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"Breakfasting: Breaking Vessels and Fasting Can Battle Obesity Mediated Metastatic Disease"

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Abstract

Current antiangiogenic and immunotherapy treatments in Breast (BC) and Pancreatic Cancer (PC) have limited efficacy, highlighting the importance of finding new strategies to improve treatment outcomes and overcome resistance to current therapy. This mini review analyses whether manipulating the vasculature via proangiogenic factors in the Tumour Microenvironment (TME) of obese Pancreatic and Breast cancer tissue will improve obesity associated inflammation, metastatic behaviour, and outcomes of anti-VEGF therapy. The effects of diet and nutrition on angiogenesis status and metastatic behaviour remains largely unknown, especially in BC and PC, therefore this article explores the current evidence for using these approaches to manipulate angiogenic pathways and further scientific effort needed to implicate these therapies in patient use. The results of this review suggest a combination approach is needed to address Bevacizumab resistance with cytokine suppressing modulators in the tumour microenvironment as well as addressing both proangiogenic and immunosuppressive effects. Holistic approaches such as fasting and natural compounds such as curcumin have a broader cytokine target range than individual pharmaceuticals against proangiogenic factors providing strong preclinical evidence for its use in clinical trials.

Keywords: Antiangiogenic therapy resistance; Breast cancer; Pancreatic cancer; Metastatic behaviour; Fasting mimicking diets and calorie restriction.

Abbreviations: EC: Endothelial Cell; VEGF: Vascular Endothelial Growth Factor; FBGF: Fibroblast Growth Factor; EMT: Epithelial Mesenchymal Transition; TME: Tumour Microenvironment; FMD: Fasting Modified Diet; BC: Breast Cancer; PC: Pancreatic Cancer; TC: Tumour Cell.

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Introduction

Importance of tumour angiogenesis in the metastatic cascade

Folkman hypothesized that proliferation of Tumour Cells (TC) and Endothelial Cells (EC) were interdependent and must include proangiogenic factors [1]. Tumour angiogenesis can be stimulated by hypoxia, hypoglycemia, and inflammation and involves perivascular cleavage, dilation of the vessel, new vessel development and recruitment of endothelial and perivascular cells [1].

A hypoxic tumour environment from excessive growth can trigger the release of growth factors from the Tumour Microenvironment (TME) such as Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FBGF), human Epidermal Growth Factor (EGF). Angiocrine endothelial cell signaling is also promoted in hypoxia, such as Thrombospondin (TS) and endostatin to stimulate EC to proliferate and loosen cell-to-cell adhesions [2]. Growth factors also facilitate the release of proteases from the Extracellular Matrix (ECM), which allow proliferating EC to migrate out of the quiescent vessels into the TME. VEGF is a popular therapeutic target, however in clinic this has had controversial success, partly thought to be due to the upregulation of pro-angiogenic factors like Thrombopoietin, FBGF, Angiopoietins (Ang) by smooth muscle peri-EC , to adapt to hypoxia and promote sprouting and migration of EC to increase oxygen exchange [3]. It is also well established that other cells in the TME secrete proangiogenic factors such as tumour-associated macrophages secreting VEGF, Matrix Metallopeptidase (MMP9), Wnt7B, and CXC motif Chemokine Ligand (CXCL12). These factors can change the permeability of the vasculature and facilitate migration of metastatic cells into the vasculature [4]. As tumour angiogenesis is an important step in the metastatic cascade, understanding resistance mechanisms to anti-angiogenesis treatments may provide better pharmacological treatment, combination strategies to prevent metastases as well as non-pharmacological approaches such as how diet and reduction of inflammation can be applied for this purpose.

Manipulation of angiogenesis activation has been attempted by treatments such as bevacizumab, sunitib, sorafenib, aflibercept in metastatic disease but result in poor response rates [5]. Phase III studies combining bevacizumab with standardized chemotherapy combinations in HER2 negative breast cancer demonstrated an improvement in progression free survival from 5 months to 7 months but no difference on overall survival. 16 Phase III trials have shown overall survival benefit and progression free survival of bevacizumab in combination with oxaliplatin based chemotherapies in metastatic colorectal cancer 17, non-small cell lung cancer, ovarian and cervical cancers 18, however this was not seen in breast and pancreatic cancer.

Targeting of tumor vasculature has been controversial as some studies have shown that hypoxia induced by antiangiogenic treatments can lead to Epithelial Mesenchymal Transition (EMT) phenotypes in TC and cause bone marrow derived cells to produce chemokines, which recruit TC to distant tissues [6]. This paradoxical hypoxia effect on tumour metastases is exacerbated in obese tissues where hypertrophic adipocytes also experience hypoxia, which leads to necrosis and adipocyte death. This can stimulate a pathogen associated molecular response on tumor-associated macrophages causing them to secrete a proinflammatory profile of cytokines changing their phenotype [7]. These proinflammatory molecules such as IL-6, TNF alpha can induce metastases by promoting the EMT phenotype and causing further angiogenesis. Therefore, the tumour microenvironment of obese tissue can greatly influence metastatic and proangiogenic capability of tumors and induce anti-VEGF therapy resistance. However, further vessel manipulation by targeting the tumor microenvironment such as secretion of growth factors, cytokines, and cell-to-cell adhesion signals may be alternative approaches at reducing proangiogenic signaling and thereby provide potential therapeutic targets of manipulation to reduce metastatic phenotypes.

Tumour Endothelial Cell (TEC) heterogeneity is a new field of research and another approach at understanding proangiogenic tumour phenotypes from the EC perspective. TEC display cytogenetic abnormalities, increased proliferation, and migration as well as many structural defects compared to normal endothelial cells with highly permeable vessels [8]. These cells also react to different stimuli such as hypoxia, tumour secreted factors such as VEGF, which is targeted by antiangiogenic therapy such as Bevacizumab. Resistance to such therapies may emerge due to the complex interaction between tumour and endothelial cells as well as poor tumour vasculature due to deficient coverage of endothelial cells, reduced perivascular cells and permeable EC junctions [9]. Subsequent leakage of protein can increase interstitial pressures in the tumour, which can collapse peripheral blood vessels, further leading to hypoxia and resulting adaptation of stromal cells through epithelial mesenchymal transition to migrate and metastasise [10]. Reports have also demonstrated that TEC abnormalities are heterogeneous within the tumour and between tumour types. TEC also upregulate genes not usually seen in endothelial cells such as Lysol oxidase, pentraxin, biglycan which are pro angiogenic and promote secretion of inflammatory factors from tumours cells. ICAM-1, VCAM-1 adhesion molecules are also overexpressed which increase migratory and intravasation abilities of tumour cells [9]. Endothelial heterogeneity and dysfunction have also been demonstrated in animal models of obesity with structural alterations and differences in angiocrine signalling [11]. Consequently as there is a wide base of preclinical evidence for obesity related tissue environments being proinflammatory and pro angiogenic, as well as tumour endothelial heterogeneity also being a target for reducing anti-angiogenesis therapy resistance and metastases prevention.

Therefore, this review summarises current limitations to anti-angiogenesis therapy in breast and pancreatic cancer and the new approaches to tumour vascular manipulation to mitigate the effects of hypoxia and inflammation mediated metastases, especially in an obese tumour tissue.

Methods

The following results were obtained using articles from PubMed published between the years 2009-2020 of preclinical models of genetically obese and diet induced mice and measurement of subsequent inflammatory secretome and its behavior of tumour metastases. Clinical trial data using fasting mimicking diets in breast cancer and pancreatic cancer patients and effects on complete response to therapy are also reviewed. Key words of angiogenesis, immunosuppression, VEGF, obesity related inflammation, fasting, and calorie restriction were used. PowerPoint applications were applied to illustrate points from the literature. The following results and discussion sections with show analysis of the preclinical models and its implications with the benefit of manipulating the tumour vasculature.

How obesity contributes to cancer metastasis in breast cancer

Obesity is associated with poor prognosis in a number of cancers including pancreatic and breast and as well as a strong influence on metastatic spread [12,13]. High vascular density of breast cancer tissue has been correlated with obesity and has demonstrated benefit from anti-VEGF therapy in reducing tumour burden [14] Adipocytes are a large component of breast tissue and the stromal compartment of BC. Therefore the risk factor of obesity and by extension adipocyte hypertrophy and hypoxia, is highly relevant in this cancer type due to the androgen and adipokine hormone profile secreted by adipocytes postmenopausally for women, which have a proliferating effect on breast tissue [15].

Overweight patients with BC are in a persistent state of inflammation and stress in adipose tissue, which leads to an increased expression of pro-apoptotic proteins. One study aimed to understand molecular mechanisms of poor prognosis in postmenopausal BC and obesity using a syngeneic mouse model of post-menopausal BC by feeding ovary-ablated mice a high fat diet [15]. To mimic human disease, they transplanted murine BC studying tumour growth and metastatic behavior. Flow cytometry and PCR of the growing tumors showed a phenotype of increased high-grade neutrophil and granulocyte interactions in the tumour as well as hypoxia in obese mice compared to their controls. These mechanisms promote the EMT and progression of the tumour type to claudin low BC, a subtype of TNBC that has increased cancer stem cells. High fat diet fed mice also had a higher frequency of lung metastases and faster rate of tumour growth. Their results describe that hypoxia driven mechanisms promote neutrophil and granulocyte expansion, which results in a metastasis initiating phenotype. Whilst ovarian ablated mice recreate the hormone microenvironment of post-menopausal BC, human BC cell lines were not used which means further studies using xenografts and in vitro studies are needed to confirm the molecular mechanisms of obesity on initiating metastases.

How obesity contributes to cancer metastasis in pancreatic cancer

Zyromski et al studied the effect of obesity on PDAC growth in mice models of lean and obese mice. Lean mice and two strains of obese mice (one homozygous for the diabetes insulin resistance gene and the other homozygous for the obesity spontaneous gene, Lep (Db) and Lep(Ob) respectively), were injected with murine PDAC cells and studied for metastatic behaviour and tumour growth after 5 weeks. Bigger, aggressive tumour cell proliferation phenotypes were observed in the obese mice compared to lean mice [16]. Moreover, 36% of the obese mice developed peritoneal metastases and 20% mortality, whereas lean mice did not. On necropsy, the cause of death in these mice dying prior to the experiment was attributed to gastrointestinal haemorrhage and diffuse peritoneal disease adding to the notion that obesity contributes to increased metastases and complications. Serum adiponectin, leptin, glucose, and insulin levels were taken as well as the homeostatic assessment score - as a measure of insulin resistance - in both obese strains. Whilst both obese mice were hyperglycaemic and had hyperinsulinemia and resistance, leptin was significantly increased in mice that were genetically diabetic whereas exceptionally low in the obese homozygous mice. This difference in tumour burden could be due to variation of TME of patients who have had established obesity for a long time versus patients who acquire obesity and insulin resistance through high fat diets. Tumour burden and growth was also negatively correlated with adiponectin plasma concentration and positively correlated with increased insulin levels. These findings indicated that insulin resistance and adipokine concentrations had important effects in promotion of pancreatic growth and metastatic spread. Overall, this model demonstrates that obese mice had different tumour growth patterns, complications, mortality, and metastatic capabilities compared to lean mice supporting the idea that obesity promotes the metastatic process.

In our view, genetically induced models are not the ideal tool in studying PDAC metastases as human cancers are genetically very heterogeneous and may have several mutations and signalling interactions that are not represented in a murine model. Future studies with human pancreatic cell lines should be used in vivo to test specific immunotherapy treatments against IL-6 and EMT phenotypes to see effects on metastases formation and increased immune infiltration. A further study testing a more comprehensive set of inflammatory cytokines in human pancreatic cell lines demonstrating, elevated IL1b, IL-6, IL10, IL17 involved in obesity related inflammation as well as infiltration of myeloid stem cells and down regulation of dendritic cells [17]. Myeloid derived stem cells can secrete VEGF promoting vascular permeability and endothelial proliferation and stimulate cancer-associated fibroblasts to express alpha smooth muscle actin and other pericyte markers to enable tumour migration through the endothelium 48. Elevated levels of TNF alpha, IL10 also decrease T helper cell differentiation 49 and IL-6 can increase proangiogenic signalling of VEGF and Fibroblast growth factor as demonstrated by the PDAC murine model 50.

A post op case control study of resected PDAC demonstrated that patients with metastatic cells in dissected lymph nodes had a significantly higher prevalence of adipose tissue in the pancreas. This presence of positive lymph nodes was also correlated with poor survival compared to node negative patients [18]. This study indicates the importance of the pancreatic tumour environment in promoting metastases mechanisms. However, data on the molecular prolife and gene expression of inflammatory cytokines were not collected to describe the metabolic basis by which this correlation between obesity and metastases occurs. Interestingly the BMI between the two cohorts of patients in the study was not significantly different, however data on visceral fat of patients were not collected, which has been linked in other studies as more prominent in producing a pathological proinflammatory environment in adipose tissue compared to subcutaneous fat, thereby indicating its relevance to a pro metastatic phenotype [15].

Obesity and endothelial heterogeneity and its effects on metastases

Endothelial dysfunction is observed in animal models of obesity and rats fed high fat diet as there is increased reactive oxygen species production from adipocytes, which can inactivate endothelia derived nitric oxide and the ability to maintain vasodilation and vascular tone. Obesity also altered endothelial cell heterogeneity in decreasing clusters of cells responsive to vasodilator agonists [11] The relevance of this is seen as low nitric oxide production in endothelial cells is a prominent feature of pulmonary endothelial dysfunction in metastatic breast cancer models of xenograft mice. Pulmonary endothelial cells in breast cancer bearing mice was observed to have decreased NO production which lead to a mesenchymal phenotype in the lungs with a reduced expression of endothelial cadherin and CD31

expression [19]. These changes increased endothelial permeability, associated with early pulmonary metastases. This study suggests that early endothelial dysfunction with nitric oxide deficiency may be exacerbated in obese tissue and therefore reversal of obesity may be a viable target to prevent early metastatic opportunities.

How obesity may hinder results of current antiangiogenic and immunotherapy

Obesity is associated with hypoxic adipose tissue, which has been correlated with resistance to anti-VEGF therapy by upregulation of proinflammatory factors in BC patients.

In a subclinical analysis of a phase 2 trial, in obese BC patients, patients with reduced response to anti-VEGF treatment had increased concentrations of FBGF and IL 6 in their BC tissues suggesting a causative mechanism of resistance. BC tissues also had lower vessel density and higher expression of hypoxic markers. The association of IL-6 production from adipocytes and resistance to anti-VEGF therapy was replicated on a cellular model by murine BC cell as well as CXCL1, IL12 and TNF [20]. Mouse models show normalisation of FGF expression in obese mice when treated with metformin, decreased tumour vessel density in mice and increased anti-VEGF sensitivity. This data indicates that obesity promotes BC resistance to anti-angiogenesis therapy via inflammatory mechanisms, but also that metformin is a potential mechanism to suppress obesity related resistance to anti-VEGF therapy.

Wan et al. also characterised immune responses in obese tissue by demonstrating non-tumour bearing Diet induced obese (DIO) mice had increased frequency of PD-1+ expression on T-cells in the liver which led to T-cell exhaustion noted by expression of Tim3 and Lag3 in peripheral blood streams, liver and spleen. This finding was supported by additional in vivo experiments conducted in 4T1 BC in DIO mice correlating with higher metastatic rates [21]. The authors also observed increased levels of leptin in serum, which correlated, with upregulation of STAT3 signaling and PD-1 expression CD8+ T cells, the leptin levels were subsequently suppressed by calorie restriction reduced T-cell exhaustion. This study is important in demonstrating a link between the inflammatory microenvironment of obese tissues and host immune dysfunction via, PD-1expression and T-cell dysfunction in murine BC. It is also suggestive of the intervention of calorie restriction and its effects of boosting the immune response, which could be applied as an anticancer strategy.

Preclinical models: Obesity and dual effects on Angiogenesis and immunosuppression

Obesity can lead to suboptimal therapeutic approaches in breast cancer as well as being a risk cancer for cancer progression and poor clinical outcomes. Kolb et al demonstrated that obesity is associated with an increase in tumour infiltrating macrophages, IL-1 production which caused increased angiogenesis and metastatic behaviour. Next generation sequencing was able to identify upregulation of angiopoietin like 4 factor as a result of IL-1 beta signalling from adipocytes in a NFKB dependent manner, which is a recognised proangiogenic factor in cancer. Subsequently blocking angiopoietin 4 via gene knock out models, obesity associated inflammation and angiogenesis was markedly reduced in obese mice tumours. Antagonising angiopoietin signalling in obese cancer tissues may be an attractive strategy at reducing obesity driven tumour angiogenesis and therefore combination treatments that address this on the tumour vasculature may be a potent synergistic strategy for enhancing anti VEGF therapies [22]. Figure 1 below summarises the associated immunosuppressive, inflammatory and proangiogenic pathways associated with obese tumour microenvironments as mentioned in the previous studies.

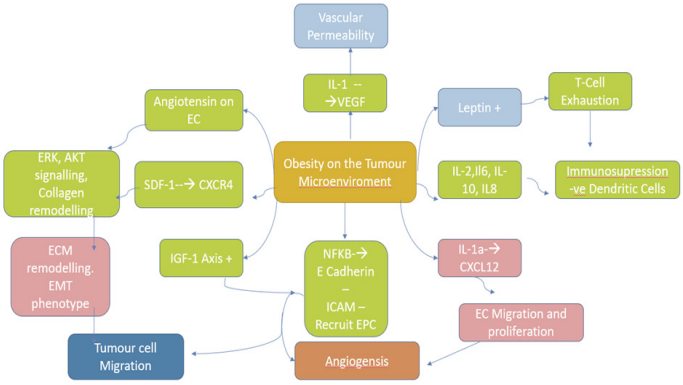

Figure 1: Summary of effects of obesity on tumour microenvironment based on these studies.

How fasting and diet can improve antigangiogenesis therapy, affect endothelial cells and metastases.

As adipose tissue is a repository for inflammatory cytokines; TNF-Alpha, NFKB, IL-8, IL 1Beta, VEGF, chemokines and interferon, these molecules are attractive targets to reverse inflammation in obese tumour tissue and reduce metastatic potential of the microenvironment. There are many pharmacological strategies as well as fasting and dietary manipulation to reduce obesity driven effects on angiogenesis and metastases in cancer. There have been concerns that calorie restriction and fasting as therapeutic strategy may have nutritional deficits hence pharmacological strategies that target similar pathways affected by calorie restriction such as metformin or hydroxy citrate have been popular therapies to gain the benefits of calorie restriction without nutritional sacrifice and compliance issues.

Pharmacological strategies

Metformin an antidiabetic drug was found to reduce IL-6 mRNA expression, a cytokine previously implicated in facilitating metastases and increase expression of IL-1 cytokines a naturally occurring anti-inflammatory, thus have some suppressive effects on these inflammatory molecules in obese patients [23]. Activation of AMPK metabolic pathway has been suggested as an important strategy for prevention of cancers associated with obesity as it stimulates fatty acid oxidation and modulates insulin secretion by pancreatic cells [24]. Metformin is a method of reactivating the AMPK pathway and reducing the inflammatory cytokine expression such as IL 6 and 8. Methods of modulating Insulin Growth Factor (IGF) expression and reducing hyperglycaemia may be methods of targeting obesity related cancer may be effective candidates for treating obesity related breast malignancies.

Dietary interventions such as curcumin which is a phytochemical extracted from turmeric has shown anti-inflammatory promise against molecules such as NF-KB, AKT, mTOR growth signalling and COX-2 pathways, present in obesity-related cancer [25]. The effect of curcumin on obese tissues includes reducing hyperglycaemia, hyperlipidemia, reducing oxidative stress, insulin resistance and fluctuating levels of adipokines all of which disrupt the homeostasis of cell proliferation and apoptosis, which is pathological in obesity related cancer [26]. The mul-

tifaceted pharmacological effects and molecular mechanisms of curcumin is under publicised however could be a useful therapeutic in the prevention of obesity related cancers as well as reducing metastatic spread[34]. Since hypoxia induced cell stressors are present in obese adipose tissue as well as promotion of NF-KB, curcumin could be a promising agent to combine with fasting mimicking diets or antiangiogenic therapy in PDAC.

Several studies have shown Antiproliferative effects of metformin, non steroidals and curcumin in breast cancer and pancreatic cancer which have been summarised in Table1.

Table 1: Antiproliferative properties of Curcumin, metformin and NSAIDS in pancreatic and breast cancer clinical trials.

Pharmaceutical Intervention	Pathways targeted	Implications for Obesity and Metastases
RCT Chemotherapy and Hormonal therapy control + Metformin 850mg Twice a day in Treatment arm [27].	Lower IGF, FBGF, insulin resistance	6months follow up were associated with decreased metastatic frequency and reduced mitosis factors, increased apoptosis due to IGFB3
RCT Addition of Metformin 1500mg between biopsy and surgery in overweight and obese patients [28].	Reduced ki67 mitosis staining, reduction of insulin resistance in tumour cells. Down regulates AKT/m TOR signaling	Therapeutic use of metformin in reducing inflammatory pathways associated with obesity driven metastases
Metformin + Gemcitabine in Xenograft mouse models using PC cells in vitro [29]. + RCT Metformin + Gemcitabine [30].	Reducing IGF levels, increase expression of BAX2 Caspaase-3, BCL-2 apoptosis proteins	Translation of this benefit has been limited in randomised trials of patients with metastatic pancreatic cancer as metformin did not significantly improve survival for patients beyond the expected 6-7 months with gemcitabine
Phase 2 Trial BC Celecoxib + Exemestane	Reduction in Aromatase and oestrogen receptor and reduction of tumour burden	Less helpful in triple negative BCs which do not express hormone receptors as well as being too selective in cytokines, missing the broader inflammatory mechanisms that are seen in obesity related inflammation
Preclinical models on EC Curcumin 100-300mg/kg/day [25].	downregulating transcription of NFKB, TGF Beta, ANG-1, intercellular adhesion molecule -1, vascular adhesion molecule present on endothelial cells	Reduces transendothelial migration, as well as induction of heme oxygenase -1, which reduces reactive oxygen species and inflammatory pathways. These popular pathways are commonly found in obesity related inflammation
Xenograft Models of PC + Curcumin Analogues UBS109 and EF31 [31].	Significant inhibition of NFKB pathways, VEGF, angiopoietin, PDGF, COX2 and TGF Beta secretions in PDAC cell lines in mice treated with curcumin showing antitumor and anti-angiogenic effects	Angiogenesis in PDAC cells would be disrupted when cells were exposed to curcumin compounds in cell cultures and blocked the vascularisation developed by mice harboring human MIA PAca-2 cell lines
Phase 2 Trial – 8g of Curcumin + Gemcitabine in Metastatic Pancreatic cancer [32].	Metastatic behavior studied	Improvement in response rates from 8months to 10months, 60% stable or reduction in disease
Phase 2 Trials Docetaxel and Curcumin in Metastatic breast cancer [33].	Metastatic behavior studied	Results yet to be published

Fasting as a strategy in manipulating the obese tumour microenvironment

The cellular response to fasting is governed by circulating glucose, growth hormone, IGFs, glucocorticoids, and adrenaline. In the initial 6-24hrs, insulin levels being to fall, and glucagon rises promoting breakdown of glycogen stores in the liver and breakdown of triglycerides stored in adipose tissue to maintain glucose concentrations [38]. Notably fasting can also reduce circulating levels of insulin, IGF, leptin and increase levels of adiponectin which has beneficial on inflammatory signaling and metastases formation as previously described. At a molecular level, reduction of IGF1 and glucose can decrease intracellular signaling cascades of AKT, mTOR, cAMP-PKA signaling which has a plethora of Antiproliferative, anti-angiogenic and immuno-complimentary effects. Fasting down regulates haem-oxygenase 1 expression in BC which makes the tumor more vulnerable to attack from cytotoxic T-cells [39]. By activating cell autophagy to recycle nutrients and amino acids, fasting can reduce levels of adenosine production, which prevents the shift of macrophages to the immunosuppressive inflammatory M2 phenotype [40]. This further prevents the context of metastatic formation, which is often activated by M2 macrophages secreting migratory cytokines and attracting EC migration.

Harvey et al hypothesised that calorie restriction by 30% of recommended intake could decrease cancer growth and target the insulin growth factor axis in xenograft mice models of human PDAC. Calorie restricted mice had decreased serum IGF-1 concentration as well as a 70% decrease in proinflammatory and 56% decrease in macrophage associated cytokines. MiaPaCa-2 human PDAC lines in nude mice had decreased expression of NF-KB transcription factor and related inflammatory downstream signalling after calorie restriction. These findings suggest that there are inhibitory effects of calorific restriction on pancreatic tumour growth and inflammatory factors such as NF-kB which are known to be upregulated in obesity and metastatic cells via IGF-1 [36].

Insulin resistance, which is prevalent in obesity, has also

been demonstrated to lead to endothelial dysfunction via nitric oxide deregulation as well as cause vasoconstriction via endothelin 1. A study on the effects of alternative day fasting on insulin resistance and endothelial function in obese non-diabetic patients, however did not see a change in endothelial function as a result of this [41]. However, this study specifically looked at flow mediated dilation properties of endothelial function rather those properties specifically relevant to metastases development such as increased permeability, reduced perivascular cells and normalisation of the tortuous structures of tumour vasculature. Therefore, future studies specifically looking at how fasting regimes and duration can affect endothelial dysfunction in tumour vasculature and its synergistic effect with antiangiogenic therapies and resistance will be valuable. It is currently unknown whether combination therapies with diet and fasting and bevacizumab can reduce metastatic behaviour in obese tissue in clinical studies, but there is preclinical evidence to suggest this would be a complimentary strategy.

Preclinical studies have suggested that fasting mimicking diets can be used in combination with chemotherapy to reduce side effects and enhance cytotoxic effects. The DIGEST trial aimed to evaluate the effects of fasting mimicking diets on tumour immunity in non-metastatic early stage breast cancer in combination with taxane chemotherapy [42]. The study utilised a low carbohydrate, low protein, calorie restricted plant-based diet for 5 days prior to surgical excision of the tumour as well as four cycles of fasting every month after surgery. The basis of this trial was derived from strong preclinical evidence that suggested plasma levels of growth factors and insulin that are known to promote metastasis and growth are significantly reduced via fasting whilst also being an attractive way of reducing chemotherapy side effects and cell toxicity. Another indirect effect of fasting is enhancing cytotoxic CD8 T cell lymphocyte infiltration which has improved tumour cell kill and reduced inflammatory signaling in many obese tumour tissue which was also demonstrated in peripheral blood samples of participants [43].

The DIRECT trial randomised Stage 2/3 HER2 Negative breast cancer patients of normal BMI, without diabetes to a fasting mimicking diet prior and concurrently to neoadjuvant chemotherapy [44]. This trial was prematurely stopped as interim analysis failed to demonstrate improved response rates in Her 2 negative breast cancer. However, the trial showed reduced DNA damage in circulating lymphocytes taken from breast cancer patients plus omission of dexamethasone during the fasting cycles in the treatment arm achieved similar complete response rates as existing control therapy arm [45]. The authors have also documented radiological decrease in tumour volumes and spread. The clinical relevance of this finding in terms of overall survival is uncertain, as many patients did not complete the fasting intervention. Patient adherence to fasting cycles included 33% patients completing 2-4 cycles of fasting out of the proposed eight. Whilst this number was sufficient in mouse models, a higher number of cycles was intended for larger biological volume in humans. The diet was also standardized in kits and therefore not an attractive option for many patients. The trial was unable to establish what effect short-term fasting had on tumour growth and spread. Another bias is that patients with earlier stages of cancer were recruited for fasting regimens to be more acceptable for patient use, however the benefit of fasting diets in early versus later stages on metastatic behavior is yet to be characterised in a study. Alternative future trials may be more beneficial as a tailored eating program with a dietician for the individual, which may help improve compliance as well as assessing response in more advanced disease.

Caffa et al hypothesised that water fasting calorie restricted plant based diets, which have been shown to reduce circulating growth factors sch as IGF1, and insulin could be used to enhance anti-oestrogen therapy of tamoxifen and fulvestrant. (reference) This was demonstrated using mouse xenografts and human breast cancer cell lines in low glucose culture conditions mimicking short-term fasting. Addition of weekly fasting cycles to treatment with tamoxifen reduced acquired resistance to tamoxifen and longer remission of tumour growth by reducing oestrogen receptor transcription [46]. Whilst the preliminary findings of low glucose cultures and BC organoids in mice is promising, these findings are yet to be replicated in patients and complex tumour environments of proinflammatory proangiogenic obese tissue. Mouse xenografts models and breast cancer cultures do not always express hormone receptor status in their studies and therefore there is a lack of knowledge on which type of breast cancers these effects are more beneficial to. In addition, whilst many clinical trials have looked at the feasibility of FMD in patients with metabolic effects on growth factors, there is no published data on the effects of FMD as an influence on metastatic behavior and tumour angiogenesis.

Approximately 75% of breast cancers express either oestrogen receptors or HER2 proteins and can have endocrine or Her2 targeted therapy to maintain disease response after surgery and chemoradiotherapy. Triple negative breast cancers are typically more aggressive with fewer targeted therapies available for reducing metastatic disease.

The BREAKFAST trial is the first known study using fasting mimicking diets in patients with triple negative breast cancer in combination with or without metformin, and chemotherapy to study metastatic behavior of tumors [47,48]. The trial started recruitment in 2020 as a randomised phase 2 trial measuring responses 3 years post intervention.

The preclinical evidence suggests cycles of fasting diets have complimentary cytotoxic effects when combined with cisplatin or doxorubicin in murine triple negative models. This is also demonstrated in combination with metformin as glycolysis is inhibited by fasting and oxidative phosphorylation reducing metabolic pathways for the cancer cell. The 5-day fasting cycle will be administered with 1700mg of Metformin capsules with views to measure how long modifications of IGF-1 concentrations will last as well as quantity of tumour percentage decrease on MRI imaging and reduction of inflammatory genetic expression profiles. Whilst the results of this study are yet to be published, the preclinical evidence provides the starting point in exploring combination therapies, which include fasting practices as a therapeutic strategy with pharmacological mimetics to address certain inflammatory pathways. Future studies in this direction will be important in determining the length of response of fasting practices on reducing angiogenesis and progression free survival as well as which type of fasting therapy is most accepted by the patient. Which stage of treatment this intervention is most useful and whether there is a difference in response in hormone, positive verses triple negative cancers [47].

Table 2 demonstrates preclinical and clinical trials involving fasting practices as therapeutic cancer strategies.

Table 2: Studies involving calorie restriction or fasting mimicking diets in pancreatic and breast cancer studies. Preclinical and human trials. Molecular Pathways in Pancreatic and Breast Cancer.

Model	Diet	Outcomes
Preclinical Models		
Human pancreatic cell lines in mice [49].	Ketone bodies (sodium hydroxybutyrate and lithium acetoacetate)	Inhibit cell survival of pancreatic cancer cells. Decrease in glucose uptake and release of lactate, reactive oxygen species. Decrease C-MYC expression. Decrease in tumour volume and weight in mice bearing tumour fed the keto diet, decreased Ki67 and desmoplasia.
Mice over expressing COX-2 spontaneously developing murine pancreatic inflammation	30% calorie reduction diet for 14 weeks	Reduced pancreatitis associated inflammation and dysplasia, reduction in Ki67proligeratie cells, VEGF expression and down regulation of the IGF-1 pathway. Suggests that modulation of the IGF pathways is a strong pancreatic cancer strategy.
Preclinical model Mice with human Triple negative breast cancer cell lines [37].	Combination treatment with Calorie restriction and radiotherapy	Calorie restriction reduced time to metastatic progression and volume and frequency of metastases and improved overall survival by down regulating the IGF-1R signaling pathway
Clinical Trial Data		
Pancreatic cancer xenografts mirrored in Phase 1 Clinical trials [50].	Fed ketogenic diet, radiation, and chemotherapy diet for 5 weeks versus standard feed.	Prolonged survival in radiation combination study of rodents. However, when patients with metastatic cancer enrolled in the clinical trials there were very low accrual rates and poor tolerance of diet.
B-AHEAD-2 Randomised Control trial [51].	Intermittent energy restriction versus chronic energy restriction during chemotherapy to promote weight loss for overweight women during therapy	Primary end point was toxicity chemotherapy reduction however results have not been published.
DIGEST Randomised Control Trial. Impact of diet on tumour immunity [42].	FMD 600kcl on day 1 and 300kcal day 2-5 on Non-metastatic breast cancer + Taxane chemotherapy	Results pending, Recruiting 2019
Direct Trial RCT HER2 negative breast cancer Stage II/III [44]	FMD. Studying pathological complete response, metabolic factors, DNA damage, long term efficacy	90-100% tumour cell loss in FMD arm reduces chemotherapy indued T-cell damage in T-lymphocytes.
BREAKFAST Trial Triple negative breast cancer –+ FMD + Metformin + Standardized chemo [47].	6months Standard anthracycline-taxane Neoadjuvant chemo + three weekly 5 day FMD. 600kcal Day 1 and 300Kcal on Day 2-5. Low carb low protein Studying Pathological complete response rate	Results pending recruiting 2019

Conclusion

The main findings from these preclinical mouse models and clinical trials indicate resistance to anti-VEGF therapy can be explained by proinflammatory proangiogenic phenotypes of obese tissue, immune suppression as well as endothelial heterogeneity within the tumour. The major signalling pathways demonstrated in these studies include secretion of TNF alpha and a range of cytokines such as Il-17, Il-6 from macrophages that promote immunosuppression and the EMT phenotype to promote tumour cell and EC migration. Upregulation of IGF-1 axis, TGF Beta activates NF Kappa B signalling which has downstream effects on further ECM remodelling, down regulation of E-cadherin on cell surface receptors of TC and upregulation of ICAM on EC, which allows cell detachment and promotes EC migration. Angiotensin 1 signalling in adipocytes can also upregulate NFKB and downstream transcription factors including the JAK/STAT pathway which can also activate angiogenesis factors such as VEGF,

FGFB and PDGF receptors in response to hypoxia. Upregulation of these proangiogenic factors in obesity can promote resistance to anti-VEGF therapy. Figure 2, illustrates the different signalling pathways involved in obesity related metastases and corresponding opportunities in targeting its proangiogenic effects.

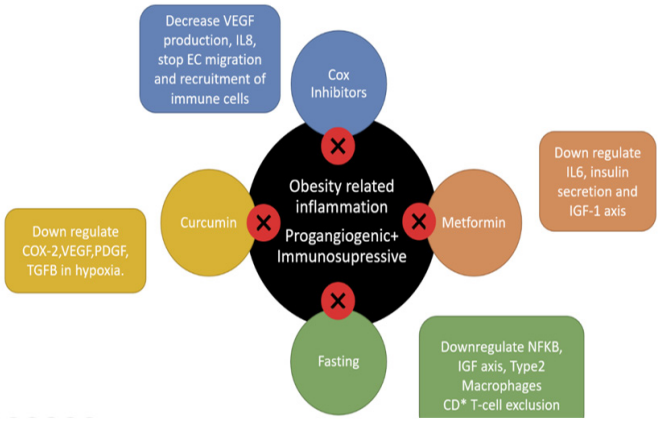

Figure 2: Proposed treatment combinations in reducing proangiogenic and immunosuppressive effects in obesity.

Changes in adipokines such as leptin and adiponectin in obesity also influences the inhibitory PD-1 expression on T-cells causing immunosuppression as well as secretion of inflammatory factors like COX2 which promotes endothelial migration in angiogenesis formation. The models and clinical evidence studied in this review suggest targeting of these inflammatory factors in obesity can manipulate the endothelial response and subsequent metastatic ability of TC, whilst also removing the immunosuppressive effects of obesity related inflammation.

One large limitation of preclinical studies described above is the lack of cell type specificity of BC and PC xenograft and genetic mutation driven cancers and therefore future studies will need to establish which cancer phenotype will most benefit from interventions such as calorie restriction and anti-inflammatory pharmacology prior to translating these effects into human trials.

As it has been shown that obesity has effects on immune suppression, angiogenesis and endothelial heterogeneity in the tumour environment, it will be important to use strategies that simultaneously address these pathologies, such as diet, calorie restriction and anti-inflammatories suggested in the studies, to manipulate metastatic behavior of tumors. Future direction of trials needs to demonstrate duration of exposure needed to change the immunophenotype of obese TMEs and whether it has an impact on outcomes such as improved survival rather than just tumour burden reduction.

Il-6 and FGF were also upregulated in visceral adipose tissue of BC and was related to poorer response to antiVEGF therapy [20]. Whilst this study did differentiate Visceral Adipose Tissue (VAT), from subcutaneous fat in their secretory profile, other studies mentioned in this thesis did not specify or measure the amount of VAT that contributed to the proinflammatory profile as cytokine concentration was taken from mouse plasma. VAT versus Subcutaneous (SCT) fat have different inflammatory secretion profiles therefore when translating the implications of this on tumour angiogenesis and metastatic behavior in human trials, BMI alone may not be the most suitable markers of indicating a proinflammatory response. VAT measurement may be a more suitable recruitment criterion to these trials.

Targeting inflammatory factors implicated in obesity driven metastatic pathways by direct pharmacological inhibitors to the IL-1alpha family is plausible, however systemic therapy and specifically affecting one group of cytokines may affect many knock-on processes in where they are needed in physiology. Non-pharmacological methods of broader suppression of these proangiogenic cytokines such as fasting and dietary manipulation in my opinion may be a more holistic way of reducing side effects by targeting obesity related inflammation.

From my literature review, we could not find any clinical trials combining antiangiogenic therapies with immunomodulatory strategies to improve treatment outcomes in pancreatic and BC. However, from the studies described in this review, combination approaches to address alternative proangiogenic factors such as TGF beta, and the interleukin 1 family may be interesting to see when used with antiangiogenic therapy in PDACs, as there is a biological basis to suggest the efficacy of bevacizumab treatments can be hindered by these inflammatory factors. Combination therapies such as immunomodulatory drugs targeting Interferon alpha 2-a and bevacizumab have already been utilised in renal cell cancer clinical trials but did not show survival benefit [52].This may be because many other proangiogenic factors were not addressed in the TME of a highly hypoxic cancer. Therefore, when choosing combination of therapies in pancreatic and breast adenocarcinomas it will be beneficial to target a broad range of inflammatory factors that are known to be upregulated in PDAC, especially in obese tissue.

Fasting and calorie restriction has been demonstrated by Zhang et al and Sun et al to suppress these same proangiogenic factors secreted by type 2 macrophages, down regulating NFKB and IGF in PDAC as well as decrease immunosuppressive phenotypes of CD8 T cell exclusion that can be brought on by proangiogenic signals like IL 6, CXCL12 and TGFBeta [53]. The benefit of using a strategy like fasting over specific cytokine inhibitors is that it has a systemic effect across the body ensuring penetration of mechanism compared to a pharmacological agent, as well as having a broader target range and removing causative factors such as hypoxia induced by hypertrophic adipocytes.

Curcumin was also demonstrated to effectively down regulate proangiogenic factors such as COX-2, VEGF, PDGF and TGF-Beta secretions in hypoxic environments of human PDAC. This could be especially potent in reducing antiangiogenic therapy resistance as many proangiogenic factors are suppressed by the compound. This is especially pertinent in obesity related angiogenesis and metastases as there is downregulation of NFKB which has both proangiogenic and tumour cell EMT phenotype promotion characteristics especially in obese tissue. Curcumin therefore could be a promising agent to combine with fasting practices alongside antiangiogenic therapy as an effective way of reducing metastatic behavior of PDAC and BC.

Dietary interventions are an inexpensive and patient empowering way of enhancing existing cancer therapies. Whilst calorie restriction is an established preventative therapy in a few inflammatory medical conditions, its ability to reduce cancer inflammation and growth factor signaling is still young in its full discovery as a cancer therapy. Cachexia is commonly observed post oncological treatment; the role of a personalized nutrition plan with careful elimination of certain amino acids may be a vital missing step in making calorie restriction as an intervention acceptable to the patient population undergoing treatment.

Existing clinical trials have utilised fasting mimicking diets or calorific restriction in combination with chemoradiotherapy to reduce tumour burden and decrease side effects of cytotoxic by inducing a differential stress response and protecting normal cells [39,35,54]. However, to date no clinical trials specifically address the question of fasting improving antiangiogenic therapy outcomes when used in combination and reducing metastatic burden, especially in the cohort of obese patients. Important questions to elicit from any future studies using this type of combination is which proinflammatory cytokines, if not all are down regulated, at what preinvasive stage is this combination most effective and how long do these benefits last.

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