Original Paper

Triggering of Suicidal Erythrocyte Death by Exemestane

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Key Words

Phosphatidylserine • Eryptosis • Oxidative stress • Calcium

Abstract

Background/Aims: The steroidal aromatase inactivator exemestane blocks estrogen biosynthesis and is thus employed for the prevention and treatment of breast cancer. Exemestane is in part effective by stimulation of suicidal cell death or apoptosis. Side effects of exemestane treatment include anemia. At least in theory, exemestane induced anemia could be secondary to stimulation of suicidal erythrocyte death or eryptosis, characterized by cell shrinkage and cell membrane scrambling with phosphatidylserine translocation to the erythrocyte surface. Signaling involved in the stimulation of eryptosis includes increase of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$), oxidative stress, ceramide, several kinases and caspases. The present study explored, whether exemestane is able to trigger eryptosis and, if so, to shed some light on the signaling involved. **Methods:** Flow cytometry was employed to quantify phosphatidylserine exposure at the cell surface from annexin-V-binding, cell volume from forward scatter, $[\text{Ca}^{2+}]_i$ from Fluo3-fluorescence, reactive oxygen species (ROS) abundance from DCF fluorescence, and ceramide abundance utilizing specific antibodies. **Results:** A 48 hours exposure of human erythrocytes to exemestane ($\geq 10 \mu\text{g/ml}$) significantly increased the percentage of annexin-V-binding cells without significantly modifying forward scatter. Exemestane significantly increased Fluo3-fluorescence (10 and 20, but not 40 $\mu\text{g/ml}$), DCF fluorescence (40 $\mu\text{g/ml}$), and ceramide abundance (40 $\mu\text{g/ml}$). The effect of exemestane (40 $\mu\text{g/ml}$) on annexin-V-binding was significantly blunted by antioxidant N-acetylcysteine (1mM), but was not significantly modified by removal or increase of extracellular Ca^{2+} , by p38 kinase inhibitor SB203580 (2 μM), casein kinase inhibitor D4476 (10 μM) and caspase inhibitor zVAD (10 μM). **Conclusions:** Exemestane triggers phospholipid scrambling of the erythrocyte cell membrane, an effect paralleled by enhanced $[\text{Ca}^{2+}]_i$, oxidative stress, and increased ceramide abundance.

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Introduction

Exemestane is a steroidal third generation aromatase inhibitor [1] utilized to block estrogen biosynthesis for the prevention and treatment of breast cancer [1-33]. Effects involved in the antitumor effect of exemestane include stimulation of suicidal cell death or apoptosis [34-38]. Side effects of exemestane treatment include anemia [39].

In theory, the anemia following exemestane treatment could result from stimulation of suicidal death of erythrocytes or eryptosis, which is characterized by cell membrane scrambling with phosphatidylserine translocation to the cell surface [40]. The cell membrane scrambling is typically paralleled by cell shrinkage [41]. Triggers of eryptosis include hyperosmotic shock [40], oxidative stress [40], energy depletion [40], or exposure to a wide variety of xenobiotics [40, 42-105]. The stimulation of eryptosis may involve increase of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$) [40], ceramide [40], caspases [40, 106, 107], casein kinase 1α [40], Janus-activated kinase JAK3 [40], protein kinase C [40], and p38 kinase [40]. Eryptosis is inhibited by AMP activated kinase AMPK, cGMP-dependent protein kinase, PAK2 kinase [40], and sorafenib/sunitinib sensitive kinases [40]. Enhanced eryptosis is observed in a variety of clinical conditions including fever [40], iron deficiency [40], dehydration [40], hyperphosphatemia [40], chronic kidney disease (CKD) [40], hemolytic-uremic syndrome [40], diabetes [40], hepatic failure [42], malignancy [40], sepsis [40], sickle-cell disease [40], beta-thalassemia [40], Hb-C and G6PD-deficiency [40], as well as Wilson's disease [40].

The present study explored, whether exemestane influences eryptosis. To this end, human erythrocytes from healthy volunteers were exposed to exemestane and phosphatidylserine surface abundance, cell volume, $[\text{Ca}^{2+}]_i$, ROS formation, and ceramide abundance determined by flow cytometry.

Materials and Methods

Erythrocytes, solutions and chemicals

Fresh Li-Heparin-anticoagulated blood samples were kindly provided by the blood bank of the University of Tübingen. The study is approved by the ethics committee of the University of Tübingen (184/2003 V). The blood was centrifuged at 120 g for 20 min at 21 °C and the platelets and leukocytes-containing supernatant was disposed. Erythrocytes were incubated *in vitro* at a hematocrit of 0.4% in Ringer solution containing (in mM) 125 NaCl, 5 KCl, 1 MgSO_4 , 32 N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES; pH 7.4), 5 glucose, 1 CaCl_2 , at 37°C for 48 hours. Where indicated, erythrocytes were exposed for 48 hours to exemestane (Selleckchem, Munich, Germany). In order to estimate the impact of Ca^{2+} entry on exemestane induced eryptosis, erythrocytes were exposed to exemestane in the presence and absence of extracellular Ca^{2+} . To test for an involvement of kinases, erythrocytes were exposed for 48 hours to a combination of exemestane and p38 kinase inhibitor SB203580 (Tocris bioscience, Bristol, UK) or casein kinase inhibitor D4476 (Tocris Bioscience, Bristol, UK). The impact of caspases was elucidated utilizing the pancaspase inhibitor zVAD (Enzo Life Sciences, Lörrach, Germany). To test for the involvement of oxidative stress, experiments were performed in the absence and presence of antioxidant N-acetylcysteine (Sigma Aldrich, Hamburg, Germany).

Annexin-V-binding and forward scatter

After incubation under the respective experimental condition, a 150 μl cell suspension was washed in Ringer solution containing 5 mM CaCl_2 and then stained with Annexin-V-FITC (1:200 dilution; ImmunoTools, Friesoythe, Germany) in this solution at 37°C for 15 min under protection from light. The annexin-V-abundance at the erythrocyte surface was subsequently determined on a FACS Calibur (BD, Heidelberg, Germany). Annexin-V-binding was measured with an excitation wavelength of 488 nm and an emission wavelength of 530 nm. A marker (M1) was placed to set an arbitrary threshold between annexin-V-binding cells and control cells. The same threshold was used for untreated and exemestane treated erythrocytes. A dot plot of forward scatter (FSC) vs. side scatter (SSC) was set to linear scale for both parameters. The threshold of forward scatter was set at the default value of "52".

Intracellular Ca^{2+}

After incubation, erythrocytes were washed in Ringer solution and loaded with Fluo-3/AM (Biotium, Hayward, USA) in Ringer solution containing 5 mM $CaCl_2$ and 5 μ M Fluo-3/AM. The cells were incubated at 37°C for 30 min. Ca^{2+} -dependent fluorescence intensity was measured with an excitation wavelength of 488 nm and an emission wavelength of 530 nm on a FACS Calibur. Afterwards, the geomean of the Ca^{2+} dependent fluorescence was determined.

Reactive oxidant species (ROS)

Oxidative stress was determined utilizing 2',7'-dichlorodihydrofluorescein (DCF) diacetate. After incubation, a 150 μ l suspension of erythrocytes was washed in Ringer solution and stained with DCF diacetate (Sigma, Schnelldorf, Germany) in Ringer solution containing DCF diacetate at a final concentration of 10 μ M. Erythrocytes were incubated at 37°C for 30 min in the dark and washed two times in Ringer solution. The DCF-loaded erythrocytes were resuspended in 200 μ l Ringer solution and ROS-dependent fluorescence intensity was measured at an excitation wavelength of 488 nm and an emission wavelength of 530 nm on a FACS Calibur (BD). Subsequently, the geomean of the DCF dependent fluorescence was determined.

Ceramide abundance

For the determination of ceramide, a monoclonal antibody-based assay was used. To this end, cells were stained for 1 hour at 37°C with 1 μ g/ml anti ceramide antibody (clone MID 15B4, Alexis, Grünberg, Germany) in PBS containing 0.1% bovine serum albumin (BSA) at a dilution of 1:10. The samples were washed twice with PBS-BSA. The cells were stained for 30 minutes with polyclonal fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG and IgM specific antibody (Pharmingen, Hamburg, Germany) diluted 1:50 in PBS-BSA. Unbound secondary antibody was removed by repeated washing with PBS-BSA. The samples were analyzed by flow cytometric analysis with an excitation wavelength of 488 nm and an emission wavelength of 530 nm. Finally, the geomean of the ceramide-dependent fluorescence was determined.

Statistics

Data are expressed as arithmetic means $\pm$ SEM. As indicated in the figure legends, statistical analysis was made using ANOVA with Tukey's test as post-test and *t* test as appropriate. *n* denotes the number of different erythrocyte specimens studied. Since different erythrocyte specimens used in distinct experiments are differently susceptible to triggers of eryptosis, comparisons were only made to erythrocytes from the same batch.

Results

The present study explored, whether exemestane influences eryptosis, the suicidal erythrocyte death. The decisive hallmark of eryptosis is phospholipid scrambling of the cell membrane with phosphatidylserine translocation to the cell surface. Phosphatidylserine exposing erythrocytes were identified utilizing annexin-V-binding to phosphatidylserine, as determined by flow cytometry. Annexin-V-binding was analysed following an incubation of the erythrocytes for 48 hours in Ringer solution without or with exemestane (10 – 40 μ g/ml). As illustrated in Fig. 1, a 48 hours exposure to exemestane significantly increased the percentage of phosphatidylserine exposing erythrocytes at all exemestane concentrations tested.

A second hallmark of eryptosis is erythrocyte shrinkage. As a measure of erythrocyte volume forward scatter was determined utilizing flow cytometry. The measurements were performed after incubation for 48 hours in Ringer solution without or with exemestane (10 – 40 μ g/ml). As a result, the erythrocyte forward scatter was similar following exposure in the absence of exemestane (496 ± 5 a.u., *n* = 24) and in the presence of 10 μ g/ml exemestane (476 ± 8 a.u., *n* = 24), 20 μ g/ml exemestane (475 ± 9 a.u., *n* = 24) and 40 μ g/ml exemestane (468 ± 8 a.u., *n* = 24). Exemestane did thus not significantly modify erythrocyte volume.

Fig. 1. Effect of exemestane on phosphatidylserine exposure. A. Original histogram of annexin-V-binding of erythrocytes following exposure for 48 hours to Ringer solution without (grey area) and with (black line) presence of 40 $\mu\text{g/ml}$ exemestane. B. Arithmetic means $\pm$ SEM ($n = 24$) of erythrocyte annexin-V-binding following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) exemestane (10 - 40 $\mu\text{g/ml}$). For comparison, the effect of the solvent DMSO is shown (grey bar). **($p < 0.01$), ***($p < 0.001$) indicates significant difference from the absence of exemestane (ANOVA).

Fig. 2. Effect of exemestane on cytosolic Ca^{2+} activity. A. Original histogram of Fluo3 fluorescence of erythrocytes following exposure for 48 hours to Ringer solution without (grey area) and with (black line) presence of 10 $\mu\text{g/ml}$ exemestane. B. Arithmetic means $\pm$ SEM ($n = 24$) of erythrocyte Fluo3 fluorescence following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) exemestane (10 - 40 $\mu\text{g/ml}$). For comparison, the effect of the solvent DMSO is shown (grey bar). **($p < 0.01$) indicates significant difference from the absence of exemestane (ANOVA).

Eryptosis could be triggered by increase of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$). Fluo3 fluorescence was employed in order to quantify $[\text{Ca}^{2+}]_i$. The erythrocytes were analyzed after 48 hours incubation in Ringer solution without or with exemestane (10 - 40 $\mu\text{g/ml}$). As illustrated in Fig. 2, 48 hours exposure to exemestane increased the Fluo3 fluorescence, an effect reaching statistical significance at 10 and 20 $\mu\text{g/ml}$, but not at 40 $\mu\text{g/ml}$ exemestane.

In order to test whether exemestane-induced translocation of phosphatidylserine required entry of extracellular Ca^{2+} , erythrocytes were incubated for 48 hours in the absence or presence of 40 $\mu\text{g/ml}$ exemestane in the presence or nominal absence of extracellular Ca^{2+} . As illustrated in Fig. 3, removal of extracellular Ca^{2+} did not significantly modify the effect of exemestane on the percentage annexin-V-binding cells. Instead, even in the nominal absence of extracellular Ca^{2+} , exemestane significantly increased the percentage of annexin-V-binding erythrocytes. Therefore, exemestane induced cell membrane scrambling did not depend on entry of extracellular Ca^{2+} . Additional experiments explored whether the stimulation of cell membrane scrambling by exemestane was influenced by increase of extracellular Ca^{2+} . As a result, exemestane (40 $\mu\text{g/ml}$) increased the percentage of phosphatidylserine exposing erythrocytes to similar values following 48 hr incubation at 1 mM extracellular Ca^{2+} (from 2.0 ± 0.2 to $9.6 \pm 1.6\%$, $n = 37$) and at 2 mM extracellular Ca^{2+} (from 2.4 ± 0.3 to $10.4 \pm 2.3\%$, $n = 12$).

Eryptosis is further stimulated by oxidative stress. Reactive oxygen species (ROS) was thus quantified utilizing 2',7'-dichlorodihydrofluorescein (DCF) diacetate. As illustrated in Fig. 4, 48 hours exposure to exemestane (40 $\mu\text{g/ml}$) increased the DCF fluorescence of erythrocytes. Apparently exemestane did induce oxidative stress. The upregulation of DCF fluorescence by exemestane was significantly blunted in the presence of antioxidant N-acetyl-cysteine (Fig. 5A). In order to test whether extracellular Ca^{2+} activity influences oxidative stress, DCF fluorescence was determined in the absence or presence of 40 $\mu\text{g/ml}$

Fig. 3. Ca^{2+} sensitivity of exemestane-induced phosphatidylserine exposure. A,B. Original histograms of annexin-V-binding of erythrocytes following exposure for 48 hours to Ringer solution without (grey areas) and with (black lines) exemestane (40 $\mu\text{g}/\text{ml}$) in the presence (A) and absence (B) of extracellular Ca^{2+} . C. Arithmetic means $\pm$ SEM ($n = 37$) of annexin-V-binding of erythrocytes after a 48 hours treatment with Ringer solution without (white bars) or with (black bars) exemestane (40 $\mu\text{g}/\text{ml}$) in the presence (left bars, $+\text{Ca}^{2+}$) and absence (right bars, $-\text{Ca}^{2+}$) of Ca^{2+} . ***($p < 0.001$) indicates significant difference from the absence of exemestane (ANOVA).

Fig. 4. Effect of exemestane on reactive oxygen species. A. Original histogram of DCF fluorescence in erythrocytes following exposure for 48 hours to Ringer solution without (grey area) and with (black line) presence of 40 $\mu\text{g}/\text{ml}$ exemestane. B. Arithmetic means $\pm$ SEM ($n = 15$) of DCF fluorescence in erythrocytes following incubation for 48 hours to Ringer solution without (white bar) or with (black bar) exemestane (40 $\mu\text{g}/\text{ml}$). **($p < 0.01$) indicates significant difference from the absence of exemestane (unpaired t test).

ml exemestane in the presence or nominal absence of extracellular Ca^{2+} . As illustrated in Fig. 5B, removal of extracellular Ca^{2+} significantly increased the effect of exemestane on DCF fluorescence. Further experiments addressed whether the effect of exemestane on cell membrane scrambling is sensitive to oxidative stress. To this end, exemestane was applied in the absence and presence of the antioxidant N-acetylcysteine (1 mM). As illustrated in Fig. 5C, N-acetylcysteine significantly blunted the effect of exemestane (40 $\mu\text{g}/\text{ml}$) on the percentage of annexin-V-binding erythrocytes.

A further stimulator of eryptosis is ceramide. Ceramide abundance at the erythrocyte surface was quantified utilizing specific antibodies. As illustrated in Fig. 6, 48 hours exposure to exemestane (40 $\mu\text{g}/\text{ml}$) increased the ceramide abundance at the erythrocyte surface. In order to test whether ceramide formation is sensitive to extracellular Ca^{2+} and/or oxidative stress additional experiments were performed in the presence and nominal absence of extracellular Ca^{2+} as well as in the absence and presence of the antioxidant N-acetylcysteine (1 mM). As a result, exemestane (40 $\mu\text{g}/\text{ml}$) increased the ceramide abundance in erythrocytes to similar values following 48 hr incubation at 1 mM extracellular Ca^{2+} (from 13.7 ± 1.2 to $19.1 \pm 2.4\%$, $n = 10$) and at 0 mM extracellular Ca^{2+} (from 18.8 ± 2.4 to $21.6 \pm 2.3\%$, $n = 10$). Moreover, exemestane (40 $\mu\text{g}/\text{ml}$) increased the ceramide abundance in erythrocytes to similar values following 48 hr incubation in the absence (from 15.6 ± 1.2 to $20.2 \pm 1.6\%$, $n = 15$) and presence (from 17.9 ± 1.5 to $21.4 \pm 2.3\%$, $n = 15$) of N-acetylcysteine (1 mM).

Fig. 5. N-acetylcysteine sensitivity of exemestane induced reactive oxygen species and cell membrane scrambling. A. Arithmetic means $\pm$ SEM (n = 12) of DCF fluorescence in erythrocytes following incubation for 48 hours to Ringer solution without (white bar) or with (black bar) exemestane (40 μ g/ml) in the absence (left bars) and presence (right bars) of N-acetylcysteine. B. Arithmetic means $\pm$ SEM (n = 20) of DCF fluorescence in erythrocytes following incubation for 48 hours to Ringer solution without (white bar) or with (black bar) exemestane (40 μ g/ml) in the presence (left bars, +Ca²⁺) and absence (right bars, -Ca²⁺) of extracellular Ca²⁺. C. Arithmetic means $\pm$ SEM (n = 10) of the percentage of annexin-V-binding erythrocytes following incubation for 48 hours to Ringer solution without (white bars) or with (black bars) exemestane (40 μ g/ml) in the absence (left bars) and presence (right bars) of N-acetylcysteine (1 mM). *** (p < 0.001), ** (p < 0.01) indicates significant difference from the absence of exemestane, # (p < 0.05), ### (p < 0.001) indicates significant difference from the absence of N-acetylcysteine (ANOVA).

Fig. 6. Effect of exemestane on ceramide abundance. A. Original histogram of ceramide abundance in erythrocytes following exposure for 48 hours to Ringer solution without (grey area) and with (black line) presence of 40 μ g/ml exemestane. B. Arithmetic means $\pm$ SEM (n = 10) of ceramide abundance in erythrocytes following incubation for 48 hours to Ringer solution without (white bar) or with (black bar) exemestane (40 μ g/ml). ** (p < 0.01) indicates significant difference from the absence of exemestane (unpaired *t* test).

To explore, whether the effects of exemestane involved kinase activity, the exemestane-induced annexin-V-binding was tested in absence and presence of p38 kinase inhibitor SB203580 and casein kinase inhibitor D4476. As a result, exemestane (40 μ g/ml) increased the percentage of phosphatidylserine exposing erythrocytes to similar values following 48 hr incubation in the absence (from 1.5 ± 0.1 to $9.1 \pm 1.4\%$, n = 13) and in the presence (from 1.4 ± 0.1 to $8.5 \pm 1.3\%$, n = 13) of SB203580 (2 μ M). Exemestane (40 μ g/ml) further increased the percentage of phosphatidylserine exposing erythrocytes to similar values following 48 hr incubation in the absence (from 1.1 ± 0.1 to $7.4 \pm 1.1\%$, n = 5) and in the presence (from 1.0 ± 0.1 to $10.5 \pm 1.3\%$, n = 5) of D4476 (10 μ M).

To test whether the effects of exemestane involved caspases, the influence of exemestane on annexin-V-binding was tested in the absence and presence of pancaspase inhibitor zVAD. As a result, exemestane (40 μ g/ml) increased the percentage of phosphatidylserine exposing erythrocytes to similar values following 48 hr incubation in the absence (from 1.8 ± 0.1 to $7.6 \pm 1.3\%$, n = 5) and in the presence (from 2.6 ± 0.3 to $13.5 \pm 3.9\%$, n = 5) of zVAD (10 μ M).

Discussion

The present observations uncover a novel effect of exemestane, i.e. the stimulation of cell membrane scrambling with phosphatidylserine translocation to the erythrocyte surface. Breakdown of phospholipid asymmetry of the cell membrane is a hallmark of both, apoptosis and eryptosis. The exemestane concentrations required for the stimulation of cell membrane scrambling are well in the range of concentrations (up to 50 µg/ml) encountered in the plasma of patients [108]. The stimulation of eryptosis may thus well explain the anemia following exemestane treatment [39].

The effect of exemestane on cell membrane scrambling was paralleled by a slight, but at 10 and 20 µg/ml statistically significant increase of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$). However, neither removal of extracellular Ca^{2+} nor increase of extracellular Ca^{2+} to 2 mM did significantly modify exemestane induced cell membrane scrambling. Thus, it appears that exemestane induced erythrocyte cell membrane scrambling was not dependent on entry of extracellular Ca^{2+} .

In any case, the exemestane induced cell membrane scrambling must involve cellular mechanisms other than increase of $[\text{Ca}^{2+}]_i$. A well known additional stimulator of eryptosis is oxidative stress [40]. Exemestane indeed significantly enhanced the abundance of reactive oxygen species. Moreover, the effect of exemestane on cell membrane scrambling was significantly blunted by the antioxidant N-acetylcysteine. Exemestane-induced cell membrane scrambling could thus in part be explained by induction of oxidative stress. Erythrocytes cell membrane scrambling could further be triggered by ceramide [40]. Exemestane treatment indeed increased the ceramide abundance at the erythrocyte surface and the substance may indeed be partially effective by influencing ceramide abundance at the erythrocyte surface. The failure of SB203580, of D4476 and of zVAD to significantly modify exemestane-induced cell membrane scrambling indicates that the effect of exemestane on cell membrane scrambling does not require activation of p38 kinase, casein kinase or caspases.

In contrast to its effect on cell membrane scrambling exemestane did not appreciably influence erythrocyte forward scatter and thus did apparently not influence erythrocyte volume. Erythrocytes are expected to shrink following increase of $[\text{Ca}^{2+}]_i$ which is known to stimulate Ca^{2+} sensitive K^+ channels with subsequent K^+ exit, cell membrane hyperpolarization, Cl^- exit and thus cellular loss of KCl with water [40]. Mechanisms accounting for the failure of exemestane to trigger cell shrinkage despite increase of $[\text{Ca}^{2+}]_i$ remained elusive. Possibly, exemestane leads to cellular energy depletion with subsequent impairment of Na^+/K^+ ATPase activity leading to decrease of cellular K^+ concentration and thus failure to hyperpolarize following activation of K^+ channels.

Phosphatidylserine exposing erythrocytes are rapidly cleared from circulation blood [40]. The physiological function of eryptosis is the clearance of defective erythrocytes from circulating blood prior to hemolysis [40]. Hemoglobin released following hemolysis may otherwise pass the renal glomerular filter, precipitate in the acidic lumen of renal tubules, occlude nephrons and thus lead to renal failure [109]. The clearance of phosphatidylserine exposing erythrocytes results in anemia if the loss of erythrocytes is not outweighed by matching stimulation of erythropoiesis [40]. Phosphatidylserine exposing erythrocytes may further interfere with microcirculation due to adherence to the vascular wall, stimulation of blood clotting and triggering of thrombosis [40].

In conclusion, exemestane triggers erythrocyte cell membrane scrambling, an effect paralleled by increase of cytosolic Ca^{2+} concentration, oxidative stress and enhancement of ceramide abundance at the erythrocyte surface. The observations provide an explanation for the exemestane-induced anemia.

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Disclosure Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

- 1 Sobral AF, Amaral C, Correia-da-Silva G, Teixeira N: Unravelling exemestane: From biology to clinical prospects. *J Steroid Biochem Mol Biol* 2016;163:1-11.
- 2 Zucchini G, Geuna E, Milani A, Aversa C, Martinello R, Montemurro F: Clinical utility of exemestane in the treatment of breast cancer. *Int J Womens Health* 2015;7:551-563.
- 3 Hortobagyi GN: Everolimus plus exemestane for the treatment of advanced breast cancer: a review of subanalyses from BOLERO-2. *Neoplasia* 2015;17:279-288.
- 4 Aktas B, Sorkin M, Pusztai L, Hofstadter EW: Uptake of exemestane chemoprevention in postmenopausal women at increased risk for breast cancer. *Eur J Cancer Prev* 2016;25:3-8.
- 5 Van Asten K, Neven P, Lintermans A, Wildiers H, Paridaens R: Aromatase inhibitors in the breast cancer clinic: focus on exemestane. *Endocr Relat Cancer* 2014;21:R31-49.
- 6 Bachelot T, McCool R, Duffy S, Glanville J, Varley D, Fleetwood K, Zhang J, Jerusalem G: Comparative efficacy of everolimus plus exemestane versus fulvestrant for hormone-receptor-positive advanced breast cancer following progression/recurrence after endocrine therapy: a network meta-analysis. *Breast Cancer Res Treat* 2014;143:125-133.
- 7 Yardley DA, Noguchi S, Pritchard KI, Burris HA, 3rd, Baselga J, Gnant M, Hortobagyi GN, Campone M, Pistilli B, Piccart M, Melichar B, Petrakova K, Arena FP, Erdkamp F, Harb WA, Feng W, Cahana A, Taran T, Lebowitz D, Rugo HS: Everolimus plus exemestane in postmenopausal patients with HR(+) breast cancer: BOLERO-2 final progression-free survival analysis. *Adv Ther* 2013;30:870-884.
- 8 Reimers L, Crew KD: Tamoxifen vs Raloxifene vs Exemestane for Chemoprevention. *Curr Breast Cancer Rep* 2012;4:207-215.
- 9 Iwata H, Masuda N, Ohno S, Rai Y, Sato Y, Ohsumi S, Hashigaki S, Nishizawa Y, Hiraoka M, Morimoto T, Sasano H, Saeki T, Noguchi S: A randomized, double-blind, controlled study of exemestane versus anastrozole for the first-line treatment of postmenopausal Japanese women with hormone-receptor-positive advanced breast cancer. *Breast Cancer Res Treat* 2013;139:441-451.
- 10 Walker G, Xenophontos M, Chen L, Cheung K: Long-term efficacy and safety of exemestane in the treatment of breast cancer. *Patient Prefer Adherence* 2013;7:245-258.
- 11 Dunn BK, Cazzaniga M, DeCensi A: Exemestane: one part of the chemopreventive spectrum for ER-positive breast cancer. *Breast* 2013;22:225-237.
- 12 Dhillon S: Everolimus in combination with exemestane: a review of its use in the treatment of patients with postmenopausal hormone receptor-positive, HER2-negative advanced breast cancer. *Drugs* 2013;73:475-485.
- 13 Litton JK, Bevers TB, Arun BK: Exemestane in the prevention setting. *Ther Adv Med Oncol* 2012;4:107-112.
- 14 Decensi A, Dunn BK, Puntoni M, Gennari A, Ford LG: Exemestane for breast cancer prevention: a critical shift? *Cancer Discov* 2012;2:25-40.
- 15 Kittaneh M, Gluck S: Exemestane in the adjuvant treatment of breast cancer in postmenopausal women. *Breast Cancer (Auckl)* 2011;5:209-226.
- 16 Lintermans A, Neven P, Paridaens R: Drug safety evaluation of exemestane. *Expert Opin Drug Saf* 2011;10:473-487.
- 17 Bertelli G, Gangadhara S: Exemestane in postmenopausal women with early or advanced breast cancer: a review. *Expert Opin Pharmacother* 2010;11:1933-1942.

- 18 Macpherson IR, Lindsay C, Canney P: Adjuvant treatment of breast cancer in postmenopausal women: role of exemestane. *Breast Cancer* (Dove Med Press) 2010;2:59-70.
- 19 Gluck S: Exemestane as first-line therapy in postmenopausal women with recurrent or metastatic breast cancer. *Am J Clin Oncol* 2010;33:314-319.
- 20 Nabholz JM, Mouret-Reynier MA, Durando X, Van Praagh I, Al-Sukhun S, Ferriere JP, Chollet P: Comparative review of anastrozole, letrozole and exemestane in the management of early breast cancer. *Expert Opin Pharmacother* 2009;10:1435-1447.
- 21 Deeks ED, Scott LJ: Exemestane: a review of its use in postmenopausal women with breast cancer. *Drugs* 2009;69:889-918.
- 22 Robinson A: A review of the use of exemestane in early breast cancer. *Ther Clin Risk Manag* 2009;5:91-98.
- 23 Untch M, Jackisch C: Exemestane in early breast cancer: a review. *Ther Clin Risk Manag* 2008;4:1295-1304.
- 24 Lonning PE, Geisler J: Experience with exemestane in the treatment of early and advanced breast cancer. *Expert Opin Drug Metab Toxicol* 2008;4:987-997.
- 25 Iddon J, Bundred NJ: To switch or not to switch: should the updated Intergroup Exemestane Study alter our decision? *Expert Rev Anticancer Ther* 2008;8:9-13.
- 26 Takei H, Suemasu K, Inoue K, Saito T, Okubo K, Koh J, Sato K, Tsuda H, Kurosumi M, Tabei T: Multicenter phase II trial of neoadjuvant exemestane for postmenopausal patients with hormone receptor-positive, operable breast cancer: Saitama Breast Cancer Clinical Study Group (SBCCSG-03). *Breast Cancer Res Treat* 2008;107:87-94.
- 27 Shetty YC, Chakkarwar PN, Acharya SS, Rajadhyaksha VD: Exemestane: a milestone against breast cancer. *J Postgrad Med* 2007;53:135-138.
- 28 Bundred N: The evolving role of exemestane in the management of breast cancer. *Br J Hosp Med (Lond)* 2006;67:427-430.
- 29 Jones SE: Exemestane as adjuvant treatment of early breast cancer: intergroup exemestane study/tamoxifen exemestane adjuvant multicenter trials. *Clin Breast Cancer* 2006;6 Suppl 2:S41-44.
- 30 Wong NS, Pritchard KI: A review of exemestane in the management of breast cancer. *Expert Opin Pharmacother* 2005;6:2353-2363.
- 31 Lonning PE: Exemestane for breast cancer prevention: a feasible strategy? *Clin Cancer Res* 2005;11:918s-924s.
- 32 Dixon JM: Exemestane and aromatase inhibitors in the management of advanced breast cancer. *Expert Opin Pharmacother* 2004;5:307-316.
- 33 Lonning PE: Exemestane: a review of its clinical efficacy and safety. *Breast* 2001;10:198-208.
- 34 Amaral C, Lopes A, Varela CL, da Silva ET, Roleira FM, Correia-da-Silva G, Teixeira N: Exemestane metabolites suppress growth of estrogen receptor-positive breast cancer cells by inducing apoptosis and autophagy: A comparative study with Exemestane. *Int J Biochem Cell Biol* 2015;69:183-195.
- 35 Shen Y, Du Y, Zhang Y, Pan Y: Synergistic effects of combined treatment with simvastatin and exemestane on MCF-7 human breast cancer cells. *Mol Med Rep* 2015;12:456-462.
- 36 Kritikou I, Giannopoulou E, Koutras AK, Labropoulou VT, Kalofonos HP: The combination of antitumor drugs, exemestane and erlotinib, induced resistance mechanism in H358 and A549 non-small cell lung cancer (NSCLC) cell lines. *Pharm Biol* 2013;10.3109/13880209.2013.841718
- 37 Amaral C, Borges M, Melo S, da Silva ET, Correia-da-Silva G, Teixeira N: Apoptosis and autophagy in breast cancer cells following exemestane treatment. *PLoS One* 2012;7:e42398.
- 38 Koutras A, Giannopoulou E, Kritikou I, Antonacopoulou A, Evans TR, Papavassiliou AG, Kalofonos H: Antiproliferative effect of exemestane in lung cancer cells. *Mol Cancer* 2009;8:109.
- 39 Mrozek E, Layman R, Ramaswamy B, Schaaf L, Li X, Ottman S, Shapiro CL: Phase II trial of exemestane in combination with fulvestrant in postmenopausal women with advanced, hormone-responsive breast cancer. *Clin Breast Cancer* 2012;12:151-156.
- 40 Lang E, Lang F: Mechanisms and pathophysiological significance of eryptosis, the suicidal erythrocyte death. *Semin Cell Dev Biol* 2015;39:35-42.
- 41 Lang PA, Kaiser S, Myssina S, Wiedert T, Lang F, Huber SM: Role of Ca²⁺-activated K⁺ channels in human erythrocyte apoptosis. *Am J Physiol Cell Physiol* 2003;285:C1553-C1560.
- 42 Alzoubi K, Egler J, Abed M, Lang F: Enhanced Eryptosis Following Auranofin Exposure. *Cell Physiol Biochem* 2015;37:1018-1028.

- 43 Bissinger R, Barking S, Alzoubi K, Liu G, Liu G, Lang F: Stimulation of Suicidal Erythrocyte Death by the Antimalarial Drug Mefloquine. *Cell Physiol Biochem* 2015;36:1395-1405.
- 44 Bissinger R, Bouguerra G, Stockinger K, Abbes S, Lang F: Triggering of Suicidal Erythrocyte Death by Topotecan. *Cell Physiol Biochem* 2015;37:1607-1618.
- 45 Bouguerra G, Aljanadi O, Bissinger R, Abbes S, Lang F: Embelin-Induced Phosphatidylserine Translocation in the Erythrocyte Cell Membrane. *Cell Physiol Biochem* 2015;37:1629-1640.
- 46 Bouguerra G, Bissinger R, Abbes S, Lang F: Stimulation of Eryptosis by Narasin. *Cell Physiol Biochem* 2015;37:1807-1816.
- 47 Briglia M, Fazio A, Faggio C, Laufer S, Alzoubi K, Lang F: Triggering of Suicidal Erythrocyte Death by Ruxolitinib. *Cell Physiol Biochem* 2015;37:768-778.
- 48 Briglia M, Fazio A, Signorello E, Faggio C, Lang F: Edelfosine Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2015;37:2221-2230.
- 49 Calabro S, Alzoubi K, Faggio C, Laufer S, Lang F: Triggering of Suicidal Erythrocyte Death Following Boswellic Acid Exposure. *Cell Physiol Biochem* 2015;37:131-142.
- 50 Egler J, Lang F: Licochalcone A Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2015;37:2060-2070.
- 51 Faggio C, Alzoubi K, Calabro S, Lang F: Stimulation of suicidal erythrocyte death by PRIMA-1. *Cell Physiol Biochem* 2015;35:529-540.
- 52 Fazio A, Briglia M, Faggio C, Alzoubi K, Lang F: Stimulation of Suicidal Erythrocyte Death by Garcinol. *Cell Physiol Biochem* 2015;37:805-815.
- 53 Jacobi J, Lang E, Bissinger R, Frauenfeld L, Modicano P, Faggio C, Abed M, Lang F: Stimulation of erythrocyte cell membrane scrambling by mitotane. *Cell Physiol Biochem* 2014;33:1516-1526.
- 54 Lang E, Jilani K, Bissinger R, Rexhepaj R, Zelenak C, Lupescu A, Lang F, Qadri SM: Vitamin D-Rich Diet in Mice Modulates Erythrocyte Survival. *Kidney Blood Press Res* 2015;40:403-412.
- 55 Lang E, Zelenak C, Eberhard M, Bissinger R, Rotte A, Ghashghaieina M, Lupescu A, Lang F, Qadri SM: Impact of Cyclin-Dependent Kinase CDK4 Inhibition on Eryptosis. *Cell Physiol Biochem* 2015;37:1178-1186.
- 56 Lupescu A, Bissinger R, Goebel T, Salker MS, Alzoubi K, Liu G, Chirigiu L, Mack AF, Qadri SM, Lang F: Enhanced suicidal erythrocyte death contributing to anemia in the elderly. *Cell Physiol Biochem* 2015;36:773-783.
- 57 Officioso A, Alzoubi K, Manna C, Lang F: Clofazimine Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2015;37:331-341.
- 58 Peter T, Bissinger R, Enkel S, Alzoubi K, Oswald G, Lang F: Programmed erythrocyte death following in vitro Treosulfan treatment. *Cell Physiol Biochem* 2015;35:1372-1380.
- 59 Stockinger K, Bissinger R, Bouguerra G, Abbes S, Lang F: Enhanced Eryptosis Following Exposure to Carnosic Acid. *Cell Physiol Biochem* 2015;37:1779-1791.
- 60 Tesoriere L, Attanzio A, Allegra M, Cilla A, Gentile C, Livrea MA: Oxysterol mixture in hypercholesterolemia-relevant proportion causes oxidative stress-dependent eryptosis. *Cell Physiol Biochem* 2014;34:1075-1089.
- 61 Waibel S, Bissinger R, Bouguerra G, Abbes S, Lang F: Saquinavir Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2015;37:1973-1982.
- 62 Zierle J, Bissinger R, Egler J, Lang F: Lapatinib Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2015;37:2275-2287.
- 63 Bissinger R, Bouguerra G, Al Mamun Bhuyan A, Waibel S, Abbes S, Lang F: Efavirenz Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2015;37:2496-2507.
- 64 Bissinger R, Waibel S, Bouguerra G, Al Mamun Bhuyan A, Abbes S, Lang F: Enhanced Eryptosis Following Exposure to Lopinavir. *Cell Physiol Biochem* 2015;37:2486-2495.
- 65 Briglia M, Calabro S, Signorello E, Alzoubi K, Laufer S, Faggio C, Lang F: Fucoxanthin Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2015;37:2464-2475.
- 66 Briglia M, Fazio A, Faggio C, Lang F: Triggering of Suicidal Erythrocyte Death by Zosuquidar. *Cell Physiol Biochem* 2015;37:2355-2365.
- 67 Fazio A, Briglia M, Faggio C, Alzoubi K, Lang F: Oxaliplatin Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2015;37:2393-2404.
- 68 Macczak A, Cyrkler M, Bukowska B, Michalowicz J: Eryptosis-inducing activity of bisphenol A and its analogs in human red blood cells (in vitro study). *J Hazard Mater* 2016;307:328-335.

- 69 Officioso A, Alzoubi K, Lang F, Manna C: Hydroxytyrosol inhibits phosphatidylserine exposure and suicidal death induced by mercury in human erythrocytes: Possible involvement of the glutathione pathway. *Food Chem Toxicol* 2016;89:47-53.
- 70 Officioso A, Manna C, Alzoubi K, Lang F: Bromfenvinphos induced suicidal death of human erythrocytes. *Pestic Biochem Physiol* 2016;126:58-63.
- 71 Qadri SM, Donkor DA, Bhakta V, Eltringham-Smith LJ, Dwivedi DJ, Moore JC, Pepler L, Ivetic N, Nazi I, Fox-Robichaud AE, Liaw PC, Sheffield WP: Phosphatidylserine externalization and procoagulant activation of erythrocytes induced by *Pseudomonas aeruginosa* virulence factor pyocyanin. *J Cell Mol Med* 2016;10.1111/jcmm.12778
- 72 Zierle J, Bissinger R, Bouguerra G, Abbes S, Lang F: Triggering of Suicidal Erythrocyte Death by Regorafenib. *Cell Physiol Biochem* 2016;38:160-172.
- 73 Pagano M, Faggio C: The use of erythrocyte fragility to assess xenobiotic cytotoxicity. *Cell Biochem Funct* 2015;33:351-355.
- 74 Al Mamun Bhuyan A, Bissinger R, Stockinger K, Lang F: Stimulation of Suicidal Erythrocyte Death by Tafenoquine. *Cell Physiol Biochem* 2016;39:2464-2476.
- 75 Al Mamun Bhuyan A, Signoretto E, Bissinger R, Lang F: Enhanced Eryptosis Following Exposure to Dolutegravir. *Cell Physiol Biochem* 2016;39:639-650.
- 76 Al Mamun Bhuyan A, Signoretto E, Lang F: Triggering of Suicidal Erythrocyte Death by Psammaphin A. *Cell Physiol Biochem* 2016;39:908-918.
- 77 Almasry M, Jemaa M, Mischitelli M, Faggio C, Lang F: Stimulation of Suicidal Erythrocyte Death by Phosphatase Inhibitor Calyculin A. *Cell Physiol Biochem* 2016;40:163-171.
- 78 Bissinger R, Bhuyan AA, Signoretto E, Lang F: Stimulating Effect of Elvitegravir on Suicidal Erythrocyte Death. *Cell Physiol Biochem* 2016;38:1111-1120.
- 79 Bissinger R, Kempe-Teufel DS, Honisch S, Qadri SM, Randrianarisoa E, Haring HU, Henes J, Lang F: Stimulated Suicidal Erythrocyte Death in Arteritis. *Cell Physiol Biochem* 2016;39:1068-1077.
- 80 Egler J, Zierle J, Lang F: Stimulating Effect of Manumycin A on Suicidal Erythrocyte Death. *Cell Physiol Biochem* 2016;38:1147-1156.
- 81 Jemaa M, Mischitelli M, Fezai M, Almasry M, Faggio C, Lang F: Stimulation of Suicidal Erythrocyte Death by the CDC25 Inhibitor NSC-95397. *Cell Physiol Biochem* 2016;40:597-607.
- 82 Lang E, Pozdeev VI, Gatidis S, Qadri SM, Haussinger D, Kubitz R, Herebian D, Mayatepek E, Lang F, Lang KS, Lang PA: Bile Acid-Induced Suicidal Erythrocyte Death. *Cell Physiol Biochem* 2016;38:1500-1509.
- 83 Lang E, Pozdeev VI, Xu HC, Shinde PV, Behnke K, Hamdam JM, Lehnert E, Scharf RE, Lang F, Haussinger D, Lang KS, Lang PA: Storage of Erythrocytes Induces Suicidal Erythrocyte Death. *Cell Physiol Biochem* 2016;39:668-676.
- 84 Mischitelli M, Jemaa M, Almasry M, Faggio C, Lang F: Stimulation of Erythrocyte Cell Membrane Scrambling by Quinine. *Cell Physiol Biochem* 2016;40:657-667.
- 85 Mischitelli M, Jemaa M, Almasry M, Faggio C, Lang F: Stimulation of Suicidal Erythrocyte Death by Rottlerin. *Cell Physiol Biochem* 2016;40:558-566.
- 86 Mischitelli M, Jemaa M, Almasry M, Faggio C, Lang F: Triggering of Erythrocyte Cell Membrane Scrambling by Emodin. *Cell Physiol Biochem* 2016;40:91-103.
- 87 Mischitelli M, Jemaa M, Almasry M, Faggio C, Lang F: Triggering of Suicidal Erythrocyte Death by Fascaplysin. *Cell Physiol Biochem* 2016;39:1638-1647.
- 88 Mischitelli M, Jemaa M, Almasry M, Faggio C, Lang F: Ca²⁺ Entry, Oxidative Stress, Ceramide and Suicidal Erythrocyte Death Following Diosgenin Treatment. *Cell Physiol Biochem* 2016;39:1626-1637.
- 89 Peter T, Bissinger R, Lang F: Stimulation of Eryptosis by Caspofungin. *Cell Physiol Biochem* 2016;39:939-949.
- 90 Peter T, Bissinger R, Liu G, Lang F: Anidulafungin-Induced Suicidal Erythrocyte Death. *Cell Physiol Biochem* 2016;38:2272-2284.
- 91 Peter T, Bissinger R, Signoretto E, Mack AF, Lang F: Micafungin-Induced Suicidal Erythrocyte Death. *Cell Physiol Biochem* 2016;39:584-595.
- 92 Pretorius E, du Plooy JN, Bester J: A Comprehensive Review on Eryptosis. *Cell Physiol Biochem* 2016;39:1977-2000.
- 93 Shan F, Yang R, Ji T, Jiao F: Vitamin C Inhibits Aggravated Eryptosis by Hydrogen Peroxide in Glucose-6-Phosphated Dehydrogenase Deficiency. *Cell Physiol Biochem* 2016;39:1453-1462.

- 94 Signoretto E, Bissinger R, Castagna M, Lang F: Stimulation of Eryptosis by Combretastatin A4 Phosphate Disodium (CA4P). *Cell Physiol Biochem* 2016;38:969-981.
- 95 Signoretto E, Castagna M, Al Mamun Bhuyan A, Lang F: Stimulating Effect of Terfenadine on Erythrocyte Cell Membrane Scrambling. *Cell Physiol Biochem* 2016;38:1425-1434.
- 96 Signoretto E, Castagna M, Lang F: Stimulation of Eryptosis, the Suicidal Erythrocyte Death by Piceatannol. *Cell Physiol Biochem* 2016;38:2300-2310.
- 97 Signoretto E, Honisch S, Briglia M, Faggio C, Castagna M, Lang F: Nocodazole Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2016;38:379-392.
- 98 Signoretto E, Laufer SA, Lang F: Stimulating Effect of Sclareol on Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem* 2016;39:554-564.
- 99 Signoretto E, Zierle J, Bissinger R, Castagna M, Bossi E, Lang F: Triggering of Suicidal Erythrocyte Death by Pazopanib. *Cell Physiol Biochem* 2016;38:926-938.
- 100 Wesseling MC, Wagner-Britz L, Huppert H, Hanf B, Hertz L, Nguyen DB, Bernhardt I: Phosphatidylserine Exposure in Human Red Blood Cells Depending on Cell Age. *Cell Physiol Biochem* 2016;38:1376-1390.
- 101 Zierle J, Bissinger R, Lang F: Inhibition by Teriflunomide of Erythrocyte Cell Membrane Scrambling Following Energy Depletion, Oxidative Stress and Ionomycin. *Cell Physiol Biochem* 2016;39:1877-1890.
- 102 Bissinger R, Modicano P, Alzoubi K, Honisch S, Faggio C, Abed M, Lang F: Effect of saponin on erythrocytes. *Int J Hematol* 2014;100:51-59.
- 103 Bissinger R, Modicano P, Frauenfeld L, Lang E, Jacobi J, Faggio C, Lang F: Estramustine-induced suicidal erythrocyte death. *Cell Physiol Biochem* 2013;32:1426-1436.
- 104 Lang E, Modicano P, Arnold M, Bissinger R, Faggio C, Abed M, Lang F: Effect of thioridazine on erythrocytes. *Toxins (Basel)* 2013;5:1918-1931.
- 105 Al Mamun Bhuyan A, Bissinger R, Cao H, Lang F: Triggering of Suicidal Erythrocyte Death by bexaroten. *Cell Physiol Biochem* 2016;40:1239-1251.
- 106 Lau IP, Chen H, Wang J, Ong HC, Leung KC, Ho HP, Kong SK: In vitro effect of CTAB- and PEG-coated gold nanorods on the induction of eryptosis/erythroptosis in human erythrocytes. *Nanotoxicology* 2012;6:847-856.
- 107 Maellaro E, Leoncini S, Moretti D, Del Bello B, Tanganelli I, De Felice C, Ciccoli L: Erythrocyte caspase-3 activation and oxidative imbalance in erythrocytes and in plasma of type 2 diabetic patients. *Acta Diabetol* 2013;50:489-495.
- 108 Vonderheide RH, LoRusso PM, Khalil M, Gartner EM, Khaira D, Soulieres D, Dorazio P, Trosko JA, Ruter J, Mariani GL, Usari T, Domchek SM: Tremelimumab in combination with exemestane in patients with advanced breast cancer and treatment-associated modulation of inducible costimulator expression on patient T cells. *Clin Cancer Res* 2010;16:3485-3494.
- 109 Harrison HE, Bunting H, Ordway NK, Albrink WS: The Pathogenesis of the Renal Injury Produced in the Dog by Hemoglobin or Methemoglobin. *J Exp Med* 1947;86:339-356.