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Review Article

The Role of TGFβ in Clinical Cancer Response

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ABSTRACT

The role of transforming growth factor beta (TGFβ) is context dependent. Even within the specific context of cancer, the functional significance of TGFβ varies according to environmental conditions. Therapies targeting TGFβ capitalize upon recent discoveries in canonical SMAD-dependent biochemical signaling networks as well as non-canonical SMAD-independent biochemical signal transduction pathways and mechanobiological signal transduction pathways. TGFβ clinical trials utilizing vaccine, small molecule inhibitor, antibody, and aptamer strategies support TGFβ as a viable anticancer target for a variety of cancer diagnoses. Combination therapies incorporating TGFβ inhibition and immune checkpoint blockade provide additional avenues for future TGFβ-based clinical intervention.

Introduction to TGFβ Signaling in Cancer

Transforming growth factor beta (TGFβ) plays multifaceted and paradoxical roles in the regulation of cell behavior. Depending on specific cellular contexts, the downstream effects of TGFβ signaling can vary dramatically. In normal adult tissue and in the early stages of tumor development, TGFβ signaling generally drives anti-proliferative, pro-apoptotic, or pro-differentiation pathways. TGFβ signaling also participates in the regulation of cell motility, immunity, and angiogenesis. The TGFβ family of proteins (TGFβ1, TGFβ2 and TGFβ3) each exert slightly different functions. In the later stages of tumor development, these activities become corrupted as TGFβ takes on oncogenic characteristics that support tumor cell growth, invasion, and metastasis. Anti-cancer therapeutics that target the TGFβ pathway attempt to abrogate these pro-tumor effects by decreasing TGFβ production, blocking TGFβ receptor interactions, or inhibiting downstream signaling proteins. Additional strategies such as those employed by Vigil, an autologous tumor vaccine, include the utilization of bi-shRNA plasmids to knock down proprotein convertase levels otherwise critical to the proteolytic processing and subsequent activation of TGFβ1 and TGFβ2.

TGFβ Signaling Pathways Relevant to Anti-Cancer Therapeutics

TGFβ is a cytokine released into the extracellular environment in order to facilitate intracellular signaling. Before being exported from the cell, TGFβ must first be translated in the cytoplasm, where it is susceptible to antisense oligonucleotide therapies such as AP 11014 [1]. Once in the extracellular matrix (ECM), TGFβ serves as a ligand for TGFβ Receptors (TGFβR). Ligand traps such as the GC1008 TGFβ antibody or soluble TGFβRs act as a sink to excess TGFβ, effectively decreasing endogenous receptor binding [2-4]. TGFβ I and II are transmembrane serine/threonine kinases directly responsible for transmitting signals into the intracellular environment; TGFβRIII is a cell-surface proteoglycan that indirectly modulates intracellular TGFβ activity by acting as a co-receptor to influence TGFβR I and II binding and subsequent signal propagation [5-7]. Once TGFβR serine/threonine kinases are activated, several signal transduction cascades are set in motion. These include biochemical signal transduction cascades involving receptor-regulated SMAD transcription factors, Ras-MAPK modulation, or TGFβ activated kinase 1 (TAK1), as well as mechanical signal transduction mechanisms mediated by latency-associated peptide (LAP) or latent TGFβ binding proteins (LTBPs) in the extracellular matrix [8].

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I Canonical SMAD-Dependent Biochemical Signal Transduction Pathways

Once TGFβ molecules bind and activate TGFβR-kinases, resultant phosphorylation events trigger assembly of SMAD protein complexes, which translocate into the nuclease where they act as transcription factors in the regulation of gene expression profiles that support tumor progression. SMAD-binding oligonucleotide aptamers such as Trx-SARA bind SMAD elements of this transcription factor complex to disrupt complex formation and subsequent transcription events [9]. Small molecules such as the TGFβR kinase inhibitor SB-431542 can prevent phosphorylation all together, inhibiting activation of canonical SMAD-dependent biochemical signal transduction pathways as well as non-canonical SMAD-independent biochemical signal transduction pathways [10].

II Non-Canonical SMAD-Independent Biochemical Signal Transduction Pathways

TGFβ signaling can propagate through a variety of intermediate proteins. Some of these proteins are downstream of TGFβR phosphorylation, such as Src homology domain 2-containing protein 1 (SHC1), which recruits growth factor receptor binding protein 2 (GRB2) and son of sevenless (SOS) proteins in order to activate Ras-Raf-MEK-ERK-MAPK pathways [11]. In fact, all three MAPKs (Erk, JNK, p38) are activated by TGFβ to some extent in mammalian cells with MAPK inhibitors representing an active area of research for TGFβ pathway suppression [12, 13]. Other non-canonical TGFβ pathways include TAK1 activation and downstream activation of NF-kB with corresponding increases in inflammatory cytokines [14]. Non-canonical TGFβ pathways have demonstrated relevance to cell apoptosis, epithelial-mesenchymal transition, migration, cell proliferation, cell differentiation, and extracellular matrix regulation [15]. As such, non-canonical TGFβ signaling networks offer additional targets for pharmacological intervention [16].

III Mechanobiological Signal Transduction Pathways

In addition to its biochemically mediated signaling networks, TGFβ cascades can proceed via mechanical transduction pathways in which extracellular matrix components inhibit inflammation and promote cancer cell proliferation by way of mechanical forces applied to adhesion receptors on the cell surface [8]. Integrins physically link cytoskeletal proteins to extracellular components of the tumor microenvironment with TGFβ increasing β1 integrin presentation on the surface of cancer cells [17]. TGFβ-mediated mechanical transduction impacts gene expression, influences DNA structural integrity, and maintains an extracellular environment conducive to cancer progression. Amplification of integrin-mediated TGFβ activation is observed in cancer and serves as a potential therapeutic target, with several therapies undergoing clinical development [18].

TGFβ Preclinical Development

Across a variety of cancers, TGFβ plays crucial roles in EMT, tumor metastasis, angiogenesis, and treatment resistance [19]. Due to the cell-type specific role of TGFβ as opposed to a cancer-specific role, understanding the pathways and modulatory factors of TGFβ are crucial to developing a broad-spectrum cancer treatment [20]. Analyzing TGFβ's role in cancer progression and the effects of a variety of methods of TGFβ inhibition through preclinical models provides an illuminating view into the future of cancer treatment.

Specifically in gastric cancer, TGFβ has been shown to activate both canonical SMAD and non-SMAD pathways to transcriptionally increase VEGF-C expression, thereby leading to lymphangiogenesis at the tumor site with increased lymph node involvement and a poor prognosis [21]. By inhibiting TGFβ signaling through a $TGF\beta_1$ receptor inhibitor, VEGF-C expression was down-regulated and lymphangiogenesis and cancer progression significantly decreased [21]. Similarly, in ovarian cancer cell lines, TGFβ was shown to induce EMT as well as hijack cancer cells in order to develop more aggressive phenotypes through the development of an ECM with increased collagen deposition that allows for a micro-environment suitable for increased cell adhesion and migration, thereby promoting tumor cell invasion [20]. When the TGFβ receptor kinase I inhibitor LY364947 was applied to ovarian cancer cell lines, cell proliferation was reduced in a dose-dependent manner, with an accompanying decrease in ATP production, oxygen consumption rate (OCR), and basal respiration, suggesting a reversal in TGFβ-mediated effects in these cell lines [20]. In a similar mouse model investigating the effects of the TGFβ receptor kinase I inhibitor LY2109761 in a prostate cancer (PCa) model, it was observed that LY2109761 inhibited SMAD activation in PCa cells, as well as ablated the effects of TGFβ on PCa cell proliferation LY2109761 [22]. Specific to this model, LY2109761 helped to stave off the unique debilitating factors of PCa by increasing osteoblast and osteoclast parameters, resulting in increased bone volume and significantly reduced bone loss [22].

At the center of recent developments in anti-cancer therapeutics are aromatic ketone chalcone-containing molecules that are precursors to flavonoids in plants and have been shown to have anti-bacterial, anti-fungal, anti-tumor, and anti-inflammatory properties [23-25]. Studies have shown that a variety of naturally-occurring chalcone compounds have inhibitory effects on $TGF\beta_1$-induced migration and invasion of tumor cells, leading to decreased tumor growth and metastasis [24, 25]. Specifically, the naturally occurring chalcone compound flavokawain A (FKA) significantly altered the molecular effects of TGFβ, including FKA-induced downregulation of MMP-9 and MMP-2, as well as upregulation of tissue inhibitor of MMP (TIMP-1) expression [25]. The matrix metalloproteinase (MMP) family of proteolytic enzymes is involved in the degradation of the ECM, while TIMP-1 inhibits ECM degradation. Therefore, the FKA-induced downregulation of MMP-9 and MMP-2 and upregulation of TIMP-1 is key in the anti-tumor effects of FKA [25].

Further, FKA was shown to inhibit the SMAD pathway by decreasing $TGF\beta_1$-mediated phosphorylation of SMAD molecules [25]. Importantly, finding a chalcone compound that is not cytotoxic yet still inhibitory of TGFβ signaling pathways has been an emerging goal of anti-cancer therapeutics. Screening a variety of novel chalcone-derivative chemical libraries provided a molecule, compound 67, with low cytotoxicity yet high inhibitory actions against $TGF\beta_1$-induced EMT and SMAD phosphorylation in a human lung cancer cell line [24]. In addition, compound 67 preserved the anti-MMP-9 and anti-MMP-2

effects of the naturally occurring chalcone compound FKA, thereby contributing to the decreased migration and invasion of human lung cancer cells [24]. With increasing research into several varieties of novel chalcone-derivative compounds, this avenue of anti-TGFβ therapeutics shows great promise in decreasing the metastasis and growth of a variety of cancers.

While TGFβ plays an important role in EMT and tumor metastasis, it can also play a crucial role in chemoresistance and decreased treatment response. In an epithelial ovarian cancer (EOC) model, inhibitory antibodies against a $TGF\beta_1$ molecule conjugated with the human IgG1 Fc domain and an IL-2 signal sequence (IL2ss) suppressed TGFβ-mediated EMT and decreased SMAD expression thereby providing evidence that the expression of EMT-related genes can be significantly reduced by targeting TGFβ. Importantly, when this $TGF\beta_1$ blockade was administered concomitantly with carboplatin in a mouse model of EOC, it was found that the blockade and carboplatin treatment significantly upregulated genes typically downregulated by TGFβ while also upregulating genes that are known inhibitory targets of the $TGF\beta_1$ signaling pathway, such as IL-6 and CXCR4, as compared to carboplatin treatment alone. This finding suggests that inhibiting the TGFβ pathway has the capability to not only inhibit EMT and tumor metastasis but also to increase therapeutic response to pre-existing treatment methods [26].

TGFβ Clinical Trials

I TGFβ Vaccines

Belagenpumatucel-L (Lucanix) is a nonviral gene-based allogeneic tumor cell vaccine that has been shown to enhance the recognition of tumor antigens following TGFβ-2 inhibition. A phase II trial was conducted using belagenpumatucel-L in patients with non-small cell lung cancer (NSCLC). This study demonstrated a survival advantage [27]. A phase III trial was subsequently conducted to evaluate the use of belagenpumatucel-L as maintenance therapy following chemotherapy in NSCLC patients. There were two arms of this trial and patients with Stage III/IV NSCLC were randomized 1:1 to either the placebo or belagenpumatucel-L treatment groups. There was no statistically significant difference in survival between the groups. However, patients who were randomized within 12 weeks of chemotherapy completion and patients who had received radiation therapy in the past demonstrated improved survival statistics [28].

Vigil (previously known as FANG) is a vaccine that targets furin convertase, resulting in decreased activation of TGFβ-1 and TGFβ-2 [29]. A phase I nonrandomized trial to evaluate the safety of the FANG vaccine in patients with advanced solid tumors without other standardized treatment options has been conducted. This study illustrated the safety of the FANG vaccine, and a phase II trial of Vigil suggested safety and evidence of efficacy in melanoma [30]. Vigil has also been tested for safety and efficacy in patients with advanced Ewing's Sarcoma and one study suggests that there is improved survival in patients who were treated with Vigil, enabling initiation of a registration trial with Vigil in second line Ewing's sarcoma[31, 32]. There was also a randomized Phase IIa trial in patients with ovarian cancer that demonstrated significant recurrence-free survival (RFS) advantage in Vigil treatments versus control [33]. A follow-up phase IIb trial involving 91 patients was recently complete and the results are currently under analysis [34].

II TGFβ Small Molecule Inhibitors

Galunisertib is a small-molecule inhibitor of TGFβ receptor I kinase (ALK5) that suppresses the phosphorylation of SMAD2, inhibiting activation of the canonical pathway [35, 36]. Galunisertib is also known as LY2157299 monohydrate. Many ALK5 inhibitors do not make it to clinical trial due to cardiotoxicity in animal models; however, galunisertib – when compared to other small molecule inhibitors – demonstrated reduced cardiotoxicity in animals [36-38]. At high continuous doses for 1 month or during continuous dosing schedule for 6 months of galunisertib, inflammatory lesion in heart valves occurred in rat and dog animal models [38]. Galunisertib overcame the cardiotoxicity issues of other ALK5 small molecule inhibitors by using an intermittent dosing regimen [36]: a dosing schedule alternating between 14 days on and off drug treatment for 3 months was well tolerated in rats allowing galunisertib to move to clinical trial [38]. In addition to the animal model studies, galunisertib demonstrated an ability to inhibit TGFβ receptor kinase in cancer models MX1 and 4T1 for breast cancer and Calu6 for NSCLC [36].

A phase I clinical study of LY2157299 monohydrate was conducted in 58 patients with glioma. Only patients with relapsed and progressive glioma were considered for this study. Clinical benefit such as partial response, complete response, or stable disease was observed in 12 patients. The study also reported safety of LY2157299 monohydrate and reported no cardiac adverse effects [39]. A phase I study was also conducted with galunisertib in 12 Japanese patients with advanced solid tumors: five patients had pancreatic cancer, three had lung cancer, and one each had lung cancer, colon cancer, esophageal cancer, anal cancer, or bladder cancer. Seven of these patients experienced adverse effects related to the treatment with the most common adverse effects being increased brain neuropeptide, leukopenia, and rash. There were no cardiovascular toxicities reported. Of the 12 patients enrolled, 10 had evaluable response data. The best response to treatment was stable disease in two patients with pancreatic cancer [40].

Galunisertib in combination with sorafenib, a multikinase inhibitor, in hepatocellular carcinoma lines and ex-vivo tumor lines demonstrated ability to increase apoptosis and inhibit proliferation [41]. A phase Ib study with galunisertib and sorafenib was conducted in Japan in 14 unresectable hepatocellular carcinoma patients. Adverse effects of the study included seven patients having abnormal QT intervals [42]. Because sorafenib has been associated with QT prolongation in the past, the abnormal cardiac findings was likely a side effect of treatment [43]. Eleven patients had a response of stable disease and one patient had a partial response [42]. A phase II clinical study was conducted using galunisertib in combination with sorafenib in a total of 47 HCC patients who had not received prior systemic therapy, from United States, Germany, France, Italy, and New Zealand. During the lead-in period, no dose-limiting or significant toxicities were seen in any of the patients. Adverse effects that occurred during the study of the drugs include one patient presenting with a grade four renal injury and seven patients developed anemia. Galunisertib dose reduction was required in two patients and dose reduction of sorafenib was required in 27 patients. Five

patients discontinued treatment due to toxicity. Although the median time to progression (TTP) of the combination therapy was no longer than monotherapy sorafenib treatment, the cohort's median overall survival of 18.8 months was longer than the normal outcome for this patient population. This suggests that TGFβ inhibition delays the resistance of sorafenib [44].

A phase Ib/II clinical study was conducted with randomized groups 2:1 to galunisertib and gemcitabine or a placebo and gemcitabine for the treatment of pancreatic cancer. Of the 156 patients enrolled in the study, 104 patients were put on a regime of galunisertib and gemcitabine, while 52 patients were put on a regime of a placebo and gemcitabine [45]. The regime of galunisertib and gemcitabine had a better median survival rate of 8.9 months compared to the placebo's median survival of 7.1 months. For the galunisertib and gemcitabine study group, it had a median overall response rate (complete response or partial response rate) of 10.6% compared to 3.8% for the placebo [46].

Galunisertib is still under active clinical investigation as a monotherapy and combination therapy in the treatment of cancer. At the time of writing, galunisertib in combination with nivolumab (anti PD-1) is in trial in a Phase Ib/II dose escalation study in 75 patients with advanced refractory tumors, non-small cell lung cancer, or hepatocellular carcinoma [47]. There is a current phase Ib trial of galunisertib in combination with paclitaxel/carboplatin for patients with carcinoma of the ovary or uterus. As of March 2020, it is still in the recruiting phase and is expected to enroll 25 total participants [48]. A phase II study of galunisertib and enzalutamide versus monotherapy of enzalutamide for patients with metastatic castration-resistant prostate cancer is in recruitment [49]. Other phase II studies involving galunisertib combinations are ongoing [50]. A phase II study comparing the results of galunisertib monotherapy, galunisertib and lomustine combination therapy, and lomustine plus placebo therapies for treatment of recurrent glioblastoma involving 180 participants revealed median overall survival for galunisertib monotherapy of 8 months, galunisertib and lomustine combination therapy 6.7 months, and lomustine plus placebo 7.5 months [51, 52].

AVID 200 is another TGFβ 1 and 3 selective inhibitor [53]. AVID200 has been shown to selectively suppress TGFβ 1 signaling associated with proliferation of mesenchymal stem cells in vivo [54]. It is currently in a Phase I trial for patients with solid tumor malignancies. As of March 2020, the trial is still in progress and recruiting patients to participate [55].

Vactosertib, also known as TEW7197, is an ALK5 inhibitor that decreases TGFβ 1 signaling. It has been shown to inhibit SMAD2 phosphorylation in a variety of hematopoietic cell lines. It also inhibits anti-tumor activity in various xenograft models such as B16/F1 melanoma, hepatocellular carcinoma and 4T1 breast cancer [56-58]. In vivo, vactosertib has also shown efficacy in TGF/alb transgenic mice [59]. A phase I trial with vactosertib was conducted in 35 patients with advanced stage solid tumors [60]. The study included six patients with colon/rectal, four patients with brain cancer, 2 patients with urothelial cancer, two patients with pancreatic cancer, and 15 patients with other type of cancer including parotid, lung, ovarian, breast, genital organ, smooth muscle, skin, and bone. This study determined once-a-day administration of vactosertib was rapidly absorbed with a median time to maximum concentration of 1.2 hours. It was, therefore determined that vactosertib treatment benefits from two to three times daily dosing schedules in order to maintain its concentration in the blood [61].

A phase Ib clinical trial in Korea for vactosertib in combination with FOLFOX is currently underway for patients with metastatic pancreatic ductal adenocarcinoma. Patients in this trial must have failed a first-line treatment of gemcitabine and nab-paclitaxel in order to become eligible to participate[62]. A 2-tiered phase II study with vactosertib is currently recruiting anemic patients with Philadelphia chromosome-negative myeloproliferative disorders [63]. A phase Ib study for vactosertib in combination with durvalumab for advanced non-small cell lung cancer is currently in progress to determine recommended phase II dosing parameters. After phase 1b testing is complete, a phase II study will investigate the anti-tumor effects and tolerability of vactosertib in combination with durvalumab [64].

III TGFβ Antibodies

Fresolimumab, or GC1008, is an anti-TGF monoclonal antibody. In total, 29 patients participated in phase I and II studies of fresolimumab for advanced malignant melanoma or renal cell carcinoma. In a phase I dose-escalation study containing 22 patients with renal cell carcinoma or melanoma, no dose limiting toxicity was observed. In phase II, a safety cohort expansion, seven additional patients were treated. Six of the patients had stable disease with a median-progressive free survival of 24 weeks. One patient with malignant melanoma had a partial response to therapy. From the phase I and II trial of fresolimumab for renal cell carcinoma or melanoma, four of the patients did develop reversible cutaneous keratoacanthomas/squamous-cell carcinomas [1]. Another phase I trial of fresolimumab was conducted with 12 patients: 10 patients with glioblastomas and one patient each with anaplastic oligodendroglioma or anaplastic astrocytoma. PET scans used to monitor treatment efficacy showed radiologic progression in all patients in the trial after one to three infusions of fresolimumab [65].

A phase II trial of fresolimumab combined with radiotherapy included 23 patients with metastatic breast cancer. The patients were randomly assigned to fresolimumab doses of 1 mg/kg or 10 mg/kg during day 1 of weeks 0, 3, 6, 9, and 12. The radiation therapy levels were kept the same for both groups. All eleven patients in the 1 mg/kg group were able to finish these treatments, while only four out of twelve patients in the 10 mg/kg group finished their course of treatment [66]. At 12-month follow-up, 20 out of the 23 participants were deceased; three patients presented with a stable disease state. Patients receiving 10 mg/kg demonstrated higher overall median survival and higher peripheral blood mononuclear cell counts compared to patients receiving 1 mg/kg [67].

There are many clinical trials of fresolimumab still underway for the treatment of other malignancies. A phase I/II trial for fresolimumab treatment combined with stereotactic ablative radiotherapy of early stage non-small cell lung cancer is ongoing, with plans to include approximately 60 participants [68]. Another phase I study of fresolimumab for the treatment of myelofibrosis enrolled three patients, but only two of these patients completed the trial due to drug procurement complications [69]. A phase II study of fresolimumab in 14

patients with relapsed malignant pleural mesothelioma also recently completed, but results have yet to be released [70].

M7824 is an anti-PD-L1/TGFβ trap fusion protein, with the anti-PDL1 moiety modeled from avelumab. Given that PD-L1 and TGFβ pathways have complementary and partially nonredundant immunosuppressive functions, it has been hypothesized that the combined inhibition of both PD-L1 and TGFβ pathways will lead to enhanced antitumor activity. Previously conducted murine studies have demonstrated that M7824 had increased antitumor activity compared to either anti-PDL1 or TGFβ trap monotherapy. A 2018 phase I study evaluated the safety and maximum tolerated dose in 19 patients with heavily pretreated advanced solid tumors. Four doses were tested, and there was evidence of efficacy with each dose, including one confirmed complete response in a patient with HPV-positive cervical cancer, as well as two durable confirmed partial responses – one in a patient with locally advanced mismatch repair-deficient pancreatic cancer, and another with HPV-positive metastatic anal cancer. The maximum tolerated dose was not reached, and no adverse events resulted in death [71]. Several dosages are currently undergoing investigation in the expansion cohorts [71, 72].

M7824 is also currently being tested for use in patients with stage II-III HER2+ breast cancer. Specifically, this ongoing phase I trial aims to evaluate the difference in tumor-infiltrating lymphocyte (TIL) percentages before and after M7824 therapy [73]. There is another ongoing phase II trial that aims to evaluate the efficacy of M7824 monotherapy in patients with advanced or metastatic biliary tract cancer who have failed treatment with first-line chemotherapy [74]. A phase Ib/II study to evaluate the safety and tolerability of M7824 and gemcitabine combination therapy against pancreatic cancer is currently suspended, awaiting further review by the FDA [75]. A phase II trial of M7824 in combination with either topotecan or temozolomide is currently recruiting patients with relapsed small cell lung cancer (SCLC) [76]. In addition, a phase Ib/II trial of M7824 in combination with chemotherapy is currently being conducted in patients with Stage IV NSCLC [77].

Ligand interactions with transforming growth factor beta receptor type I (TGFβRI) and type II (TGFβRII) complexes are involved in a multitude of cellular processes may play an essential role in tumorigenesis. LY3022859, also known as TR1 or IMC-TR1, is a human anti-TGFβRII immunoglobulin G, subclass 1 (IgG1) monoclonal antibody. The mechanism of this drug ultimately relies upon prevention of ligand-receptor complex formation thereby inhibiting receptor-mediated signaling activation. A phase I multicenter and nonrandomized dose-escalation study was performed in order to evaluate the safety and tolerability of LY3022859 in patients with advanced solid tumors. Due to infusion-related reactions despite prophylaxis, the study was unable to achieve its primary objective, as a safe dose without infusion-related reactions was unable to be determined [78, 79].

IV TGFβ Aptamers

Trabedersen, also known as AP 12009, is a synthetic antisense phosphorothioate oligodeoxynucleotide that targets the mRNA of the human TGFβ2 gene. It has undergone three phase I/II dose escalation studies and is aimed at treating patients with recurrent anaplastic astrocytoma or glioblastoma multiforme (GBM). During these studies, the maximum tolerated dose was not reached, and complete remission was seen with 10 and 80 uM doses of trabedersen. A multinational and randomized phase IIb trial was conducted afterward, aiming to evaluate the safety and efficacy of 10 and 80uM trabedersen compared to standard chemotherapy. At the primary endpoint of 6 months, there were no statistically significant differences between treatment groups. However, through the results of this study, the clinical benefits of trabedersen was shown to increase over time, and this effect was more obvious in the 10uM trabedersen group. As a result, Bogdahn *et al*. concluded that the benefits can only be accurately assessed at later time points. At the same time, they report that the optimal dose of trabedersen is 10 uM, as its safety and efficacy results were superior to that of the 80 uM dose. Although the mechanism is not fully understood, in vitro studies using human GBM cells have demonstrated that the 10 uM dose of trabedersen has an increased inhibitory effect upon the secretion of TGFβ2 compared to the 80 uM dose of trabedersen [80, 81].

A phase III trial was subsequently conducted to compare the 14-month progression and 24-months survival rates of patients who received 10 uM trabedersen compared to those who received standard chemotherapy [80]. However, the study was terminated as they were unable to recruit the projected number of patients [82]. In addition, a phase I dose-escalation study was conducted to evaluate the safety and tolerability of trabedersen in adult patients with pancreatic neoplasms, colorectal neoplasms, or melanoma [83].

TGFβ Signaling and Checkpoint Inhibition

Checkpoint inhibition has produced durable responses in a subset of patients. However, there remains a proportion that do not respond despite having numerous suggestive biomarkers. Calon *et al*., identify a stromal gene signature in colorectal cancer that is predictive of poor response [84]. Genes associated with this signature are involved in stromal cell TGFβ signaling and TGFβ inhibitors were able to stop disease progression. Analysis of non-responding patients treated with checkpoint inhibitors revealed members of the TGFβ pathway that were significantly upregulated [85]. In murine models of mammary and colon carcinoma, TGFβ inhibition and PD-L1 blockade resulted in tumor regression and increased CD8+ T cells in the tumor microenvironment [85]. Another study in colorectal cancer found that combined treatment with galunisertib and anti-PD-L1 antibody increased response rate and CD8+ T cell infiltration in the tumor [86]. These results suggest that TGFβ inhibition may have clinical efficacy. The combination with checkpoint inhibitors may increase durable response rates in previously nonresponsive patients and will need to be evaluated in clinical trials.

Conclusion

Extensive preclinical and clinical data support TGFβ as a viable target associated with anticancer activity. Vigil may be furthest along based on registration trial opportunity in Ewing's sarcoma. Moreover, pending the results of the recently completed Phase IIb trial, activity in ovarian cancer appears encouraging. Several other methods of controlling TGFβ expression are also underway and generally demonstrate safety and evidence of positive effect in several cancer types. The relationship of

TGFβ inhibition in cancers with high TGFβ expression needs to be studied more extensively.

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