Can Some Anticancer Treatments Preserve the Ovarian Reserve?

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CAN SOME ANTICANCER TREATMENTS PRESERVE THE OVARIAN RESERVE?

Short title: Targeted therapies and fertility preservation

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ABSTRACT

Background: Preventing premature ovarian failure (POF) is a major challenge in oncology. With conventional regimens, cytotoxicity-associated POF involves primordial follicles (PF) pool depletion by apoptosis or overactivation mechanisms, notably mediated by the ABL/TAp63 and PI3K/Akt/mTOR pathways. New anticancer treatments have been designed to target pathways implicated in tumor growth. Although, concerns regarding fertility arise with these targeted therapies, we hypothesized that targeted therapies may exert off-tumor effects on PF that might delay POF. We provide an overview of evidences concerning these off-tumor effects on PF. Limitations and future potential implications of these findings are discussed.

Design: PubMed was searched by combining Boolean operators with the keywords: fertility, ovarian, follicle, anti-tumoral, cancer, targeted, cytotoxic, chemotherapy.

Results: Cisplatin-related PF apoptosis via the ABL/TAp63 pathway was targeted with a tyrosine kinase inhibitor, imatinib, in mice, but effects were recently challenged by findings on human ovarian xenografts in mice. In cyclophosphamide-treated mice, PI3K/Akt/mTOR pathway inhibition with mTOR inhibitors and AS101 preserved the PF pool. Proteasome and GSK3 inhibitors were evaluated for direct and indirect follicle DNA damage prevention. Surprisingly, evidence for cytotoxic drug association with PF pool preservation were found. We also describe selected non-anticancer molecules which may minimize gonadotoxicity.

Conclusions: Not all anticancer treatments are associated with POF, particularly since the advent of targeted therapies. The feasibility of associating a protective drug targeting PF exhaustion mechanisms with cytotoxic treatments should be evaluated, as a way of decreasing the need for conventional fertility preservation techniques. Further evaluations are required for transfer into clinical practice.

IMPLICATIONS FOR PRACTICE

Anticancer therapies are associated with infertility in 10% to 70% patients which is the result of primordial follicles pool depletion. Alone or associated with gonadotoxic treatments, some targeted therapies may exert favorable off-targets effects on primordial follicle pool by slowing down their exhaustion. Current evidences of these effects rely on murine models or human in vitro models. Evaluations of these protective strategies in humans is challenging but if these results are confirmed with clinical and biological data not only it could be a new approach to female fertility preservation, but it will change standard fertility strategies.

INTRODUCTION

Fertility preservation during cancer treatment is challenging [1]. In female patients, premature ovarian failure (POF) is associated not only with infertility, but also with hormonal disorders, leading to osteoporosis and cardiovascular diseases, which can impair both quality of life (QOL) and survival [2]. Cytotoxic treatments are associated with POF in 10% to 70% patients, depending on the alkylating agents or ovarian irradiation doses used and the age of the patient at treatment [3].

Fertility preservation based on the freezing of embryos, oocytes or ovarian tissue requires ovarian stimulation and/or surgical approaches that may not be appropriate for use in emergency conditions or in patients with hormone-sensitive cancers [1,4]. Autologous grafts of cryopreserved ovarian cortex tissue can restore fertility and ovarian endocrine functions, but concerns have been raised about tissue contamination with minimal residual disease, which may contraindicate transplantation [1,5,6].

The primordial follicle (PF) pool, also known as the ovarian reserve, is fixed and determined at time of birth. Menopause, or POF in young patients is related to the depletion of this reserve. It occurs with successive PF activation, a process in which individual primordial follicles leave their dormant state and enter a growth phase. PF activation is mediated, in particular, by the phosphatidylinositol 3-kinase(PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway, following KIT activation [7,8]. Cytotoxic agents, such as platinum salts, have been shown to promote apoptosis via the ABL/TAp63 pathway [9].

Over the last 20 years, a growing number of anticancer treatments have been designed to target proteins involved in tumor growth or survival pathways, including some involved in PF activation, such as the ABL protein kinase, mTOR complex 1 (mTORC1) or mTORC2 (Figure 1). We hypothesized that these anticancer drugs might have on-target/off-tumor effects on PF activation or death pathways that might preserve the ovarian reserve. In this review, we provide an overview of current evidence concerning the mechanisms by which targeted or conventional anticancer treatments may target PF activation or apoptosis pathways, thereby contributing to protection of the ovarian reserve.

In April 2019, we searched the PubMed database for studies reporting a specific association of anticancer treatments with signs of ovarian reserve protection. We used the following keywords,

together with Boolean operators: fertility, ovarian, follicle, anti-tumoral, cancer, targeted, cytotoxic, chemotherapy. The initial search of PubMed was then complemented with targeted queries. No limitations relating to study methodology were applied, and ovarian reserve protection was defined as increases in primordial follicles count, pregnancy rate and time-to-POF.

CELLULAR PATHWAYS INVOLVED IN PRIMORDIAL FOLLICLE ACTIVATION

The constitution of the PF pool and the number of follicles it contains are determined during intrauterine development. This pool, also known as the ovarian reserve, and the rate of PF loss, determine the duration of ovarian activity. The mean number of PFs at birth ranges from one to two million then decreases with age, ultimately resulting in the menopause (Figure 2) [10]. The rate of decrease in the ovarian reserve depends on activating and maintenance signals. The pathways involved in PF activation have already been reviewed in detail and are summarized below, highlighting targetable pathways (Figure 3) [7,8].

PI3K signaling is the cornerstone of differentiation and proliferation signaling. The PI3K pathway is triggered by the activation of the KIT receptor by its ligand (KIT-L), which is secreted by granulosa cells and oocytes. PI3K activates Akt, in turn leading to the activation of mTORC1 and mTORC2. Downstream, this pathway leads to the activation of 4E-BP1 and ribosomal protein S6 (RPS6), which are involved in cell survival and growth [7]. Conversely, PI3K/Akt/mTORC signaling is downregulated by phosphatase and tensin homolog (PTEN). In *Pten-null* mice, the PI3K pathway is constitutively activated, leading to PF exhaustion [7,11]. KIT-L expression is controlled by oocytes and granulosa cells, which secrete molecules, such as platelet-derived growth factor (PDGF), basic fibroblast growth factor and leukemia inhibitory factor, that have been shown to promote the primordial to primary follicle transition [12]. The JAK/STAT pathway is also involved in the inhibition of PF growth, notably through JAK1 activation [13], and stromal cells can activate follicles by secreting bone morphogenetic protein-4 (BMP-4) and keratinocyte growth factor (KGF) [7]. In addition, ovarian cell-to-cell contact promotes the inactivation of Hippo signaling, which has been shown to promote follicle activation through PI3K/Akt/mTOR signaling [14].

MECHANISMS INVOLVED IN PREMATURE OVARIAN FAILURE AFTER CYTOTOXIC TREATMENT

The pathogenesis of ovarian damage after chemotherapy has been reviewed in detail elsewhere [15]. The mechanisms underlying PF exhaustion involve apoptosis or PF overactivation, and depend on the cytotoxic agents used [15]. The ABL/TAp63 and PI3K/Akt/mTOR pathways are the principal pathways implicated in POF. Ovarian stroma damage may also lead to POF, but the interaction between follicles and the ovarian stroma remains poorly understood (Figure 4A).

Signaling pathways involved in cytotoxicity-related effects on follicles

In humans, comparisons of patients on chemotherapy regimens including alkylating agents with age-matched healthy controls demonstrated a decrease in the PF pool in the treated patients [16]. TUNEL analyses of human ovaries after CY administration in a xenografted mouse model have also revealed follicle apoptosis [17]. TUNEL analyses of the ovaries in CY-treated mice did not reveal signs of apoptosis for the ovarian cortex, suggesting that PF loss might not result from direct injury through apoptosis [18]. Independent research teams have shown that the PF loss in CY-treated mice is associated with an increase in growth rate. This overactivation is responsible for PF exhaustion. Higher levels of p-Akt, p-mTOR and p-RPS6 expression have been demonstrated in the ovaries of CY-treated mice [18-20].

Ovaries from xenografted SCID mice treated with DOX display an increase in apoptosis, particularly in cortical cells potentially corresponding to PF [21]. In *ex vivo* cultures of mouse ovaries, DOX has been shown to be associated with granulosa cell apoptosis [22]. This apoptosis results from double-strand breaks caused by the ATM/ABL/TAp63 pathway in mice xenografted with human ovarian tissue [21]. Oxidative damage may also be involved in the ovarian damage caused by DOX [23].

In a mouse model, CIS has been shown to activate cell death mechanisms via the TAp63 protein (a P53 homolog). The TAp63 protein may be activated by a pathway involving ABL, although this remains a matter of debate [9,24-26]. Following CIS administration, TAp63 promotes ABL expression, leading to TAp73-BAX-mediated apoptosis [26]. Studies of *ex vivo* cultures of mouse

ovaries have suggested that CIS may preferentially damage oocytes rather than granulosa cells, through apoptosis mechanisms [22].

Effects mediated by alterations to the ovarian stroma

Follicle growth homeostasis requires functional stromal cells and vascularization. Fabbri *et al.* showed that DOX and CIS induce apoptosis in ovarian stromal cells through BAX expression and Bcl overexpression, in studies of cultured adherent *ex vivo* cells from cryopreserved human ovarian tissues [27]. DOX also affects microvessel formation and blood flow through these vessels [21,28].

EVIDENCE THAT SOME ANTICANCER TREATMENTS CAN PROTECT THE OVARIAN RESERVE

Tyrosine kinase inhibitors

Imatinib

Imatinib is a well-known tyrosine kinase inhibitor (TKI) specifically targeting the ABL kinase domain of the BCR-ABL1 oncogenic protein in chronic myeloid leukemia [29]. However, it has been shown to have off-target effects [30]. In particular, it inhibits the platelet-derived growth factor receptor (PDGFR) and KIT tyrosine kinases, particularly in the contexts of gastrointestinal stromal and oligodendroglial tumors [31-33]. PF apoptosis and activation are mediated by the ABL and KIT pathways, respectively, suggesting that imatinib may prevent ovarian damage due to these pathways.

Imatinib alone was found to have no impact on the PF pool in five-week-old mice into which BCR-ABL1 cells were injected. However, ovarian morphology was disorganized in these mice [34]. These findings were confirmed following the treatment of postnatal day 2 (PND2) rats with imatinib for three days [35].

The effect of imatinib on oocyte damage after chemotherapy was investigated with an *in vitro* model of cultured mouse ovaries. Ovaries were cultured with DOX or CIS, alone or together with imatinib. In this study, imatinib was associated with a smaller number of morphologically classified unhealthy follicles after CIS treatment, whereas the effect of DOX was not affected by the addition of imatinib [22]. As ABL could potentially induce TAp63 after DNA damage in CIS-treated mice, combined treatment with imatinib was evaluated *in vivo*. In cultured ovaries from PND5 mice, combined treatment with imatinib was associated with lower levels of ABL and TAp63 expression and lower rates of apoptosis [9]. The longer term impact of imatinib and CIS co-administration was

evaluated in three-week-old ovariectomized mice into which *in vitro*-treated mouse ovaries were grafted [26]. PF numbers were larger in mice receiving grafts of ovarian tissue subjected to the combined treatment than in mice receiving grafts of ovaries treated with CIS only, which displayed no signs of ovarian tissue recovery [26]. Pooled primordial and primary follicle counts, mean number of pups and pregnancy rates were also higher in mice receiving ovarian tissue subjected to the combined treatment than in mice receiving ovarian tissue treated with CIS alone [9]. However, this findings were challenged by Kerr *et al.*, who reported that the combined treatment was not associated with higher PF counts or a larger number of pups in a comparable mice model [24]. Kerr *et al.* also reported an association between imatinib treatment and a decrease in the number of PF [24]. They evaluated the effects of imatinib injected with or before CIS, whereas Gonfloni *et al.* administered imatinib and CIS at the same time [9]. Finally, the differences in the results obtained by these two team may reflect differences in the type of CIS used. Kerr *et al.* used hospital-grade CIS and reported higher follicle toxicity at the same dose than was reported for SIGMA-CIS in the work of Gonfloni *et al.* [9,24]. Together, these results highlight the importance of CIS dose in experimental models. ABL was thought to be targeted in these experiments, but it remains possible that the effects of imatinib were due to KIT inhibition. Thus, imatinib may prevent follicle activation by cytotoxic agents, as illustrated by the low Ki67 levels observed in PF granulosa cells from imatinib-treated rats [35].

But, studies of human ovarian xenografts in mice and *ex vivo* cultures have reported different effects of imatinib in human ovarian tissues [36]. The use of a combination of CIS (Eli Lilly and Company) and imatinib to treat xenografted mice and *ex vivo* cultures of healthy human ovarian tissues was associated with a PF count similar to that obtained following treatment with CIS alone. Small follicles lacking oocytes and with a bizarre shape were observed in imatinib-treated ovaries [36].

Sunitinib

Sunitinib is a multikinase inhibitor targeting proteins involved in PF activation, such as PDGFR and KIT, that is used in kidney cancers [37]. In three-week-old mice, sunitinib treatment was associated with lower oocyte levels after superovulation tests. In unstimulated six-week-old mice, the PF pool of sunitinib-treated mice was similar to that in controls. Litter size was also found to be similar for treated mice and controls. In parallel, in cultured granulosa cell lines, p-ERK1/2 (a marker of proliferation) expression was found to be lower in the granulosa cells and oocytes of treated mice [38]. Thus,

sunitinib may counteract PF activation after the administration of cytotoxic agents. This hypothesis has not been tested in co-administration studies.

mTOR inhibitors

mTOR inhibitors are immunosuppressors used in the contexts of breast cancer treatment and solid organ or hematopoietic stem cell transplantation [39-41]. The PI3K/Akt/mTOR pathway is overactivated in CY-treated mice [18-20]. *Pten-null* mice were used to model PI3K/Akt/mTOR overactivation as PTEN downregulates this pathway [7]. Rapamycin was the first mTOR inhibitor to be tested in *Pten-null* mice; it was found to lead to a decrease in p-RPS6 and higher PF counts than in untreated mice [42]. The administration of rapamycin in eight-week-old rats led to a doubling of the proportion of PFs, whereas the proportions of antral, atretic and corpora lutea decreased [43]. Immunoblots revealed that mTOR and p-PS6K1 (the RPS6 activation enzyme) levels were lower in the ovaries of treated rats than in those of untreated rats [43].

Combined treatment with rapamycin and CY decreases PF exhaustion through the PI3K/Akt/mTOR pathway. In eight-week-old mice, CY treatment is not associated with an increase in PF apoptosis. By contrast, the proliferation index was found to be higher for the PF of treated mice, which had lower PF counts. In mice receiving the combined treatment, PF counts were higher, and lower levels of mTOR and p-RPS6 expression were observed [18]. This effect is not specific to rapamycin. The PF pool was also preserved following the co-administration of everolimus or sapanisertib (INK128), this combined treatment being associated with lower levels of p-AKT and p-PS6K1 detection in PFs. The inhibition of p-4EBP1 in PFs was observed only with sapanisertib cotreatment. Interestingly, in the remaining fertile animals given CY alone, the number of pups per litter was lower than that for mice receiving the combined treatment [19].

The effect of mTOR inhibitors has also been studied *ex vivo,* with cultures of thawed human ovarian tissue from 19- to 29-year-old female patients (the indications for tissue preservation were not described). Everolimus limited PF loss and decreased the number of growing follicles. The effect of everolimus on p-RPS6 observed in mice was also demonstrated in human follicles [14]. This suggests that the mouse model is of relevance to humans. mTOR inhibitors may, therefore, help to protect the ovarian reserve, by blocking PF activation without impairing ovarian hormonal function.

AS101

AS101 is a low-molecular weight organic tellurium compound with immunomodulatory activities. It has been shown to decrease p-Akt levels in cultured mouse multiple myeloma cell lines [49]. AS101 has been evaluated in CY-treated mice, in which it was associated with a lower ratio of growing to non-growing follicles. Co-treatment was also associated with lower levels of p-rpS6, whereas p-Akt levels were similar to those observed after treatment with CY alone, suggesting that AS101 might act downstream in the PI3K/Akt signaling pathway. Co-treatment preserved ovarian function, as demonstrated by its effects on litter size and cumulative pup numbers, which were similar to those in controls and greater than those for CY alone [20].

Proteasome inhibitor

Bortezomib is a proteasome inhibitor approved for use in the treatment of multiple myeloma [44]. Following the demonstration that the proteasome transports anthracycline to the nucleus, leading to DNA damage, the potential of bortezomib to protect against the damaging effects of anthracycline was studied in mice [45]. Mice received either bortezomib or a control followed by a DOX injection. Mice treated with bortezomib and DOX had fewer DNA breaks per ovarian cell. Co-treatment with bortezomib was also associated with a decrease in the rate of secondary follicle cell apoptosis and a larger litter size than treatment with DOX alone [46]. The DOX-induced apoptosis of PFs was prevented by bortezomib administration, but this study did not assess apoptosis in the follicles.

Glycogen synthase kinase 3 (GSK3) inhibitors

GSK3 is a serine/threonine protein kinase that interacts with Akt in the PI3K/Akt/mTOR pathway [47]. It is involved in reactive oxygen species (ROS) detoxification through mechanisms decreasing nuclear factor erythroid 2-like 2 (Nrf2) translocation to the nucleus, favoring ROS-induced cell death [48]. GSK3 inhibitors are currently under evaluation in phase I and II studies for use in the treatment of cancer (the NCT03678883 and NCT04239092 trials in particular). DOX induces cell damage through ROS. The effects of GSK3 inhibitors on folliculogenesis after DOX administration were therefore studied in mice. DOX administration with these inhibitors were associated with lower levels of ROS markers (malondialdehyde) and higher levels of antioxidant protein synthesis and expression (superoxide dismutase and glutathione peroxidase). Nrf2 expression and synthesis levels were also

higher in co-treated mice. In parallel, PF counts were higher in co-treated mice than in mice treated with DOX alone [23].

Cytotoxic drugs

ABVD (adriamycin [doxorubicin], bleomycin, vinblastine, dacarbazine) is a polychemotherapy regimen frequently used in the first-line treatment of Hodgkin lymphoma. McLaughlin *et al.* reported that ABVD-treated patients (*n*=8) had a higher proportion of non-growing follicles than controls [50]. The controls for this study included untreated patients (*n*=3), patients treated with OEPA-COPDAC (vincristine, etoposide, doxorubicin, prednisone-cyclophosphamide, vincristine, prednisone, dacarbazine; *n*=3), and women undergoing elective cesarean section (*n*=12). In parallel, mean follicle density (MFD) increased in ABVD-treated patients to levels even higher than the expected MFD for age [50].

The cytotoxic agents included in ABVD chemotherapy were studied individually. Adriamycin is a brand name for the anthracyline DOX. In a retrospective setting, DOX was found to be associated with an increase in the risk ratio of pregnancy [1.22 (95%CI: 1.04-1.45)]. This case-controlled study included female cancer survivors, treated before the age of 21 years, without fertility preservation. Exposure to cytotoxic drugs was assessed on the basis of medical records [51]. This surprising observation may reflect an uncontrolled bias or alpha risk inflation, given that DOX has been reported to be associated with PF loss in mice [21,22]. The ABVD regimen also contains vinblastine, an alkaloid agent like vincristine. The impact of vincristine on the ovarian reserve was evaluated in eight-week-old and six-month-old mice. PF counts were unaffected by vincristine administration, whereas pre-antral and antral follicle counts decreased. Remarkably, atretic follicle counts were higher in treated mice than in controls [52], suggesting an impact of vincristine on growing follicles but not on PFs. Nevertheless, these results are not consistent with a hypothetical protective effect of ABVD on the ovarian reserve.

OTHER MOLECULES MAY MINIMIZE CHEMOTHERAPY-RELATED GONADOTOXICITY

Non-anticancer molecules were also reported to minimize chemotherapy-related gonadotoxicity possibly by targeting apoptosis or PF activation pathways.

Sphingolipids

Sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P) are two sphingolipid compounds that were shown to impact cell survival [53,54]. In mice xenotransplanted with human ovarian cortical pieces co-administration of S1P with DOX and CY was associated with lower rate of apoptosis measured in PF by immunohistochemistry assays for activated capsase-3 [55]. This result was also reported with busulfan by an independent team which also reported higher PF count with co-administration procedure [56]. C1P associated with CY was associated with higher PF count. The effect may be also related to inhibition of apoptosis as illustrated by a higher BCLX-L/BAX ratio on whole ovarian lysed tissues. In addition, C1P was associated with rescue of fertility [57].

Anti-Müllerian hormone (AMH)

AMH, a member of transforming growth factor beta family, is known for its role in inducing the regression of Müllerian ducts in male sex differentiation [58]. Lower rate of developing follicles were observed in *in vitro* cultures of human ovaries cultured with AMH, suggesting a role in PF dormancy [59]. Co-administration of DOX, carboplatin or CY with recombinant human AMH (rhAMH) in peripuberal 6- to 7-week-old mice was associated with higher rate of PF [60]. Additionally number of eggs following gonadotropins injections and number of pups after mating from co-treated mice was comparable to control [61]. Co-administration of CY and AMH were associated with a slight decrease in pPS6K compared to CY-alone in 6-week-old Swiss mice. This suggests that AMH impact on PF growth is mediated through mTOR pathway [61].

Granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF)

G-CSF (filgrastim) alone or with SCF was concomitantly administered with CY and busulfan in 6-week-old mice. Higher number of PF was found in mice which received G-CSF alone or with SCF. After cytotoxic treatment, microvessel density measured by immunohistochemical staining for PECAM-1/CD31 increased when G-CSF alone or with SCF were administered. It was associated with higher rate litters and pups [62].

Dexrazoxane

Dexrazoxane is known for prevention of cardiologic and extravasation injuries from anthracyclines [63]. In mice, associated with DOX, dexrazoxane reduced double strand breaks in ovarian cells more particularly granulosa cells [64,65]. TUNEL assay revealed an effect on secondary follicles but not PF. PF pool was not reported but co-administration was associated with reduced infertility index [65].

Curcumin capsaicin

Curcumin and capsaicin, two dietary compound [66,67], were studied after CY injection in Wistar rats. Oxidative stress markers in rats were comparable to controls and histological analyses revealed lower rate of ovarian damage. But effects of these compound on PF pool and fertility were not reported [68].

Shilajit

Shilajit, a complex mixture of organic compounds which exudes from mountain rocks [69], administered after 8.3 Gy total body irradiation in Wistar rats was associated with higher PF counts [70].

Manganese dipyridoxyl diphosphate (mangafodipir)

Mangafodipir is used in magnetic resonance imaging and has some antioxidant activities [71]. CIS and paclitaxel treatment with mangafodipir in 6-week-old mice was associated with higher count of PF with a reduced expression of Ki67 illustrating the inhibition PF growth. Concomitantly, a reduction in oxidative stress, analyzed by lipid peroxidation assays, was observed notably in granulosa cells and follicles [72], suggesting that mangafodipir may prevent PF loss by preventing ROS-mediated damages.

Resveratrol

Resveratrol is a polyphenolic phytoalexin found in grapes and raisins. It is thought to exert antioxidant activities [73]. Resveratrol rescued viability and decreased levels of cellular ROS of rat granulosa cells cultured with CY [74]. But cultures of ovarian sheep tissues revealed that resveratrol alone was associated with lower PF count possibly mediated by PI3K pathway activation [75].

DISCUSSION AND PERSPECTIVES

Not all anticancer treatments are associated with POF. In this review, we highlight the ovarian reserve-protecting effects of some such treatments, through their impact on PF activation and apoptosis pathways. In mouse models, combinations of certain targeted therapies and classic cytotoxic treatments has been shown to prevent exhaustion of the PF pool (Figure 4B).

The PI3K/Akt/mTOR pathway is the cornerstone of PF activation. mTOR inhibitors may promote PF quiescence during cytotoxic treatments. The level of proof for this finding currently extends to *ex vivo* models of cultured human ovaries, suggesting feasibility for use in humans [14,18,19]. Nevertheless, adding an immunosuppressive drug to the treatment regimen may increase the risk of infection in patients who are already immunocompromised. New PI3K inhibitors, such as idelalisib, may also target PI3K pathways and could be evaluated *in vitro* [76].

KIT and ABL are involved in PF activation and apoptosis after chemotherapy, respectively [9,12]. The impact of ABL and KIT inhibitors on PF pathways remains a matter of debate. Gonfloni *et al.* demonstrated that imatinib prevents PF death from CY-induced apoptosis in a mouse model [9]. However, this blocking of apoptosis by imatinib in the context of double-strand breaks could potentially results in the conservation of oocytes with chemotherapy-related acquired cytogenetic abnormalities. But Kim *et al.* reported better tissue recovery in mice receiving grafts of co-treated ovaries than in those receiving grafts treated with CIS alone [26]. Imatinib inhibits KIT and might therefore be useful for blocking PF activation, and therefore limiting PF loss [32]. However, in a different model of CIS-treated mice, Kerr *et al.* found that imatinib had no beneficial effects for ovarian reserve protection, suggesting that the effects of imatinib may not be reproducible in different conditions [24]. Another study showed that the dose and manufacturer of the CIS used for treatment affected the results obtained [25]. Sunitinib was found to block PF activation in mice after superovulation, but its impact on mice receiving chemotherapy is unknown [38]. Inhibition profiles differ between TKIs, so explorations of other KIT inhibitors are warranted [77].

Other approaches have been evaluated, including the prevention of DOX transport into the nucleus by proteasome inhibition, for example [46]. According to the hypothesis underlying this approach, bortezomib might be expected to decrease the anti-tumoral efficacy of DOX. However, adding bortezomib to DOX-containing polychemotherapy for diffuse large B-cell lymphoma did not

shorten progression-free or overall survival [78]. GSK3 inhibitors have been studied as ROS modulators, probably exerting their effects by improving the antioxidant activities of Nrf2 after DOX administration [23]. GSK3 is inactivated by pArk, suggesting that GSK3 inhibitors might affect the PI3K/Akt/mTOR-mediated activation of PF growth after treatment with CY [47].

Surprisingly, two independent groups using different methods observed that some cytotoxic agents protected the ovarian reserve in humans [50,51]. However, these results might also be explained by uncontrolled confusion bias; synergistic effects of certain chemotherapy agents on the ovarian reserve; possible false-positive results; publication bias. In studies based on the same methodology, no PF pool preservation after ABVD was observed in our institution (unpublished results).

Precautions must be taken when using targeted therapies. Current evidence concerning their impact on the ovarian reserve mostly relates to *in vivo* murine or *ex vivo* human ovarian culture models. Imatinib has been studied preferentially in young mice, whereas mTOR inhibitors have been studied mostly in eight-week-old mice. Results for cultured human ovaries treated with imatinib have suggested that the findings for the ABL/TAp63 pathway in mice are not relevant to humans [9,25,36]. Conversely, the effect of the mTOR inhibitor everolimus on the PI3K/Akt/mTOR pathway seems to be reproducible in humans [14]. Murine models can be used to study the pharmacodynamics of the molecules, but the protocols used for evaluations have been heterogeneous (Table 1). The targeted therapy was administered at the same time as the cytotoxic agent in some studies, and before it in others. Furthermore, mice of different ages were used for these experiments. Similarly, the effects of targeted treatments were evaluated during only one cycle of cytotoxic treatment, at a particular time point. The effect of supposedly protective treatments on the ovarian reserve after multiple cycles of cytotoxic treatment therefore remains unknown. The dose of cytotoxic agent may also have a major impact on the protective effect of the drug. In CIS-treated mice, high doses of CIS abolished the effects of imatinib on apoptosis [24,25]. Similarly, chemotherapy protocols often combine multiple agents with different effects on the cell cycle over a specific time sequence. The additive, synergic or antagonist effects on the PF pool of the targeted therapy in association with cytotoxic agents require evaluation.

Data for humans are therefore warranted, to find new targetable pathways and to evaluate the safety and feasibility of these co-administration procedures. Given the potentially large number of

pathways involved in cytotoxicity-related PF exhaustion, multi-omics approaches associating transcriptomic and proteomic studies might facilitate the identification of new targetable pathways modified by chemotherapy. An analysis of tissues from patients included in prospective studies cryopreserved for the purposes of fertility preservation, involving a single randomized targeted treatment, could provide preclinical data for the evaluation of such treatments. The subsequent design of prospective trials is likely to be a major challenge. The bioavailability of these molecules in ovarian tissues is unknown. The administration sequence must be defined, as there is increasing evidence for pharmacological interactions affecting the pharmacodynamics of anticancer treatments [79]. Potential interactions will, therefore need to be assessed before administration with cytotoxic agents, to prevent toxicity and decreases in efficacy.

Apart from anticancer treatments, other molecules like sphingolipids, AMH, G-CSF, dexrazoxane, curcumin, capsaicin, shilajit, mangafodipir, resveratrol may target PF exhaustion mechanisms by preventing stroma injury or by the inhibition of apoptosis, ROS generation or PI3K pathway. Data regarding the POF preventive effect of these molecules are restricted to mice models and little is known on their respective mechanisms of action. Some of these approach may be limited by their feasibility in human. In example: *(i)* G-CSF dose in mice was 10 times the dose used in febrile neutropenia prevention [62] *(ii)* the equivalent dose of curcumin and shilajit for a 70 kg female patient would be 7 kg (100 mg/kg used in rats) [68,70].

Fertility preservation strategies that rely on embryos, oocyte cryopreservation are not appropriate in emergency conditions or in patients with hormone-sensitive cancers. In addition, surgical procedures for ovarian tissue cryopreservation may not be suitable in the context of hematological disorders [1,4,80]. Hormone suppression with gonadotropin-releasing hormone agonist (GnRHa) is indicated for premenopausal breast cancer patients [80,81] but its effect on other cancer subtypes remains controversial [82,83]. If the co-administration of a targeted drug with cytotoxic treatment proves to be feasible with no increase in toxicity it could be used in fertility preservation regardless the cancer subtype. Co-administration protocols might slow PF exhaustion, and could be used alone or in association with other strategies, such as ovarian tissue transplantation, which is more effective at high follicle density [84]. By stopping follicle activation, the co-treatment strategy might also increase the follicular content of the ovary and improve ovarian tissue quality, thereby promoting the recovery of ovarian tissue function after transplantation. Co-treatment strategies may

also be useful in emergency situations in which cryopreservation is impossible. Another strategy could consider the use of targeted therapies preventing PF activation may be used after cancer treatment, to delay POF further.

CONCLUSIONS

Cytotoxic agents, including alkylating agents in particular, are the most damaging anticancer treatments, but other drugs may not reduce the PF pool. The studies performed to date have highlighted the prevention of PF activation by the co-administration of mTOR inhibitors with cytotoxic treatment. These results should stimulate reflections about alternative fertility preservation strategies. Nevertheless, the available evidence was mostly obtained from mouse models, and there is, therefore, still a need for more clinical data based on studies of human tissues before evaluations of these protective studies in humans can be envisaged.

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REFERENCES

1. Salama M, Anazodo A, Woodruff TK. Preserving fertility in female patients with hematological malignancies: a multidisciplinary oncofertility approach. Ann Oncol. 2019;30(11):1760-1775.
2. Podfigurna-Stopa A, Czyzyk A, Grymowicz M, et al. Premature ovarian insufficiency: the context of long-term effects. J Endocrinol Invest. 2016;39(9):983-990.
3. Chemaitilly W, Li Z, Krasin MJ, et al. Premature ovarian insufficiency in childhood cancer survivors: A report from the St. Jude lifetime cohort. J Clin Endocrinol Metab. 2017;102(7):2242-2250.
4. Kim SY, Kim SK, Lee JR, et al. Toward precision medicine for preserving fertility in cancer patients: existing and emerging fertility preservation options for women. J Gynecol Oncol. 2016;27(2):e22.
5. Poirot C, Abirached F, Prades M, et al. Induction of puberty by autograft of cryopreserved ovarian tissue. Lancet. 2012;379(9815):588.
6. Poirot C, Fortin A, Dhédin N, et al. Post-transplant outcome of ovarian tissue cryopreserved after chemotherapy in hematological malignancies. Haematologica. 2019;104(8):e360-e363
7. Adhikari D, Liu K. Molecular mechanisms underlying the activation of mammalian primordial follicles. Endocr Rev. 2009;30(5):438-464.
8. Reddy P, Liu L, Adhikari D, et al. Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. Science. 2008;319(5863):611-613.
9. Gonfloni S, Tella LD, Caldarola S, et al. Inhibition of the c-Abl-TAp63 pathway protects mouse oocytes from chemotherapy-induced death. Nat Med. 2009;15(10):1179-1185.
10. Hansen KR, Knowlton NS, Thyer AC, et al.. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod. 2008;23(3):699-708.
11. Reddy P, Zheng W, Liu K. Mechanisms maintaining the dormancy and survival of mammalian primordial follicles. Trends Endocrinol Metab. 2010;21(2):96-103.
12. Nilsson EE, Detzel C, Skinner MK. Platelet-derived growth factor modulates the primordial to primary follicle transition. Reproduction. 2006;131(6):1007-1015.
13. Sutherland JM, Frost ER, Ford EA, et al. Janus kinase JAK1 maintains the ovarian reserve of primordial follicles in the mouse ovary. Mol Hum Reprod. 2018;24(11):533-542.

14. Grosbois J, Demeestere I. Dynamics of PI3K and Hippo signaling pathways during *in vitro* human follicle activation. Hum Reprod. 2018;33(9):1705-1714.
15. Morgan S, Anderson RA, Gourley C, et al. How do chemotherapeutic agents damage the ovary? Hum Reprod Update. 2012;18(5):525-535.
16. Oktem O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. Cancer. 2007;110(10):2222-2229.
17. Oktem O, Oktay K. A novel ovarian xenografting model to characterize the impact of chemotherapy agents on human primordial follicle reserve. Cancer Res. 2007;67(21):10159-10162.
18. Zhou L, Xie Y, Li S, et al. Rapamycin prevents cyclophosphamide-induced over-activation of primordial follicle pool through PI3K/Akt/mTOR signaling pathway *in vivo*. J Ovarian Res. 2017;10(1):56.
19. Goldman KN, Chenette D, Arju R, et al. mTORC1/2 inhibition preserves ovarian function and fertility during genotoxic chemotherapy. Proc Natl Acad Sci USA. 2017;114(12):3186-3191.
20. Kalich-Philosoph L, Roness H, Carmely A, et al. Cyclophosphamide triggers follicle activation and “burnout”; AS101 prevents follicle loss and preserves fertility. Sci Transl Med. 2013;5(185):185ra62.
21. Soleimani R, Heytens E, Darzynkiewicz Z, et al. Mechanisms of chemotherapy-induced human ovarian aging: double strand DNA breaks and microvascular compromise. Aging (Albany NY). 2011;3(8):782-793.
22. Morgan S, Lopes F, Gourley C, et al. Cisplatin and doxorubicin induce distinct mechanisms of ovarian follicle Loss; imatinib provides selective protection only against cisplatin. PLoS One. 2013;8(7):e70117.
23. Niringiyumukiza JD, Cai H, Chen L, et al. Protective properties of glycogen synthase kinase-3 inhibition against doxorubicin-induced oxidative damage to mouse ovarian reserve. Biomed Pharmacother. 2019;116:108963.
24. Kerr JB, Hutt KJ, Cook M, et al. Cisplatin-induced primordial follicle oocyte killing and loss of fertility are not prevented by imatinib. Nat Med. 2012;18(8):1170-1172.
25. Maiani E, Di Bartolomeo C, Klinger FG, et al. Reply to: cisplatin-induced primordial follicle oocyte killing and loss of fertility are not prevented by imatinib. Nat Med. 2012;18(8):1172-1174.

26. Kim SY, Cordeiro MH, Serna VA, et al. Rescue of platinum-damaged oocytes from programmed cell death through inactivation of the p53 family signaling network. Cell Death Differ. 2013;20(8):987-997
27. Fabbri R, Macciocca M, Vicenti R, et al. Doxorubicin and cisplatin induce apoptosis in ovarian stromal cells obtained from cryopreserved human ovarian tissue. Futur Oncol. 2016;12(14):1699-1711.
28. Bar-Joseph H, Ben-Aharon I, Tzabari M, et al. *In vivo* bioimaging as a novel strategy to detect doxorubicin-induced damage to gonadal blood vessels. PLoS One. 2011;6(9):e23492.
29. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996;2(5):561-566.
30. Bantscheff M, Eberhard D, Abraham Y, et al. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. Nat Biotechnol. 2007;25(9):1035-1044.
31. Buchdunger E, Cioffi CL, Law N, et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits *in vitro* signal transduction mediated by c-kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther. 2000;295(1):139-145.
32. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med. 2002;347(7):472-480.
33. Jaeckle KA, Anderson SK, Twohy EL, et al. Phase I-II trial of imatinib mesylate (Gleevec; STI571) in treatment of recurrent oligodendroglioma and mixed oligoastrocytoma. North Central Cancer Treatment Group Study N0272 (ALLIANCE/NCCTG). J Neurooncol. 2019;143(3):573-581.
34. Schultheis B, Nijmeijer BA, Yin H, et al. Imatinib mesylate at therapeutic doses has no impact on folliculogenesis or spermatogenesis in a leukaemic mouse model. Leuk Res. 2012;36(3):271-274.
35. Asadi-azarbaijani B, Santos RR, Jahnukainen K, et al. Developmental effects of imatinib mesylate on follicle assembly and early activation of primordial follicle pool in postnatal rat ovary. Reprod Biol. 2017;17(1):25-33.
36. Bildik G, Acilan C, Sahin GN, et al. C-Abl is not activated in DNA damage-induced and Tap63-mediated oocyte apoptosis in human ovary. Cell Death Dis. 2018;9(10):943.
37. Nassif E, Thibault C, Vano Y, et al. Sunitinib in kidney cancer: 10 years of experience and development. Expert Rev Anticancer Ther. 2017;17(2):129-142.

38. Bernard V, Bouilly J, Kramer P, et al. The tyrosine kinase inhibitor sunitinib affects ovulation but not ovarian reserve in mouse: a preclinical study. PLoS One. 2016;11(4):e0152872.
39. Saunders RN, Metcalfe MS, Nicholson ML. Rapamycin in transplantation: A review of the evidence. Kidney Int. 2001;59(1):3-16.
40. O'Shaughnessy J, Thaddeus Beck J, Royce M. Everolimus-based combination therapies for HR^+, $HER2^-$ metastatic breast cancer. Cancer Treat Rev. 2018;69:204-214.
41. Xhaard A, Launay M, Sicre de Fontbrune F, et al. A monocentric study of steroid-refractory acute graft-versus-host disease treatment with tacrolimus and mTOR inhibitor. Bone Marrow Transplant. 2020;55(1):86-92.
42. Adhikari D, Risal S, Liu K, et al. Pharmacological inhibition of mTORC1 prevents over-activation of the primordial follicle pool in response to elevated PI3K signaling. PLoS One. 2013;8(1):e53810.
43. Zhang XM, Li L, Xu JJ, et al. Rapamycin preserves the follicle pool reserve and prolongs the ovarian lifespan of female rats via modulating mTOR activation and sirtuin expression. Gene. 2013;523(1):82-87.
44. Scott K, Hayden PJ, Will A, et al. Bortezomib for the treatment of multiple myeloma. Cochrane Database Syst Rev. 2016;4:CD010816.
45. Kiyomiya K, Matsuo S, Kurebe M. Mechanism of Specific Nuclear Transport of Adriamycin: The Mode of Nuclear Translocation of Adriamycin-Proteasome Complex. Cancer Res. 2001;61(6):2467-2471.
46. Roti Roti EC, Ringelstetter AK, Kropp J, et al. Bortezomib prevents acute doxorubicin ovarian insult and follicle demise, improving the fertility window and pup birth weight in mice. PLoS One. 2014;9(9):e108174.
47. Hermida MA, Dinesh Kumar J, Leslie NR. GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. Adv Biol Regul. 2017;65:5-15.
48. Rojo AI, Sagarra MR, Cuadrado A. GSK-3beta down-regulates the transcription factor Nrf2 after oxidant damage: Relevance to exposure of neuronal cells to oxidative stress. J Neurochem. 2008;105(1):192-202.

49. Hayun M, Naor Y, Weil M, et al. The immunomodulator AS101 induces growth arrest and apoptosis in multiple myeloma: association with the Akt/survivin pathway. Biochem Pharmacol. 2006;72(11):1423-1431.
50. McLaughlin M, Kelsey TW, Wallace WH, et al. Non-growing follicle density is increased following Adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) chemotherapy in the adult human ovary. Hum Reprod. 2017;32(1):165-174.
51. Green DM, Kawashima T, Stovall M, et al. Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol. 2009;27(16):2677-2685.
52. Winship AL, Carpenter M, Griffiths M, et al. Vincristine chemotherapy induces atresia of growing ovarian follicles in mice. Toxicol Sci. 2019;169(1):43-53.
53. The Human Metabolome Database, Sphingosine-1-phosphate. Available at https://hmdb.ca/metabolites/HMDB0000277. Accessed November 26, 2020.
54. The Human Metabolome Database, Ceramide-1-phosphate. Available at https://hmdb.ca/metabolites/HMDB0010699. Accessed November 26, 2020.
55. Fang L, Turan V, Lierman S, et al. Sphingosine-1-phosphate prevents chemotherapy-induced human primordial follicle death. Hum Reprod. 2014;29(1):107-113.
56. Tan SJ, Lee LJ, Tzeng CR, et al. Targeted anti-apoptosis activity for ovarian protection against chemotherapy-induced ovarian gonadotoxicity. Reprod Biomed Online. 2014;29(5):612-620.
57. Pascuali N, Scotti L, Di Pietro M, et al. Ceramide-1-phosphate has protective properties against cyclophosphamide-induced ovarian damage in a mice model of premature ovarian failure. Hum Reprod. 2018;33(5):844-859.
58. Moolhuijsen LME, Visser JA. Anti-Müllerian Hormone and Ovarian Reserve: Update on Assessing Ovarian Function. J Clin Endocrinol Metab. 2020;105(11):3361-3373.
59. Carlsson IB, Scott JE, Vissier JA et al. Anti-Müllerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro. Hum Reprod. 2006;21(9):2223-2227.
60. Kano M, Sosulski AE, Zhang L, et al. AMH/MIS as a contraceptive that protects the ovarian reserve during chemotherapy. Proc Natl Acad Sci U S A. 2017;114(9):E1688-E1697.
61. Sonigo C, Beau I, Grynberg M, et al. AMH prevents primordial ovarian follicle loss and fertility alteration in cyclophosphamide-treated mice. FASEB J. 2019;33(1):1278-1287.

62. Skaznik-Wikiel ME, McGuire MM, Sukhwani M, et al. Granulocyte colony-stimulating factor with or without stem cell factor extends time to premature ovarian insufficiency in female mice treated with alkylating chemotherapy. Fertil Steril. 2013;99(7):2045-2054.e3.
63. Doroshow JH. Dexrazoxane for the prevention of cardiac toxicity and treatment of extravasation injury from the anthracycline antibiotics. Curr Pharm Biotechnol. 2012;13(10):1949-1956.
64. Roti Roti EC, Salih SM. Dexrazoxane ameliorates doxorubicin-induced injury in mouse ovarian cells. Biol Reprod. 2012;86(3):96.
65. Kropp J, Roti Roti EC, Ringelstetter A et al. Dexrazoxane Diminishes Doxorubicin-Induced Acute Ovarian Damage and Preserves Ovarian Function and Fecundity in Mice. PLoS One. 2015;10(11):e0142588.
66. The Human Metabolome Database, Curcumin. Available at https://hmdb.ca/metabolites/HMDB0002269. Accessed November 26, 2020.
67. The Human Metabolome Database, Capsaicin. Available at https://hmdb.ca/metabolites/HMDB0002227. Accessed November 26, 2020.
68. Melekoglu R, Ciftci O, Eraslan S, et al. Beneficial effects of curcumin and capsaicin on cyclophosphamide-induced premature ovarian failure in a rat model. J Ovarian Res. 2018;11(1):33.
69. Agarwal SP, Khanna R, Karmarkar R, et al. Shilajit: a review. Phytother Res. 2007;21(5):401-405.
70. Kececi M, Akpolat M, Gulle K, et al. Evaluation of preventive effect of shilajit on radiation-induced apoptosis on ovaries. Arch Gynecol Obstet. 2016;293(6):1255-1262.
71. Spath NB, Thompson G, Baker AH, et al. Manganese-enhanced MRI of the myocardium. Heart. 2019;105(22):1695-1700.
72. Qin Y, Iwase A, Murase T, et al. Protective effects of mangafodipir against chemotherapy-induced ovarian damage in mice. 2018;16(1):106.
73. The Human Metabolome Database, Resveratrol. Available at https://hmdb.ca/metabolites/HMDB0003747. Accessed November 26, 2020
74. Nie Z, Zhang L, Chen W, et al. The protective effects of pretreatment with resveratrol in cyclophosphamide-induced rat ovarian granulosa cell injury: In vitro study. Reprod Toxicol. 2020;95:66-74.

75. Bezerra MA, Gouveia BB, Barberino RS, et al. Resveratrol promotes in vitro activation of ovine primordial follicles by reducing DNA damage and enhancing granulosa cell proliferation via phosphatidylinositol 3-kinase pathway. Reprod Domest Anim. 2018;53(6):1298-1305.
76. Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med. 2014;370(11):997-1007.
77. Karaman MW, Herrgard S, Treiber DK, et al. A quantitative analysis of kinase inhibitor selectivity. Nat Biotechnol. 2008;26(1):127-132.
78. Davies A, Cummin TE, Barrans S, et al. Gene-expression profiling of bortezomib added to standard chemoimmunotherapy for diffuse large B-cell lymphoma (REMoDL-B): an open-label, randomised, phase 3 trial. Lancet Oncol. 2019;20(5):649-662.
79. Min JS, Bae SK. Prediction of drug–drug interaction potential using physiologically based pharmacokinetic modeling. Arch Pharm Res. 2017;40(12):1356-1379.
80. Lambertini M, Peccatori FA, Demeestere I, et al. Fertility preservation and post-treatment pregnancies in post-pubertal cancer patients: ESMO Clinical Practice Guidelines. Ann Oncol. 2020 (in press).
81. Lambertini M, Moore HCF, Leonard RCF, et al. Gonadotropin-Releasing Hormone Agonists During Chemotherapy for Preservation of Ovarian Function and Fertility in Premenopausal Patients With Early Breast Cancer: A Systematic Review and Meta-Analysis of Individual Patient-Level Data. J Clin Oncol. 2018;36(19):1981-1990.
82. Demeestere I, Brice P, Peccatori FA, et al. Gonadotropin-releasing hormone agonist for the prevention of chemotherapy-induced ovarian failure in patients with lymphoma: 1-year follow-up of a prospective randomized trial. J Clin Oncol. 2013;31(7):903-909.
83. Turan V, Bedoschi G, Rodriguez-Wallberg K, et al. Utility of Gonadotropin-Releasing Hormone Agonists for Fertility Preservation: Lack of Biologic Basis and the Need to Prioritize Proven Methods. J Clin Oncol. 2019;37(1):84-86.
84. Poirot C, Fortin A, Lacorte JM, et al. Impact of cancer chemotherapy before ovarian cortex cryopreservation on ovarian tissue transplantation. Hum. Reprod. 2019;34(6):1083-1094.

FIGURE LEGENDS

Figure 1. Indexed references in PubMed identified with the keywords 'cancer targeted therapies' from 1980 to 2018.

Figure 2. Change in primordial follicle count over time, and influence of cytotoxic agents on ovarian decay. In black: the pool of primordial follicles (ovarian reserve) is fixed at birth. In healthy women, the number of primordial follicles decreases with age, resulting in menopause. In red: cytotoxic treatment may lead to apoptosis or trigger follicle activation, leading to ovarian reserve exhaustion and premature ovarian failure (POF).

Figure 3. Main pathways involved in follicle activation leading to the initiation of follicular growth. The PI3K/Akt/mTOR pathway is triggered by the binding of KIT-ligand (KIT-L) to its receptor (KIT). Environmental factors also regulate follicular growth: cell-to-cell contact (Hippo signaling), stromal signals (BMP-4 and KGF) or cytokine-like platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and leukemia inhibitory factor (LIF).

Figure 4. Impact of cytotoxic agents on pathways involved in primordial follicle pool exhaustion. (A) Main mechanisms involved in primordial follicle pool exhaustion during chemotherapy. (B) Hypothetical targetable pathways for inhibiting apoptosis or follicle activation in patients treated with cytotoxic agents.

Table 1. Evidence for protective effects of anticancer drugs on the ovarian reserve.

Class	Molecule	Reference	Model		Administration	Dose/day	Co-administered cytotoxic agent	Administration sequence	Effects on ovarian reserve relative to control	Effect on *in vivo* fertility
Tyrosine kinase inhibitor	**Imatinib**	Kim et al. [26]	*In vitro* culture then grafted into mice	CD57BL/6 PND 5 (ovaries) and 3-week-old mice (grafted)	Medium	5 µM	CIS SIGMA (4 µM)	In culture: cisplatin day 1 imatinib days 2 to 4 then grafted into syngeneic ovariectomized mice	Day+14 after graft: better tissue recovery and higher PF count	NR
		Schultheis et al. [34]	*In vivo*, mice	C3H/Hej 5-week-old mice injected with BCR-ABL cell lines	Oral (water)	3 mg (150 mg/kg) 50 days	No	N/A	After 2 months of continuous treatment: PF pool similar to control Ovary architecture disorganized	NR
		Gonfloni et al. [9]	*In vivo*, mice	CD1 PND 5 and 7	IP	7.5 mg/kg once	CIS SIGMA (5 mg/kg)	Simultaneous	PND 10: higher counts of pooled primordial and primary follicles	Larger number of pups in litters from co-treated mice
		Kerr et al. [24]	*In vivo*, mice	CD1 & C57BL/6 PND 5 and 7	IP	7.5 mg/kg once	CIS HG (5 mg/kg)	Simultaneous	PND 10 and PND 49: similar depletion of PF	No difference between CIS- and co-treated mice
			In vivo, mice	C57BL/6 PND 5 and 7	IP	7.5 mg/kg once	CIS HG (5 mg/kg)	Imatinib 2 hours before CIS	PND 10 and PND 49: similar depletion of PF	NR
		Asadi-Azarbaijani et al. [35]	*In vivo*, rats	Sprague Dawley PND 2	Intracavitary (stomach) injections	150 mg/kg 3 days	No	N/A	PND 5: increase in number of oogonia, induce in the quiescence of granulosa cells	NR
		Morgan et al. [22]	*Ex vivo* ovarian cultures, mice	C57BL/6J newborn	Medium	N/A	DOX CIS SIGMA	Imatinib days 1 to 3 cytotoxic agent day 2	After 6 days of culture: smaller numbers of unhealthy follicles in CIS group	N/A
		Bildik et al. [36]	*Ex vivo* cultures, human	Female patients with benign ovarian cysts (mean age 27 years)	Medium	10 µM	CIS Eli Lilly and Company 20 µM	Imatinib 2 hours before CIS	At 24h, similar depletion of PFs and bizarre-shaped primordial follicles	N/A
			Human ovaries xenografted, mice	Nude mice 8 weeks old	IP	7.5 mg/kg	CIS Eli Lilly and Company 5 mg/kg	Imatinib 2 hours before CIS	At 24h, similar depletion of PFs and bizarre-shaped primordial follicles	NR
	Sunitinib	Bernard et al. [38]	*In vivo*, mice	NR 3 to 6 weeks old	oral	50 mg/kg 35 days	no	N/A	less response to superovation test	No difference relative to untreated mice

mTOR inhibitors	**Rapamycin**	Adhikari et al. [42]	*In vivo,* mice	B57BL/6J *OoPten*$^{-/-}$ PND 4	IP	5 mg/kg 19 days	No	N/A	PND 23: increase in PF pool	NR
		Zhang et al. [43]	*In vivo,* rats	Sprague Dawley 8 weeks old	IP	5 mg/kg 10 weeks	No	N/A	After 10 weeks: increase in PF pool	NR
		Zhou et al. [18]	*In vivo*, mice	BALB/c 8 weeks old	NR	5 mg/kg once	CY (75-100-150 mg/kg)	Rapamycin 1 week before and after CY	7 days after last CY injection: increase in PF pool	NR
	Sapanisertib	Goldman et al. [19]	*In vivo,* mice	C57BL/6 8 weeks old	NR	0.3 mg/kg	CY (75 mg/kg)	Sapanisertib daily days 1-5 of weeks 1-3, and days 1-4 of week 4; CY on day 3 of weeks 1-3	1 week after last CY injection: increase in PF pool	More pups and no infertility in co-treated mice
	Everolimus	Goldman et al. [19]	*In vivo,* mice	C57BL/6 8 weeks old	NR	2.5 mg/kg	CY (75 mg/kg)	Everolimus daily days 1-5 of weeks 1-3, and days 1-4 of week 4; CY on day 3 of weeks 1-3	1 week after last CY injection: increased PF pool	More pups and no infertility in co-treated mice
		Grosbois et al. [14]	*Ex vivo* cultures, human	19-29 years old female	medium	N/A	No	N/A	Higher PF count	N/A
Proteasome inhibitor	**Bortezomib**	Roti Roti et al. [46]	*In vivo,* mice	CD1 4 weeks old	IP	0.143 mg/kg once	DOX (20 mg/kg)	Bortezomib 1 hour before DOX	Not assessed	Longer fertility and more pups per litter
GSK3 inhibitor	**SB216763**	Niringiyumukiza et al. [23]	*In vivo*, mice	ICR 7-8 weeks old	IP	5-10 mg/kg	DOX (10 mg/kg)	10 mg/kg before single-dose DOX and 5 mg/kg 3 times per week for 2 weeks	3 weeks after DOX injection: increase in PF pool	N/A
Cytotoxic agents	**DOX**	Green et al. [51]	Human, retrospective study	Female < 21 years old	N/A	N/A	N/A	N/A	N/A	RR of pregnancy: 1.22 (95%CI: 1.04-1.45)
	ABVD	McLaughlin et al. [50]	Human, retrospective study	Female patients	N/A	N/A	N/A	N/A	Higher PF count	NR
	Vincristine	Winship et al. [52]	*In vivo,* mice	C57BL/6 8 weeks old 6 months old	IP	1 mg/kg	N/A	Days 1, 4 and 8	No effect on PF pool	NR
Other	**AS101**	Kalich-Philosoph et al. [20]	*In vivo,* mice	BALB/c 8 weeks old	IP	10 µg	CY (75-100-150 mg/kg/week, 75 mg x4 mg/kg/week)	1 week before and after chemo	1 week after CY injection: increase in PF pool	More pups and larger litter size

ABVD: Adriamycin, bleomycin, vinblastine, dacarbazine; CY: cyclophosphamide; CIS: cisplatin; DOX: doxorubicin; HG: hospital-grade; PND: postnatal day IP: intraperitoneal; NR: not reported; PF: primordial follicles; RR: risk ratio

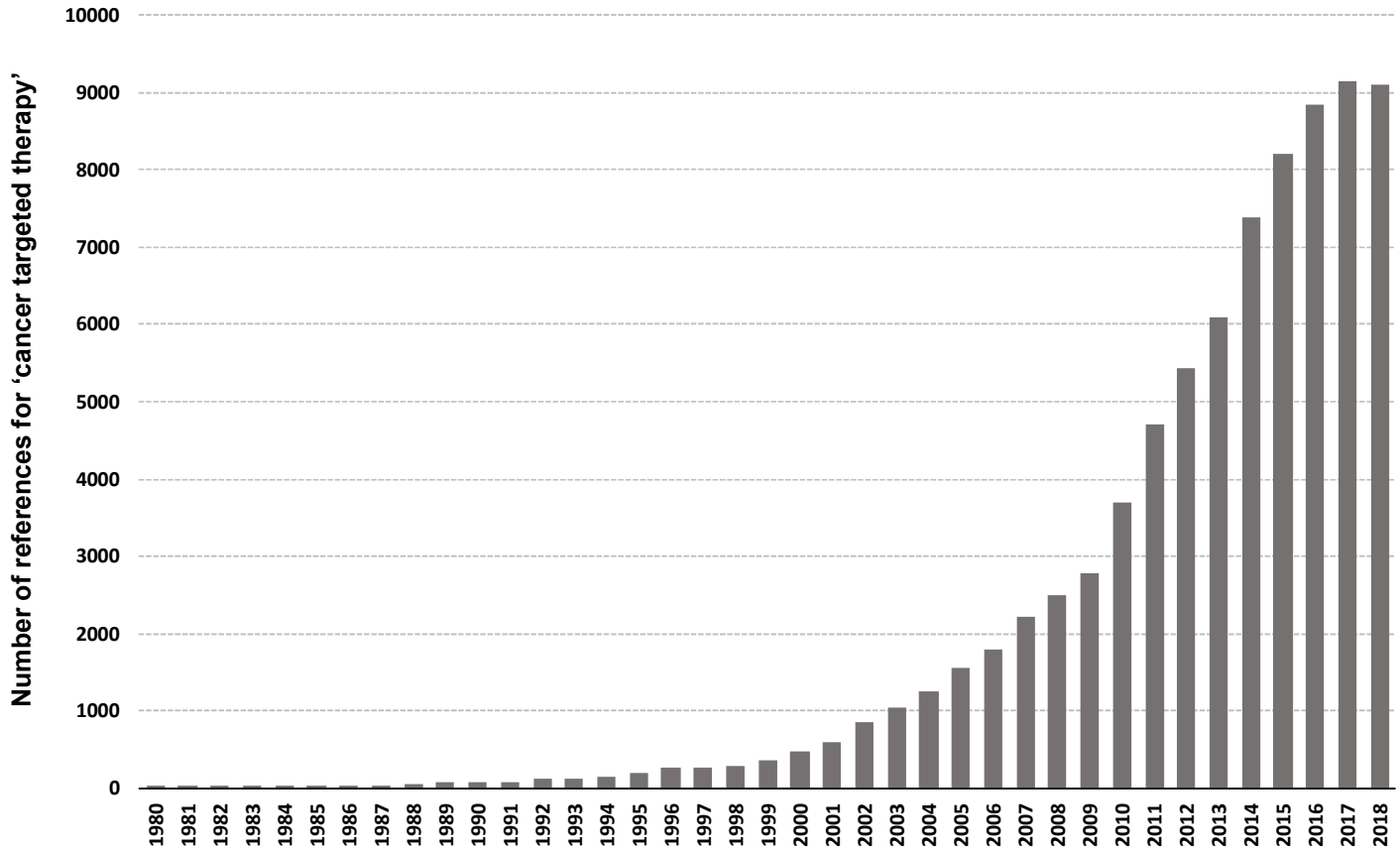
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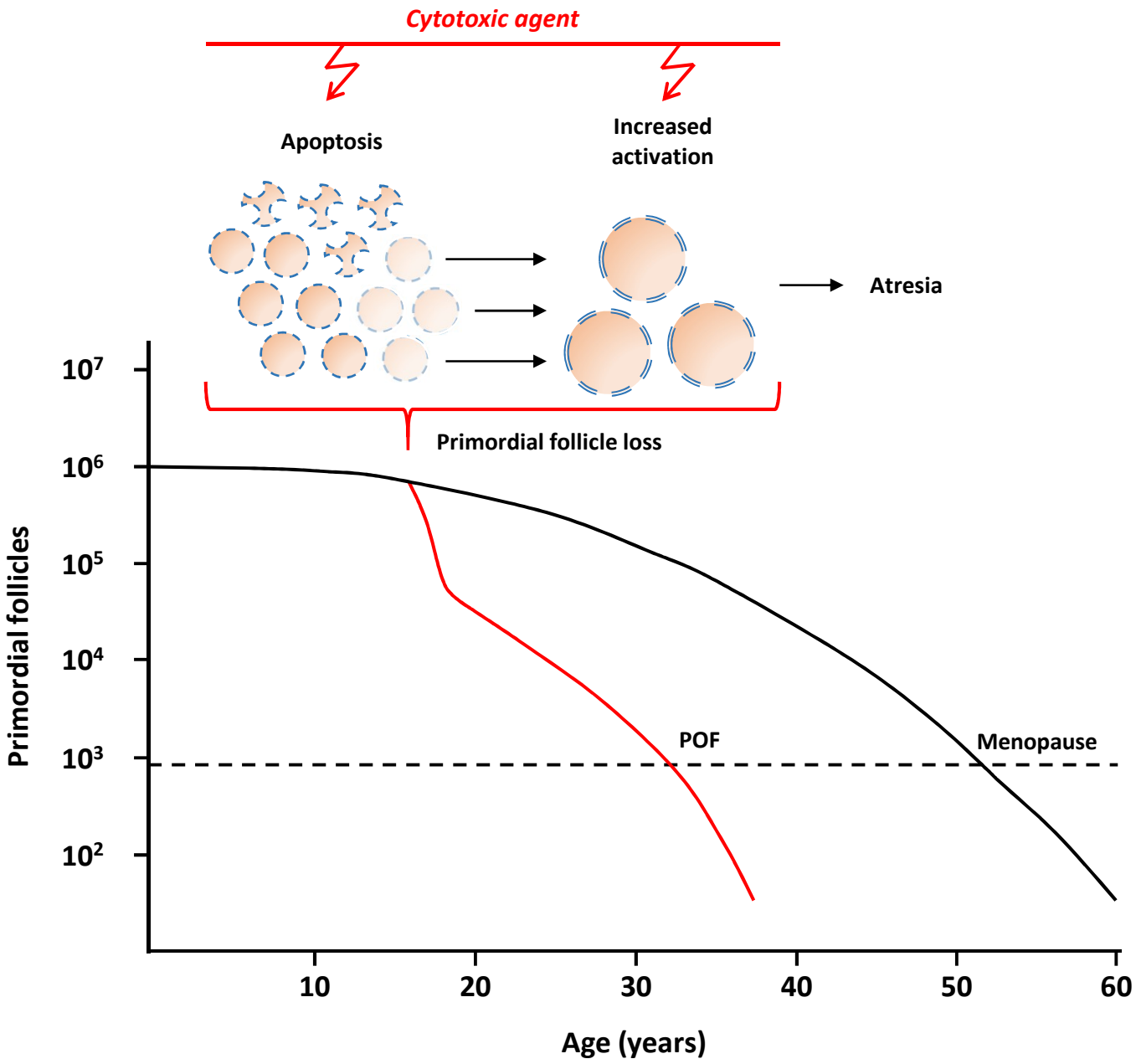
Figure 1


Cytotoxic agent
Apoptosis
Increased activation
Atresia
Primordial follicle loss
Primordial follicles
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POF
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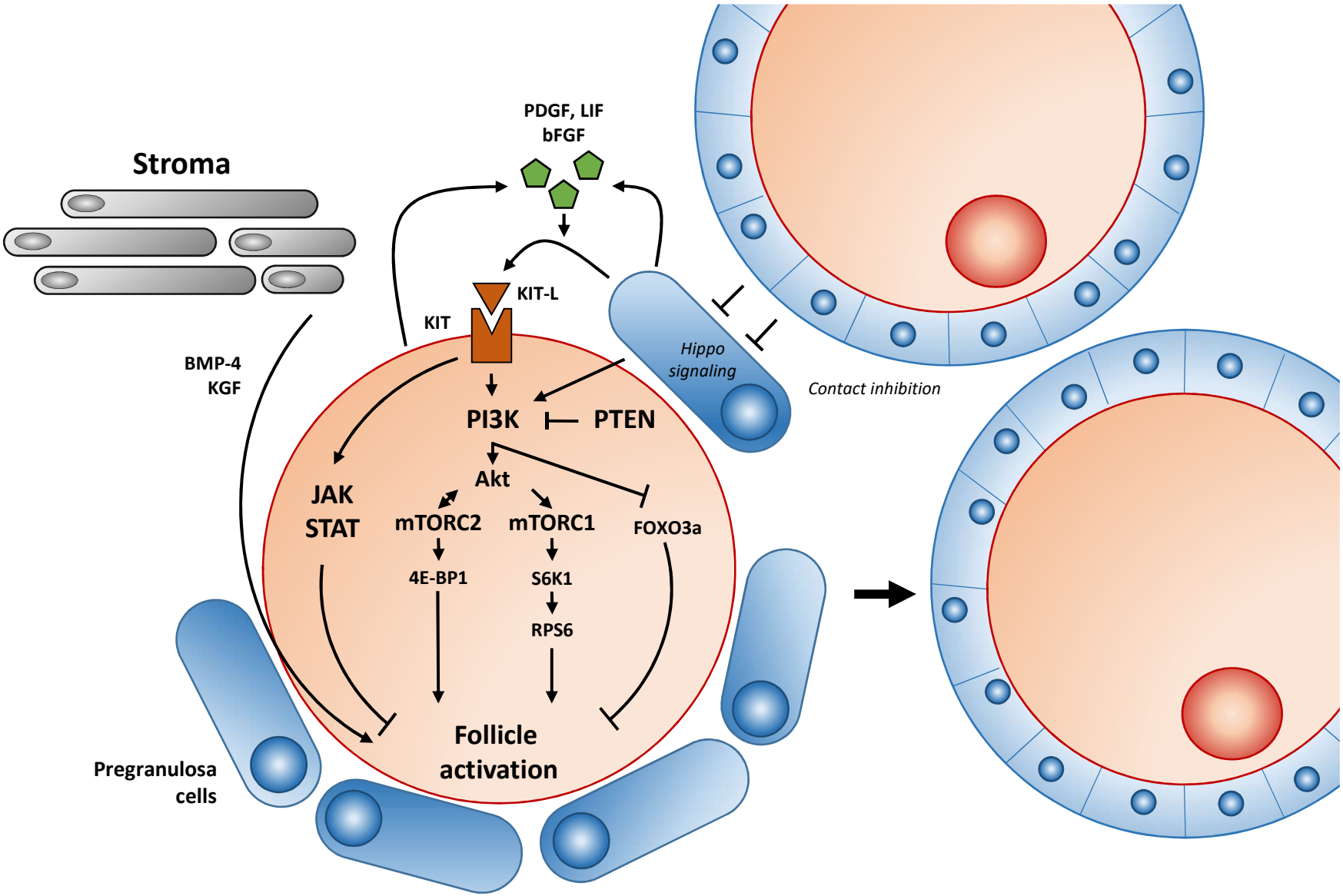
Age (years)

Figure 2


Stroma
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bFGF
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BMP-4
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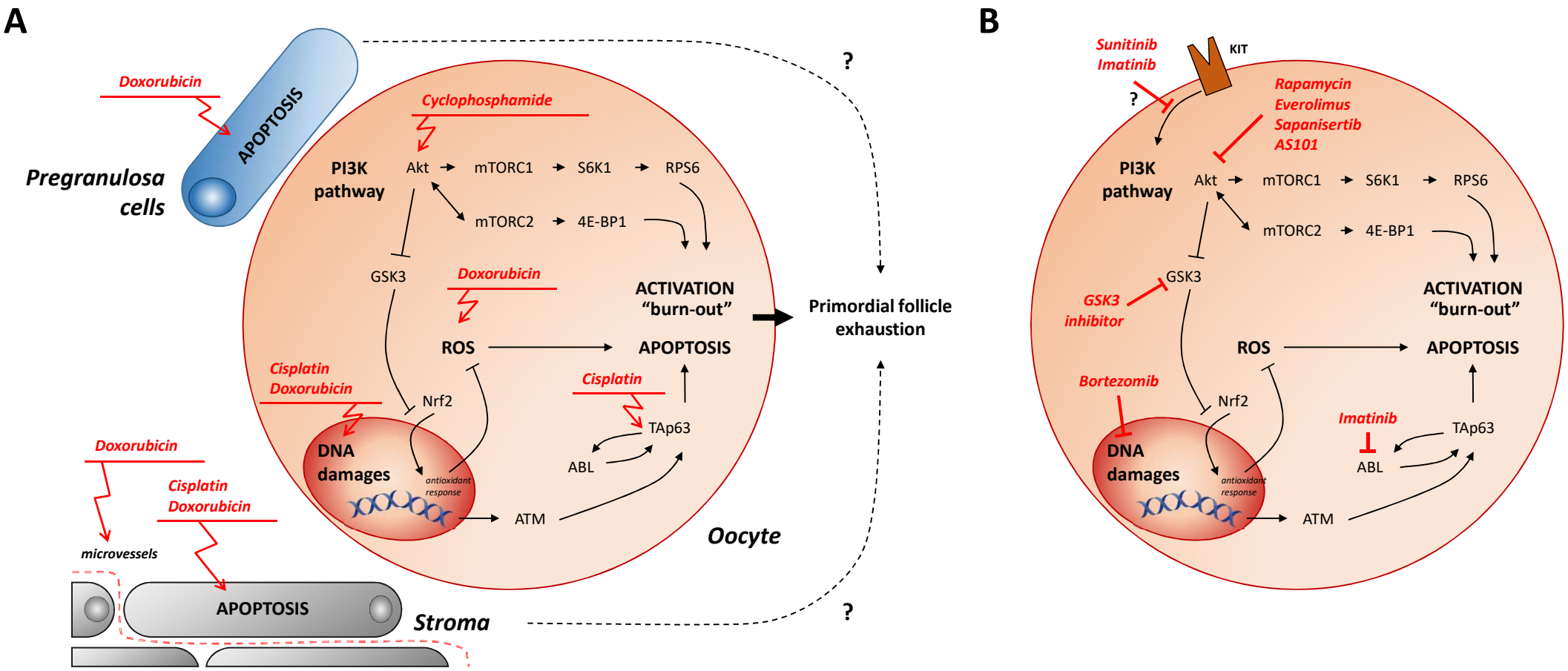
Hippo
signaling
Contact inhibition
PI3K
PTEN
Akt
JAK
STAT
mTORC2
mTORC1
FOXO3a
4E-BP1
S6K1
RPS6
Follicle
activation
Pregranulosa
cells
Primordial follicle
Primary follicle

Figure 3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Doxorubicin
ACTIVATION "burn-out"
ROS
APOPTOSIS
Primordial follicle exhaustion
Cisplatin
Doxorubicin
Nrf2
Cisplatin
TAp63
DNA damages
antioxidant response
ABL
ATM
Oocyte
Doxorubicin
Cisplatin
Doxorubicin
microvessels
APOPTOSIS
Stroma
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B
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Imatinib
KIT
?
Rapamycin
Everolimus
Sapanisertib
AS101
PI3K pathway
Akt
mTORC1
S6K1
RPS6
mTORC2
4E-BP1
GSK3
GSK3 inhibitor
ACTIVATION "burn-out"
ROS
APOPTOSIS
Bortezomib
Nrf2
Imatinib
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DNA damages
antioxidant response
ABL
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Figure 4