescence.

The changes in CYP3A4 expression modulate C-1305-induced cellular response in HepG2 cells, despite the fact that CYP3A4 did not influence C-1305 metabolism. In contrast, C-1748 metabolism was influenced by increased CYP3A4 expression, but no significant impact on the modulation of cellular response was seen in these cells. This indicates for "no-metabolic" role of CYP3A4 expression in cellular response.

3. Imidazoacridinone derivative C-1311 is an effective inhibitor of receptor tyrosine kinase FLT3 in human acute leukemia AML cells. Moreover, this compound more profound inhibited FLT3 activity in FLT3-ITD mutant cells and this effect was correlated with cytotoxic activity of C-1311. Leukemia cells with mutant FLT3-ITD were twice more sensitive for C-1311 than cells with wild-type FLT3 or FLT3-negative.

Triazoloacridinone C-1305 was also FLT3 kinase inhibitor, however compare to C-1311, it inhibited autophosphorylation of FLT3 in much higher concentration. Inhibition of FLT3 potentiated apoptosis induced by C-1305, especially in mutated leukemia FLT3-ITD cells. Thus, we have shown that receptor tyrosine kinase FLT3 can be a new molecular target for searching new, active acridine derivatives.

The importance of the results.

As I mentioned above, C-1311 derivative reached phase II clinical trials and showed antitumor activity against human breast cancer. Triazoloacridinone C-1305 and 4-methyl-1-nitroacridine are qualified for extended preclinical trials. The mechanism of action of these derivatives indicated DNA damage, what is similar for that observed for clinical used chemotherapeutics. Therefore, the studies performed on the basis of this achievement concerning molecular and biochemical mechanisms of inhibition of tyrosine kinase FLT3 by imidazoacridinone C-1311 and triazoloacridinone C-1305 identified a new, more selective mechanism of action of both derivatives, what will raise their attractiveness among potential cancer drugs. Furthermore, at least

for clinical tested imidazoacridinone C-1311, our results may provide relevant evidence to begin clinical trials in patients with acute myeloid leukemia with overexpression of kinase FLT3.

In turn, the studies concerning the mechanism of action of antitumor derivatives in tumor cells depending on expression of cytochrome P450 genes, can be crucial to enhance the knowledge about potential drugs. These studies indicate how biological effect of studied compounds may change in particular patients, whose characterized by different expression of cytochrome P450 genes. Furthermore, because of increasing knowledge of differential expression of these enzymes in normal and cancer cell, may help to develop new methods of anticancer therapy directed selectively for cancer cell.

In conclusions, obtained results on the basis of this achievement broadened the knowledge about mechanisms of action of new antitumor drugs, which can be used in the design of therapy with studied compounds. This is particularly important due to recent trends chemotherapy in combination with other therapeutic methods that should be developed individually for each patient. Personalized therapy (focused on the individual patient) take into account not only the condition of the patient carrying the disease, but also the individual features of its genome, which controls its own specific response to chemotherapeutics. Obtained results may help in designing new acridine derivatives with favorable antitumor properties.

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5. Overview of other scientific achievements.

After I finished my studies at the Faculty of Biology and Earth Science at the University of Gdańsk (1986), I was employed at the Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, at first as a Chemist, then as a Specialist, from 1999 as a Assistent, Assistant Professor and from 2011 as a Senior Lecturer. From the beginning of my research I was involved in the studies concerning the mechanisms of action on the cellular level, of new acridine derivatives, synthesized in our laboratory. In the Team of Chemistry and Biochemistry of Anticancer Drugs headed by Prof. Jerzy Konopa, the search for new drugs with high antitumor activity among acridine derivatives are conducted from decades. As I mentioned above, the first polish original antitumor drug - Ledakrin (Nitracrine^{R}) was synthesized in the Department of Pharmaceutical Technology and Biochemistry, in the Team headed that time by Prof. Andrzej Ledochowski. In turn, the studies headed by Prof. Jerzy Konopa led to find among others, imidazoacridinones, among them derivative C-1311 (Symadex^{TM}), reached the phase II of clinical trials for the patients with malignant breast tumor. This compound became the first object of my research related to the mechanism of action of acridine derivatives, especially the research concerning the influence of imidazoacridinones on the cell cycle progression and induction of apoptosis in tumor cells. In 1992 I was 3 months on scientific internship in Scotland, at the University of Aberdeen, Department of Cell Pathology headed by prof. Denys Wheatley, where I conducted the research in mentioned above topics and obtained results are published in the paper in which I am coauthor (paragraph 5.1, item 1). In 1975 I had scientific internship in Gustave Roussy Institute in Villejuif in France, where I conducted the research concerning the induction of apoptosis by imidazoacridinone derivatives in tumor cells. At the same time, together with prof. Zofia Mazerska, I performed studies on structure-activity relationship of imidazoacridinones (paragraph 5.1, item 2). In 1998 I obtained doctoral degree in biology at the

Faculty of Biology, Geography and Oceanology, at University of Gdańsk. My doctoral thesis concerned the influence of imidazoacridinone derivatives and some anticancer drugs (melphalan, mitoxantrone, mitomycin C, CCNU) on the cell cycle progression of L1210, HT29 cells and on induction of apoptosis in these cells. My supervisor was prof. Jerzy Konopa.

Further, I continued the studies concerned the mechanism of action of acridine derivatives in terms of cell death. In 1999 I was a Leader in Project (KBN 1999 – 2001) related to cellular effects induced by other group of acridine derivatives synthesized by prof. Jerzy Konopa team – triazoloacridinones . In 2002 I was a Leader in other project from Polpharma related to the mechanism of action induced by triazoloacridinones of II generation – acylotriazoloacridinones. These projects resulted in two papers (paragraph 5.1, item 6 and paragraph 4, item 1 in scientific achievement). I showed that acylotriazoloacridinones, like triazoloacridinones, induced apoptosis in human leukemia MOLT4 and HL60 cells which involved mitochondria and caspase 3. Obtained results were presented also on 3 international and 4 national conferences.

At the same time I took active part in the studies on the mechanisms of action of acridine derivatives at the cellular level conducted in our Team. I worked on the project funded by a grant from the Xanthus (US), concerned the cancer cells blocked in G2 phase of the cell cycle by imidazoacridone C-1311. I am co-author in the paper related to induction of apoptosis proceeded by mitotic catastrophe by imidazoacridinone C-1311 in human leukemia MOLT4 cells (paragraph 5.1, item 3). The most important achievement of this work was that mitotic catastrophe induced by C-1311 in leukemia MOLT4 cells is not death itself, and leads to apoptosis, both caspase-dependent or indepedent. Studies on the mechanism of action of C-1311 in tumor cells in the aspect of induction of various types of cell death, they have been and are continuing in our team. In 2013 we published the paper in which I am co-author (paragraph 5.1, item 11) related to high cytotoxic activity of C-1311 against human non-small lung carcinoma cells A549 and H460. We showed for the first time, that C-1311 induced both autophagy and senescence in these cells. Our results concerned the cellular effects induced by C-1311 were presented on 5 international and 8 domestic conference. I also took part in the research related to induction of different type of cell death by 4-methyl-1-nitroacridine derivative C-1748. In the paper published in 2010 (paragraph 5.1, item 4) we showed that C-1748 at biological active concentrations induced different biological response in human colon carcinoma HT29 and HCT9 cells. HCT8 cells accumulated in the G2/M followed by apoptosis. These cells which did not die, underwent senescence. HT29 cells did not undergo senescence and they died by apoptosis in a limited extent following C-1748 treatment. They massive died by necrosis. The results were presented also on 1 international and 3 national conferences.

During the further research I started with new research project, connected with the studies described above, related to the influence of metabolic enzymes expression on cell cycle progression and the type of final cellular response induced by acridine derivatives in tumor cells. The results of research conducted within my own projects, which became part of the achievement submitted for evaluation was described in detail in Chapter 4, Section C. In other studies conducted in our team, we showed that overexpression of CYP3A4 isoenzyme in CHO cell model system had significant impact on type of cellular response (apoptosis, necrosis, cellular senescence) induced by imidazoacridinone derivative C-1311 despite the fact that this enzyme did not influence C-1311 metabolism (paragraph 5.1, item 13). Previous studies in our laboratory have shown that the triazoloacridinone C-1305 and imidazoacridinone C-1311 which were not a substrates of cytochrome P450, underwent metabolic transformation by flavin monooxygenase FMO, mainly types 1 and 3 [40, 48], as well as these compounds were the substrate of UDP-glucuronylotransferases UGT, especially form UGT1A10 [49]. In the next paper in which I am co-author (paragraph 5.1, item 8) we determined the role of isoenzyme UGT in cellular response induced by C-1305 and C-1311 in tumor cells. Our studies revealed that C-1305 and C-1311 induced capsase 3-dependent apoptosis in human KB-3 carcinoma cells. What was very important, UGT1A10 overexpression affects the cytotoxicity of C-1305, but not C-1311 against KB-3 cells. Moreover, overexpression of UGT1A10 potentiated apoptosis induced by C-1305, and decreased apoptosis induced by C-1311. Summing up, UGT1A10 isoenzyme plays a crucial role in cytotoxicity and in mode of action of triazoloacridinone C-1305 in tumor cells. Expression systems of cytochrome P450 proteins play an important role in studies of drug metabolism. Therefore, together with PhD student we wrote a paper about expression systems of cytochrome P450 proteins in mammalian cells, including human (paragraph 5.1, item 5). The studies concerned the influence of expression of metabolizing enzymes on cellular response induced by acridine derivatives were sponsored by 3 projects, in which I was and I am a principal investigator (KBN 2004 – 2006; MNiSW 2007 – 2009; NCN OPUS 5 2014 – 2017). I am also co-author of the paper related to metabolism of 4-methyl-1-nitroacridine derivative C-1748 in rat liver microsomes and in HepG2 cells (paragraph 5.1, item 7). We demonstrated in this work that metabolism of C-1748 under normoxia gave metabolites of low concentrations, whereas hypoxia stabilised high concentrations of metabolites of this compounds. I would like to mention that the results of research on the role of overexpression of CYP3A4 and UGT1A10 isoenzymes were presented on 7 national and 2 international conferences.

I was also participated in the project sponsored by NCBR concerning the development of the new original antitumor drug with new mechanism of action. I am a co-author of the patent application based on results obtained in the framework of this project (Appendix No. 3, point IIB).

At the same time within my research skills I collaborate with different scientific teams. The results of these cooperations were published in the 4 papers in which I am co-author. (paragraph 5.1, item 9, 10, 12 and 14). In the paper item 9, together with researchers from University of Virginia, US, we showed that inhibition of T-type calcium channels by mibefradil reduced cell viability and decreased resistance for therapy in glioblastoma malignant tumors. In turn, we cooperate with prof. Zbigniew Stojek from the University of Warsaw in investigating the biological properties of the conjugates of magnetic nanoparticles with doxorubicin. My contribution in this study was to examine the cytotoxic activity of doxorubicin - nanoparticles conjugates against human urinary bladder cells. I demonstrated that these conjugates exhibited much more higher cytotoxicity than doxorubicin alone. Furthermore, conjugates doxorubicin - nanoparticles induced apoptosis in tumor cells in a greater extent than doxorubicin alone (paragraph 5.1, item 10). In performing research to determine cytotoxic activity of copper complexes with substituted imidazole against lymphoma cells I cooperate with dr. Anna Dołęga from Department of Inoorganic Chemistry, Chemical Faculty of Gdańsk University of Technology. We published a paper (paragraph 5.1, item 12) and we presented our experimental data on 3 conferences, national and international. In the framework of cooperation with Grzegorz Gorczyca from the team of prof. Sławomir Milewski from our Department, I studied cytotoxic activity of chitosan-collagen-gelatin scaffolds against mice fibroblasts NIH-3T3 (paragraph 5.1, item 14).

My scientific work has been awarded several times by Rector of the Gdańsk University of Technology (Appendix No. 3, point C). I am also invited to reviewing manuscripts in: *Biochemical Pharmacology, ChemMedChem, Current Pharmaceutical Analysis, Cell Biology International, Journal of Integrative Medicine* (Appendix No. 3, point I). For many years I have been involved in research conducted by graduate students in our Department. I was informal tutor for 5 doctoral dissertations and actually I am secondary promoter of doctoral thesis of Barbara Borowa - Mazgaj (Appendix No. 3, point G).

Since 1999 I conducted teaching activity, first worked as an assistant, then assistant professor and now as a senior lectures at the Department of Pharmaceutical Technology and Biochemistry, Chemical Faculty of Gdańsk University of Technology. As part of this activity I give lectures, seminars, exercises and laboratories, as well as looking after graduate students. In 2002 I elaborated new subject for Biotechnology, bachelor degree, entitled: "Tissue culture" and I give the lectures and laboratories. I wrote a script entitled: "Some aspects of plant and animal cell cultures", collective work edited by Ewa Augustin, Gdańsk 2010. Academic Handbook. I arranged a special room for plant cell culture *in vitro* with controlled temperature, humidity and lighting, which is used by

biotechnology students. I also give a lecture for students of biotechnology, master degree, entitled: "Biology of tumor cells", and since 3 years, I gave this lectures and exercises for students of Biomedical Engineering, Chemistry in medicine. I also participate in teaching of biochemistry, laboratories and seminars for students of Biotechnology, bachelor degree. I would like to add, that I am responsible for all laboratories in the Department of Pharmaceutical Technology and Biochemistry.

Since 2000, I was a supervisor of 32 master's thesis and 19 bachelor's thesis (51 together) on specialization Biotechnology. I was also a reviewer of 66 master's thesis and 21 bachelor's thesis (87 together) on specialization Biotechnology. From 1999 to 2004 I was a tutor for students of Biotechnology and I received Awards from Rector of Gdańsk University of Technology for the "Best Tutor of the Year". I also cooperate with the Students Biotechnology Society, Gdańsk University of Technology, in the field of culture of plant and animal cells *in vitro.* In 2011 I received a National Education Committee Medal. My teaching and the organization activities is described in detail in Appendix No. 3.

In summary, all my academic achievements include:

papers - 21 (**including selected as a scientific achievement - 7**)
conference reports - **73**
patent application - **1**
Sum of the impact factor IF - **58,945** (**including selected as a scientific achievement IF - 16,746**)
Sum of MNiSW points - **605** (**including selected as a scientific achievement - 185**)
Sum of the times cited Web of Science - **168**

Sum of the times cited including database Scopus **- 186**

h-index (Index Hirsch'a): **7** (Web of Science)

5.1. List of other publications not included in the scientific achievements

1. **E. Augustin**, D.N. Wheatley, J. Lamb, J. Konopa. Imidazoacridinones arrest cell cycle progression in the G2 phase of L1210 cells. 1996. *Cancer Chemotherapy and Pharmacology,*38, 39-44. IF 1,261; 5-years IF 2,79; MNiSW 25; number of citations 28

2. Z. Mazerska, **E. Augustin**, J. Dzięgielewski, M.W. Chołody, J. Konopa. 1996. QSAR of acridines, III. Structure-activity relationship for antitumor imidazoacridinones and intercorrelations between in vivo and in vitro tests. *Anti-Cancer Drug Design*, 11, 73-88. IF 1,69; IF 2013/2014 - 2,38; MNiSW 20; number of citations 30

3. A. Skwarska, **E. Augustin**, J. Konopa. 2007. Sequential induction of mitotic catastrophe followed by apoptosis in human leukemia MOLT4 cells by imidazoacridinone C-1311. *Apoptosis*, 12 (12), 2245-2257. IF 3,043; 5- years IF 4,16; MNiSW 30; number of citations 23

4. **E. Augustin**[*], A. Moś-Rompa, D. Nowak-Ziatyk, J. Konopa. 2010. Antitumor 1-nitroacridine derivative C-1748, induces apoptosis, necrosis or senescence in human colon carcinoma HCT8 and HT29 cells. *Biochemical Pharmacology,* 79, 1231-1241. IF 4,889; 5-years IF 4,772; MNiSW 40; number of citations 11

5. M. Pawłowska, **E. Augustin**[*]. Systemy ekspresyjne białek cytochromu P450 w badaniach metabolizmu leków. 2011. *Postępy Higieny i Medycyny Doświadczalnej,* 65, 367 –376. IF 0,654; 5-years IF 0,761; MNiSW 15; number of citations 5

6. **E. Augustin.** 2011. Indukcja apoptozy w komórkach białaczek ludzkich MOLT4 i HL60 przez acyloksytriazoloakrydony – nową grupę związków o właściwościach przeciwnowotworowych. *Postępy Polskiej Medycyny i Farmacji,* 1 (1), 31 – 38.

7. A. Wiśniewska, M. Niemira, K. Jagiełło, A. Potęga, M. Świst, C. Henderson, A. Skwarska, **E. Augustin**, J. Konopa, Z. Mazerska. 2012. Diminished toxicity of C-1748, 4-methyl-9-hydroxyethylamino-1-nitroacridine, compared with demethyl analog, C-857, corresponds to its resistance to metabolism in HepG2 cells. *Biochemical Pharmacology,* 84, 30-42. IF 4,576; 5-years IF 4,772; MNiSW 40; number of citations 2

8. M. Pawłowska, R. Chu, B. Fedejko, **E Augustin**, Z. Mazerska, A. Radomińska-Pandya, T. Chambers. 2013. Metabolic transformation of antitumor acridinone C-1305 but not C-1311 via selective cellular expression of UGT1A10 increases cytotoxic response: implication for clinical use. *Drug Metabolism and Disposition,* 41: 414-421. IF 3,334; 5-years IF 3,827; MNiSW 35; number of citations 3

9. N.C.K. Valerie, B. Dziegielewska, A.S. Hosing, **E. Augustin**, L.S. Gray, D. Brautigan, J.M. Larener, J. Dziegielewski. 2013. Targeting of T-type calcium channels inhibits the Akt pro-survival pathway and promotes apoptosis in glioblastoma cells. *Biochemical Pharmacology,* 85:888-897. IF 4,65; 5-years IF 4,772; MNiSW 40; number of citations 7

10. A.M. Nowicka, A. Kowalczyk, A. Jerzebinska, M. Donten, P. Krysinski, Z. Stojek, **E. Augustin**, Z. Mazerska. 2013. Progress in targeting tumor cells by using drug-magnetic nanoparticles conjugate. *Biomacromolecules,* 14:828-833. IF 5,788; 5-years IF 5,75; MNiSW 40; number of citations 11

11. J. Polewska, A. Skwarska, **E. Augustin,** J. Konopa. 2013. DNA-damaging imidazoacridinone C-1311 induces autophagy followed by irreversible growth arrest and senescence in human lung cancer cells. *The Journal of Pharmacology and Experimental Therapeutics,* 346:393-405. IF 3,855; 5-years IF 3,959; MNiSW 35; number of citations 4

12. S. Godlewska, J. Jezierska, K. Baranowska, **E. Augustin**, A. Dołega. 2013. Copper(II) complexes with substituted imidazole and chlorido ligands: X-ray, UV-Vis, magnetic and EPR studies and chemotherapeutic potential. *Polyhedron,* 65:288-297. IF 2,047; 5-years IF 2,068; MNiSW 30; number of citations 2

13. M. Pawłowska, **E. Augustin**[*] and Z. Mazerska. 2014. CYP3A4 is not involved in C-1311 metabolism, but affects the cellular response induced by this drug in CHO cells overexpressing CYP3A4 isoenzyme and P450 reductase. *Acta Pharmacologica Sinica,* 35(1):98-112. IF 2,496; 5-years IF 2,673; MNiSW 30; number of citations 0

14. G. Gorczyca, R. Tylingo, P. Szweda, **E. Augustin,** M. Sadowska, S. Milewski. 2014. Fabrication and characterization of a porous chitosan – protein scaffold for biomedical application. *Carbohydrate Polymers,* 102:901-911. IF 3,916; 5-years IF 3,94; MNiSW 40; number of citations 3

[*]Corresponding author.

Ewa Augustin