C2AZ as Treatment Adjunct - A Review

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Abstract
This paper is a technical note showing how the established pharmacodynamic actions of four non-oncology drugs happen to inhibit several established pathophysiological mechanisms active in driving or facilitating breast cancers' growth. The resulting four repurposed drug regimen, C2AZ, uses the analgesic drug celecoxib; the antifungal drug clotrimazole; a drug used to treat rheumatoid arthritis, auranofin; and a drug used to treat asthma, zileuton. All four have a large database showing that they inhibit one or more growth driving pathways of breast cancer. The four drugs of C2AZ have been well tolerated in general medical practice and no drug-drug interaction is predictable. All four are old, cheap, generic drugs. They are predicted to be growth retarding rather than directly cytotoxic so C2AZ would best be studied in an adjunctive role.

Keywords: Auranofin; Breast cancer; Celecoxib; Clotrimazole; Nrf2; Zileuton

Abbreviations: COX: Cyclooxygenase; KCa3.1: Intermediate-conductance Ca++ activated K+ channel; 5-LO: 5-lipoxygenase; Nrf2: NF-E2-related factor 2

Introduction
This paper presents the rationale for addition of an antifungal drug, clotrimazole, an analgesic drug, celecoxib, an anti-rheumatoid arthritis drug, auranofin, and an anti-asthma drug that inhibits 5-lipoxygenase (5-LO), zileuton - the C2AZ Regimen - as adjunctive treatment of breast cancer. As things now stand (as of Summer 2020), breast cancer, once it has metastasized, is incurable. The four drugs of C2AZ have been well tolerated when used individually in general medical practice and, surprisingly, all have robust preclinical database supporting their adjunctive use in treating breast cancer. They have never been used clinically for breast cancer.

Nests of metastatic breast cancer cells can remain quiescent for years, then for as yet to be identified reasons, they exit the dormant state, start dividing, actively growing, and further metastasizing [1-3]. One could infer that dormant breast cancer nidi are actually the predominant breast cancer form from the fact that punctilious autopsy of middle-aged women dying from non-cancer causes, 18% had occult in situ breast cancer as did elderly women dying of non-cancer causes [4]. It is the aim of C2AZ to increase the chance of breast cancer cell nests to remain dormant. Details follow:

Literature Review
High dose celecoxib
Celecoxib is an analgesic drug, generically available and widely used in low doses for treating pain. It is a selective cyclooxygenase-2 (COX-2) inhibitor that also inhibits several isoforms of carbonic anhydrase. 99% of metastatic breast cancers grossly overexpress COX-2 [5]. Although COX-2 has commonly been called “... a ubiquitous driver of mammary carcinogenesis...” [5], this must be understood as one driver among many cross-covering drivers.

The role of COX-2 and its eicosanoid prostaglandin product PGE2 in breast cancer growth and its associated immunosuppression has been the subject of several recent reviews [5-10]. People dying of metastatic breast cancer had grossly elevated RNA transcripts levels of both 5-LO and COX-2 [11]. Note the particular value of combined inhibition of PGE2 formation by COX-2 inhibition with simultaneous leukotriene synthesis inhibition during breast cancer treatment [9,12-16]. C2AZ aims to achieve this by combining high dose celecoxib with zileuton (vide infra). Giving a leukotriene inhibitor with any COX inhibitor when treating cancer is important to obviate shunting from COX inhibition to increased leukotriene synthesis [15,16].

Celecoxib inhibits CA IX with a Ki of ~ 16 nM, comparable to the
traditional, clinically used, archetypal, nonselective carbonic anhydrase inhibitor acetazolamide, that has Ki variously reported as 5 to 25 nM [17-21]. Celecoxib’s plasma concentration-time curve is dose proportional between 200 and 800 mg, T_max is 2 to 4 hours, T_1/2 is 11 hours, C_max after an 800 mg dose is ~ 2.9 ± 0.4 mg/L. Celecoxib is 98% albumin bound. Celecoxib is metabolised primarily by hepatic CYP2C9 [22]. IC50 is 15 ± 1 μg/L for COX-1 and 0.04 ± 0.01 μg/L for COX-2 giving a selectivity ratio, COX-1: COX-2 of 375 [22]. Although celecoxib is a sulfonamide, allergic reactions in people who are sulfonamide allergic are not common. A curiosity of COX-2 is, that while it is traditionally termed an inducible isoform and COX-1 the constitutive form, COX-2 is constitutively expressed in the human brain and renal macula densa and adjacent cortical thick ascending limb [23].

In searching for drugs that might augment auranofin’s selective cytotoxicity to cancer cells, a high-throughput drug screening identified celecoxib [24]. The combination generated greater oxidative stress than either alone did mitochondrial hexokinase inhibition with catastrophic decrease in intracellular ATP without seeming to impair non-malignant tissue function or vitality [24]. Celecoxib together with auranofin (vide infra) are particularly effective in increasing damaging intracellular ROS to which cancer cells are more sensitive than non-malignant cells [24,25]. In short, inhibition of carbonic anhydrase and COX-2 with high dose celecoxib is eminently worth trying in metastatic breast cancer.

**Clotrimazole**

Clotrimazole is a broad-spectrum antifungal drug, most commonly used today as oral troches to treat mucositis, or topical 1% cream to treat tinea corporis, pedis, or intercrurals. Clotrimazole has excellent oral bioavailability. Since the potential advantages of adding clotrimazole to current treatments of GB in 2010 [26], much new data on the general cancer-growth inhibiting effects of clotrimazole has accrued. Clotrimazole inhibited proliferation of human breast cancer cell lines MCF-7, MDA-MB-231 and T47D [27-29].

**Clotrimazole and KCa3.1**

The outer cell membrane intermediate-conductance Ca++ activated K+ channel (KCa3.1) is inhibited by clotrimazole in an intermediate nM range [30-32]. Well-functioning KCa3.1 channels drive breast cancer growth and KCa3.1 inhibition impairs breast cancer cells’ growth [33-37].

KCa3.1 opening triggers or induces the secretion of IL-1beta, an effect blocked by clotrimazole [38]. IL-1beta also promotes growth in breast cancers and contributes to the characteristic local immunosuppression [39-44]. IL-1beta also enhances or induces a shift towards glycolysis via glycerol-3-phosphate dehydrogenase phosphorylation [45].

**Phosphofructokinase inhibition and inhibiting glycolysis: Potential for reversing Warburg effect**

Clotrimazole directly inhibits function of a key glycolytic enzyme - phosphofructokinase [46-50]. Part of glycolysis, aerobic or anaerobic - occurs free in the cytosol, but partly occurs where the enzymes mediating the steps are held in cytosol but on an actin filament scaffolding. Clotrimazole unthters these, predominantly phosphofructokinase, resulting in its inhibition [28,51,52]. Clotrimazole decreases glycolysis in other cancer model systems with consequent retardation of growth. Direct inhibition of breast cancer growth by clotrimazole has been demonstrated [48,53].

The Warburg effect of increased aerobic glycolysis has been widely demonstrated in the common cancers and specifically in breast cancer [54-60]. This makes phosphofructokinase a particularly attractive target for inhibition in that phosphofructokinase is a rate limiting enzyme in aerobic (and anaerobic) glycolysis.

It is precisely the cancer stem cell subpopulation that is particularly associated with increased glycolysis and relative radio resistance. Indeed, when forced to switch from reliance upon glycolysis by clotrimazole inhibition of phosphofructokinase, stem-like features of glioblastoma are reduced [61]. We can expect the same in breast cancer [62-64]. Whether or not clotrimazole could be similarly helpful in human patients remains to be formally evaluated. In short, inhibition of KCa3.1 and phosphofructokinase with clotrimazole is eminently worth trying in metastatic breast cancer.

**Auranofin**

Auranofin is an inhibitor of cathepsin B and thioredoxin reductase [65,66]. It is FDA/EMA approved and marketed since the 1980s to treat rheumatoid arthritis [67,68]. Auranofin is now seeing a renaissance as treatment adjunct in several cancers, including breast cancer, by virtue of its inhibition of thioredoxin reductase [69-76]. Thioredoxin reductase recharges [reduces] oxidized 12 kDa thioredoxin, a process essential to coping with mitochondrial reactive oxygen species (ROS). Thioredoxin donates reducing equivalents (electrons) to oxidized metabolic intermediates using an intermolecular cysteine thiol-disulfide exchange. 

**BRCa1** protein is part of a multimeric protein complex that repairs double strand DNA breaks. Mutations in BRCa1 predispose women to breast and ovarian cancer. Breast cancer cells with mutated BRCa1 are more sensitive to auranofin [77]. Auranofin was effective in reducing triple-negative breast cancer growth in *in vitro* and in xenograft models at auranofin levels that did not affect non-transformed breast cell viability [78,79]. Similar results from auranofin were seen in estrogen-progesterone receptor expressing breast cancer [80,81].

NRF2 is one of the main transcription factor regulators of the antioxidant responses. p53 is often mutated in breast cancer [82]. Thioredoxin is a mutant p53-activated NRF2 target with pro-survival and pro-migratory functions in breast cancer cells under oxidative stress [83].

Nrf2 is a transcription factor of genes coding for antioxidant proteins. Nrf2 is kept in cytosol by Kelch like-ECH-associated protein 1 (KEAP1) and Cullin 3, [84]. Kept in cytosol, Nrf2 tends to be degraded quickly. Unbound Nrf2 translocates to nucleus where it triggers transcription of its antioxidant target genes. Nrf2 is a transcriptional regulator that targets heme oxygenase-1 (HMOX1), ferritin (FTH) genes, and that coding for thioredoxin (TXRD1). Nrf2-driven metabolic reprogramming promotes...
breast cancer cells’ exit from dormancy [85]. Human breast cancers overexpress Nrf2 and patient survival is shorter in those with higher Nrf2 expression, longer in those with lesser Nrf2 expression [86,87]. Higher Nrf2 expression facilitates tumor neoangiogenesis as well [88].

Although there are doubtless a coalition of intracellular forces that together give rise to a cancer cells’ dormant state, Nrf2 is one, a consequence of which we might be able to inhibit. Auranofin inhibits thioredoxin reductase, thereby reversing a core effect of Nrf2 [24,78,79,89,90]. Thioredoxin and thioredoxin reductase tend to be elevated in a variety of the common lethal cancers [91]. Auranofin in high nanomolar concentration increased radiation induced cell death in MDA-MB-231 breast cancer cells [92].

Thioredoxin and thioredoxin reductase is located in both cytoplasmic and nuclear compartments of breast cancer cells [93]. Breast cancers with higher thioredoxin levels are more resistant to docetaxel [94]. By immunohistochemistry on 224 breast cancers, high expression of cytoplasmic peroxiredoxin-I was a negative prognostic sign [95]. Thioredoxin 1 is increased in breast cancer tissue and in-patient sera even in those with stage 1 disease [96,97]. In short, inhibiting thioredoxin reductase with auranofin is eminently worth trying in metastatic breast cancer.

Zileuton

Zileuton is an inhibitor of 5-LO, inhibiting formation of 5-LO products - leukotrienes LTB4, LTC4, LTD4, and LTE4. It is marketed and FDA/EMA approved to treat asthma [98,99]. Zileuton and inhibition of 5-LO decreases growth in a wide variety of cancers in preclinical study [100-110]. There has been no reported clinical study of zileuton in any cancer. Let C2AZ be the first.

5-LO sits at the branchpoint in the metabolism of arachidonate to leukotriene synthesis from prostaglandin synthesis [111]. 5-LO by itself is inactive. It requires 5-lipoxygenase activating protein (FLAP). FLAP is elevated in breast cancer tissue and degree of elevation was inversely correlated with survival [111].

Human breast cancers overexpress 5-LO [112,113]. Serum levels of 5-LO are elevated in breast cancer patients [114]. Untreated breast cancer cell lines synthesize large amounts of leukotriene LTB4 [115]. Insulin-like growth factor 1 stimulated growth and leukotriene synthesis in breast cancer cell line MCF-7 [116]. In vitro growth of human breast cancer cell line MDA-MB-231 was inhibited by non-marketed 5-LO inhibitors [117].

Human breast cancer exhibits a strong predilection for metastasis to bone. A nidus of bone resorption is required for that process to occur. In experimental models of breast cancer-mediated bone resorption, 5-LO inhibition inhibited that process [118,119].

5-LO product LTB4 stimulated breast cancer growth by a positive feedback loop with fatty acid synthase [120]. Agonism at the LTB4 receptor mediated paclitaxel resistance in MCF-7 breast cancer [121]. Expression of BLT2, a LTB4 receptor, is upregulated in human breast cancer cells, suggesting an autocrine growth loop mediating aggressiveness [122-124]. Higher breast cancer tissue expression of BLT2 is associated with shorter survival [123]. This work also indicated that survival under high ROS load was partly dependent on LTB4-BTL2 signaling, leading to the conclusion in the current paper on C2AZ, that zileuton plus auranofin would be a particularly felicitous combination.

Serum alanine aminotransferase (ALT) must be monitored during zileuton treatment as this reflects occasional liver injury with zileuton. ALT elevations usually resolve upon zileuton discontinuation. Standard zileuton dosing in asthma would be two 600 mg extended-release tablets twice daily within one hour of morning and evening meals [125].

In a murine breast cancer study, zileuton decreased metastasis and circulating breast cancer cells [126]. Leukotrienes are instrumental in new vessel formation associated with breast cancer growth [127]. 5-LO products, specifically LTB4 contribute to the immunosuppression and Treg generation in breast cancer [128]. Neutrophils are active in 5-LO leukotriene generation that facilitate establishment of breast cancer metastases [129]. In short, inhibiting 5-LO with zileuton is eminently worth trying in metastatic breast cancer.

Discussion

Two genes are synthetic lethal if mutation of either gene alone is compatible with viability but mutation of both leads to death. The inverse corollary would be our principle of multiple cross-covering growth drive pathways in cancers generally, and in breast cancer specifically. We therefore believe a polypharmacy approach will be needed for long-term control of breast cancer. Adjunctive use of C2AZ during breast cancer treatment is a step in that direction.

The combination of celecoxib and zileuton has been used and shown to be effective in inhibiting growth in a variety of animal cancer models [130-133]. Note also that no untoward interactions were seen when using simultaneous celecoxib and zileuton in these cancer models.

C2AZ regimen is not without risks. The unknown risks of combining the all C2AZ drugs together are partly mitigated by absence of any predictable risk of the combination beyond additive liver irritation potential. Patients must take zileuton 600 mg every 6 hours and 4% develop elevated hepatic transaminases [134]. Although these elevations were reversible, this means monitoring of liver function will be required.

Two leukotriene receptor blockers are approved for use in humans in treating asthma - zafirlukast and montelukast [135-137]. They block receptors for cysteinyi leukotrienes LTC4, LTD4, and LTE4 and so might be substituted for zileuton in jurisdictions where zileuton is not marketed or in case of transaminase elevations with zileuton. Both zafirlukast and montelukast inhibited in vitro viability of breast cancer cell line MDA MB-231, with concentrations near those clinically achievable [138].

Conclusion

Given that metastatic breast cancer is incurable (as of Summer 2020) an adjunctive four drug protocol, C2AZ, was designed to
keep the small paucicellular metastases dormant. C2AZ uses the documented attributes of four drugs repurposed to treat breast cancer. How these three drugs constructively intersect with and inhibit the relevant known growth-driving aspects of breast cancer was reviewed in this paper. In short, the projected safety of the C2AZ regimen, it is eminently worth trying in metastatic breast cancer.

**Declarations**

**Ethics approval and consent to participate**

No human or animal subjects were used in this work.

**Consent for publication**

The sole author, REK, consents for publication.

**Availables of data and materials**

All data has been presented in the manuscript.

**Competing interests**

The author has no competing interest.

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**Authors' contributions**

REK is the sole author.

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**References**


71 Oomen D, Yiannakis D, Jha AN (2016) BRCA1 deficiency increases the sensitivity of ovarian cancer cells to auranofin. Mutat Res 785: 8-15.


Myeloid Leukaemia: Implications for CML Treatment. Antioxidants (Basel) 9: 207.


