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PERSPECTIVES

The ABC7 regimen: a new approach to metastatic breast cancer using seven common drugs to inhibit epithelial-to-mesenchymal transition and augment capecitabine efficacy

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Abstract: Breast cancer metastatic to bone has a poor prognosis despite recent advances in our understanding of the biology of both bone and breast cancer. This article presents a new approach, the ABC7 regimen (Adjuvant for Breast Cancer treatment using seven repurposed drugs), to metastatic breast cancer. ABC7 aims to defeat aspects of epithelial-to-mesenchymal transition (EMT) that lead to dissemination of breast cancer to bone. As add-on to current standard treatment with capecitabine, ABC7 uses ancillary attributes of seven already-marketed noncancer treatment drugs to stop both the natural EMT process inherent to breast cancer and the added EMT occurring as a response to current treatment modalities. Chemotherapy, radiation, and surgery provoke EMT in cancer generally and in breast cancer specifically. ABC7 uses standard doses of capecitabine as used in treating breast cancer today. In addition, ABC7 uses 1) an older psychiatric drug, quetiapine, to block RANK signaling; 2) pirfenidone, an anti-fibrosis drug to block TGF-beta signaling; 3) rifabutin, an antibiotic to block beta-catenin signaling; 4) metformin, a first-line antidiabetic drug to stimulate AMPK and inhibit mammalian target of rapamycin, (mTOR); 5) propranolol, a beta-blocker to block beta-adrenergic signaling; 6) agomelatine, a melatonergic antidepressant to stimulate M1 and M2 melatonergic receptors; and 7) ribavirin, an antiviral drug to prevent eIF4E phosphorylation. All these block the signaling pathways – RANK, TGF-beta, mTOR, beta-adrenergic receptors, and phosphorylated eIF4E – that have been shown to trigger EMT and enhance breast cancer growth and so are worthwhile targets to inhibit. Agonism at MT1 and MT2 melatonergic receptors has been shown to inhibit both breast cancer EMT and growth. This ensemble was designed to be safe and augment capecitabine efficacy. Given the expected outcome of metastatic breast cancer as it stands today, ABC7 warrants a cautious trial.

Keywords: ABC7, breast cancer, agomelatine, capecitabine, metformin, pirfenidone, propranolol, quetiapine, repurposing, ribavirin, rifabutin, TGF-beta

Plain language summary

This article presents the rationale and thinking behind the ABC7 regimen for metastatic breast cancer. Since there is currently no cure for breast cancer once it has spread to bone and other organs beyond the breast itself, the ABC7 regimen was designed to take advantage of ancillary attributes of seven common and readily available noncancer treatment drugs that, in theory, should make current traditional cytotoxic chemotherapy with capecitabine more effective. The ABC7 regimen has not been shown to be safe or effective yet. In the current article, we discuss an untested proposal for a new treatment approach to metastatic breast cancer.

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Introduction

Estrogen-positive metastatic breast cancer cannot be cured currently.[1] One major metastatic site of breast cancer is bone. Once breast cancer metastasizes to bone, the survival rate declines despite recent advances in local treatments of breast cancer. Current treatment strategies for bone metastasis, including bone-targeted agents (bisphosphonate and denosumab), provide only palliation. New and effective therapeutic strategies for this still incurable disease are therefore urgently needed.

This article reviews the attributes of seven currently marketed drugs that, as indicated by prior research data, will block or partially block the escape pathways from current traditional treatments. The seven drugs of ABC7 were chosen by first identifying the basic pathways by which EMT is initiated and maintained. We then reviewed 1000 of the most commonly used drugs[2] for which we have both usual plasma levels and published data showing potential inhibitory interaction with these pathways. The resulting list was reduced by semi-subjective evaluation of the strength of data on their EMT inhibition benefit versus the drugs' expected tolerability. The better the tolerability, the weaker the data had to be for inclusion.

This ABC7 regimen is designed to block several core breast cancer growth signals in a coordinated manner, thereby augmenting the cytotoxicity of a currently used cytotoxic chemotherapy drug, capecitabine. Figure 1 shows an overview schematic of the biochemistry that ABC7 is designed to influence. This is explained in detail in the respective drug discussions in the "Drugs to inhibit EMT" section. Table 1 gives an overview of the ABC7 drugs and their intended targets in treating breast cancer.

The ABC7 regimen follows the approach of previous cancer treatment regimens, for example, MTZ regimen,[3] COMBAT regimen,[4] MEMMAT regimen, a current trial of Peyrl et al's seven-drug cocktail (ClinicalTrials.gov Identifier: NCT01356290), and CUSP9 regimen.[5,6] In all of the studies, extensive use is made of drugs not primarily marketed to treat cancer but that have ancillary attributes that research data indicate would enhance the anticancer effect of a cytotoxic, traditional cancer treatment drug. The ancillary drugs exert anticancer effects by blocking various growth-enhancing survival pathways used by the target cancer or as for agomelatine are agonists at growth-retarding receptors.

Similar to other cancers, breast cancer has heterogeneous regions within the same tumor – different areas that depend on or use different growth-signaling pathways. This is related to but distinct from the idea of clonal evolution driven by cytotoxic chemotherapy selection. Both forms of heterogeneity exist in a typical breast cancer, proteomic and genomic. ABC7 aims to inhibit breast cancer by pharmacological manipulation of what genes are expressed and what genes are not, as well as by targeting different clonal variants of the original breast cancer clone.

Because of these limitations, we do not expect testing for molecular markers to be predictive or useful. In addition, further intensifying the spatial and temporal diversity of the molecular status, particularly for EMT markers, are the diversity-driving effects of chemotherapy[7] and discussed in greater detail in the following sections.

Cytotoxic chemotherapy also induces important receptor status changes in a large minority of breast cancer cases.[8,9] Typical findings are as follows: 13% changed from HR+ to HR−, 5% changed from HR− to HR+, 6% changed from HER2+ to HER2, 3% changed from HER2 to HER2+, and 13% changed to triple negative.[8]

Multiple signaling systems have been identified that drive metastatic breast cancer.[1,9–11] These growth-driving receptors can cross cover for each other.[1,9–11] When one is pharmacologically blocked, several parallel growth-driving pathways can become active, taking the place of the blocked pathways. Growth factor signaling converges from a wide variety of outer membrane receptors to more restricted, fewer, intracellular pathways. This is, more elegantly stated, the spatial–temporal genomic and proteomic range, the "genetic collectives [that] dominate the landscape of advanced-stage (malignant) disease."[11,12] We see this as mandating an integrated, coordinated polypharmacy to successfully address these malignancy attributes.

Capecitabine is intracellularly metabolized to 5-FU; the details are given in the "Capecitabine: 359 Da, half-life <1 hour" section. ABC7 drugs are designed to make 5-FU more effective.

The results of several recent ER+ metastatic breast cancer studies are listed in Table 2. These studies cannot be judged simply by overall survival in that entry requirements were different, with different kinds and number of prior treatments. These numbers in Table 2 are for general idea only.[1] One cannot conclude that one of these is better than another.

In general, post-progression survival durations in recent Phase III studies of combination therapy ranged from approximately 16 to 33 months.[1]

EMT is a feature of cancers generally[13] and breast cancer specifically.[14,15]

Table 3 lists several features and behaviors associated with the two (epithelial and mesenchymal) states. Interestingly, a transcription factor ZEB1, known to control EMT,

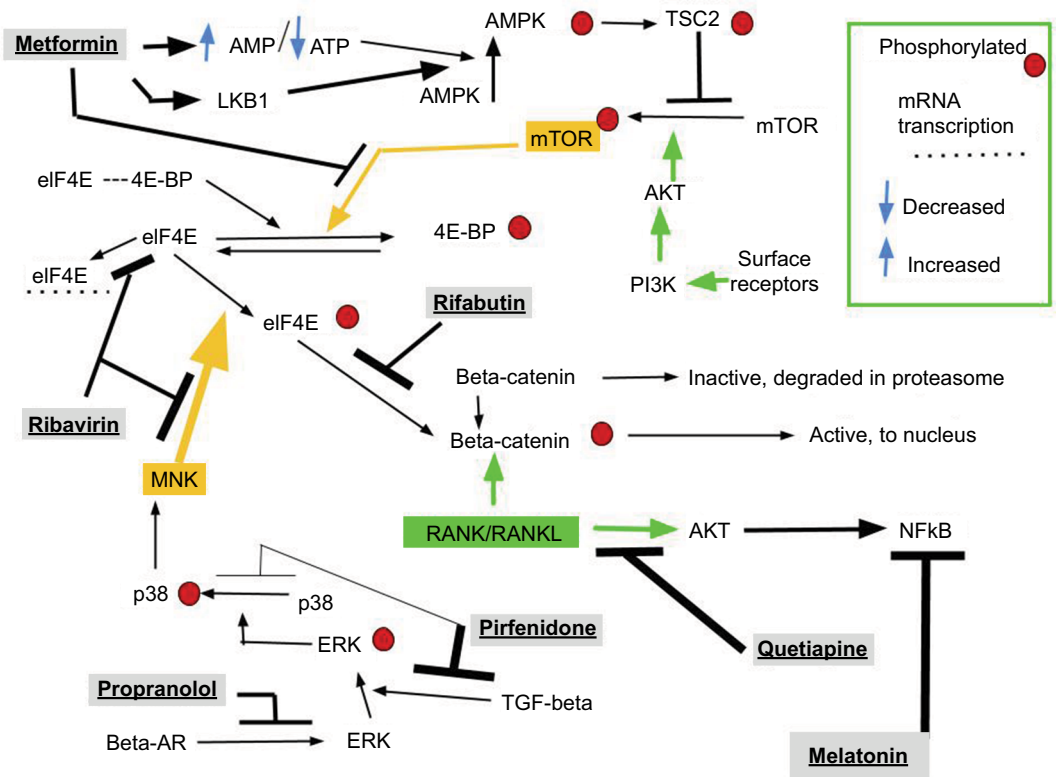

Figure 1 The basic biochemical, intracellular, and receptor pathways relating to interventions of the ABC7 regimen for advanced breast cancer.
Notes: This is the basic breast cancer intracellular circuitry that the ABC7 regimen attempts to address. The two major controllers or stimuli for eIF4E activation are indicated by yellow arrows, the p-mTOR and the MNK paths.

Table 1 The drugs of ABC7, their targets during treatment of breast cancer, and suggested doses

Drug	Target in breast cancer treatment	Starting dose	Target dose
Capecitabine	DNA synthesis	600 mg/m^2 twice daily. 7 days on, 7 days off	1250 mg/m^2 twice daily. 7 days on, 7 days off or 14 days on, 7 off
Quetiapine	RANK/RANKL	50 mg once at bedtime	300–600 mg once at bedtime
Pirfenidone	TGF-beta	200 mg three times daily	600 mg three times daily
Rifabutin	BCL-6; beta-catenin	150 mg/day	300 mg/day
Metformin	AMPK*; mTOR; mitochondria oxphos	500 mg once daily	1000 mg twice daily
Propranolol	Beta-adrenergic receptors	10 mg twice daily	Uptitrate as tolerated
Agomelatine	Melatonergic receptors*	25 mg once at bedtime	50 mg once at bedtime
Ribavirin**	eIF4E; MNK; IMPDH	600 mg/day	1200 mg/day

Notes: The drugs are listed in a suggested order of addition. Pace of drug addition is individualized per patient and physician estimations of risk/benefit. *Note that all entries denote inhibition of named target except for metformin that activates AMPK and agomelatine that stimulates melatonin receptors. All drugs, except capecitabine, are given continuously without interruption. Capecitabine is given on 7 days on, 7 days off, or 14 days on, 7 days off cycles. **Ribavirin is likely to give unpleasant side effects and depressed mood-but is potentially a beneficial enough drug to try.

Table 2 Representative recent trials in metastatic HR+ breast cancer

Intervention	Months survival	Trial
Anastrozole + fulvestrant	48	SWOG
Anastrozole + fulvestrant	38	FACT
Anastrozole + fulvestrant	21	SoFEA
Letrozole + fulvestrant	52	LEA
Everolimus + exemestane	31	BOLERO 2

contributes to breast cancer osteolytic bone metastasis, but not brain or lung metastasis.[16,17]

EMT is a phase transition, where flat, sessile, mutually adherent epithelioid cells take on a rounded, non-adherent, motile mesenchymal shape and behavior.[18–20] The reverseless transient state and process, MET, also occurs and is also a feature of robust or aggressive cancer growth.[21] Post-EMT cells tend to be invasive but proliferation restricted. Post-MET cells tend to be proliferative but have limited invasiveness.[18–20] Breast cancers develop in proximity to adipose tissue. Adipocytes are a further trigger to EMT.[22] The relationship between stem cell subpopulations within a cancer and EMT

process is also unclear,[23] and the two populations probably largely overlap.

Perhaps our deepest insight into the EMT process in breast cancer came from a study by Bulfoni et al.[24] They showed that all patients with metastatic breast cancer had CTCs. These circulating cancer cells split into four groups: those with epithelial, those with mesenchymal, those with both, and those with neither marker. Patients with higher numbers of circulating cancer cells that expressed both markers had shorter overall survival.

Survival as a function of E-cadherin expression, representative of epithelial state, and fibronectin, representative of mesenchymal state, was examined by immunohistochemistry on 1495 breast cancer biopsies.[25] More E-cadherin and less fibronectin are associated with longer survival.[25] Breast cancer patients whose tissue expresses greater EMT-related protein have shorter survival.[26] EMT drives chemotherapy resistance and other poor prognosis features in breast cancer.[27–29] In addition, a greater degree of metabolic changes characteristic of EMT in breast cancer predicts shorter overall survival.[30]

Medical research discusses vimentin, fibronectin, and N-cadherin as markers of EMT process, but these proteins would be more accurately viewed as mediators of the attributes we designate EMT.

As indicated in Table 3, fibronectin is a characteristic marker of EMT. Higher breast cancer tissue expression of fibronectin correlates with shorter survival.[31,32] A range of other characteristic behavioral and morphological attributes of EMT and MET states is also outlined in Table 3.

Table 3 Characteristic protein markers and mediators of EMT in breast cancer

Marker	Epithelial state	Mesenchymal state
E-cadherin	Increased	Decreased
N-cadherin	Decreased	Increased
ZO-1	Increased	Decreased
Occludin	Increased	Decreased
Vimentin	Decreased	Increased
Fibronectin	Decreased	Increased
MMP-2	Decreased	Increased
MMP-9	Decreased	Increased
Phenotype	Epithelial state	Mesenchymal state
Motility	Sessile	Motile
Shape	Elongated	Rounded
Adherence	Adherent to neighbors	Non-adherent to neighbors
Invasion	Noninvasive	Invasive
Proliferation	Higher proliferation	Lower proliferation
Microtentacles	Absent	Present

Abbreviation: EMT, epithelial-to-mesenchymal transition.

Chemotherapy triggers EMT

Paclitaxel triggers EMT in breast cancer, increasing mesenchymal markers, vimentin and fibronectin, and decreasing epithelial marker, ZO-1.[33] Experimental inhibitors of TGF-beta signaling block paclitaxel-induced EMT and suppress paclitaxel-induced CSC properties.[33] Paclitaxel also increases EMT markers in mouse breast cancer cell line, MCF-7/PAX.[34] Doxorubicin exposure enhances gastric cancer's EMT marker expression.[35]

These reports, combined with similar findings in other cancers, allow a general statement of a core principle of oncology: cytotoxic chemotherapies tend to provoke EMT. Such a conjecture is amply supported by the recent work of Yoshimasu et al[36] who reported that cisplatin, 5-FU, gemcitabine, paclitaxel, and vinorelbine show hormesis when tested individually.

Surgical trauma or fine-needle biopsy triggers EMT

Of concern in current medical practice, there is a tendency for cancers generally, and breast cancer specifically, to be triggered by any kind of tissue disruption – including fine-needle biopsy – to undergo EMT with consequent cancer cell shedding to circulation. Such hematogenous tumor cell dissemination could be the origin of later overt metastases.

Breast cancers in mice release a flood of CTCs after simple fine-needle biopsy.[37] Clinical needle biopsy of breast cancer triggers recruitment of inflammatory cells to the biopsy site and causes increased tumor cell mitoses in the biopsied area.[38] In a second murine breast cancer study, both fine-needle biopsy and surgical resection resulted in the release of a flood of CTCs, but noteworthy in this work is that biopsy resulted in greater and longer lasting appearance of circulating cancer cells than did surgical resection.[39] These murine data were replicated by Kaigorodova et al[40] who showed that simple fine-needle biopsy of human breast cancers releases a flood of breast cancer cells into the general circulation. These authors found that although some released CTCs had CSC markers and attributes and some did not, none of them had the particular EMT markers for which they were tested.

This worrisome situation in breast cancer is similar to data collected in other cancers. For example, treatment with radioactive needle insertion into prostate cancer results in significant hematological shedding of tumor cells post procedure.[41] Standard transrectal ultrasound-guided prostate needle biopsy results in detectable prostate cancer cells in the circulation in half of patients.[42] Oral squamous cell carcinoma biopsies result in 16% of patients having post-biopsy

CTCs.[43] Simple wide excision that does not disrupt the tumor tissue integrity did not result in postoperative CTCs, whereas incisional biopsy did.[44]

A study is therefore required comparing the long-term outcome potential difference between those having fine-needle biopsy versus those having initial wide lesion excision. If initial excision that leaves the suspicious mass intact does result in fewer later disseminated metastases, it might be worth the iatrogenic morbidity incurred by the consequent excision of some benign masses.

Radiation triggers EMT

Above we reviewed some evidence that chemotherapy and mechanical tissue disruption give rise to CTC and EMT in surviving cells. Below we review data showing radiation causes CTC and EMT as well.[45,46] For a few specific examples, as in other cancers,[47] breast cancers synthesize GM-CSF that then functions as a growth factor for them.[48] Clinically used, radiation treatment not only kills breast cancer cells and prolongs survival in breast cancer but also triggers exposed residual cells that are not killed to undergo EMT, to start migrating, and to synthesize increased amounts of autocrine growth factor, GM-CSF.[49] Radiation also increases IL-6, migration, and EMT markers in murine and human breast cancer cell lines.[50] The subject of radiation-induced EMT and radiation-induced increase in CTCs was recently reviewed by Lee et al.[51]

Clinically, finding greater post-EMT CTCs confers a worse prognosis with more aggressive disease course and greater metastatic proclivity in colon cancer[52] and finding circulating clusters of vimentin-positive gastric cancer cells confers a worse prognosis[53] as did finding circulating cancer cell clusters and vimentin-positive CTC in colon cancer.[54] Surgery for epithelial ovarian cancer causes an increase in both EMT-positive and EMT-negative CTCs, but there is a disproportionate increase in EMT positive. The increase in EMT-positive CTCs was even stronger after platinum-based chemotherapy.[7] As Kolbl et al[55] point out, EMT precedes the release of CTCs but after entering circulation CTC can revert to epithelial or partial epithelial phenotype.

Based on all these evidences, it seems that inhibiting EMT is a worthwhile goal during breast cancer treatment and that current common cancer treatments have elements of cancer growth stimulation inherent to them, or as Niccolo Machiavelli (born 1469–died 1527) said in 1513:

> People should either be caressed or crushed. If you do them minor damage they will get their revenge; but if you cripple them there is nothing they can do. If you need to injure someone, do it in such a way that you do not have to fear their vengeance.

ABC7 regimen was crafted with that in mind.

Drugs to inhibit EMT

Quetiapine: 384 Da, cyp3A4 to norquetiapine, 6-hour half-life

The RANK, its ligand (RANKL), and the soluble decoy receptor OPG (or bone protector) are central elements in breast cancer's establishment of metastases to bone.[56] Early indications are that quetiapine inhibits the RANK/RANKL signaling system.[57]

Several forms of pro-RANKL are expressed on osteoblasts. After proteolytic release, RANKL binds to RANK leading to osteoclast syncytium formation then osteoclasts' resorption of bone. Osteopetrosis results when RANK/RANKL system is nonfunctional. RANK/RANKL also functions in guiding normal breast gland ontogeny. There occurs an ebb and flow of RANK expression in mammary duct epithelial cells during the menstrual cycle, the increase occurring in late luteal phase. RANK/RANKL function is essential to the luminal epithelial proliferation seen particularly where ducts branch.[56] Higher levels of RANK/RANKL in human breast cancer biopsy tissue correlate with higher metastasis likelihood and shorter survival.[58]

PR-negative cells are affected through RANKL-induced paracrine actions leading to proliferation of mammary epithelial PR-negative cells.[59]

RANK/RANKL is a core physiologic signaling system allowing circulating breast cancer cells to metastasize to bone.[60,61] RANK/RANKL is a principal part of the complex signaling giving rise to breast cancer's propensity to metastasize to bone.

Since breast cancer commonly metastasizes to bone with consequent bone pain, pathological fractures, vertebrae compressions, and hypercalcemia, this process is important to block. Breast cancer cells are continuously shed into circulation from the primary and metastatic sites. Why then do these CTCs choose to establish growth preferentially in bone? This is because these CTCs can establish growth-enhancing communication with bone cells, specifically osteoblasts, and they do so primarily via RANK/RANKL.[62] Muscle, skin, liver, spleen, fat, and other sites of less common breast cancer metastasis cannot so reciprocally communicate.

Osteoblasts receiving RANKL signaling transform to syncytial osteoclasts that resorb bone and increase TGF-beta. TGF-beta is also stored in bone, then released with any bone

dissolution. This creates room for the CTCs to grow and free TGF-beta signaling prompting them to do so,[62,63] making the TGF-beta blocking drug pirfenidone, which is discussed in the following sections, an ideal partner drug for quetiapine during metastatic breast cancer treatment.

An initial dose can be 50 mg once at bedtime, uptitrating to a target dose of quetiapine 300 mg or more as tolerated, given once at bedtime. Tiredness for a few hours on awakening is common upon starting quetiapine. It then abates after a week or so but reappears after each dose increase. Some weight increase due to increased appetite can be expected. Otherwise, side effects are not common.

Pirfenidone: 185 Da, cyp1A2, 3-hour half-life

Pirfenidone is a 185 Da drug approved and marketed to treat idiopathic pulmonary fibrosis.[64] Mild-to-moderate, reversible, nausea, dyspepsia, and rash are side effects in about one-third of treated patients, but these often resolve with continued use. Approximately 2403 mg/day divided into three equal doses is a common pirfenidone dose in treating its marketed indication, idiopathic pulmonary fibrosis.[64,65]

Pirfenidone blocks TGF-beta signaling.[66–70] TGF-beta is a 25 kDa signaling protein proteolytically clipped from a larger precursor protein. Carboplatin induces elevation of TGF-beta and triggers EMT in NSCLC, as given in the "Chemotherapy triggers EMT" section,[71] both effects blocked by coadministration with pirfenidone.[71]

TGF-beta signaling is a major driver of EMT in cancer generally[72–74] and in breast cancer EMT specifically.[75–78] TGF-beta is a facilitating element of many cancers by promoting angiogenesis and differentiation, by immune suppression, by promoting loss of cell-to-cell contact, and particularly by promoting EMT. Pirfenidone inhibits TGF-beta-induced phosphorylation of SMAD3, p38, and AKT. TGF-beta provides a "get up and go" signal for breast cancer.[79] In a murine breast cancer model, TGF-beta exposure also enhances normal lung's ability to better support establishment of breast cancer metastases.[80] TGF-beta drives breast cancer's EMT and various biochemical, morphological, and behavioral changes characteristic of EMT.[10,81–83] The manifold paths by which TGF-beta signaling leads to or enhances EMT specifically in breast cancer were outlined by Tan et al,[18] Chen et al,[81] Nooshinfar et al,[84] and Felipe Lima et al.[75]

TGF-beta dependency for taking on typical mesenchymal morphology, increased motility, and increased vimentin expression after radiation exposure was shown in breast, colon, and lung adenocarcinoma cell lines.[85]

Preclinical studies have shown activity in pirfenidone's enhancing cisplatin cytotoxicity to NSCLCs.[86] In addition, pirfenidone enhances radiation and sunitinib cytotoxicity in Lewis lung cancer cells[87] and reduces desmoplasia in pancreatic cancer.[88] Growth of human TNBC tissue (ER negative, PR negative, HER2 negative) xenografted to nude mice was inhibited more by pirfenidone and doxorubicin than by doxorubicin alone.[89] In another murine breast cancer model, pirfenidone reduced intratumoral collagen and hyaluronan by TGF-beta inhibition with consequent improvement of doxorubicin efficacy.[90]

Pirfenidone disrupts Hh signaling in parallel with TGF-beta inhibition, a worthwhile added benefit during breast cancer treatment.[91]

The starting dose of pirfenidone is 267 mg three times a day. This is gradually increased at 14-day intervals as tolerated to 801 mg three times daily. Pirfenidone at 400 mg three times daily (1200 mg/day) used to treat potential progression of hepatitis C-related fibrosis reduced circulating TGF-beta and IL-6.[92] Abdominal pain, rash, and nausea were seen in a half of treated patients, but these side effects tended to subside within a month or two and no patient dropped out due to them.

Ribavirin: 244 Da, 6-day half-life for a single oral dose, up to 12 days after continuous use

Since its introduction to clinical practice in the late 1970s, ribavirin had been used to treat various viral infections, later becoming central to a now-outmoded hepatitis C treatment. Ribavirin remains useful in treating human respiratory syncytial virus infections and selected other rarer virus infections such as those of the hantavirus group.[93] Ribavirin is currently being investigated in numerous clinical trials for its therapeutic activity in various cancers, particularly acute myeloid leukemia (NCT02109744, NCT02073838), head and neck cancer (NCT01268579), and notably for ABC7, metastatic breast cancer (NCT01056757).

Although ribavirin's mechanisms of antiviral and anticancer action are uncertain and probably will vary between viruses, several potential mechanisms of action have been identified. One proposes that ribavirin enters the cell via a nucleoside transport mechanism, intermingling itself within the viral RNA, thus inhibiting/altering viral RNA synthesis. However, ribavirin, particularly when paired with interferon-alpha, activates anti-inflammatory responses in various other ways. Alternatively, due to the fact that ribavirin is structurally analogous to GTP, a purine nucleoside, ribavirin can be incorporated into the cell passively, thereafter competitively

binding to, and inhibiting, RNA polymerase, and RNA synthesis as a whole; ribavirin often achieves this via blocking the IMPDH pathway, among other pathways such as the eIF4E pathway. Ultimately, five major mechanisms of action have been proposed:[94–96]

1. Immunostimulation by upregulating cytokines to shift Th1/2 cell balance to Th1 dominance.
2. Inhibition of 24 kDa eIF4E function, thereby inhibiting mRNA capping and translation initiation.
3. Modulation of interferon-alpha-related gene expression.
4. Direct inhibition of IMPDH with consequent depletion of intracellular GTP.
5. After triphosphorylation, ribavirin triphosphate is incorporated into replicating RNA viral RNA polymerases with consequent induction of viral mutagenesis.

How ribavirin acts vis-a-vis eIF4E is as follows:

eIF4E forms part of the multimeric cap-dependent mRNA translation initiation complex. Mammalian cap-dependent translation starts with that complex binding to an RNA methyl-nucleotide. eIF4E has many positive and negative control points, two of which are 1) posttranslational phosphorylation and 2) 4E-BPs.[97–99] There are several variants of 4E-BP protein, hereafter designated simply as 4E-BP. 4E-BP is in turn controlled by its phosphorylation status.

eIF4E non-covalently bound to 4E-BP is inactive in translation initiation. Both currently recognized complexes of mTOR (mTORC1, loosely associated with growth and mTORC2, loosely associated with cell survival and apoptosis resistance) can phosphorylate 4E-BP.[100] Unphosphorylated 4E-BP has non-covalent affinity to and prevents transcription initiation activity of eIF4E. When phosphorylated, 4E-BP1 loses that affinity and separates from eIF4E, thereby allowing eIF4E to function in cap-dependent mRNA translation.[97,99–101]

MAP kinase interacting kinases (hereafter referred to as MNK) can also phosphorylate 4E-BP, releasing it from eIF-4E.[99,102–105] A wide variety of internal and extracellular events converge on mTOR and/or MNK to enhance or inhibit their activity. Development of resistance to mTOR inhibitors such as everolimus is often caused by eIF4E amplification or MNK upregulation.[97,105]

eIF4E overexpression has been identified in 30% of human cancers generally,[97,106–110] including in invasive breast cancer where the degree of eIF4E, both gene and protein overexpression, has been positively correlated with occurrence, recurrence, and metastasis.[111–118] eIF4E protein expression was associated with shorter survival, higher tumor mitotic index, and higher-grade breast cancer.[115] Increased phosphorylated 4E-BP confers a worse prognosis and faster disease progression in breast, ovary, and prostate cancers.[103]

A crucially important oddity of eIF4E in breast cancer is the homogenous spatial uniformity of phosphorylated eIF4E protein overexpression in breast cancer tissues, both metastatic and primary.[116] This is particularly notable given the spatial heterogeneity of ER, PR, HER2, mTOR, and other commonly overexpressed markers in breast cancer.

In addition, in 200 patients with Stage 4 breast cancer, immunohistochemistry analysis revealed that greater increase in eIF4E phosphorylation in response to chemotherapy with doxorubicin, cyclophosphamide, or FU was correlated with shorter median overall survival,[114] 4.7 years in patients with a two- to fourfold increase in eIF4E phosphorylation versus 3.1 years in patients with a 9–11-fold increase. A second study a few years later found similar results.[118] Among patients undergoing primary debulking for a node-positive breast cancer when nodes were positive, after 4-year follow-up, systemic recurrence occurred in 22% of women with low eIF4E protein expression, 27% of the intermediate group, and in 49% expressing large amounts of eIF4E.[119] Even more serious was the presence of multiple distant metastases in 60% of women whose primary expressed large amounts of eIF4E but in 15% of women whose primary expressed low amounts of eIF4E, again after 4-year follow-up.[119]

In an unusually exciting and instructive study, Li et al[120] studied breast cancer biopsy tissue by immunohistochemistry both before and after chemotherapy. After cytotoxic chemotherapy with doxorubicin, or cyclophosphamide or 5-FU, the expression of phosphorylated eIF4E increased in the posttreatment biopsy material, as given in the "Chemotherapy triggers EMT" section, and chemotherapy-activated Wnt/beta-catenin, as given in the "Rifabutin: 847 Da, 2-day half-life" section, signaling in a phosphorylated eIF4E-dependent manner.[120]

Although the significance of eIF4E phosphorylation or its range of functions is not fully understood, some aspects are predominantly the empirical data in the abovementioned paragraph. Regulation of eIF4E function is partly achieved through this phosphorylation process. Untreated GBMs show an excess of phosphorylated (unbound) 4E-BP.[98,102] Inhibition of 4E-BP phosphorylation with consequent retention of its association with 4E-BP leads to inhibition of protein synthesis, inhibition of glioma cell proliferation in vitro, and tumor growth in vivo, in an orthotopic GBM mouse model.[98,102] We know ribavirin gets good brain tissue levels based on the psychiatric morbidities associated with its use in treating hepatitis C. Volpin et al[121] suggested using ribavirin to treat GBM based on these considerations.

That metformin inhibits 4E-BP1 phosphorylation via mTOR inhibition[122] makes metformin a good coordinated partner drug to ribavirin. That ribavirin also inhibits MNK and since MNK phosphorylation of eIF4E is an alternate eIF4E activation pathway particularly used during the development of resistance to the mTOR inhibitor everolimus,[98,99,102,123,124] ribavirin might be combined with everolimus or metformin to advantage. This would be a good example of the phenomenon mentioned in the "Introduction" section that when one growth pathway is pharmacologically blocked other parallel growth-driving pathways can become active, taking the place of blocked paths. mTOR phosphorylates 4E-BP1, or if mTOR is inhibited then MNK can take over, phosphorylating 4E-BP1. This would also explain why and how mTOR inhibitors have not been successful in treating some tumors such as GBM even though they express an overabundance of mTOR. MNK simply takes over when mTOR is blocked.

TGF-beta promotion of EMT that occurs largely through phosphorylation of eIF4E by MNK (with multiple intermediates between the two)[103] makes pirfenidone a good partner drug for both metformin and ribavirin.

In addition, experimental MNK inhibitors decrease eIF4E phosphorylation levels in breast cancers,[120] and GBM,[98] where MNK inhibition enhanced temozolomide cytotoxicity. In parallel fashion, in 103 cases of astrocytomas, high expression of phosphorylated eIF4E was significantly correlated with shorter overall survival rates.[107]

All treated breast cancers were found to overexpress phosphorylated (activated) eIF4E,[116,120] a remarkable and unique finding in any cancer. Decreased eIF4E phosphorylation in breast cancer also resulted in increased E-cadherin and beta-catenin protein levels[125] reflecting a shift from mesenchymal toward epithelial attributes. The abovementioned combined data suggest that ribavirin could be of potential benefit by inhibiting eIF4E in breast cancer. Kentsis et al[126,127] have demonstrated that ribavirin inhibits m^7G mRNA cap binding to eIF4E. Ribavirin directly bound to eIF4E with a micromolar affinity at the functional site used by m^7G mRNA cap, reducing eIF4E/mRNA binding and disrupting the translation process. Of note, not all mRNA translation is eIF4E dependent, but important mRNAs in breast cancer are, for instance, the one coding cyclin D1/3, c-Myc, VEGF, FGF2/4, and MCL-1. Some preclinical studies in several murine models of breast cancer revealed that ribavirin inhibits breast cancer cell proliferation through eIF4E blockage.[128,129] Moreover, in these studies, multiple-aspect characteristics of EMT were reversed or diminished by ribavirin.[128,129]

More recently, two studies demonstrated significant glioma cell killing by ribavirin,[121,130] confirming a 2014 study showing that ribavirin induced G0/G1 arrest in seven glioma cell lines at a median 55 μM IC_{50} (range 28–664).[131] This latter study positively correlated mRNA expression of PDGF receptor-alpha, a major driver of GBM growth, with better glioma cell sensitivity (lower IC_{50}) to ribavirin. That PDGF receptor is also a major driver of breast cancer[132,133] and can cross cover for the ER[134,135] forming one of the many escape paths from aromatase inhibitor suppression of breast cancer growth. This fact favors the possibility of this path contributing to ribavirin's inhibitory effect in breast cancer as well.

Similar to the abovementioned data on breast cancer, targeting eIF4E using ribavirin to block migration and EMT in NSCLC has been highlighted.[136] In this study, inhibition of eIF4E after ribavirin treatment led to decreased migration, differentiation, and expression of several EMT-related genes such as *ERα, SMAD5, NF-κB, cyclin D1, c-MYC, or HIF-1α*.[136] As we expect to do but using ribavirin, an engineered short hairpin RNA interfering with eIF4E transcription inhibited breast cancer cell migration, primary tumor growth, and metastasis establishment.[137]

TGF-beta-induced eIF4E phosphorylation enhanced metastases, invasion, and EMT in a mouse breast cancer model, all of which were inhibited when an un-phosphorylatable eIF4E was present.[138]

IMPDH is a pivotal enzyme for biosynthesis of GTP and is frequently increased in tumor cells.[139] It has been shown that ribavirin via IMPDH inhibition was effective against chronic lymphocytic leukemia cells.[140] Recently, Isakovic et al[130] demonstrated in glioma cells that ribavirin inhibits IMPDH activity and induces autophagy inhibiting the activity of mTORC1 and the SRC/AKT pathway.

Of deep significance for understanding breast cancer growth and the ABC7 regimen to inhibit it, is the study by Decarlo et al,[141] where they demonstrated a feed-forward amplification loop between TGF-beta and eIF4E (that we intend to block with pirfenidone and ribavirin, respectively). In addition, TGF-beta agonism drives eIF4E activation[138] confirming pirfenidone as a good partner drug for ribavirin.

Ribavirin has also been shown to inhibit mTOR/eIF4E signaling increasing paclitaxel and imatinib activity in squamous cell carcinoma[142] and leukemic cells,[124] respectively.

Discussion here of ribavirin strikes at the heart of why pharmaceutical mTOR inhibitors such as everolimus have not been as clinically useful as the biochemistry of cancer indicates it should be. The data in this section paint a consistent picture of eIF4E as a central element in breast cancer malignancy degree and as such a worthwhile target to inhibit. Ribavirin can be expected to do this effectively but it will be the most difficult of the ABC7 drugs to tolerate. Ribavirin is

the problematic drug of ABC7. When used over months to treat hepatitis C, 1000 mg/day would have been a common dose. Depressed mood, anemia, weight loss, and a severe but ill-defined malaise were common side effects and not rarely required dose reduction or even stopping ribavirin entirely.[143] Given ribavirin's propensity to give unpleasant side effects, it should be increased with caution from starting dose of 100 mg once daily with frequent mood and CBC evaluations.

Rifabutin: 847 Da, 2-day half-life

Rifabutin is an old antibiotic closely related to the even older drug rifampin (same as rifampicin). Rifabutin is active against *Mycobacterium tuberculosis*, atypical mycobacteria, staphylococci, group A streptococci, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Haemophilus ducreyi*, *Campylobacter* spp., *Helicobacter pylori*, chlamydia, and *Toxoplama gondii*.[144,145]

In 2016, rifabutin was reported to have blunted the growth of a patient's NSCLC, subsequently studied in vitro and found to be active in inhibiting lung cancer cell growth and suppressing Ki67 staining.[146] Rifabutin suppressed eIF4E phosphorylation with consequent decreased beta-catenin phosphorylation and increased beta-catenin destruction consequent to that.[146] Thus, rifabutin could coordinate to advantage with ribavirin to thoroughly block eIF4E.

Erlotinib inhibits epidermal growth factor receptors (HER1, EGFR) and is effective initially in stopping some lung cancers' growth. As resistance to erlotinib develops, EGFR mutations resulting in EGFR affinity to beta-catenin, thereby shifting growth drive to beta-catenin system.[147,148] eIF4E–beta-catenin axis is inhibited by several of the ABC7 drugs.

BCL6 is a 95 kDa protein transcription factor of selected genes, inhibiting expression of some and triggering transcription of others' in cancers generally[149] and in breast cancer specifically.[150–152] The result is a BCL-6-mediated anti-apoptosis effect. Breast cancer cells' survival is enhanced by BCL6.[151] Rifabutin binds to BCL6, preventing its function in translation inhibition.[153] This would be expected to be of benefit on multiple accounts during breast cancer treatment. Interestingly, miR-544 inhibition of BCL6 in TNBC cells inhibited proliferation, migration, and invasion in vitro.[154] BCL6 promoted invasion, migration, and EMT marker expression in breast cancer with indication that greater expression of BCL-6 correlates with shorter overall survival in breast cancer.[150]

Not all malignant cells within a strongly ER+ breast cancer will express ERs. The minority population not expressing ERs is relatively chemotherapy resistant with some of that extra chemotherapy resistance mediated by upregulated BCL-6 specifically in that subpopulation.[155]

Metformin: 129 Da, not metabolized, 6-hour half-life

Metformin is the most prescribed initial drug treatment for type 2 diabetes worldwide. Despite 60 years of use, the mechanism of action in lowering average glucose is not entirely clear.[156] Hepatic gluconeogenesis is decreased by metformin and insulin sensitivity is increased but how that occurs is uncertain. Metformin in vivo and in vitro increases AMPK, a major regulator of energy homeostasis, metabolism, and protein synthesis.[156] Thus, activated AMPK results in inhibition of mTOR. Breast cancer cell expression of beta-catenin was decreased by metformin concomitantly and proportionately to AMPK phosphorylation.[157]

Decreased insulin/insulin-like growth factor-I signaling and inhibition of mitochondrial electron transport chain complex are other documented actions of metformin. Across many cancers, a large chart review has shown decreased mortality in patients treated with metformin.[158] Experimental data support the notions that increased lactate secretion, reduced oxygen consumption, and activated AMPK signaling are plausible mechanisms for metformin's anticancer effects.[159] Metformin also decreased breast cancer cells' intracellular adenosine triphosphate, viability, and anti-apoptotic protein BCL6 concomitant with increased intracellular ROS,[160] the conclusion being that metformin acts primarily on mitochondria, other effects being secondary to that. That work confirmed a related earlier breast cancer study where the mode of viability loss mediated by metformin was found to be by oxidative stress increase and BCL-2 decrease.[161]

Silvestri et al[162] showed that metformin was indeed cytotoxic to breast cancer cells but 1) only in low glucose conditions – high glucose in vitro could subvert metformin's growth inhibition and 2) although AMPK activation was a requirement for cytotoxicity, mTOR was not. However, Wu et al[163] showed that metformin both increased lifespan of the nematode *Caenorhabditis elegans* and showed growth inhibition of pancreatic cancer and melanoma cells by an AMPK-independent interference with mitochondrial respiration mechanism. Likewise, Ben Sahra et al[164] demonstrated that metformin cytotoxicity to androgen-sensitive human prostate adenocarcinoma cells was AMPK independent but mTOR inactivation dependent. Furthering complicating delineation of metformin's mechanism of action in treating cancer, Gui et al[165] showed that metformin's anticancer effect

was by inhibiting mitochondrial regeneration of oxidized NAD+ regeneration and lowering aspartate levels.

Just in 2016, five extensive reviews appeared recounting evidence favoring the use of metformin as treatment adjunct in cancer generally.[166–170]

In a study particularly relevant to ABC7 regimen considering that capecitabine metabolizes into 5-FU within cancer cells, Qu et al[171] showed that breast cancer cells that had become resistant to 5-FU regained cytotoxic sensitivity to 5-FU by simultaneous exposure with metformin. Metformin synergy with 5-FU could also be demonstrated to breast cancer cells in both the stem and non-stem subpopulations.[172] Of central importance to the ABC7 regimen, IC_{50} of 5-FU to esophageal cancer cells was lowered by metformin[173] and correlated with increased AMPK activation and decreased mTOR function and lactate production. Metformin plus 5-FU combination was also active in slowing esophageal cancer growth in a xenotransplant model more than either agent alone.[174]

YAP is a small protein transcription factor promoting the growth of many cancers. When phosphorylated, it is retained in cytoplasm and therefore nonfunctional in promoting growth or inhibiting apoptosis. Metformin treatment of hepatocellular carcinoma patients increased YAP phosphorylation via AMPK phosphorylation and prolonged survival, half deceased at ~31 months without compared to ~44 months with metformin.[175] Adding metformin to exemestane also increased survival in ER+ breast cancers that overexpressed IGF1R.[176]

An ongoing trial (ClinicalTrials.gov Identifier NCT01589367) is studying potential survival benefits of adding metformin 2000 mg/day to standard antiestrogen aromatase inhibitor, letrozole 2.5 mg/day, in nondiabetic postmenopausal women with ER+ breast cancer.

Preoperative treatment of breast cancer patients with metformin has given mixed results. Some studies showed reduced mitotic rate after metformin 2000 mg/day[177] and 1500 mg/day,[178] while others showed no reduction using 1500 mg/day.[179] A similarly designed study using 1700 mg/day found marginally lower Ki67 only in women with increased insulin resistance.[180]

A pivotal study supporting metformin use during the treatment of breast cancer was reported back in 2011. In women undergoing primary resection for breast cancer, 1 g twice daily metformin was given 14 days prior to surgery.[177] By immunohistochemistry, the diagnostic biopsy was compared to resected tissue for p-AMPK, p-AKT, insulin receptor, cleaved caspase-3, and Ki67. In metformin pretreated, increased p-AMPK and decreased p-AKT were seen compared to those not treated with metformin in the interval between biopsy and surgery. Ki67 and cleaved caspase-3 were diminished in metformin-treated women compared to those not so treated. These changes were not large but were statistically significant and large enough to expect some clinical benefit.[177]

Although metformin decreases breast cancer cell survival in vitro,[160–162,181] the clinical benefit would seem small given the equivocal human trials and evidence that the small benefit seen tended to be restricted to diabetic/prediabetic people. However, small benefit is not no benefit.

Metformin despite being hydrophilic achieves approximately equal plasma and brain tissue levels. In rats, after single-dose oral metformin administration, 28 μmol/L plasma and 14 nmol/g brain tissue (14 μM) were seen.[182] Average metformin plasma levels typically seen in asymptomatic diabetes patients were 2.7±7.3 mg/L, ~3 μM. The unusually wide drug range seen, ±7.3 mg/L (±57 μM), reflects metformin's safety.[183] Metformin's side effects are limited to diarrhea, nausea, and vomiting. Some cases of lactic acidosis could occur but at a low frequency and when metformin is implicated as the cause of lactic acidosis, metformin plasma levels greater than 5 μg/mL are generally found. Target dose of metformin is the standard dose used in past breast cancer studies of metformin – 1700–2000 mg/day.

Propranolol: 259 Da, cyp 1A2, 2D6, 9-hour half-life

Propranolol was the first beta-blocker introduced to clinical practice. Introduced in the 1960s, it is still in wide use to treat hypertension, migraine, angina, selected arrhythmias, essential tremor, resolution of infantile hematomas, and in reducing the cardiac effects (tachycardia) due to acute anxiety. Propranolol's general cancer process inhibiting attributes were recently reviewed.[184] Below are selected data supporting propranolol's use specifically as adjunct in breast cancer treatment.

A study of 404 breast cancer patients to compare the proliferation rates of breast cancers in women who had taken beta-blockers compared to those who had not found a clear reduction in Ki67 only in those with Stage 1 disease.[185] A single ER+, HER2− patient was treated with 25 days of propranolol 1.5 mg/kg per day after diagnostic biopsy but before resection. Resection of tumor tissue showed a 23% reduction in Ki67 staining compared to biopsy tissue 25 days earlier, before any propranolol.[185] Of important note, beta-1-selective beta-blockers did not work to reduce Ki67, only

nonselective beta-blockers did. However, a large European epidemiological study found no survival benefit from propranolol use after a breast cancer diagnosis.[186]

Of particular interest to ABC7 regimen, Rico et al[187] examined the effects of metformin and propranolol singly and combined in several preclinical TNBC models, finding additive to synergistic growth-inhibiting effects.

In a cohort of 800 women with early TNBC, 9% used beta-blockers. The beta-blocker use and nonuse groups were well matched. At 5 years, 19% of the nonusers had died of breast cancer while 8% of beta-blocker users had died of breast cancer.[188]

In examining a cohort of 1971 multiple myeloma patients, those who took any beta-blocker, had a 24% disease-specific mortality at 5 years. Those who took a beta-blocker plus other cardiac drugs had 32% while those on no cardiac or blood pressure medicines had 41% myeloma-specific mortality at 5 years.[189]

An interesting study from Choy et al[190] showed that among 1000 breast cancer patients those on a beta-blocker had a lower recurrence rate, and specifically TNBC expressed particularly high levels of beta-adrenergic receptors. Their brain metastases expressed more beta-adrenergic receptors per cell than did the primary tumors.[190] This study also gave evidence of propranolol's inhibition of proliferation and migration in breast cancer cells expressing the beta-adrenergic receptor.

In reviewing seven epidemiological studies prior to 2015 on beta-blocker use in breast cancer, Childers et al[191] concluded that, although results were mixed between these studies, slightly lower risk of death was associated with beta-blocker use. Beta-blocker use is associated with improved relapse-free survival (but not in overall survival) also in patients with TNBC.[192]

Bone is richly supplied with sympathetic nerve endings. When specifically osteoblasts' beta-adrenergic receptor is stimulated by norepinephrine from these nerve endings, the osteoblasts secrete RANKL.[193] Thus, propranolol should harmonize with quetiapine (vide supra) in treating and preventing bone metastases in breast cancer. CA125 is a high molecular weight mucin commonly elevated in ovarian cancer. Patients given perioperative propranolol showed an 83% CA125 decrease on postoperative day 7 when those given placebo had a 72% decrease.[194]

Although the data were mixed, a review of 10 studies completed by 2015 of beta-blocker use in breast cancer concluded that specifically propranolol use was indeed associated with slightly reduced breast cancer-specific mortality.[195]

Propranolol-blocked beta-adrenergic agonist induced increased migration and decreased breast cancer cell-to-cell adhesion.[196] Propranolol inhibited breast cancer cell migration in vitro.[197] Breast cancer cells express beta-adrenergic receptors. Blocking these with propranolol lowers their glucose uptake.[198]

Campbell et al[199] demonstrated that beta-adrenergic stimulation of bone increased osteoblasts' RANKL expression. That induced RANKL increased breast cancer establishment of metastases in bone.[199] Thus, the combination with quetiapine might be particularly beneficial.

Beta-adrenergic stimulation did not change the growth of an orthotopic murine breast cancer but did induce a remarkable 30-fold increase in metastases, an effect partially blocked by propranolol.[200] Of clinical importance to ABC7, Shaashua et al[201] showed that combining propranolol with a COX-2 inhibitor in perioperative breast cancer decreased EMT, serum IL-6, and C-reactive protein levels.

There is risk of symptomatic iatrogenic hypotension with propranolol. The propranolol dose must therefore be slowly uptitrated as tolerated, monitoring blood pressure.

Capecitabine: 359 Da, half-life <1 hour

Capecitabine is a 359 Da pro-drug giving rise to intracellular release of 130 Da 5-FU.[202,203] 5-FU inhibits thymidylate synthase, which mediates the synthesis of thymidine monophosphate, the active form of thymidine required DNA synthesis.

Despite ~20 years of clinical use in treating breast cancer, there remains some unclarity on the ideal dosing schedule for capecitabine.[204–206] A comparison of cycles of 1000 mg/m^2 twice daily for 14 days, 7 days off with 1250 mg/m^2 twice daily for 14 days, and 7 days off indicated lower side effect burden with 1000 mg/m^2 twice daily.[207,208]

Several reports indicate that dosing capecitabine at just high enough level to generate palmar–plantar erythrodysesthesia might be most effective dosing regimen.[206,209] This would be analogous to erlotinib dosing where titrating to rash might be most effective.[210]

Capecitabine is best given with Coke™ or fresh squeezed lemon juice to assure low enough gastric pH for adequate and uniform absorption. This would be particularly important for those on proton pump inhibitors.

Principle toxicity is palmar–plantar erythrodysesthesia (synonyms hand-foot syndrome or chemotherapy induced acral erythema), diarrhea, and nausea, although cytopenias, fatigue, dyspnea, or cardiac abnormalities can be seen.[211] The common dose for capecitabine in breast cancer is 1250 mg/m^2 orally twice daily for 14 days, none for 7 days, every 21 days.

Agomelatine: 243 Da, 2-hour half-life

Agomelatine is a 243 Da pharmaceutical melatonergic agonist at both melatonin's receptors, M1 and M2.[212] It has many advantages over the use of melatonin itself[213,214] In short, these advantages are: 1) agomelatine is Health Canada and EMA approved and marketed as an antidepressant. As such, it is a well-standardized product, as opposed to over-the-counter melatonin preparations which are exempt from the strict standards of approved medicines; 2) agomelatine has considerably tighter affinity to both M1 and M2 receptors than does the natural ligand (melatonin); 3) agomelatine has a much longer dwell time in the body than does melatonin, and; 4) absorption is more uniform and reliable than is absorption of melatonin.

Although agomelatine is available for import into the USA, it is not FDA approved. Ramelteon is an equally potent melatonergic agonist at M1 and M2 as is agomelatine. Ramelteon is FDA approved and marketed in the USA. It has similar actions and advantages over melatonin as does agomelatine[213,215] and can be substituted for agomelatine in the ABC7 regimen.

Elevation of hepatic transaminases is of potential concern when using agomelatine. This requires regular monitoring. Elevation is dose dependent, occurring in ~3% of those receiving 50 mg once at bedtime.[216] It is usually reversible.

Work pointing to diminished breast cancer cell malignant behavior during exposure to melatonin dates back at least 3 decades.[217] There are numerous studies about oncostatic effects of melatonin on several tumors as well as recent reviews summarizing the different mechanisms of cancer inhibition by melatonin.[84,218–221] These include regulation of estrogen pathway, melatonin as SERM and SEEM, modulation of the cell cycle, differentiation and the induction of apoptosis, inhibition of telomerase activity, inhibition of oxidative stress, inhibition of angiogenesis, regulation of circadian rhythms, avoidance of circadian disruption, inhibition of tumor metastasis, invasiveness and motility decline, and enhancement of immune system and epigenetic regulation.[218,221]

Briefly and empirically, melatonin has readily demonstrable growth-inhibiting effects in both in vivo animal models, with chemically induced mammary tumors in rodents, and in vitro assays in estrogen-positive human breast cancer cells.[221–224] Melatonin inhibits invasive and metastatic properties of human breast cancer cells in different xenograft models.[225–228] Due to the broad spectrum of melatonin's actions, the mechanisms through which it interferes with metastases are varied. These include modulation of cell–cell and cell–matrix interaction, extracellular matrix remodeling by matrix metalloproteinases, cytoskeleton reorganization, EMT, and angiogenesis.[229]

Melatonin shifts human breast cancer cells to a lower invasive status by upregulating E-cadherin and β1-integrin expression and decreasing OCT4, N-cadherin, and vimentin.[219,227,228,230] These findings suggest that melatonin modulates both cell–cell and cell–matrix interactions in breast cancer and reduces the metastatic potential of the tumor. Melatonin also has regulatory actions on matrix metalloproteinases in breast cancer. It has been described that melatonin inhibits the induction, catalytic activity, and expression of MMP-9 and MMP-2.[229,231] In addition to modulating the metalloproteinase activity, melatonin reduces cancer cell migration through the downregulation of ROCK-1 and MCLCK, two kinases that control the cytoskeletal rearrangement associated with cell–cell and cell–matrix adhesion.[229,232] The attenuation of HER2-Rsk2 signaling by melatonin plays a main role in the melatonin-mediated suppression of EMT and late-staged metastasis in breast cancer cells.[226,229]

In tumor angiogenesis, there is a crosstalk between cancer cells and surrounding endothelial cells. Melatonin interferes in the paracrine interactions between malignant epithelial cells and proximal endothelial cells through a downregulatory action on VEGF expression in human breast cancer cells, which decrease the levels of VEGF around endothelial cells.[230,231] In addition, melatonin directly exerts antiangiogenic actions by reducing endothelial cell proliferation, invasion, migration, and tube formation, through a downregulation of VEGF expression.[219,233–235] Melatonin also impedes the EMT process and cancer cell dissemination through downregulatory actions of the p38 pathway[227] and interferences with NF-κB signaling in tumor cells.[217,229,236]

Recently, a review of the effects of melatonin and chemotherapeutic agents in combination in cancer treatments has been published.[237] Although the information available is limited, the results obtained suggest that melatonin sensitizes tumor cells to the cytotoxic effects of chemotherapeutic agents.

In addition, in a rat ER+ breast cancer model, melatonin reduced tumor weight, prolonged survival, and increased E-cadherin without giving apparent side effects.[238] In this model, doxorubicin cytotoxicity to the breast cancers was augmented by giving simultaneous melatonin.[238] Melatonin reduced in vitro migration and in vivo growth, proliferation index, and metastases in a murine xenograft model.[232]

Of particular relevance to ABC7, earlier in year 2017, melatonin was shown to increase 5-FU inhibition of colon cancer cell proliferation, in vitro colony formation, migration,

and invasion, showing a corresponding in vivo synergy with 5-FU in colon cancer tumor growth inhibition in a xenograft model.[239]

Similarly, melatonin moderately enhanced cytotoxicity to cisplatin and doxorubicin, while slightly but significantly enhancing 5-FU cytotoxicity to HeLa cells.[240] In an in vitro study, in rat pancreatic adenocarcinoma, melatonin augmented cytotoxicity of 5-FU, cisplatin, and doxorubicin.[241] Melatonin decreased pancreas cancers in hamsters given a carcinogen (*N*-nitrosobis (2-oxopropyl) amine), as did capecitabine. Giving both melatonin and capecitabine decreased this incidence further.[242] Melatonin augmented doxorubicin cytotoxicity to lymphocytic leukemia cells without having cytotoxicity to normal lymphocytes.[243]

Melatonin sensitized human breast cancer cells to radiation via 1) reduction in estrogen-synthesizing proteins, and 2) induction of a twofold change in p53 expression, and 3) downregulation of proteins involved in double-strand DNA break repair, such as RAD51 and DNA-PKcs.[244] Melatonin enhanced cytotoxicity of 5-FU to esophageal squamous carcinoma cells both in vitro and in a xenograft model.[245] These authors used 20 mg/kg per day melatonin in the xenograft model, corresponding to a nominal 1400 mg/day for a 70 kg adult human. The common over-the-counter melatonin used is 3–20 mg once at bedtime. The tighter affinity to melatonin receptors and much longer half-life of agomelatine compared to melatonin would go some of the way toward generating a stronger agonist signal to M1 and M2 than today's commonly used melatonin doses. Another felicitous aspect of melatonergic agonism is a potential increase in NK cell numbers and function.[246]

A remarkable epidemiological study of cancer-free postmenopausal women showed that higher urinary melatonin levels were associated with a slightly reduced risk of later developing breast cancer,[247] although these data are not uncontested. A review of all studies on urinary melatonin would indicate that this matter remains unsettled.[248]

The suggested dose of agomelatine is 50 mg once at bedtime, twice the EMA and Health Canada recommended dose for treating depression. If ramelteon is used instead 16 mg at bedtime, twice the FDA-approved dose is recommended.

Conclusion

Once breast cancer has metastasized to bone, liver, or lungs, the prognosis becomes poor. No current treatment has a reliable and robust disease control rate at that point.

Animal study of the complete ABC7 regimen would be advisable. Based on clinical experience with these drugs individually and in pairs in general medical practice, the predicted safety and tolerability of the ABC7 regimen should be safe. As a further safety measure, the ABC7 drugs should be added one at a time at weekly intervals, thereby catching any unwanted interactions early and the offending drug more easily identified.

In this article, we propose that seven common and already FDA-approved drugs, such as agomelatine (or ramelteon), metformin, pirfenidone, propranolol, quetiapine, ribavirin, and rifabutin, can have the ability to reduce EMT and breast cancer cell tumorigenesis. These ancillary drugs have demonstrated that attributes that we have reason to believe will inhibit EMT and enhance capecitabine's efficacy. The predicted safety and tolerability of the ABC7 regimen is good. A clinical trial is warranted given the fatal outcome of metastatic breast cancer as things now stand.

Abbreviations

ABC7, Adjuvant for Breast Cancer treatment using seven repurposed drugs; AMPK, AMP-activated protein kinase; BCL-6, B-cell lymphoma-6; CBC, complete blood count; COMBAT regimen, combined oral metronomic biodifferentiating antiangiogenic treatment regimen; CSCs, cancer stem cells; CTCs, circulating tumor cells; CUSP9 regimen, coordinated undermining of survival paths with nine regimens; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; EMT, epithelial-to-mesenchymal transition; ER, estrogen receptor; ER+, ER positive; 4E-BP, eIF4E-binding protein; 4E-BP1, eIF4E-binding protein-1; FDA, Food and Drug Administration; 5-FU, 5-fluorouracil; GBM, glioblastoma; GM-CSF, granulocyte–monocyte colony-stimulating factor; GTP, guanosine-5′-triphosphate; HER2, human epidermal growth factor receptor 2; Hh, hedgehog; HR, hormone receptor; HR+, hormone receptor positive; HR−, hormone receptor negative; IGF1R, insulin-like growth factor type 1 receptor; IL-6, interleukin-6; IMPDH, inosine monophosphate dehydrogenase; MCLCK, myosin light-chain kinase; MEMMAT regimen, metronomic and targeted anti-angiogenesis therapy regimen; MET, mesenchymal-to-epithelial transition; MNK, MAP kinase-interacting kinase; mTOR, mammalian target of rapamycin; MTZ regimen, minocycline telmisartan and zoledronic acid regimen; m^7G, 7-methylguanosine; NAD+, nicotinamide adenine dinucleotide; NCATS, National Center for Advancing Translational Sciences; NK, natural killer; NSCLC, non-small cell lung cancer; OPG, osteoprotegerin; PDGF, platelet-derived growth factor; PR, progesterone receptor; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; ROCK-1, rho-associated protein kinase; ROS, reactive oxygen species;

SEEM, selective estrogen enzyme modulator; SERM, selective estrogen receptor modulator; TGF-beta, transforming growth factor-beta; Th, T helper; TNBC, triple-negative breast cancer; ZO-1, zonula occludens-1.

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Disclosure

The authors report no conflicts of interest in this work.

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