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Review

At the Intersection of Cardiology and Oncology: TGFβ as a Clinically Translatable Therapy for TNBC Treatment and as a Major Regulator of Post-Chemotherapy Cardiomyopathy

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Simple Summary: Specific/targeted therapies have been shown to be effective in the treatment of certain cancers. Unfortunately, there currently is no specific therapy for the treatment of triple negative breast cancer (TNBC) which is attributed to why this subtype of breast cancer is associated with reduced patient prognosis. While there is an immense focus on development on new therapies the issue of cardiotoxicity following chemotherapeutic treatment is commonly overlooked despite its role as a leading cause of mortality in cancer survivors. This review aims to discuss the connection of TGF-β signaling, and its role in modulating cardiac fibrosis and remodeling as well as its role in TNBC tumor progression, cancer stem cell enrichment, chemoresistance and relapse. Together we highlight the modulation of TGF-β as a method to target two of the greatest causes of morbidity and mortality in breast cancer patients.

Abstract: Triple-negative breast cancer (TNBC) is a subtype of breast cancer that disproportionally accounts for the majority of breast cancer-related deaths due to the lack of specific targets for effective treatments. While there is immense focus on the development of novel therapies for TNBC treatment, a persistent and critical issue is the rate of heart failure and cardiomyopathy which is a leading cause of mortality and morbidity amongst cancer survivors. In this review, we highlight mechanisms of cardiotoxicity post-chemotherapeutic exposure, assess how this is assessed clinically and highlight the transforming growth factor-beta family (TGF-β) pathway and discuss its role as a mediator of cardiomyopathy. We highlight recent findings demonstrating TGF-β inhibition as a potent method to prevent cardiac remodeling, fibrosis and cardiomyopathy. We describe how dysregulation of the TGF-β pathway is associated with negative patient outcomes across 32 types of cancer including TNBC. We then highlight how TGF-β modulation may be a potent method to target mesenchymal (CD44+/CD24-) and epithelial ($ALDH^{high}$) cancer stem cell (CSC) populations in TNBC models. CSCs are associated with tumorigenesis, metastasis, relapse, resistance, and diminished patient prognosis; however, due to plasticity and differential regulation these populations remain difficult to target and persist as a major barrier barring successful therapy. TGF-β inhibition represents an intersection of two fields: cardiology and oncology. Through inhibiting cardiomyopathy, cardiac damage and heart failure may be prevented and through CSC targeting, patient prognosis may be improved. Together, both approaches, if successfully implemented would target the two greatest causes of cancer-related morbidity in patients and potentially lead to a breakthrough therapy.

Keywords: TNBC TGF-β; Cardiology; Oncology; CSC

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1.0 Introduction

Breast cancer is the most frequent cancer affecting women and accounted for over 2 million breast cancer diagnoses and approximately 600,000 related mortalities in 2018[1]. TNBC only accounts for the minority of breast cancer incidences (15-20%); however, TNBC is disproportionally associated with reduced patient prognosis compared to the other breast cancer subtypes[2,3]. TNBC in contrast with other breast cancer subtypes lacks expression of the estrogen receptor, progesterone receptor and HER-2. The presence of these receptors are associated with the usage of specific therapies; thus non-specific chemotherapies and radiotherapies are mainstays for the treatment of TNBC which overall is associated with reduced patient prognosis.

As such, there is immense focus on the development of targeted therapies to target TNBC. However, a critical issue garnering increased attention in preclinical research is the high incidence of cardiotoxicity following therapy leading to increased rates of heart failure and cardiomyopathy[4]. CVD and its related complications is a leading cause of morbidity and mortality in cancer survivors[5]. In a observational study, Patnaik *et al* demonstrated in 63,566 breast cancer patients, that while there were increased adjusted relative hazards of comorbidities such as cardiovascular disease, COPD and diabetes; it was found that cardiovascular disease was the primary cause of death amongst the patients (15.9%) exceeding mortality due to breast cancer (15.1%)[6].

Moreover, Bardia *et al* demonstrated in a clinical trial using a 10-year recurrence risk prediction model in breast cancer patients with early stage breast cancer (stage I-III with 67.5% having stage I) which calculated CVD and breast cancer recurrence risk [7]. It was found that the risk of a CVD event exceeded the risk of breast cancer relapse in 37% of the patients and 43% possessed a risk equal to that of breast cancer recurrence[7]. These studies highlight that not only is the development of therapeutics for primary tumor management important for patient prognosis, but that the cardiovascular health of the patient must be protected due to sensitivity following chemotherapy treatment.

To highlight this point, a recent study by Sturgeon *et al*, 3,234,256 US cancer survivors from the year 1973-2012 were assessed and mortality ratios stemming from CVD (consisting of a grouping of heart disease, hypertension, atherosclerosis, cerebrovascular disease, aortic aneurysm or aortic dissection) and cancer related causes were assessed[8]. The patients were separated by cancer type and CVD mortality was found to highly elevated in patients diagnosed with breast, prostate or bladder cancer (together accounting for 61% of all CVD mortality) and also in patients diagnosed at an earlier age (<35 years old)[8]. Importantly, this study identified that CVD is highly prevalent in breast cancer and that the risk of CVD mortality is continually elevated throughout the follow ups of the study and does not dissipate[8].

Due to the essential inclusion of cardiotoxic agents such as anthracyclines, taxanes and antimetabolites for the treatment of breast cancer combined with the CVD issues plaguing patients post-chemotherapeutically there is a drastic need for cardio-oncology research into mechanisms promoting chemotherapy induced cardiotoxicity and methods to alleviate this process. This review will discuss mechanisms of chemotherapy induced cardiomyopathy in TNBC patients and also highlight TGF-β signaling as an emerging pathway of therapeutic interest for the prevention of chemotherapy induced cardiotoxic effects. Additionally, this review will highlight the anti-tumorigenic properties of TGF-β modulation in targeting the TNBC bulk tumor and its CSC populations. Clinically translatable mediators of TGF-β signaling involved in breast cancer and cardiac disease related clinical trials will be described and listed for future investigation.

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2.0 Post-Chemotherapeutic Cardiomyopathy

Due to the aforementioned lack of specific cellular targeting in TNBC treatment, there is a strong reliance on standard cytotoxic chemotherapeutic agents in clinical practice.[9] These regimens often involve the use of anthracycline or taxane class chemotherapeutic agents.[10] Unfortunately, chemotherapy often induces very severe side effects, with cardiotoxicity at the forefront of dose limiting toxicity.[11]

Cardiotoxicity is a broad term which includes both early and late onset forms as well as effects ranging from subclinical impairment of cardiac function to cardiac death.[12] Early onset, also called "acute"/"subacute" cardiotoxicity develops immediately after chemotherapeutic infusion or up to 2-4 weeks after completion and is typically characterized by reversible arrhythmias, abnormalities in ventricular repolarization, prolongation of the QT interval, acute coronary syndrome, pericarditis/myocarditis-like syndromes or altered myocardial function.[13] Late onset cardiotoxicity can be divided into either early-chronic or late-chronic subtypes. Early chronic cardiotoxicity occurs within 1 year after termination of chemotherapy while late-chronic cardiotoxicity occurs more than 1 year after termination.[14] Late-onset cardiotoxicity can result in systolic/diastolic left ventricular dysfunction that leads to congestive cardiomyopathy which can transition towards cardiac death.[14] Additionally, cardiomyopathy can be classified into two subtypes: type I (caused by cardiomyocyte death and is irreversible) and type II (caused by cardiomyocyte impairment of cardiac function and is reversible).[15] This concept was originally proposed by Ewer *et al* and these subtypes can differentiate the effects of various chemotherapeutic agents; for example, doxorubicin (an anthracycline class chemotherapeutic) induces type I cardiotoxicity while the biologic trastuzumab (an anti HER-2 chemotherapeutic agent) induces type II cardiotoxicity.[15]

3.0 Anthracycline and Taxane Mechanisms of Cardiotoxicity

Doxorubicin, an anthracycline, is one of the most frequently prescribed chemotherapeutic agent for the treatment of TNBC. The toxicity of doxorubicin on cardiac tissue is mediated through multifactorial mechanisms which remain convoluted. One commonly proposed mechanism is that anthracycline agents such as doxorubicin are prone to the generation of reactive oxygen species (ROS) during their metabolism.[11] Specifically, the univalent reduction of the anthracycline class quinone moiety by mitochondrial complex I in the electron transport chain (ETC) results in the formation of semiquinone radicals which rapidly undergo auto-oxidation to form superoxide anions (O2-), thereby also regenerating the quinone moiety. [16,17] This cycle can then continue under aerobic conditions, producing additional ROS. This process may shed light on the correlation between anthracycline class chemotherapeutics and the induction of cardiotoxicity as the cardiomyocytes experience large demand for ATP produced by ETC, and therefore have a greater density of mitochondria (and subsequently complex I) than other cell types[18]. The high rate of ROS production in the mitochondrion of the cardiomyocyte can then interfere with iron reduction, damaging the cells via ROS mediated reactions which result in the formation of reactive nitrogen species leading to mitochondrial/cardiomyocyte dysfunction which ultimately promotes apoptosis [19-21].

Another proposed mechanism for the cardiotoxicity of anthracyclines is via its intended anti-tumor mechanism of DNA-topioisomerase2 (Top2) intercalation, wherein the anthracycline forms a Top2-doxorubicin-DNA ternary complex. In humans Top2 is expressed as the isoenzymes Top2α and Top2β, with the former expressed in proliferative cells (including cancer cells) and the latter in quiescent cells[22]. Top2 β positive malignant cells demonstrate ternary complex formation upon anthracycline therapy promoting the inhibition of DNA replication leading to G1/G2 arrest and apoptosis in cancerous cells.

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Unfortunately, Top2β is also the primary form expressed in adult cardiac tissue promoting anthracycline binding and cardiotoxicity resulting in mitochondrial and cellular dysfunction.[23],[24]

Paclitaxel (a taxane) is another commonly used chemotherapeutic agent in the treatment of breast cancer, especially in anthracycline-resistant breast cancer.[25] Although it was thought that taxanes have negligible cardiotoxicity when compared to anthracyclines, phase I and II clinical trials revealed acute cardiac reactions upon paclitaxel infusion such as: cardiac rhythm disturbances, atrioventricular conduction abnormalities, sinus bradycardia and ventricular tachycardia [26],[27]. Importantly, the majority of cardiac disturbances were not associated with clinical symptoms and were found incidentally during cardiac monitoring. Moreover, these cardiac issues were common in taxane treated patients with 29% of patients having asymptomatic bradycardia when administered the maximal tolerable dose of taxane therapy(110-250 mg/m2)[28]. One proposed mechanism for taxane cardiotoxicity is the mediated not by the taxane agent itself but rather through its formulation vehicle, Cremophor EL (a vehicle to enhance the solubility). It has been proposed that Cremophor EL induces massive histamine release causing acute cardiovascular reactions.[29] Interestingly, taxanes such as paclitaxel are often used in combination with anthracyclines; however, it was found in clinical trials that the combination produced unacceptably high rates of heart failure (18% of patients)[30]. This is thought to be due to pharmacokinetic interference, where paclitaxel interferes with the clearance of doxorubicin possibly through competition for biliary clearance promoting cardiotoxicity.[31]

4.0 Clinical assessment of cardiotoxicity

The severity of cardiomyopathy is important not only for determining therapeutic course but also for the manifestations of CVD later in life, especially in regards to pediatric populations who were administered chemotherapeutic agents. The gold standard for anthracycline class cardiotoxicity determination is a cardiac biopsy; however, due to the impracticality of this as a clinical assessment it is not typically considered. Rather, cardiac imaging can be used to monitor cardiac deterioration, where the left ventricle ejection fraction (LVEF) is used to track progression. LVEF can be determined via TC-99 Multiple Gated Acquisition Scan (MUGA), also called radionuclide ventriculography[32,33]. Current guidelines place cardiotoxicity as one or more of the following: 1) Reduction of LVEF, either globally or more within the septum. 2) The onset of symptoms associated with heart failure. 3) A reduction of >5% to < 55% in regards to the ejection fraction (EF) alongside symptoms of heart failure, or a >10% to <55% decline in EF without symptoms of heart failure[34,35]. It's thought that through patient monitoring, cardiotoxic effects of anthracycline therapy can be discovered early and its effects minimized. Research by Swain *et al* however challenge this notion via demonstrating that doxorubicin-related CHF may occur at a lower dosage, at a greater frequency (26% compared to the 7% at a cumulative dose of at 550 mg/m2) and outside guideline parameters[36]. These findings challenge LVEF tracking and highlight the importance in mitigating chemotherapy induced cardiotoxicity. In addition to this finding, radionuclide ventriculography emits a high dose of radiation and is relatively expensive, making it a poor choice for longitudinal cardiac monitoring.

In contrast, echocardiogram is a radiation free, cheap, and readily available alternative for measurements of LVEF as compared to MUGA; however, it was found by Hoffmann *et al* that unenhanced echocardiography resulted in a slight underestimation of EF as compared to radionuclide ventriculography or MRI assessment.[37]. This disappointing result was however improved upon the use of contrast, where contrasted echocardiography was found to be comparable to MRI and even exceed the capabilities of radionuclide

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ventriculography [37]. Additionally, echocardiography can evaluate for adverse structural effects, such as valvular disease or pericardial constriction[38,39].

Cardiovascular magnetic resonance imaging (CMR) is another imaging technique for the evaluation of cardiomyopathies induced by cardiotoxic therapies which has the advantage of being radiation free[40]. CMR has the ability to detect subclinical cardiac dysfunction prior to detectable LVEF changes in addition to the ability to detect myocardial edema (a marker of myocardial injury). The high cost and low availability of CMR in contrast to echocardiography make it less widely utilized as a screening tool.[38]

The utilization of electrocardiogram (ECG) for cardiac monitoring circumvents the above problems associated with imaging and has the added benefit of being inexpensive and readily available. Horacek *et al* found statistically significant correlation between decreased QRS voltage, corrected QT interval (QTc) prolongation and left ventricular dysfunction as visualized by echocardiography[41]. ECG also has the added benefit of being able to correlate with malignant ventricular arrhythmias via QTc, an important indicator of acute cardiotoxicity[41]. Additionally, Fukumi *et al* found that signal-averaged ECG was able to detect acute and chronic cardiotoxicity from anthracycline chemotherapeutics at lower cumulative doses than echocardiography based imaging. Such a finding could allow for earlier insight into cardiac dysfunction[42].

Many well established biomarkers are used to investigate the cardiomyocyte damage. Not only can troponins serve as an indicator of damage, but their levels correlate with the clinical severity of the damage that occurs from the insult[43]. This allows for risk stratification during an infarct or other cardiac insults[44]. A study by Cardinale *et al* found that elevation in Troponin I in patients undergoing high dose chemotherapy (anthracyclines) preceded and was able to accurately predict the development of future cardiac dysfunction (via lowered LVEF)[45]. As the elevation of cardiac troponin I is a very specific and sensitive marker for cardiac damage and is one that many hospitals utilize in their practice its adoption into chemotherapy related cardiac monitoring remains a popular proposition[46]. Other markers of interest include natriuretic peptides such as Brain Natriuretic Peptide (BNP), its preprohormone and cleavage product (NT-proBNP) and Atrial Natriuretic Peptide (ANP). These substances serve to regulate blood pressure and circulating blood volume and are released from cardiomyocytes in response to atrial stretching/volume overload[47]. Similar to troponins, BNP may allow for the early detection of cardiotoxicity, although they may have the added advantage of being detectable for longer periods of time. While troponin was detectable within 4-15 hours until 10-14 days, natriuretic peptides were detectable within 24 hours and for as long as 2 years[48-52]. Routine assessment of patients exposed to chemotherapy with the detection methods described above would serve to identify early signs of CVD post-treatment allowing for increased opportunity to mitigate negative effects and increase prognosis in patients post therapy.

5.0 TGF-β Overview

Extensive studies have shown that transforming growth factor beta (TGF-β) is a major mediator which modulates multiple cellular steps that promote cardiovascular disease, cardiac hypertrophy, arrhythmia, fibrosis and cardiac failure [53]. In brief, various proteins/conditions have been found to activate TGF-β secretion [54]. Initially, TGF-β ligand is bound by the TGF-β binding protein which is activated via binding of αv integrin to the prodomain of TGF-β1/2 mediated by a myriad of conditions including myofibroblast induced contraction [55-57]. Activated TGF-β signaling is primarily mediated via two distinctive downstream effectors: the SMAD pathway and the non-canonical pathway. SMAD signaling is mediated through activated TGF-β interaction with type I (TβRI) and type II receptor (TβRII) via trans-phosphorylation of multiple serine/threonine residues

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of TβRI GS domain [58]. The activated TGF-β type I receptor then activates SMAD2 and SMAD3 via phosphorylation. Following SMAD2/3 activation, the complex trimerizes with SMAD4 forming the activated SMAD complex which translocate into the nucleus to regulate transcription for a variety of downstream effectors including the COL1A1/COL3A1 genes that facilitate production/deposition of collagens, plasminogen activator inhibitor-1 that builds matrix, and connective tissue growth factor that upregulates the expression of fibronectin or heparan sulfate proteoglycans (HSPG) (Figure1) [59,61].

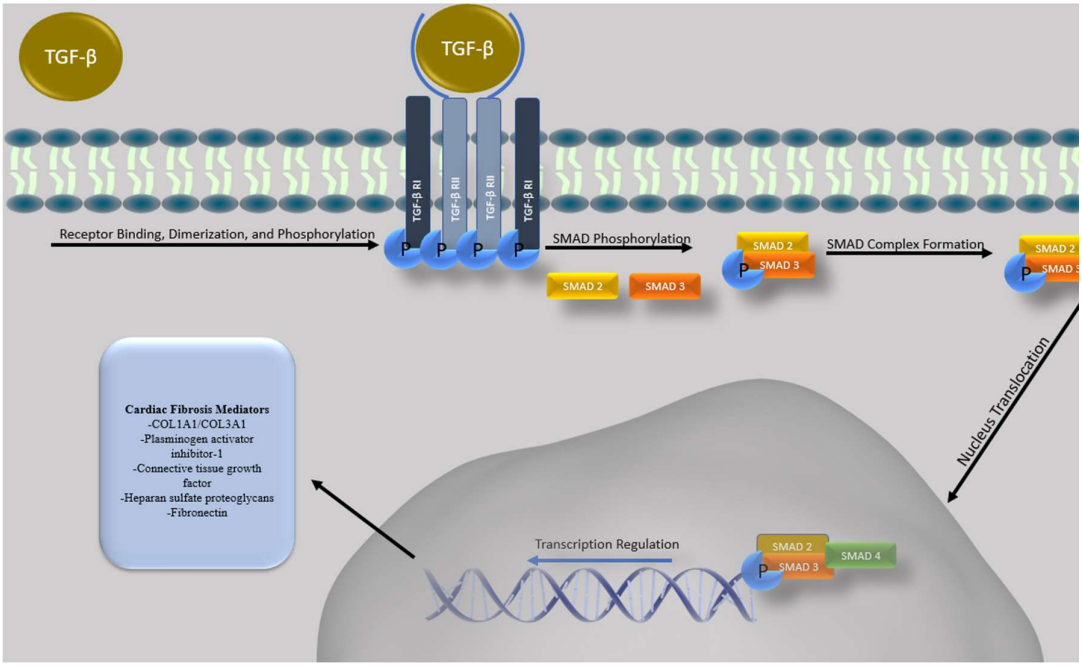

Figure 1: Overview of Conventional TGF-β Signaling. A schematic overview of conventional (SMAD mediated) TGF-β signaling occurring after TGF-β ligand binding which leads to the activation of TGF-β type II and TGF-β type II receptor heteromeric complex which can induce the phosphorylation of SMAD2 and 3, promoting complex formation with co-SMAD (SMAD4). This trimeric complex can translocate into the nucleus and induce transcription of numerous genes including those involved in cardiac remodeling and fibrosis as well as cellular differentiation, survival, invasion and apoptosis.

SMAD-independent pathways are broadly referenced as non-canonical pathways and can mediate TGF-β signaling independently or work in conjunction with SMAD-dependent pathways to facilitate/repress the TGF-β pathway [62,63]. Amongst the various non-canonical mediated intercellular signals, mitogen activated protein (MAP) kinase is one of the mechanistic pathways which showed growing evidence of its roles in mediating TGF-β induced cardiac fibrosis [64]. Activated TGF-β receptors can interact with TNF Receptor Associated Factor 6 (TRAF-6) to induce ubiquitination [62]. Subsequently, ubiquitinated TRAF-6 recruits TGF-β activated kinase (TAK-1). TAK-1 activation consists of the kinase domain of TAK-1 forming a complex with TAK1-binding protein (TAB1). The active TAK1-TAB1 heterometric complex can then upregulate non-canonical mediating effectors such as MKK4/7 and MKK3/6 via phosphorylation [65]. Phosphorylated MMK4/7 upregulates the expression of JNK which in turn recruits transcriptional factor c-jun. Similarly, phosphorylated MMK3/6 can upregulate the expression of p38 which in turn increases the expression of ATF-2 [62,63]. These non-canonical pathways induce c-jun and ATF-2 co-transcription factors which can regulate the expression of SMAD dependent fibrosis via phosphorylation, signifying the intricate cellular interplays between the SMAD dependent and non-canonical induced fibrosis in cardiac models [62,63,66].

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6.0 TGF-β as a Mediator of Cardiac Fibrosis and Remodeling

Cardiac fibrosis is a hallmark response to injuries of the heart and its onset has been associated with myocardial infarction, ventricular remodeling, arrhythmia, dilated cardiomyopathy and heart failure [67-69]. Cardiac fibrosis is characterized by the differentiation of cardiac fibroblasts into myofibroblasts [70,71]. TGF- β is a crucial mediator in the differentiation of myofibroblasts and resistance to apoptosis via activation of the Smad3 pathway which promotes α-SMA (alpha-smooth muscle actin) transcription in fibroblasts and induces extracellular matrix protein deposition and myofibroblast differentiation[72-75].

Dobaczewski *et al* demonstrated via a closed-chest model of coronary occlusion/reperfusion to induce reperfused myocardial infarction in Smad3 null mice that ablation of SMAD-mediated signaling was associated with a reduction of α-SMA transcription in fibroblasts. Furthermore, upon TGFβ1 stimulation, while wild-type mice demonstrated increased α-SMA and fibrosis, Smad3 null mice did not, highlighting the association between TGFβ-SMAD signaling and the cardiac fibrosis[73]. In another similar study, a closed-chest model of reperfused myocardial infarction in Smad3 null mice demonstrated that TGF-β1 stimulation was associated with upregulation of procollagen III but not in Smad3-null mice, which indicates that TGF-β mediated Smad3 signaling plays an important role in the extracellular matrix protein synthesis[76]. Using mice subjected to cardiac pressure overload stimulation via transverse aortic constriction surgery, Khalil *et al* showed that TGF-β treated Smad3 and Smad2/3-deleted fibroblasts had a significant reduction in fibroblast marker genes (*POSTN, COL1A1*, and *COL3A1*) in primary cardiac fibroblasts, indicating that deletion of Smad3 from newly activated fibroblasts may significantly attenuate the cardiac fibrosis response[77].

Additionally, angiotensin II of the Renin-Angiotensin-Aldosterone System (RAAS) has been associated with the onset of cardiac fibrosis. Research has demonstrated the correlation between angiotensin II expression and TGF-β expression in cardiac fibroblasts[78-80]. Wang *et al* stimulated mouse primary aorta vascular smooth muscle cells (VSMCs) with angiotensin II *in vitro* and demonstrated that angiotensin II can mediate Smad2/3 signaling pathway in a TGF-β dependent manner[81]. Furthermore, Zhang *et al* demonstrated that chronic angiotensin II infusion upregulates human c-reactive protein (CRP) in CRP transgenic mice, leading to a 5-fold increase in serum CRP, a biomarker associated with cardiovascular diseases and events. As angiotensin II-induced cardiac TGF- β1 expression and activation of the Smad signaling were enhanced in CRP transgenic mice as well this highlights that angiotensin II mediated activation of TGF-β plays a pathogenic role in cardiac remodeling[82].

TGF-β can also mediate non-canonical signaling to promote pathological cardiac remodeling via activation of TGF-β Activated Kinase 1 (TAK1) as a delayed response to mechanical stress. Transgenic mice that expressed TAK1DN (a constitutive active form of TAK1) under the control of cardiac specific aMHC promoter (aMHC-TAK1DN) had a 46% increase in cardiac mass at 9-11 days after aortic banding and selective activation of p38 in myocardium at 9 days (up to 400%). Hearts of mice 9-10 days old showed hypertrophied myocytes with hyperchromatic nuclei, interstitial fibrosis, and other signs seen in load-induced hypertrophy and heart failure[83]. Constitutive overexpression of the human tumor suppressor A20 suppressed TAK-1 induced collagen synthesis and TAK-1 dependent Smad 2/3/4 activation in murine hearts, protecting against cardiac hypertrophy and fibrosis[84]. Thus, TGF-β mediated TAK-1 activity plays an important role in myocardial hypertrophy and heart failure.

Thus, TGF- β through SMAD dependent and independent signaling is associated with the onset of adverse cardiac pathologies and negative clinical outcomes making preclinical

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research into TGF-β modulation for the treatment of cardiac disease as potential target for further clinical consideration. This is highlighted in a study by Laviades *et al* which demonstrated that hypertension and microalbuminuria in patients was associated with left ventricular hypertrophy and higher levels of serum TGF- β1 compared to normotensive participants. In the same hypertensive patient group, treatment with Losartan (a clinically approved angiotensin II receptor antagonist with TGF-β inhibitory activity) decreased TGF-β1 levels in patients which correlated with a reduction of microalbuminuria and left ventricular hypertrophy [85]. To further highlight the importance of TGF-β in cardiac function, using sequence specific oligonucleotide probing (SSOP), Holweg *et al* studied genomic DNA samples from heart transplant recipients and found that Leu>Pro (codon 10) polymorphism in the *TGFB1* gene is associated with end-stage heart failure caused by dilated cardiomyopathy[69]. Thus, TGF-β through SMAD dependent and independent signaling is associated with the onset of adverse cardiac pathologies and negative clinical outcomes making preclinical research into this pathway for the treatment of cardiac disease an unmet medical need.

7.0 TGF-β Inhibition to Prevent Cardiomyopathy

It has been demonstrated that TGF-β exerts physiologic effects on embryonic development, cardiac development and cellular growth and it has been further highlighted that dysregulated TGF-β signaling is associated with a host of unwanted pathologic conditions such as fibrosis, cardiac hypertrophy and inflammation [86-89]. Thus, modulation of TGF-β through pharmacologic agents may be of therapeutically benefit patients with post-chemotherapy fibrosis, heart failure and cardiomyopathy.

Oliveira *et al*, demonstrated that GW788388 (a TGF-β inhibitor specific for TβRI/ALK5) can reduce cardiac fibrosis [90]. This was demonstrated through injecting Swiss mice with Trypanosoma Cruzi parasites to induce Chagas disease and cardiac fibrosis which was measured via fibronectin and collagen type I deposition [90]. It was found that this model induced substantial indications of cardiac fibrosis; however, upon treatment with GW788388, deposition of fibronectin and collagen type I was reduced in cardiomyocytes and cardiac electrical conduction was improved [90]. In a separate study by Ferreira *et al*, these results were repeated in a chronic Chagas *in vivo* mouse model consisting of C57BL/6 mice injected with Trypanosoma Cruzi and treated with GW788388 [91]. Mice receiving treatment demonstrated reduced fibrosis of cardiac tissue indicated by reduced levels of collagen type I and fibronectin deposition in cardiac tissue. Moreover, GW788388 inhibited TGF-β/pSmad2/3 expression and activity which was correlated with reduced CD3+ inflammatory lymphocyte cell migration into cardiac tissue [91]. Interestingly, these effects were correlated with increased stem cell antigen-1 (Sac-1+) cardiac cells following treatment. As Sca-1+ is a marker for cardiac stem cells it was suggested that TGF-β inhibition can not only inhibit fibrosis but also promote the enrichment of cardiac stem cells which could promote cardiac recovery [91].

TGF-β has also demonstrated translatability in the treatment of myocardial infarction (MI). Myocardial infarctions lead to cardiomyocyte death through ischemia, fibrosis and eventual heart failure. During a MI, there is a well-documented upregulation of TGF-β isoforms which facilitate healing and repair [87,92,93]. This process however also leads to fibroblastic extracellular matrix protein deposition and an upregulation of TIMPs (Tissue inhibitors of metalloproteinases) which inhibits matrix degeneration and ultimately stimulates fibrosis [94]. Khalil *et al* highlights the importance of TGF-β signaling in the fibrotic response via deletion of TGF-β receptors TGFβR1/2 and Smad3 in cardiac fibroblasts which reduced TGF-β–induced gel contraction indicating a disruption in myofibroblast differentiation. Moreover, a novel *in vivo* mouse model was used with Periostin-GFP reporter tracking of myofibroblasts of the heart in combination with TGFβR1/2, Smad2,

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Smad3 and Smad2/3 knockouts [95]. This model then induced cardiac pressure overload via aortic constriction (an *in vivo* methodology to induce cardiac hypertrophy and heart failure) which found that deletion of Smad3, Smad2/3, or TGFβR1/2 was able to inhibit cardiac fibrosis following aortic constriction [95]. Moreover, 12 weeks after aortic constriction TGFβR1/2 knockout mice demonstrated reduced ventricular fractional shortening, preserved diastolic function and reduced cardiac hypertrophy highlighting the targeting of the TGF-β pathway as a viable strategy to reduce cardiac fibrosis [95]. Importantly it was also found that the inhibition of Smad2/3 led to reduced fibroblast proliferation, differentiation and activity which correlated with a reduction of cardiac fibrosis although it did not lead to altered hypertrophy [95]. Thus, this study demonstrated differential effects upon targeting different parts of TGF-β pathway and suggests that inhibition of Smad2/3 can inhibit fibrosis while TGFβR1/2 inhibition can affect fibrosis but also hypertrophy and other aspects of cardiac signaling.

TGF-β1 has also been shown to induce cardiomyocyte hypertrophy and post-MI remodeling through the activation of TGF-β 1/TAK-p38MAPK signaling within non-infarcted myocardium after acute MI [96]. Thus, inhibition of the TGF-β signaling cascade is an attractive target for the prevention of cardiac remodeling and cardiomyopathy post-MI. In this regard, Ellmers *et al* demonstrated using SD-208 (a TGF- β receptor kinase 1 inhibitor) that deleterious cardiac remodeling post-infarction could be inhibited [97]. MI was induced in mice via left coronary artery ligation and were treated with SD-208 for 30 days. While there was no difference recorded in ventricular TGFβ gene expression, there was increased TAK-1 (a downstream effector of TGFβ) in the control which was inhibited upon treatment with SD-208. The blockade of TGF-β signaling after MI resulted in reduced ventricular expression of TGF-β -activated kinase-1, decreased collagen 1 and decreased cardiac mass highlighting TGF-β inhibition as a potent method to reduce cardiac remodeling post-MI [97].

Additionally, as diabetic mortality is primarily due to cardiovascular complications recent studies have sought to investigate whether TGF-β inhibition can affect diabetic cardiomyopathy [98]. A study by Zhang *et al* demonstrated in Sprague-Dawley rats which were induced to become diabetic through the injection of streptozotocin that Matrine (an inhibitor of the TGF-β/Smad pathway) administration to rats could prevent diabetic cardiomyopathy as indicated through reduced fibrosis, recovery of LV function and heart compliance [99].

Together these reports demonstrate that inhibition of TGF-β signaling via pharmacologic modulation may reduce cardiac fibrosis, improve heart function, and decrease cardiomyopathy in a wide variety of preclinical models. The ultimate goal is to translate these findings to the clinic and improve patient prognosis; however, much work remains to be done to identify effective TGF-β inhibitors which can be translated for effective patient therapy. As such, we have identified potential TGF-β inhibitors for this purpose which are currently in active and interventional clinical trials for the treatment of cardiotoxicity or heart disease (including heart failure, cardiovascular disease, ischemic heart disease, coronary heart disease and arrhythmia) from the Clinicaltrials.gov database are summarized in Table 1. Identified potential TGF-β inhibitors seem to be safe for the usage in clinic and have been demonstrated to suppress the TGF-β signaling pathway in preclinical studies; however, further studies will be needed to determine clinical efficacy in combination with chemotherapy as well as the underlying mechanism.

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Table 1: Potential TGF-β inhibitors in Active Cardiotoxicity and Cardiac Disease Related Clinical Trials. The Clinicaltrials.gov database was used to assess active, interventional clinical trials for the treatment of heart disease and cardiotoxicity within phase 1, 2, 3, or 4 of development. Following inhibitor identification, literature was consulted to determine any hypoxia modulating effects. Clinical Trial Search link (accessed on 1 August 2021): https://clinicaltrials.gov/ct2/results?cond=Cardiotoxicity&term=&type=Intr&rslt=&recrs=d&age_v=&gndr=&intr=&titles=&outc=&spons=&lead=&id=&cntry=&state=&city=&dist=&locn=&phase=0&phase=1&phase=2&phase=3&rsub=&strd_s=&strd_e=&prcd_s=&prcd_e=&sfpd_s=&sfpd_e=&rfpd_s=&rfpd_e=&lupd_s=&lupd_e=&sort= ; https://clinicaltrials.gov/ct2/results?cond=Cardiac+Disease&term=&type=Intr&rslt=&recrs=d&age_v=&gndr=&intr=&titles=&outc=&spons=&lead=&id=&cntry=&state=&city=&dist=&locn=&phase=0&phase=1&phase=2&phase=3&rsub=&strd_s=&strd_e=&prcd_s=&prcd_e=&sfpd_s=&sfpd_e=&rfpd_s=&rfpd_e=&lupd_s=&lupd_e=&sort=

Inhibitor	Clinical Trial Number	Mechanism	References
Enalapril	NCT01968200	ACEI with antifibrotic activity via inhibition of TGFB1 and p-SMAD2/3 expression	[100,101]
Carvedilol	NCT02177175 NCT01347970	Suppression of myocardial fibrosis by inhibiting TGFB1 mRNA expression	[102,103]
Simvastatin	NCT02096588	Downregulates TGFb1 mediated phosphorylation of Smad 2/3 via activation of PP2A and PP2C/PPM1A phosphatases.	[104,105]
Rivaroxaban	NCT02303795 NCT01776424 NCT02066662	Downregulates mRNA expression of TGFB in the infarcted area following an MI potentially via suppression of PAR-1 and PAR-2 pathways.	[106]
Clopidogrel	NCT02044250 NCT02317198	Platelet blocker that inhibits the expression of TGFB mRNA and the protein levels preventing cardiac fibrosis	[107]
Rituximab	NCT03072199	Monoclonal antibody against CD20 inhibits fibrotic signaling of TGF-β1 and p-Smad2/3	[108]
LCZ696	NCT02816736	Angiotensin receptor–neprilysin inhibitor that improves cardiac function by downregulates cardiac fibrosis via suppression of	[109,110]

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	NCT03190304 NCT02468232 NCT02924727	TGF-β expression primarily through its specific inhibition of neprilysin	
Spironolactone	NCT03409627 NCT02673463	SP prevents cardiac fibrosis cause by inhibiting the production of TGFβ1 and phosphorylation of Smad2/3.	[111,112]
Macitentan	NCT03153111	Dual endothelin receptor antagonist (ETA and ETB) that suppresses expression of TGFβ, esp. in DM patients where TGFβ is upregulated.	[113,114]
Ivabradine	NCT04448899 NCT04308031	Hyperpolarization-activated pacemaker current (If) channel inhibitor ivabradine inhibits the expression of TGFb1 and Smad-2 post MI suppressing collagen synthesis and pro-fibrotic activity.	[115,116]
Empagliflozin	NCT03128528 NCT03030222 NCT03057977 NCT03057951 NCT03485092 NCT02998970	Inhibits the fibrotic activity of TGFb in the heart by suppressing the expression of TGFb1, p-Smad2/3 and upregulating TGFb inhibitor Smad7. Further resulting in decreased expression of collagen I and II mediated by TGFb/Smad pathway.	[117,118]
Pirfenidone	NCT02932566	Inhibits Ang II induced expression of TGFb1 and suppresses myocardial interstitial fibrosis.	[119,120]
Atorvastatin	NCT02679261	Suppresses cardiac fibrosis by attenuating TGFb1 mediated phosphorylation of Smad3, PI-3 kinase, Akt, collagen I and endoglin expression.	[121]
Eplerenone	NCT01857856	Inhibits the expression of TGFb1 and collagen I resulting in downregulation of cardiac remodeling induced by cardiomyopathy	[122]
Olmesartan	NCT04174456	Angiotensin II type 1 receptor blocker that reduces the expression of TGFb in pressure overloaded, diabetic, obese pts. preventing cardiovascular injury.	[123,124]

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Tadalafil	NCT03049540	cGMP mediated inhibition of TGFb1 expression	[125]
Berberine	NCT04434365	Antifibrotic activity by inhibition of TGFb1 secretion potentially by upregulation of AMPK phosphorylation and downregulation of mTOR and p70S6K phosphorylation.	[126]
Melatonin	NCT02099331	Antifibrotic by suppressing TGFb1expression.	[127]
N-Acetylcysteine (NAC)	NCT02750319 w/ Amiodarone NCT01878669 NCT01878344	Antioxidant that inhibits TGFb1 mediated signaling involved in fibrosis potentially by suppressing its interaction with TGB1R, downregulating phosphorylation of Smad2/3 and upregulating Smad7 mRNA.	[128,129]
Colchicine	NCT02594111 NCT01709981 NCT02624180 NCT04382443	Antifibrotic activity by inhibiting expression of TGFb1 mRNA.	[130]
Ticagrelor	NCT02539160 NCT03437044 NCT01944800	Antifibrotic by inhibiting the expression of TGFb	[131]
Valsartan	NCT01912534	Inhibition of Ang II type I (AT 1) receptor resulting in suppression of AT 1 mediated action of TGFb/Smad pathway.	[132]
Metformin	NCT03629340	Suppression of cardiac fibrosis by inhibiting TGFb1 production and phosphorylation of Smad-3.	[133]
Nitrite	NCT03015402 NCT02980068	Downregulation of cardiac remodeling by suppressing AT II and AT 1R inhibiting TGFb1.	[134]
Nebivolol	NCT02053246 NCT01648634	Attenuated profibrotic activity and prevents vascular remodeling by downregulating the expression of TGFb1and MMP-2/9.	[135]
Riociguat	NCT01065454	Guyanalate cyclase stimulant with antifibrotic activity by inhibiting TGFb1 mediated collagen synthesis	[136]

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8.0 TGF-β as a Therapeutic Target in TNBC

TGF-β signaling has been associated with disease progression and negative patient prognosis in a wide number of cancer models including breast, colon and small cell lung cancers [137-139]. To highlight the clinical importance of TGF-β dysregulation; using the cbioportal clinical database in our own analysis we assessed the impact of genomic TGF-β alterations (Alterations defined as TGF-β genomic mutations, structural variants and copy number variations; see methods for specific genes assessed) in relation with overall patient survival across 32 TCGA, PanCancer Atlas datasets which 10,610 patients [140,141]. 38% of patients were found to have an alteration in at least one TGF-β gene and patients with an alteration in TGF-β signaling demonstrated a dramatic reduction in progression free survival compared to patients without TGF-β signaling alteration (Figure 1A-B, TGF-β altered patients: 4047 cases and 47.60 median month progression free survival; TGF-β unaltered patients: 6563 cases and 75.48 median month progression free survival). Thus, our findings demonstrate the importance of TGF-β in patient outcomes across a broad spectrum of tumor types and datasets (Supplemental Table 1 for detailed list of studies/cancer studies used for analysis) and in over 10,000 patients. Notably, this analysis does not take into account treatment, age, disease sub-type and other critical factors influencing patient prognosis.

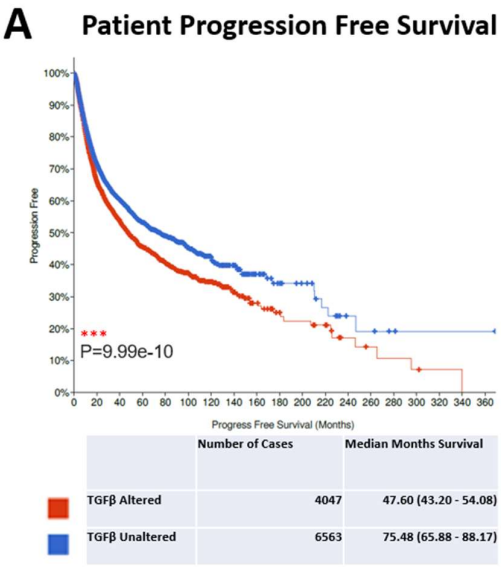

Figure 2: Database Analysis of Patients with TGF-β Altered/Unaltered gene expression and Survival. Kaplan–Meier curves for progression free survival of the patients with Alterations in TGF-β signaling in cancer samples (red curve) in comparison with patients with unaltered expression (blue curve). n = 10,610, ***P=9.99e-10, log-rank test.

While TGF-β alterations are significant in a wide variety of cancer models, it has been found in a study by Ding *et al* that 52.5% of TNBC patients were found to have elevated TGF-β expression which was associated with increased rates or metastasis, increased tumor grade and negative disease free survival[137]. Moreover, our own previous database analysis revealed similar findings using cbioportal to assess a cohort of 1082 breast cancer patients [142]. It was found that increased TGF-β signaling was correlated with diminished overall prognosis and median month survival (122.83 median month survival in patients with TGF-β high gene expression versus 140.28 median month survival in

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patients without increased TGF-β gene expression)[142]. Moreover, our assessment found that TNBC patients possessed increased levels of *TGFBRA* mRNA expression and reduced disease-free survival compared to other breast cancer subtypes highlighting the importance of TGF-β modulation for prospective treatment [142]. As dysregulated TGF-β signaling is associated with increased CSC enrichment, chemoresistance and decreased patient survival in TNBC it highlights TGF-β modulation as a potential therapeutic target [143-146].

It has been demonstrated that within breast cancer tumors, the cellular hierarchy is not uniform and a small population (known as cancer stem cells, CSCs) maintains self-renewal and differentiation capabilities regulating tumor composition and heterogeneity. Conversely to differentiated tumor cells, CSCs have demonstrated robust resistance to conventional chemotherapy and are thought to persist following therapy/intervention and are a major cause of relapse [147-149]. A wide number of breast cancer models currently support two distinct sub-populations of CSCs: a mesenchymal CSC population defined by $CD44^{+}/CD24^{-}$ markers and an epithelial CSC population with $ALDH^{high}$ markers[150]. Famously Al Hajj *et al* demonstrated through fractionation experiments of breast tumors that $CD44^{+}/CD24^{-}$ populations were capable of forming a tumor with as little as 100 cells in comparison with the tens of thousands of cells within the different populations required to achieve a similar tumorigenicity[150]. Further characterization experiments demonstrated that $CD44^{+}/CD24^{-}$ mesenchymal CSCs reside at the tumor edge, have diminished E-cadherin and increased vimentin, N-cadherin, YAP signaling and EMT-related migratory pathway enrichment [151-154]. Importantly, this population was found to be associated with increased migration away from the original tumor and markedly increased resistance and quiescence upon exposure to chemotherapy [155]. Conversely, the $ALDH^{high}$ epithelial CSC population is localized within the tumor core and is characterized by E-cadherin expression, low EMT-related signal enrichment, increased Wnt, HIF1α, glycolytic and proliferative pathway enrichment [151,154]. $ALDH^{high}$ CSCs also demonstrate increased tumorigenicity where as little as 1500 cells are required to form a tumor [156].

It has also been demonstrated that these CSC populations are able to interconvert making therapeutic approaches difficult as simply targeting one population would just lead to reconstitution by the surviving CSCs[154]. Unfortunately, due to the non-specific, toxic nature of conventionally used chemotherapeutic agents such as paclitaxel, doxorubicin, 5-FU or a plethora of other conventional chemotherapeutic agents; administration is associated with resistance and CSC enrichment over time which promotes increased tumorigenicity [137,157,158]. Overcoming this obstacle represents a currently unmet medical need and recent findings highlighting TGF-β as a mediator of CSC enrichment and resistance is providing valuable insight into how this process may be inhibited. It was found that even short term exposure of TNBC cells to epirubicin (a cytotoxic chemotherapy used for the treatment of TNBC) promoted robust TGF-β protein expression which in turn enriched the $CD44^{+}/CD24^{-}$ mesenchymal CSC population, increased apoptotic resistance and malignancy[159]. Likewise Asiedu *et al* demonstrated using mouse mammary carcinoma cells (an epithelial tumor cell line) that upon exposure to TGF-β /TNF-α promoted a mesenchymal phenotype, increased EMT signature as well as an enrichment of $CD44^{+}/CD24^{-}$ CSCs and mammosphere formation. To determine whether TGF-β /TNF-α could transform normal mammary human epithelial cells, MCF10a cells were exposed to TGF-β /TNF-α and a similar transformation was observed alongside increased migration and tumorigenicity. These transformed cells were then treated with oxaliplatin, paclitaxel and etoposide and its was found that mammary cells post-TGF-β /TNF-α exposure were found to be resistant to chemotherapy[160]. These studies may partially explain the findings of Zhang *et al* who described that amongst 180 TNBC patients, TGFβ1 expression was elevated within 37.2% and associated with a higher histologic tumor grade, lymph node

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status and reduced disease-free survival (hazard ratio 1.796, 95% CI 0.995-3.242, P = 0.052) [161]. Together these studies highlight TGF-β signaling as a potent mediator of chemotherapy-induced chemoresistance and tumorigenicity via CSC enrichment. Thus, the development of novel therapies to target TGF-β may provide a tangible approach towards patient treatment.

Interestingly, TGF-β signaling has been found to regulate the secretion of IL8 cytokines although the exact mechanism remains convoluted [145,162,163]. Jia *et al* found using TNBC cell lines *in vitro* that upon treatment with paclitaxel, doxorubicin or 5-FU, there was robust enrichment in $CD44^{+}/CD24^{-}$ CSCs, mammospheres and cytokine secretion such as IL6 and IL8 through enrichment of NF-κB and STAT3 signaling [164]. These effects were reproduced in a TNBC mouse xenograft model which demonstrated increased tumorigenicity following treatment via serial dilution analysis; however, through NF-κB/STAT3 inhibition in conjunction with chemotherapy, these effects and chemotherapy induced-cytokine mediated CSC enrichment was alleviated [164]. Interestingly, other reports have also demonstrated that paclitaxel induces TGF-β, IL6 and IL8 transcription in TNBC which in turn promotes increased CSCs and tumorigenicity. Further experiments demonstrated that through siRNA knockdown of SMAD4 or through small molecule inhibition of TGF-β, chemotherapy induced enrichment of IL8 and subsequent tumorigenicity could be inhibited[145,165]. This association was found to be maintained in breast cancer patients correlating the expression of IL8 and TGF-β with diminished patient prognosis making these findings of great clinical importance and highlighting the potential benefit of TGF-β inhibitors in combination with conventional chemotherapy[166]. Importantly, when compared to other breast cancer subtypes, TNBC has been found to express increased levels of proinflammatory chemokines (CXCL1,2,3 and 8) compared to the other breast cancer subtypes highlighting the potential sensitivity of TNBC towards anti-TGF-β/IL6/IL8 targeted therapy [167].

A recent study highlighting the potential clinical application of targeting TGF-β regulated cytokine secretion in TNBC demonstrated that comparison amongst TNBC breast cancer biopsies before and after chemotherapy revealed a marked increase in TGF-β signaling [145]. Moreover, TGF-β expression was associated with increased mammosphere formation and CSC markers ($CD44^{+}/CD24^{-}$ and $ALDH^{high}$) which were associated with increased tumorigenicity[145]. Mechanistic analysis in paclitaxel treated tumors revealed that subsequent TGF-β mediated CSC enrichment was through the upregulation and secretion of IL-8 and its binding to the CXCR1/2 receptors. Moreover, upon addition of a TGF-βR1 serine/threonine kinase small molecule inhibitor (LY2157299) in combination with paclitaxel inhibited IL8 expression which correlated with a reduction in both CSC populations following co-therapy. This was highlighted using the gold-standard for tumorigenicity- an *in vivo* serial dilution assay where compared to the vehicle it was found that Paclitaxel increased the rates of tumor formation while combinational treatment with LY2157299 not only prevent paclitaxel induced tumorigenicity but reduced tumor formation compared to the control. Together this work highlights the therapeutic implications of targeting TGF-β signaling in the context of anti-tumorigenic and long-term patient prognosis[145].

Downstream effector inhibition of TGF-β signaling has also demonstrated preclinical efficacy. As TGF-β has been classically associated in TNBC with metastasis and tumor invasion through facilitation of epithelial to mesenchymal transition (EMT)- a process which can be typically characterized via induction of *SNAI1/TWIST1/TWIST2/ZEB1* gene expression[168]. These factors in turn inhibit E-cadherin and its associated signaling; reduce adhesion and promote dissemination[169]. Park *et al* demonstrated using TNBC tumor xenograft *in vivo* models that paclitaxel treatment was found to increase TGF-β signaling and demonstrated increased *SNAI1* gene and protein expression following treatment (~4 fold

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increase). This correlated with a marked increase in $ALDH^{high}$ and $CD44^{+}/CD24^{-}$ CSCs following paclitaxel exposure as well as CSC associated genes (*OCT4*, *NANOG*, *KLF4*, *c-MYC* and *SOX2*); however, these effects were reversed upon combinational treatment with the TGF-β/ALK5 inhibitor EW-7917. siRNA knockdown of *SNAI1* also prevented paclitaxel-induced CSC enrichment supporting that *SNAI1* inhibition via TGF-β targeting may prevent paclitaxel mediated CSC enrichment in TNBC[170].

More recently, Wardhani *et al* using a TMEPAI KO TNBC cell model (TMEPAI- Transmembrane prostate androgen-induced protein which involved TGF-β signaling via Smad-dependent and independent mechanisms and has been found highly expressed in a wide number of cancer models, including breast cancer) found that upon TMEPAI KNO, there was a substantial sensitization towards doxorubicin and paclitaxel treatment reducing the IC50 from approximately 12.5nM in the control to approximately 4nM for doxorubicin and from ~30nM to ~12nM for paclitaxel treatments[171]. TMEPAI is a TGF-β target gene and is highly expressed in TNBC. Moreover, TMEPAI was found to be positively stimulated upon increased TGF-β signaling and sensitive to its inhibition[172]. Knockdown of TMEPAI in TNBC led to robust inhibition of *in vivo* tumor growth accompanied by reduced VEGF and HIF1α tumor promoters and enhanced levels of PTEN and p27 tumor suppressors [172]. Thus, TMEPAI is thought to affect a wide number of oncogenic pathways in TNBC and be directly mediated through TGF-β signaling.

Together these reports highlight the impact of TGF-β signaling in conventional chemotherapy resistance generation and CSC enrichment in TNBC. Moreover, these reports highlight TGF-β inhibition as a clinically translatable approach to reduce chemotherapeutic-induced CSC enrichment following therapy warranting further investigation. Such a combination may lead to the development of combinational strategies to improve short and long-term efficacy in TNBC patients. In this regard, Active and interventional clinical trials in Clinicaltrials.gov database for the treatment of patients with TNBC are summarized in Table 2. These potential TGF-β inhibitors seem to be safe for the usage in clinic and have been demonstrated to suppress the TGF-β signaling pathway in preclinical studies.

Table 1: Potential TGF-β inhibitors in Active TNBC Clinical Trials. The Clinicaltrials.gov database was used to assess active, interventional clinical trials for TNBC treatment within phase 1, 2, 3, or 4 of development. Following inhibitor identification, literature was consulted to determine any hypoxia modulating effects. Clinical Trial Search link (accessed on 1 August 2021): https://clinicaltrials.gov/ct2/results?cond=Triple+Negative+Breast+Cancer&term=&type=Intr&rslt=&recrs=d&age_v=&gndr=Female&intr=&titles=&outc=&spons=&lead=&id=&cntry=&state=&city=&dist=&locn=&phase=0&phase=1&phase=2&phase=3&rsub=&strd_s=&strd_e=&prcd_s=&prcd_e=&sfpd_s=&sfpd_e=&rfpd_s=&rfpd_e=&lupd_s=&lupd_e=&sort=

Inhibitor	Clinical Trial Number	Mechanism	References
Sorafenib	NCT02624700 - w/ Pemetrexed	-Suppression of TGFb1 mediated EMT via epigenetic modification of TGFb1 and Smad2/3 promoters through loss of active histone markers (H3K4me3 and/or H3K9ac). - Has also been shown to disrupt the phosphorylation of Smad2/3	[173,174]

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		-Suppression of TGFb signaling in hepatocellular carcinoma	
Halaven (eribulin mesylate)	NCT01372579 - w/ Carboplatin NCT02120469	Suppresses metastasis by inhibiting TGFb mediated phosphorylation of Smad2/3 (Potentially by altering the interactions between Smad proteins and microtubules following erlubin binding)	[175,176]
Pembrolizumab (MK-3475)	NCT02644369 NCT02730130 NCT02734290 NCT03036488 NCT02555657 NCT02819518 NCT02981303 - w/ Imprime PGG NCT03567720 NCT02657889 - w/ Niraparib NCT02971761- w/ Enobosarm NCT01676753 - w/ Dinaciclib NCT02178722	Decreased the production of TGFb in tumor microenvironment	[177,178]
Apatinib	NCT03075462 NCT03394287	Downregulates TGFb1 pathway	[179]

9.0 Conclusion and Future Directions

Heart disease is a leading cause of mortality amongst breast cancer patients due to the reliance on cardiotoxic, non-specific chemotherapies for treatment [6]. While chemotherapy is an essential part of therapy, the development of novel methods to modulate its cardiotoxic effects are critical. TGF-β has been demonstrated to be upregulated post-chemotherapeutic exposure in patients which is in turn associated with increased fibrosis, cardiac hypertrophy and inflammation impacting both short and long-term patient prognosis [86-89,180]. Moreover, it has been found that through inhibition of TGF-β these adverse effects can be limited, thus TGF-β inhibitors combined with chemotherapy may be a tangible approach to increase patient prognosis and reduce cardiovascular disease.

Additionally, TGF-β has been associated with post-chemotherapeutic enrichment of $CD44^{+}/CD24^{-}$ mesenchymal and $ALDH^{high}$ epithelial CSCs which are a major barrier against successful long-term patient survival through promotion of tumorigenicity, metastasis and resistance. TGF-β inhibition in preclinical models have demonstrated promising results in regards to inhibition of both CSC populations and prevention of chemotherapy induced CSC enrichment following combinational treatment. This is important as treatment of CSCs are essential for effective treatment of TNBC and prevention of chemotherapy-induced CSCs may reduce the rate of metastasis, relapse and increase patient

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prognosis. Therefore, the investigation into TGF-β inhibition as a treatment for TNBC CSCs remains of great importance and of great clinical translational value.

Together TGF-β inhibition represents an intersection in two fields: cardiology and oncology. On one side, cardiomyopathy, cardiac fibrosis, cardiac damage and heart failure may be prevented and on the other side, tumor efficacy may be enhanced and chemotherapeutically induced CSCs may be inhibited. Together both of these approaches, if successfully implemented would target the two greatest causes of cancer-related morbidity in patients and potentially lead to a breakthrough therapy.

10.0 Materials and Methods

Clinical Database Analysis

Pan-cancer datasets from the Cancer Genome Atlas PanCancer Atlas (TCGA, https://www.cell.com/pb-assets/consortium/pancanceratlas/pancani3/index.html) were used and analyzed with cBioportal (http://www.cbioportal.org/index.do). Altered TGF-β were defined as mutations, structural variants and/or copy number alterations in one of the following genes composing the TGFB superfamily: *TGFB1 TGFB2 TGFB3 TGFBR1 TGFBR2 TGFBR3 BMP2 BMP3 BMP4 BMP5 BMP6 BMP7 GDF2 BMP10 BMP15 BMPR1A BMPR1B BMPR2 ACVR1 ACVR1B ACVR1C ACVR2A ACVR2B ACVRL1 NODAL GDF1 GDF11 INHA INHBA INHBB INHBC INHBE SMAD2 SMAD3 SMAD1 SMAD5 SMAD4 SMAD9 SMAD6 SMAD7 SPTBN1 TGFBRAP1 and/or ZFYVE9*. Kaplan–Meier survival curves were generated using the datasets compiled by January 2022 from the following database IDs: https://bit.ly/2BngXkv

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: List Cancer Types Across 32 Studies Used for Database Analysis

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