**Table 1. Natural product derived inhibitors with potential synergy with cisplatin**

| Class | Compounds | Source | Mode of action |
|---|---|---|---|
| HSP90 inhibitors | 17DMAG<br>17AAG<br>Novobiocin | *Streptomyces* Species | Inhibit *AKT, B-Raf, etc*... via direct downregulation of proteins due to lack of chaperone mediated folding |
| Flavone & Isoflavones | Geninstein | Plant Derived | Induce apoptosis |
| | Tangeretin | Citrus Peels | Potentiate or synergize with cisplatin in BCa<br>Genistein is a phytoestrogen and agonist of estrogen receptor |
| Napthoquinones | Shikonin<br>Alkannin | *Lithospermum erythrorhizon* | Inhibition of *PKM2* reverses Warburg Effect and produces antiproliferative & pro-apoptotic changes associated with pyruvate metabolism |
| Anthrocyclines | Adriamycin<br>Epirubicin | *Streptomyces* Species | Inhibits topoisomerase II resulting in catastrophic DNA damage |
| | Evodiamine | Plant Derived | Evodiamine is a dual inhibitor of topoisomerase I and II |
| Allyl disulfides | (di & tri) allylsulfides | Garlic Derived | Induce apoptosis, activates caspases, reduce phosphorylation of CHK proteins, prevents resolution of double -strand breaks through an *ATR/ATM* dependent mechanism |
| Curcumin and curcumin derivatives | Curcumin | Plant (Turmeric) Derived | Induces apoptosis<br>Downregulating anti-apoptotic proteins such as Bcl2 & survivin.<br>Upregulating Pro-apoptotic protein e.g., Bax.<br>Prevent upregulation of *COX2*<br>Reduction in PGE2 |
| Vitamin C | Ascorbic Acid | Typically plant derived | ROS-induced cell death<br>May also downregulate *HIF1A* |

Summary table of natural products, their source and mechanism of action. Numerous natural products have potential use in BCa. COX-2: cyclooxygenase; PGE2: prostaglandin E2; CHK: checkpoint kinase; PKM2: pyruvate kinase M2; AKT: protein kinase B; B-Raf: serine/threonine-protein kinase B-Raf; ATM: ataxia telangiectasia mutated; ATR: serine-threonine protein kinase ATR; ROS: reactive oxygen species; HIF1A: hypoxia inducible factor 1A

mechanisms, especially those with recently developed novel compounds. We have provided a Table [Table 1] to given a broad overview of the compound described in this section as well as their proposed methods of action and sources. This table should serve as an introductory guide to following compounds.

## HSP90 inhibitors

Heat-shock protein 90 (HSP90) is a chaperone protein that stabilizes proteins necessary for tumor growth including protein kinase B (AKT), B-raf, and more[83]. HSP90A has routinely been demonstrated to be critical to cancer growth. Geldanamycin was the initial HSP90 inhibitor and was originally isolated from *Streptomyces* strains of bacteria and is thus a bacterially derived natural product. Natural product derived compounds such as geldanamycin and its analogues 17DMAG, 17AAG, and the more targeting HSB90 have proven successful in laboratory models but have limited potential clinically due to on-target toxicity associated with inhibition of all HSP90 isoforms[83,84]. Efforts aimed at specifically inhibiting HSP90 have yielded novel compounds that can inhibit BCa proliferation, but unfortunately led to the understanding that this induces a heat-shock response that is deleterious to the anti-cancer activity associated with HSP90 inhibition. In contrast, efforts to inhibit the C-terminus of HSP90, or efforts to inhibit the N-terminus of the ß form of HSP90 specifically have yielded inhibitors that may circumvent the heat shock response while also yielding efficacious therapeutics[84-86]. Novobiocin is a bacterially derived HSP90 inhibitor that targets the C-terminus of HSP90[87]. Novobiocin and derivatives do not initiate the heat shock response to the same degree and thus may be more viable means for inhibiting HSP90 long-term[84,87]. These compounds are derived from current HSP90 inhibitors and as such, the relative improvement seen with additional medicinal chemistry efforts may result in beneficial compounds. This is evidenced by prior studies indicating 17-DMAG can effectively combat cisplatin resistance in $CD44^+$ BCa causing stem-like cells[88]. The exact mechanism remains undetermined though, as HSP90 inhibitors can affect multiple pathways simultaneously given their broad range of protein targets. Further studies examining whether these compounds can functionally improve cisplatin-based therapy are warranted in HSP90 inhibitors with reduced toxicity.

eventually leading to cellular apoptosis due to uncontrolled DNA strand breakages. Adriamcyin improves cisplatin presumably through amplification of the apoptotic signal and enhanced DNA stress associated with DNA damage. Other anthracycline compounds such as epirubicin and 2"R)-4'-O-tetrahydropyranyl-doxorubicin (THP) have extensively been investigated for the potential use as an instillation in superficial BCa tumors[101,102]. Attempts at using intravesical epirubicin demonstrated that it did not improve Bacillus-Calmette-Guerin monotherapy[103]. Similarly, epirubicin alone fails to outperform BCG monotherapy on its own, although some effect with epirubicin alone was noted and the drug was well tolerated[104-106]. Given the prior success with adriamycin and known toxicity associated with topoisomerase II inhibitors future efforts should be aimed at developing inhibitors that are potent but with reduced toxicity. A number of new topoisomerase I and II inhibitors are currently being developed, some of which are derivatives of natural products[107]. Evodiamine is a plant derived dual inhibitor of topoisomerase I and II, demonstrating efficacy against a number of tumor cell types including BCa; although notably, the proposed mechanism in BCa was attributed to inhibition of mTOR/S6K1 resulting in apoptosis[108,109]. *In vitro* IC50s for evodiamine are fairly high though, and thus improved compounds may also improve clinical merit. Molecular efforts aimed at understanding how to reduce toxicity, and synthetic efforts aimed at developing improved compounds with increased potency and reduced toxicity are necessary.

### Allyl disulfide and other garlic derivatives

Compounds derived from garlic have potent anti-cancer activity, particularly diallyl disulfide, and triallyl disulfide. Early studies using non-orthotopically implanted murine tumors indicate large doses of garlic derivatives block cancer growth with dose limiting toxicity[110]. This activity is likely attributable to diallyl and triallyl disulfide as they directly induce apoptosis in BCa though activation of caspases[111,112]. In addition to apoptosis induction; allyl disulfides also reduce expression of metallomatrix proteinases which slows proliferation and invasion of BCa EJ cells which may contribute to their anti-tumorigenic effect[113]. Perhaps more importantly, diallyl sulfide induces phosphorylation of CHK proteins and potentially prevents resolution of double strand breaks through an ATR/ATM dependent mechanism[114,115]. As cisplatin also works through the formation of double strand breaks; and *ERCC1* mutation is known to enhance cisplatin therapy, it is possible that synergy could be observed between diallyl/triallyl disulfide and cisplatin. Garlic derivatives and cisplatin were found to synergize in gastric cancer; although, this was postulated to function through attenuation of Nrf2, a mechanism which has not been investigated in BCa with regards to garlic or garlic derivatives[116]. Even still, diallyl disulfide and other garlic derivatives may be highly efficacious, especially in cancers that are already known to be sensitive to cisplatin in human patients if the mechanism is ERCC1 dependent. Given the wide range of currently stated mechanisms, it is unclear what the exact mechanisms of diallyl disulfide induced apoptosis is, but it is consistently noted to block cancer proliferation induce apoptosis. Furthermore, relevant concentrations take extremely high doses that may not be reachable clinically in patients before the onset of toxicity.

### Curcumin and Curcumin derivatives

Curcumin is a plant-based compound derived from turmeric and a potent inhibitor of BCa cancer growth. Curcumin induces apoptosis directly by downregulating anti-apoptotic proteins such as Bcl-2 and survivin, and upregulating pro-apoptotic proteins such as Bax[117]. Perhaps more importantly, curcumin inhibits the formation of superficial tumors in a rat model of orthotopic BCa carcinogenesis[117]. Similar to other compounds derived from natural products, mechanisms other than apoptosis have been suggested. Recent work in the BCa field indicates curcumin may block BCa progression through mechanisms not previously appreciated. Curcumin can prevent upregulation of cyclooxygenase-2 (COX-2) which results in reductions in prostaglandin E2 (PGE2) as COX-2 is the primary metabolizing gene for the production of PGE2[118,119]. PGE2 levels promote tumor formation in BCa and other drugs targeting PGE2 production have demonstrated efficacy in BCa, including potentiation or improvement of cisplatin-based therapy[8,120-123]. Importantly, whether this is directly due to inhibition of PGE2 production or due to

other factors remains debatable, and thus, it is likely that if curcumin does inhibit PGE2 this is not the only mechanism through which it can block BCa proliferation. Blockade of PGE2 may be an important means through which curcumin could be used therapeutically in BCa as studies have demonstrated PGE2 is both involved in BCa tumor progression and cisplatin chemoresistance. Specifically, PGE2 levels contribute to a cancer stem cell like phenotype that is known to be resistant to cisplatin-based therapy though multiple mechanisms, as listed above[8]. Evidence indicates curcumin can directly prevent BCa proliferation in addition to the indirect evidence that curcumin-mediated blockade of PGE2 production would also have beneficial effects[8,124,125]. Curcumin improves both cisplatin based chemotherapy and gemcitabine based therapy as well[126,127]. Advancement of curcumin is unlikely as curcumin pharmacokinetics indicate it is rapidly metabolized when given orally and bioavailability is poor[128,129]. Novel curcumin derivatives with improved bioavailability are actively being developed, but must overcome major issues with bioavailability while retaining their anti-apoptotic effects if they are to be used successfully clinically. These studies may also be useful for determining the effects of PGE2 on BCa.

### Vitamin C

A number of essential vitamins derived from different natural products have been investigated for anti-cancer activity. Oral doses of ascorbic acid (Vitamin C) do not produce systemic levels of Vitamin C consistent with anti-cancer activity due to active uptake by SLC23A1/SLC23A2 and high levels of excretion of non-absorbed Vitamin C[130,131]. Vitamin C is known to kill cancer cells at levels obtained observed during intravenous delivery of vitamin C, however[130-132]. The mechanism is thought to be through generation of hydrogen peroxide via an ascorbate radical formed during Vitamin C metabolism[133]. This radical is only minimally formed in blood which spares many blood cells normally damaged by chemotherapeutics. Alternate mechanisms including downregulation of hypoxia inducible factor 1α have also been proposed; although, how these mechanisms would directly kill cancer cells remains undetermined, whereas, increased levels of ROS are known to induce cell death[134]. Pharmacological levels of vitamin C are toxic to BCa cell lines which may occur in part through methylation of DNA and increased levels of 5-hydroxymethylcytosine which prevents malignancy in addition to established effects on ROS production[135]. No study has directly tested synergy between cisplatin and Vitamin C as of yet; however, prior work indicates vitamin C can improve carboplatin-based therapy for ovarian cancer in laboratory models and was shown to prolong overall survival and time to disease recurrence in a small trial, although the prolonged survival did not meet statistical significance[132]. Clearly, given the solid safety profile of Vitamin C and its prior successes, it may be a viable means of improving cisplatin-based chemotherapy, or alternatively, improving therapy for patients that need less toxic chemotherapy regimens such as those that are ineligible for cisplatin.

It should be noted that many of the above chemicals are present in higher quantities in some specific diets designed to promote human health. One of the more common diets containing high quantities of plants and plant derivatives that includes isoflavones, garlic and garlic derivatives and other natural products is the Mediterranean Diet. This diet is associated with reduced risk of BCa in some analyses[136,137]. While oral consumption of these agents is likely to result in extensive metabolism and produce far lower systemic concentrations than direct administration, some benefit may be yielded from consistent intake of the diet. If this intake is related to natural product consumption and chemo-preventative effects, then understanding and isolating these specific agents or understanding their combined effects may be beneficial to treating BCa patients.

### Natural products and other forms of chemotherapy

While cisplatin remains the mainstay chemotherapy for muscle invasive disease, it should be noted that immunotherapy in the form of BCG and immunotherapy in the form of checkpoint inhibitors are critical treatments for BCa in the modern era. Checkpoint inhibitors function through removing the blockade on T

cells and re-establishing immunity; however, dysregulated inflammation is a known promoter of BCa[138,139]. As checkpoint inhibition as a therapeutic remains in its infancy, little information is available in this regard. Notably though, garlic derivatives are known to promote BCG effects and may improve the effects of other immunotherapy agents as well[140]. Similarly, reductions in PGE2 by curcumin may reduce the immunosuppressive environment and improve checkpoint inhibition. Given the diverse set of mechanisms associated with natural products, it will be imperative to define any potential benefit these agents may provide with immunotherapy.

## CONCLUSION

Natural products have been a major source of chemotherapeutic drugs and will likely continue to be so. The number of therapeutics or chemotypes derived from natural products currently under study is substantial and far outweighs those discussed here. Both cisplatin and many of the discussed natural products have multiple mechanisms which may complicate our understanding of how to effectively dose these compounds and generate synergy with cisplatin. One thing missing from many of these agents are solid, high quality pharmacokinetic/pharmacodynamic studies that generate the data required for proper dosing of these compounds. These studies may further illuminate how we can alter structure-activity relationships of these compounds to improve their efficacy *in vivo* and duplicate the positive affects we have observed. The establishment of effective *in vivo* doses will then allow for more detailed mechanistic studies that target those concentrations that are achievable and effective *in vivo*. Increasing our understanding of how these compounds work is critical to developing synthetic derivatives that have improved pharmacokinetics and greater efficacy. Moreover, understanding the molecular basis of chemoresistance will allow us to position new drugs appropriately in those patients most likely to be responsive to chemotherapy. In summary, future studies aimed at refining current natural product derived compounds towards those that target specific aspects of cisplatin chemoresistance will be the most likely route to generate novel compounds that can improve patient chemotherapy.

## DECLARATIONS

### Authors' contributions

Writing, conception, figure generation: Rajendran G
Writing, editing: Taylor III JA
Writing, conception, figure generation, editing: Woolbright BL

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