The difficulty in translating the preclinical success of combined TGFβ and immune checkpoint inhibition to clinical trial

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Summary

Immune checkpoint inhibitors (ICIs) have transformed the treatment paradigm for solid tumors. However, even in cancers generally considered ICI-sensitive, responses can vary significantly. Thus, there is an ever-increasing interest in identifying novel means of improving therapeutic responses, both for cancers in which ICIs are indicated and those for which they have yet to show significant anti-tumor activity. To this end, Transforming Growth Factor β (TGFβ) signaling is emerging as an important barrier to the efficacy of ICIs. Accordingly, several preclinical studies now support the use of combined TGFβ and immune checkpoint blockade, with near-uniform positive results across a wide range of tumor types. However, as these approaches have started to emerge in clinical trials, the addition of TGFβ inhibitors has often failed to show a meaningful benefit beyond the current generation of ICIs alone. Here, we summarize landmark clinical studies exploring combined TGFβ and immune checkpoint blockade. These studies not only reinforce the difficulty in translating results from rodents to clinical trials in immune-oncology but also underscore the need to re-evaluate the design of trials exploring this approach, incorporating both mechanism-driven combination strategies and novel, predictive biomarkers to identify the patients most likely to derive clinical benefit.

Keywords: Immunotherapy; Tumor immunology; Immune checkpoint inhibitors; Transforming growth factor β; Drug resistance

Introduction

Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment in the last decade and are now the preferred first-line treatment for several solid tumors. ICI-based immunotherapy consists of neutralizing antibodies against negative immune checkpoints, with most targeting either programmed cell death protein 1 (PD-1) or PD-1 ligand 1 (PD-L1), thereby impeding the ability of tumor cells to evade the cytotoxic immune program.[1] In monotherapy, these medications are sufficient to produce objective clinical responses and achieve satisfactory disease control for many cancer types.[2] However, despite the rapid progress in immunotherapy in recent years, several cancers appear largely refractory to ICIs, particularly as monotherapy.[3] Thus, there is an ever-increasing interest in developing new, effective combination strategies to further enhance the efficacy of ICIs, particularly for cancers that have demonstrated poor therapeutic responses to ICIs in clinical trials.

To this end, Transforming Growth Factor β (TGFβ) is a potent and pleiotropic cytokine with complex, often contradictory roles in tumorigenesis.[4] While the effects of TGFβ on tumor cells are varied and context-specific,[4] the role for TGFβ signaling in immune evasion appears to be somewhat similar across a wide range of tumor types.[5] TGFβ is a central mediator of immune tolerance, and its immunosuppressive effects are well-documented.[6] Accordingly, TGFβ is emerging as an important and clinically actionable means of immune evasion within the tumor microenvironment (Fig. 1). Several preclinical studies have explored the combination of TGFβ and PD-1/PD-L1 inhibition as cancer therapy with uniformly positive results. However, as this approach has begun to emerge in clinical trials, progress has been difficult, with most trials failing to recapitulate the successes observed in animal models[7–34] (Table 1). Here, we summarize key clinical trials exploring concomitant TGFβ and PD-1/PD-L1 signal inhibition in solid tumors. Additionally, we discuss potential reasons for the relative lack of success in translating this approach from the bench to the bedside, as well as potential strategies to improve response rates in subsequent trials.

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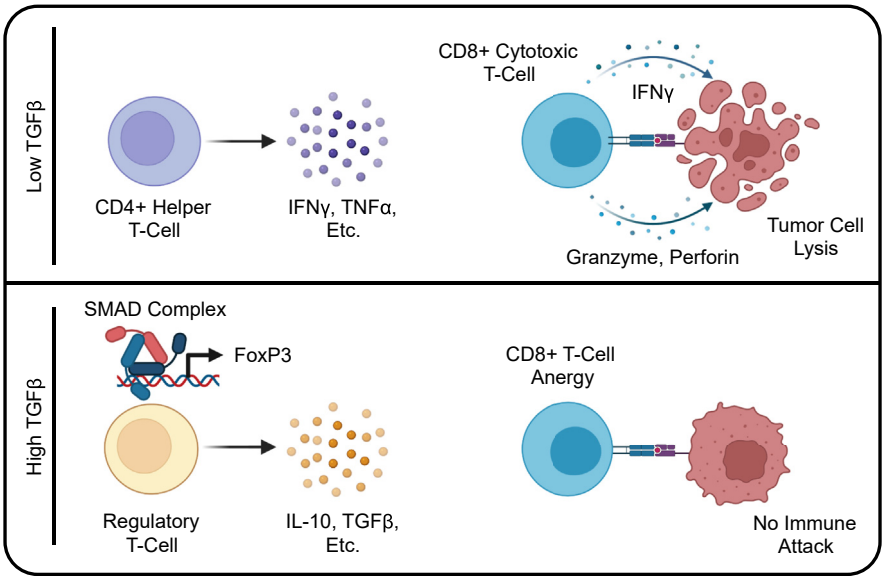

Fig. 1: **Abbreviated mechanisms of TGFβ-mediated immune suppression**. The immunosuppressive effects of TGFβ signaling are well documented, particularly with respect to T-lymphocytes. For instance, in the absence of TGFβ, CD4+ T-cells can mount functional Th1 or Th2 responses, producing a variety of effector cytokines to enhance local immune function. However, in response to TGFβ, these cells undergo SMAD-mediated upregulation of the transcription factor FoxP3, causing differentiation into suppressive regulatory T-cells (Tregs), which act to impede sterilizing immunity and facilitate immune escape. Similarly, TGFβ signals can act on CD8+ cytotoxic T-cells to directly suppress their effector function and promote anergy, thereby preventing an anti-tumor immune attack.

Clinical studies exploring combined TGFβ and immune checkpoint inhibition

Given the encouraging anti-tumor effects of combined TGFβ and immune checkpoint inhibition in preclinical testing, these approaches are beginning to emerge in clinical trials, many of which have shared early results (Table 2). Here, we summarize select major trials exploring combined TGFβ and immune checkpoint. Specifically, we prioritize those with early efficacy and safety data, with a particular emphasis on the treatment-related outcomes data used to determine FDA approval and/or guide clinical decision-making.

Colorectal cancer

The combined inhibition of TGFβ and PD-1/PD-L1 signaling has shown promising anti-tumor activity in preclinical murine models of colon cancer. Accordingly, several such approaches are under clinical investigation in colorectal cancer (CRC) patients. An open-label phase 2 study is evaluating the combination of Vactosertib and the anti-PD-1 antibody Pembrolizumab in patients with previously treated microsatellite stable (MSS) CRC (NCT03724851). Though this study is ongoing, preliminary data have been reported from 33 patients. At the interim analysis, the authors noted partial responses in 5/33 patients (15.2%), with 7/33 (21.2%) showing stable disease. This approach was generally well tolerated, with only three serious adverse effects reported in the form of pneumonitis (3%), nausea (3%), and vomiting (3%). Moderate side effects were more common, including an increase in serum amylase (21.2%), pruritus (21.2%), rash (21.2%), and increased serum lipase (18.2%).[35]

The bifunctional anti-PD-L1/TGFβ trap fusion protein Bintrafusp alfa is also under clinical investigation in CRC (NCT03436563). A recent phase 2 study of patients with microsatellite instability-high (MSI-H) metastatic solid tumors who had progressed on prior ICI has posted early results from 15 patients, 12 of whom (80%) had CRC. Of the 14 evaluable patients, three (21.4%) had stable disease, with only one deriving long-term clinical benefit. The remaining 11 (78.6%) patients had progressive disease. Safety data were available from all 15 patients, with one showing grade 3 adrenal insufficiency and one dying from hepatic failure.[36] Interestingly, a recent study also explored Bintrafusp alfa in metastatic CRC patients with MSS, liver-limited disease, and detected circulating tumor DNA (ctDNA) following complete resection and standard-of-care therapy. Compared to a historical cohort, patients treated with Bintrafusp alfa had increased new metastases and greater tumor volumes. It is important to note that only four of 15 planned patients received Bintrafusp alfa, as this study was terminated given the above results.[37] Hence, caution may be required when advancing Bintrafusp alfa and similar targets without a guiding

Anti-TGFβ and ICI Medications	Additional therapy	Model system(s)	Results	Notes	References
Colon cancer					
Galunisertib + anti-PD-1	–	Genetically reconstituted colon cancer metastasis	Improved anti-tumor cytotoxicity, extended survival, reduced liver metastases		7
	–	CT26 MC38	Improved tumor growth inhibition, increased rate of complete regressions		8
LY734947 + anti-PD-L1	–	MC38	Enhanced CTL infiltration and improved survival		9
anti-TGFβ + anti-PD-L1	–	MC38	Enhanced CTL infiltration, improved survival, increased rate of complete regressions		10
SAR439459 + anti-PD-1	–	MC38	Reduced tumor growth, extended survival, and enhanced T-Cell responses		11
Bintrafusp alfa	–	MC38	Reduced tumor burden and metastasis, improved survival		12,13
YM101	–	CT26	Increased tumor infiltrating lymphocytes and dendritic cells, and enhanced cytokine production		14
anti-GARP:TGF-β1 + anti-PD-1	–	CT26	Increased cytotoxic immune responses and improved survival		15
Bintrafusp alfa	Oxaliplatin 5-Fluorouracil Radiation Therapy	MC38	Increased CTL activation, reduced t umor burden	Synergized with chemotherapy or radiation	13
Bintrafusp alfa	NHS-muIL12	MC38	Reduced tumor burden, improved survival	Protected from tumor rechallenge	16
Pancreatic cancer					
Galunisertib + anti-PD-1	–	KPC	Significantly improved survival and increased T-cell infiltration	Highly variable responses	17
LY364947 + anti-PD-L1	–	KPC1	Addition of anti-PD-L1 did not improve results beyond LY364947 alone		9
Galunisertib + siPD-L1	–	Panc02	Enhanced anti-tumor immunity and restrained tumor growth	Nanoparticle delivery	18
Galunisertib + anti-PD-1	Gemcitabine	KPC	Increased CTL infiltration/activation, reduced tumor burden, improved survival	More effective than Galunisertib + anti-PD-1 alone	19
LY364947 + anti-PD-L1	Gemcitabine	KP16	Improved survival		20
Bintrafusp alfa	Localized Radiotherapy	Orthotopic KPC	Reduced tumor growth		21
Lung cancer					
Bintrafusp alfa	–	HCC4006	Transient responses observed	Reverted TGFβ-Induced EMT	22,23
	Radiation Therapy	Lewis Lung Carcinoma	Reduced tumor burden and improved survival		21
anti-TGFβ antibodies + anti-PD-1	HPV16 $E7_{43-77}$ Vaccine GM-CSF α-Galactosylceramide	TC1	The combination of anti- TGFβ and anti-PD-1 further enhanced the efficacy of the therapeutic tumor vaccine		24
YM101	–	Lung Cancer Cell Lines and 3LL Lewis Lung Carcinoma murine model	Increased complete responses and promoted immune-mediated tumor regression	Reduced TGFβ-Induced EMT *in vitro*	14
anti-TGFβ + anti-PD-L1	–	Lewis Lung Carcinoma	Increased rates of complete responses		14
Breast cancer					
anti-TGFβ + anti-PD-L1	–	EMT-6	Increased rates of complete responses, enhanced CTL-mediated tumor regression		10
Bintrafusp alfa	–	EMT-6	Enhanced activation of Innate and adaptive immune systems		13
	Radiation Therapy	4T1	Reduced tumor burden, eradicated lung metastases, improved survival	Cooperation with radiation	21
	NHS-muIL12	EMT-6 4T1	Restrained tumor growth, improved survival	Protected from tumor rechallenge	16
YM101	–	EMT-6	Reduced tumor burden, restored anti-tumor immune responses	Increased M1 Polarization	14
SRK-181-mIgG + anti-PD-L1	–	EMT-6	Restored anti-tumor immune responses, improved Survival		25

(Table 1 continues on next page)

Anti-TGFβ and ICI Medications	Additional therapy	Model system(s)	Results	Notes	References
(Continued from previous page)					
anti-CTLA-4-TGFBR2	–	Partially humanized mice w/HLA-matched MDA-MB231 xenografts	Reduced tumor growth, restored anti-tumor immune responses		26
anti-PD-L1-TGFBR2	–		Improved efficacy compared to inhibition of either TGFβ or PD-L1	Reduced Tregs, increased IFNγ production	26
Melanoma and non-melanoma skin cancers					
SRK-181-mIgG + anti-PD-1	–	S91	Reduced tumor burden, improved survival		25
anti-PD-L1-TGFBR2	–	Partially humanized mice w/HLA-matched melanoma xenografts	Restrained tumor growth	Superior to anti-PD-1 monotherapy	26
TGFβ depletion + anti-PD-1	LSD1	B16	Restored cytotoxic responses, eradicated tumors	Protected from tumor rechallenge	27
Vactosertib + anti-PD-1	–	$Braf^{V600E}Pten^{-/-}$ model	Failed to improve responses beyond anti-PD-1 monotherapy	Improved responses when administered at escape from anti-PD-1	28
anti-TGFβ + anti-PD-1	–	Syngeneic tumor models derived from SCC cell lines	Increased rates of complete responses		29
LY2109761 + anti-PD-L1	–	A223	Improved efficacy in combination	Protected from tumor rechallenge	30
Bintrafusp alfa	–	A223	Enhanced CD8-mediated tumor regression		30
LY2157299 + anti-CTLA-4	–	$Braf^{V600E} \times Pten^{-/-}$ *Melanoma*	Reduced primary tumor growth, inhibited distant metastases		31
anti-CTLA-4-TGFBR2	–	Partially Humanized Mice Bearing HLA-matched Melanoma Xenografts	Improved efficacy compared to CTLA-4 monotherapy		26
Vactosertib + anti-CTLA-4	–	$Braf^{V600E} \times Pten^{-/-}$ *Melanoma*	Improved efficacy compared to CTLA-4 monotherapy		28
Urothelial cancer					
SRK-181-mIgG + anti-PD-1	–	MBT-2	Reduced tumor burden	Protected from tumor rechallenge	25
Bintrafusp alfa	–	Human cell lines	Facilitated immune-mediated lysis		32
Prostate cancer					
CAR-T anti-TGFβ and anti-PD-1 Trap Cells	–	Modified PC3 cell-derived tumors	Increased CTL infiltration, reduced tumor burden	Protected from tumor rechallenge	33
Glioblastoma multiforme					
Bintrafusp alfa	Localized Radiotherapy	GL261	Improved survival		21
Multiple myeloma					
anti-TGFβ or Galunisertib + anti-PD-1	–	Patient-Derived Myeloma Cells and Bone Marrow-MNCs	Increased CD8+ T-cell proliferation/activation		34

Abbreviations: Transforming Growth Factor β (TGFβ); Immune checkpoint inhibitor (ICI); Programmed cell death protein 1 (PD-1); PD-1 ligand 1 (PD-L1); Cytotoxic T-Lymphocyte (CTL); Glycoprotein A repetitions predominant (GARP); Epithelial-to-mesenchymal transition (EMT); Human leukocyte antigen (HLA); Interferon gamma (IFNγ); Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4); Type 2 TGFβ Receptor (TGFβR2); Lysine-specific demethylase 1 (LSD1); Squamous cell carcinoma (SCC); Chimeric antigen receptor-modified T cells (CAR-T); Mononuclear cells (MNCs).

Table 1: **Abbreviated results for preclinical studies exploring combined TGFβ and immune checkpoint inhibition.**

biomarker, in part due to the innate tumor suppressive effects of TGFβ signaling in colon cancer.[38]

Pancreatic cancer

TGFβ is an established driver of immune evasion in PDAC,[39,40] and TGFβ inhibition has been shown to augment therapeutic responses to ICIs in preclinical models of disease.[17,19] Accordingly, combined TGFβ and PD-1/PD-L1 inhibition is now emerging in clinical trials for PDAC. A phase 1b study (NCT02734160) recently explored the combination of Galunisertib and the anti-PD-L1 antibody Durvalumab in 32 patients with metastatic PDAC that had progressed on ≤2 prior systemic regimens. In this group, one patient (3.1%) demonstrated a partial response, and seven (21.9%) had stable disease for a disease control rate of 25%. This corresponded to a median overall survival of 5.72 months and median progression-free survival of 1.87 months. This approach was generally well tolerated, with five patients (15.6%) experiencing a grade 3/4 treatment-related adverse events, including elevated serum AST/ALT,

Anti-TGFβ and ICI Medications	Additional therapy	NCT identifier	Phase	Sample size	Response rate	Median OS (%)	Median OS (Months)	Notes
Colorectal cancer								
Vactosertib + Pembrolizumab	–	NCT03724851	2	33	15.2%	NR	NR	Pre-treated, ICI-naïve, MSS, metastatic CRC
NIS793 + Spartalizumab	–	NCT02947165	1b	40	5%	NR	NR	Advanced, Pre-treated, MSS CRC
Bintrafusp alfa	–	NCT03436563	2	15	0%	NR	9.1	MSI-H CRC
		–	1	4	0%	NR	NR	Liver-limited, ctDNA-positive, MSS mCRC
Pancreatic cancer								
Galunisertib + Durvalumab	–	NCT02734160	1b	32	3.125%	NR	5.72	Heavily pre-treated, Recurrent/refractory, metastatic PDAC
Bintrafusp alfa	–	NCT02517398	1	5	20%	NR	NR	Heavily pre-treated
Biliary tract cancers								
Bintrafusp alfa	–	NCT02699515	1	30	20%	NR	12.7	Pre-treated
Gastroesophageal cancers								
Bintrafusp alfa	–	NCT02699515	1	31	22.6%	NR	NR	Recurrent, locally advanced/metastatic gastric/gastroesophageal junction cancer
Bintrafusp alfa	–	NCT02517398	1	30	20%	NR	NR	Platinum-refractory esophageal adenocarcinoma
	–	NCT02699515	1	30	10%	NR	11.9	Heavily pre-treated esophageal SCC
Primary lung cancers								
Galunisertib + Nivolumab	–	NCT02423343	2	25	24%	NR	11.99	Recurrent/refractory NSCLC
Bintrafusp alfa	–	NCT02517398	1	40	27.5%	18-month: 49.7% 24-month: 39.7%	NR	Advanced, platinum-treated NSCLC
Cervical cancer								
Bintrafusp alfa	–	NCT02517398 NCT03427411	1 2	39	30.77%	NR	13.4	Pre-treated, recurrent/metastatic cervical cancer
Head and neck cancer								
Bintrafusp alfa	–	NCT02517398	1	32	12.5%	18-month: 44.0% 24-month: 36.0% 36-month: 24.0%	9.1	Heavily pre-treated, advanced HNSCC
	–	NCT04247282	1	14	[a]86%	NR	NR	Previously untreated, surgically resectable HPV-unrelated HNSCC
HPV-associated cancers								
Bintrafusp alfa	–	NCT02517398 NCT03427411	1 2	39	32%	12-month: 59.7% 18-month: 51.5%	NR	Advanced, pre-treated HPV-associated cancers
	NHS-IL12 PDS0101	NCT04287868	2	14	71%	NR	NR	HPV-positive, relapsed or refractory advanced cancers
Neurologic malignancies								
Bintrafusp alfa	–	NCT02517398	1	35	5.71%	–	5.3	Recurrent GBM following radiation and Temozolomide treatment
Multi-cancer studies								
NIS793 + Spartalizumab	–	NCT02947165	1b	120	3.33%	NR	NR	Advanced or metastatic solid tumors

Abbreviations: Transforming Growth Factor β (TGFβ); Immune checkpoint inhibitor (ICI); Overall survival (OS); Not reported (NR); Microsatellite stable (MSS); Colorectal cancer (CRC); Metastatic CRC (mCRC); Microsatellite instability-high (MSI-H); circulating tumor DNA (ctDNA); Pancreatic ductal adenocarcinoma (PDAC); Squamous cell carcinoma (SCC); Non-small cell lung cancer (NSCLC); Head and neck squamous cell carcinoma (HNSCC); Human papillomavirus (HPV); Glioblastoma multiforme (GBM). [a]Denotes pathologic response rate.

Table 2: **Select clinical trials exploring combined TGFβ and immune checkpoint inhibition that have shared preliminary results.**

neutropenia, anemia, and lymphopenia.[41] Though efficacy was limited in this heavily pre-treated population, given the lack of available subsequent-line therapies for PDAC patients, this approach warrants continued investigation, particularly as an earlier line of therapy and in combination with other treatments.

Data regarding Bintrafusp alfa is limited, though a recent phase 1 trial of 19 heavily pre-treated cancer patients included five with PDAC (NCT02517398). Only one patient with MSI-H, locally advanced PDAC demonstrated a partial response that persisted until disease progression after 10.5 months.[42] Bintrafusp alfa has also been evaluated in combination with Gemcitabine. A phase 1b/2 trial included a small number of patients with heavily pre-treated PDAC. Unfortunately, all patients in this study experienced grade 3/4 adverse events, 66% in the form of anemia, 33% thrombocytopenia, and 16% developing upper GI hemorrhage, pleural effusion, or thromboembolism. This study was terminated after one patient died due to treatment-induced hepatitis (NCT03451773). Several other trials are ongoing but have yet to share results.

Biliary tract cancers

Despite a lack of preclinical data, several studies are now investigating TGFβ and PD-1/PD-L1 inhibition in biliary tract cancer, with most using Bintrafusp alfa. Phase 1 data has been encouraging, including a recent study evaluating Bintrafusp alfa in 30 patients with pre-treated biliary tract cancer (NCT02699515). Here, the objective response rate was 20% (6/30), with median progression-free and overall survival of 2.5 and 12.7 months, respectively. Interestingly, clinical activity was observed independent of either PD-L1 expression or MSI-H status. Safety data were consistent with previous reports, with 11/30 (36.7%) patients experiencing grade 3 or worse adverse events, including three patients with treatment-related death attributed to septic shock or interstitial lung disease.[43]

However, in a phase 2 study of 159 patients (NCT03833661), the objective response rate was 10.5%. Although there was single-agent activity in some patients, this trial did not meet the pre-specified threshold for regulatory filing as a second-line treatment of patients with biliary tract cancer, marking the first major failure for Bintrafusp alfa in large-scale testing. Another flagship phase 2 trial also compared Bintrafusp alfa in combination with Gemcitabine and Cisplatin as a first-line treatment for locally advanced or metastatic biliary tract cancer (NCT04066491). Though data has not been made available to the public, per a recent press release, Bintrafusp alfa failed to meaningfully improve outcomes compared to the control arm of Gemcitabine and Cisplatin alone. Thus, this study was also discontinued, given that it was unlikely to meet its primary trial objective of improving overall survival. This marked another major failure for Bintrafusp alfa in larger-scale testing, with some calling this trial the drug's "third strike" following another high-profile failure in NSCLC (discussed in a subsequent section). Despite this, several similar studies are ongoing.

Gastroesophageal cancers

Though not evaluated in preclinical studies, several ongoing clinical trials have also evaluated the concomitant inhibition of TGFβ and PD-1/PD-L1 in gastric and esophageal cancers. To date, only two have shared interim results. The first is a multi-cancer phase 1 trial (NCT02699515) evaluating Bintrafusp alfa monotherapy, which included 31 patients with recurrent, locally advanced, or metastatic gastric/gastroesophageal junction cancer for whom standard of care therapies have either failed or do not exist. At the time of reporting, 7/31 (22.6%) patients had objective responses, 2 (6.5%) of which were complete. Bintrafusp alfa was generally well tolerated, with 6/31 (19.4%) patients experiencing grade 3 adverse events, though one treatment-related death was attributed to the rupture of a pre-existing thoracic aortic aneurysm.[44]

Also nested within a phase 1 pan-cancer trial (NCT02517398), Bintrafusp alfa is under evaluation in esophageal cancer patients. In this study, 30 esophageal adenocarcinoma patients were treated with Bintrafusp alfa following failure on platinum-based chemotherapy. This study reported a response rate of 20% (6/30 patients), with a median duration of 1.3–8.3 months. Importantly, 83.3% of responses were observed in tumors exhibiting an immune-excluded phenotype. This study reported safety data consistent with other trials, with 19/30 (63.3%) patients experiencing treatment-related adverse events and 7/30 (23.3%) experiencing grade 3 toxicities. No grade 4 events or treatment-related deaths were observed.[45] A recent phase 1 study of 30 heavily pre-treated patients with esophageal SCC has also reported initial results (NCT02699515). The objective response rate in this study was 10% (3/30), with a duration of 2.8–8.3 months. Similar to the previous study, all responses were observed in patients with immune-excluded tumors, with nearly identical safety data.[46] Additional studies are ongoing, many also evaluating Bintrafusp alfa.

Lung cancer

Following promising data in preclinical studies, the combination of TGFβ and PD-1/PD-L1 blockade is now under investigation in lung cancer. Several early-stage trials have shown promise, including a recent phase 2 study that explored the combination of Galunisertib with Nivolumab in 25 patients with recurrent/refractory NSCLC (NCT02423343, also referenced in the HCC section). This study reported an objective response rate of 24% (6/25 patients), with a median time to response of

4.2 months and a median duration of 9.0 months. Median progression-free survival and overall survival were 5.3 months and 12.0 months, respectively (https://clinicaltrials.gov). Similarly, a phase 1 pan-cancer study also evaluating Bintrafusp alfa monotherapy has recently shared data from a group of 40 patients with advanced, platinum-treated NSCLC (NCT02517398). Eleven patients (27.5%) demonstrated an objective response, with a median duration of 18 months. After two years, the authors reported progression-free and overall survival rates of 11% and 39.7%, respectively. Of the 7 patients with high PD-L1 expression, 6 (85.7%) were alive at the study endpoint.[47,48] Similar trials are ongoing in NSCLC patients, though these have yet to share results.

However, these studies are all predominantly in very early-stage testing. Evaluation for Bintrafusp alfa has been more extensive in NSCLC, both as a monotherapy and in combination with other treatments. Though an ongoing phase 2/3 study is investigating neoadjuvant Bintrafusp alfa in resectable, untreated NSCLC patients (NCT04560686), this approach has recently experienced a major setback in a recent phase 3 study that has marked one of the most significant failures for Bintrafusp alfa thus far (NCT03631706). In this study, Bintrafusp alfa was directly compared with Pembrolizumab in patients with advanced, PD-L1-expressing NSCLC. Bintrafusp alfa failed to show any improvement over Pembrolizumab, though it is not known whether Bintrafusp alfa was equal to or inferior to Pembrolizumab. This study was discontinued following a preliminary assessment of patient data indicating that the co-primary endpoint of progression-free survival was unlikely to be achieved (https://www.emdgroup.com). Though much of this data has not been made public, this study marks yet another major failure for Bintrafusp alfa. It raises important questions regarding both the usefulness of combined TGFβ and PD-1/PD-L1 inhibition in lung cancer or whether Bintrafusp alfa is a truly effective means of neutralizing either target in the clinic. As with biliary cancer, despite this major setback, several trials are nevertheless still exploring Bintrafusp alfa in NSCLC.

Cervical, head & neck, and other HPV-associated cancers

Despite a lack of preclinical studies, several clinical trials are also exploring combined TGFβ and PD-1/PD-L1 inhibition in human papillomavirus (HPV)-associated cancers, particularly cervical cancer and HNSCC. Though most are early stages, some are beginning to show promise. For example, early data are now available from 39 patients with pre-treated, recurrent/metastatic cervical cancer receiving Bintrafusp alfa. This cohort was a combination of patients from a phase 1 (NCT02517398) and phase 2 study (NCT03427411), all treated with single-agent Bintrafusp alfa. In the combined cohort, the authors observed two complete (5.1%) and nine partial responses (23.1%), with a median duration of 11.7 months. One additional delayed partial response was also observed, with a duration of 23.7 months. Interestingly, responses were independent of either tumor histology or prior treatment with Bevacizumab or radiation. The median overall survival in this study was 13.4 months, with a two-year overall survival rate of 33.2%. Safety data were similar to previous studies, with 8/39 (20.5%) patients experiencing a grade 3 treatment-related adverse event and one patient (2.6%) experiencing grade 4 toxicity in the form of asymptomatic hypokalemia secondary to grade 3 gastroparesis.[49]

As with lung cancer and melanoma, ICIs have rapidly advanced in the treatment of head and neck squamous cell carcinoma (HNSCC).[50] Accordingly, there are several ongoing trials exploring TGFβ and PD-1/PD-L1 inhibition in HNSCC patients, most often using Bintrafusp alfa. Recently, long-term survival data has been posted from the expansion cohort of a phase 1 study evaluating Bintrafusp alfa in patients with advanced, heavily pre-treated HNSCC (NCT02517398). Of the 32 patients enrolled, 4 (12.5%) had objective responses with a median duration of 21.4 months. This corresponded to a three-year overall survival rate of 24.0%.[51] No new grade 3 or worse toxicities were reported, with earlier data from this cohort reporting 11 (34.4%) patients had experienced a grade 3 adverse event.[52] Interestingly, responses were observed largely independent of PD-L1 status, but more commonly in HPV-positive tumors.[52]

Similar studies are exploring the efficacy of combined TGFβ and PD-1/PD-L1 inhibition in patients with any HPV-associated cancers, irrespective of the tumor site. While several are ongoing, others have shared results, with many showing promise. Data from a combination of patients from a phase 1 (NCT02517398) and phase 2 study (NCT03427411) have now been shared. These trials explored Bintrafusp alfa in patients with any advanced, pre-treated, HPV-associated cancers. Though early results were shared in 2020,[49] long-term survival data has now been posted. The combined cohort included 39 cervical cancer patents, 19 HNSCC patients, 9 anal cancer patients, and 8 patients with non-specified HPV-associated cancers. The combined objective response rate was 28% (21/75 patients), with a median duration of 17.3 months. An additional three patients had a delayed response, increasing the overall response rate to 32%. This was associated with encouraging 12- and 18-month overall survival rates of 59.7% and 51.5%, respectively. Bintrafusp alfa was well tolerated, with five patients (6.7%) developing grade 3 anemia.[53]

Interestingly, a recent study explored the peripheral immunome of 65 patients across these two trials, both before and 14 days following treatment with Bintrafusp alfa.[54] The authors identified several factors that were associated with therapeutic responses. Specifically, they found that higher pre-treatment sCD27:sCD40L

expression ratios and lower levels of TGFβ1 were favorable biomarkers. Similarly, lower levels of soluble factors associated with tumor mesenchymalization also correlated with improved responses, as did a higher CD8+ T-cell:MDSC ratio. Two weeks after initiating treatment, patients who would eventually develop clinical responses had fewer increases in both IL-8 levels and the neutrophil to lymphocyte ratio, as well as increased levels of HPV-16 specific CD8+ T-cells.[54] Hence, these biomarkers warrant continued exploration in other cohorts.

Bintrafusp alfa is also under phase 2 investigation in patients with advanced, HPV-associated cancers in combination with NHS-IL12 and PDS0101, a micellar multi-peptide-based therapeutic vaccine targeting HPV16 E6/E7 (NCT04287868). At the time of the initial report, 10/14 patients (71.4%) achieved objective responses, five of which were ICI-naïve and five ICI-refractory. Additionally, after five months of follow-up, 90% of these responses were ongoing. This approach was generally well-tolerated, with 4/14 patients (28.6%) experiencing grade 3 treatment-related toxicities in the form of hematuria and/or AST/ALT elevation. There was a single grade 4 toxicity in the form of transient and asymptomatic neutropenia.[55] This marks the most successful trial exploring combined TGFβ and PD-1/PD-L1 inhibition to date, though this approach has yet to advance to phase 3 testing.

Biomarkers, resistance mechanisms, & limitations of current studies

There have been unprecedented advances in cancer immunotherapy in the last decade. ICIs have largely replaced broad-spectrum chemotherapy as the preferred treatment for several tumor types, with many patients achieving either complete responses or long-term disease control.[2,56,57] Despite these many successes, there are still several cancer types for which ICIs have yet to demonstrate significant therapeutic efficacy. Further, therapeutic responses can be highly variable from patient to patient, even in cancers generally considered ICI-responsive.[58–62] As discussed, TGFβ signaling is emerging as a central means of immune evasion in several cancers.[5,6,63] Accordingly, several anti-TGFβ therapies are emerging in clinical trials,[64] often in combination with ICI-based immunotherapy. Though the combination of anti-TGFβ therapy and ICIs has shown near-uniform efficacy in preclinical studies, progress for such approaches in clinical trials has been more difficult. While several trials have shown encouraging results, others appear to suggest that the efficacy of this approach may be limited (recently terminated trials summarized in Table 3).

For example, despite the initial excitement surrounding Bintrafusp alfa, which was at the center of a $4.2 billion venture between Merck KGaA and GlaxoSmithKline (GSK), Bintrafusp alfa has yet to either show efficacy in phase 3 study or earn approval by the FDA for any cancer. In fact, Bintrafusp alfa has lost considerable momentum in recent years, with Merck KGaA discontinuing its phase 2 trial in biliary cancer in 2021 due to poor efficacy, as described previously. Following the failure of Bintrafusp alfa in a large-scale phase 3 trial of NSCLC patients (NCT03631706), Merck KGaA and GSK announced a mutual decision to end their deal in 2021. Similarly, despite its promise as an adjuvant to ICI in animal studies, the development of Galunisertib by Eli Lilly was halted in January 2020. Though trials using these medications are still ongoing (summarized in Table 4), these strategic realignments leave their future in question.

Anti-TGFβ and ICI medications	Additional therapy	NCT identifier	Phase	Reason for termination	Notes
Pancreatic cancer					
Bintrafusp alfa	Gemcitabine	NCT03451773	1b/2	One treatment-related death	
Biliary tract cancer					
Bintrafusp alfa	Gemcitabine Cisplatin	NCT04066491	2/3	Unlikely to meet survival objective	
Hepatocellular carcinoma					
Galunisertib + Nivolumab	–	NCT02423343	2	HCC cohort terminated due to low enrollment	Part of a larger multi-cancer trial
Lung cancer					
Bintrafusp alfa	–	NCT03631706	3	Unlikely to meet survival objective	
Bintrafusp alfa	Aerosolized Azacytidine	NCT04648826	1/2	Increased frequency of early progression/death	Metastatic lung lesions from other cancers
Breast cancer					
Bintrafusp alfa	BN-Brachyury Entinostat Ado-trastuzumab Emtansine	NCT04296942	1	Slow accrual and safety concerns	

Abbreviations: Transforming Growth Factor β (TGFβ); Immune checkpoint inhibitor (ICI); Hepatocellular carcinoma (HCC).

Table 3: **Recently terminated clinical trials exploring combined TGFβ and immune checkpoint inhibition.**

Anti-TGFβ and ICI medications	Additional therapy	NCT identifier	Phase	Notes
Colorectal cancer				
Vactosertib + Pembrolizumab	–	NCT03844750	2	Post-chemotherapy, pre-operative treatment for patients with resectable liver metastases
Bintrafusp alfa	CV301 N-803 NHS-IL12	NCT04491955	2	Locally advanced or metastatic MSS small intestine or colorectal adenocarcinoma ICI-naïve
Pancreatic cancer				
SHR-17011	Gemcitabine/Albumin-Paclitaxel	NCT04624217	1b/2	Advanced PDAC
NIS793 + Spartalizumab	nab-Paclitaxel/Gemcitabine	NCT04390763	2	Untreated metastatic PDAC
Bintrafusp alfa	Stereotactic Body Radiation Therapy M9241	NCT04327986	1/2	Advanced PDAC
Biliary tract cancer				
Bintrafusp alfa	–	NCT03833661	2	Second-line treatment in advanced disease
	–	NCT04727541	2	Neoadjuvant treatment for resectable disease
	Hypofractionated Radiation Therapy	NCT04708067	1	Advanced intrahepatic cholangiocarcinoma
Gastroesophageal cancers				
Vactosertib + Durvalumab	–	NCT04893252	2	Heavily pretreated, metastatic disease
Bintrafusp alfa	Paclitaxel	NCT04835896	1b/2	Second-line treatment for recurrent/metastatic disease
	Paclitaxel Carboplatin External Beam Radiotherapy	NCT04595149/ NCT04481256	2	Nonresectable esophageal or gastro-esophageal junction SCC
Primary lung cancers				
Vactosertib + Durvalumab	–	NCT03732274	1b/2a	Advanced, platinum-treated NSCLC
Vactosertib + Pembrolizumab	–	NCT04515979	2	Advanced, untreated, PD-L1-expressing NSCLC
Bintrafusp alfa	–	NCT04560686	2	Resectable, untreated NSCLC
	Cisplatin/Etoposide Carboplatin/Paclitaxel Cisplatin/Pemetrexed Intensity Modulated Radiation Therapy	NCT03840902	2	Nonresectable Stage III NSCLC
	Cisplatin Carboplatin/Pemetrexed/Etc.	NCT03840915	1/2	Stage IV NSCLC
	Docetaxel	NCT04396535	2	Advanced NSCLC that has progressed on prior anti-PD-1/PD-L1 and chemotherapy
	Pemetrexed Carboplatin Cisplatin	NCT04971187	2	Nonresectable locally advanced or metastatic, Tyrosine Kinase Inhibitor-Resistant, EGFR-Mutant NSCLC
	Topotecan Temozolomide	NCT03554473	2	Relapsed small cell lung cancer
	–	NCT05005429	2	Previously treated, advanced or metastatic malignant pleural mesothelioma
	Standard of Care Chemotherapy	NCT04297748	1/2	Advanced NSCLC, bio-distribution study
Breast cancer				
Bintrafusp alfa	–	NCT04489940	2	HMGA2-expressing TNBC
	–	NCT03620201	1	Stage II/III HER2+ breast cancer
	Radiation Therapy	NCT03524170	1	Metastatic, HR+, HER2- breast cancer
	Eribulin Mesylate	NCT03579472	1	Metastatic TNBC
	NHS-IL12 Radiation Therapy	NCT04756505	1	Metastatic, HR+, HER2- breast cancer
Gynecologic malignancies				
Bintrafusp alfa	–	NCT04246489	2	Non-resectable, platinum-treated advanced cervical cancer
	Cisplatin Carboplatin Paclitaxel Bevacizumab Radiation Therapy	NCT04551950	1	Locally advanced/advanced disease
	Carboplatin Paclitaxel	NCT05145569	1	Advanced ovarian cancer

(Table 4 continues on next page)

Anti-TGFβ and ICI medications	Additional therapy	NCT identifier	Phase	Notes
(Continued from previous page)				
HNSCC				
Galunisertib + Unspecified anti-PD-1 antibodies	Gemcitabine Cisplatin Radiation Therapy	NCT04605562	2	High-risk, locoregionally advanced nasopharyngeal carcinoma
Bintrafusp alfa	–	NCT04428047	2	Untreated, resectable HNSCC
	TriAd vaccine N-803	NCT04247282	1/2	Untreated, resectable, non-HPV-associated HNSCC
	Stereotactic Body Radiation Therapy	NCT04220775	1/2	Recurrent or second primary HNSCC
	–	NCT04396886	2	Previously treated, recurrent or metastatic non-keratinizing nasopharyngeal carcinoma
Other HPV-associated cancers				
Bintrafusp alfa	PRGN-2009	NCT04432597	1/2	Locally advanced or metastatic HPV-positive cancers
Urologic cancers				
Vactosertib + Durvalumab	–	NCT04064190	2	Urothelial cancer refractory to anti-PD-1/PD-L1
Bintrafusp alfa	–	NCT04349280	1b	Locally advanced or metastatic, platinum-treated urothelial cancer
	–	NCT04878250	2	Neoadjuvant treatment for resectable urothelial carcinoma
	NHS-IL12 Stereotactic Body Radiation Therapy	NCT04235777	1	Metastatic, non-prostate genitourinary malignancies
	NHS-IL12 Docetaxel	NCT04633252	1/2	Metastatic prostate cancer
	CV301 PROSTVAC-V PROSTVAC-F	NCT03315871	2	Recurrent prostate cancer
	BN-Brachyury Vaccine N-803 Epacadostat	NCT03493945	1/2	Castration-resistant prostate cancer
Neurologic malignancies				
Bintrafusp alfa	–	NCT05012098	2	Previously treated, recurrent or metastatic olfactory neuroblastoma
	Pimasertib	NCT04789668	1/2	Metastatic brain lesions originating from extracranial tumors
Thymus cancer				
Bintrafusp alfa	–	NCT04417660	2	Platinum-treated thymoma or thymic carcinoma
Sarcoma				
Bintrafusp alfa	Doxorubicin	NCT04874311	2	Advanced soft-tissue sarcoma
	NHS-IL12	NCT04303117	1/2	Advanced Kaposi sarcoma
Multi-cancer studies				
SAR439459 + Cemiplimab	–	NCT03192345	1	Nonresectable, advanced or metastatic solid tumors
Bintrafusp alfa	–	NCT02517398	1	Locally advanced or metastatic solid tumors
	SX-682 CV301	NCT04574583	1/2	Locally advanced or metastatic solid tumors
	Entinostat NHS-IL12	NCT04708470	1/2	Phase 1: locally advanced or metastatic HPV-associated malignancies or MSS small bowel or colorectal cancers Phase 2: locally advanced or metastatic checkpoint-refractory HPV-associated malignancies or MSS small bowel or colorectal cancers
	M6223	NCT04457778	1	Nonresectable, locally advanced or metastatic solid tumors
	Feladilimab	NCT02723955	1	Advanced or recurrent solid tumors
	–	NCT05061823	3	Prospective long-term safety study
	–	NCT04267861	–	Retrospective, observational long-term safety study

Abbreviations: Transforming Growth Factor β (TGFβ); Immune checkpoint inhibitor (ICI); Programmed cell death protein 1 (PD-1); PD-1 ligand 1 (PD-L1); Microsatellite stable (MSS); Pancreatic ductal adenocarcinoma (PDAC); Squamous cell carcinoma (SCC); Non-small cell lung cancer (NSCLC); High-mobility group AT-hook 2 (HMGA2); Triple negative breast cancer (TNBC); Human epidermal growth factor receptor 2 (HER2); Hormone receptor (HR); Head and neck squamous cell carcinoma (HNSCC); Human papillomavirus (HPV).

Table 4: **Ongoing clinical trials exploring combined TGFβ and immune checkpoint inhibition that have yet to post results.**

There is little explanation for the discrepancies between preclinical and clinical studies evaluating combined TGFβ and PD-1/PD-L1 inhibition in cancer. Hence, these studies underscore the difficulty of transitioning from animal models to clinical trials and affirm the need to both refine the *in vivo* systems used for studying immune-oncology and incorporate complementary model systems such as *ex vivo* slice cultures, patient-derived xenografts in partially humanized mice, and large animal models. Additionally, given the high degree of variation in responses observed in most trials, these observations also raise important questions regarding potential predictive biomarkers and combination approaches.

For example, as discussed above, a recent phase 1 study (NCT02517398) demonstrated that NSCLC patients with high PD-L1 expression were more likely to derive a long-term survival benefit from Bintrafusp alfa, with 85.7% of these patients being alive at the study endpoint compared to 39.7% for all patients.[47,48] However, PD-L1 expression may not be a universal biomarker given data from biliary cancer[43] or HNSCC[52] patients, where responses to Bintrafusp alfa were unrelated to PD-L1 expression. However, in the latter study, responses to Bintrafusp alfa were closely related to HPV expression. Similarly, one of the most successful trials for Bintrafusp alfa was observed in patients with HPV-positive tumors.[55] However, the utility of HPV expression as a biomarker may be context-specific, particularly given the early results from a cohort of 14 patients with HPV-unrelated HNSCC treated with neoadjuvant Bintrafusp alfa in which the objective pathologic response rate was 86%. Interestingly, in this cohort, the detection of neoepitope-specific tumor T cell responses correlated with the development of a pathologic response. Additionally, neoepitope-specific and pathologic responses in tumors did not correlate with genomic features or tumor antigenicity but were associated with limited pre-treatment myeloid cell tumor infiltration.[65] Hence, future trials would likely benefit from a careful re-evaluation of patients who derived clinical benefit from combined TGFβ and PD-1/PD-L1 inhibition in earlier studies.

Beyond identifying candidate biomarkers for therapeutic responses, it is also essential to further explore mechanisms of resistance to combined TGFβ and PD-1/PD-L1 blockade in order to advance more effective combination strategies in clinical trials. Though mechanistic data regarding resistance is limited, these studies are beginning to emerge, shedding new light on the potential reasons for the failure of combined TGFβ and PD-1/PD-L1 inhibition in patients. For instance, a recent preclinical study demonstrated that while the combination of TGFβ and PD-L1 inhibition was highly effective in both MC38 and EMT-6 tumor models, the combined treatment led to the upregulation of several immune response genes, including the cytokine CCL5. Intratumoral administration of CCL5 similarly enhanced responses to an anti-PD-L1 antibody in MC38 tumors, offering a potential strategy to augment responses to combined TGFβ and PD-1/PD-L1 inhibition in the clinic.[66]

Importantly, emerging data suggest that select immune phenotypes may dictate responses to cancer immunotherapy. Though we have discussed the importance of immune excluded phenotype in select trials/tumor types, it now appears that select immune phenotypes also involve the tumor stroma. For example, a landmark study has recently identified a unique subset of TGFβ-driven, LRRC15-expressing CAFs, which predict poor responses to anti-PD-L1 antibodies across over 600 patients across six different tumor types.[67] This is consistent with observations supporting three non-redundant barriers to the therapeutic efficacy of ICI-based immunotherapy in urothelial cancers, notably (1) the degree of pre-existing immunity represented by PD-L1 expression on immune cells as well as surrogate biomarkers of immune function, e.g., IFNγ expression, (2) tumor mutational burden, cell proliferation, proliferation, and DNA damage responses, and (3) the degree of active TGFβ-pathway signaling measured by a gene expression profiling and SMAD2/3 phosphorylation.[10] However, it should be noted that SMAD2/3 phosphorylation is not unique to TGFβ signaling and overlaps with the Activin signaling network,[68] and non-canonical TGFβ signaling overlaps significantly with MAP Kinase and Receptor Tyrosine Kinase signaling pathways.[69–71]

Though imperfect, the clinical trials described in this article have underscored the importance of patient selection. Hence, the above factors may be informative in identifying patients most likely to derive clinical benefit from combined TGFβ and PD-1/PD-L1 inhibition. For example, in EMT-6 mice, mice that underwent TGFβ and PD-L1 inhibition demonstrated a significant redistribution of tumor-infiltrating T-cells, which had an increased distance from the stromal border and a decreased distance from the tumor center, a phenomenon that was not observed in mice treated in either single agent arm. Additionally, mice receiving the combination treatment had a global alteration to gene expression in peritumoral CAFs, with significant repression of genes involved in canonical fibroblast function and extracellular matrix remodeling, enhancing CD8-effector function and promoting disease regression.[10] Therefore, patients with a TGFβ-driven tumor microenvironment and associated CAF-mediated immune exclusion may be particularly sensitive to this approach, which warrants prospective evaluation. In addition to helping to identify patients who may most benefit from the addition of a TGFβ inhibitor, these and other studies may also help identify patients in which TGFβ inhibition is not necessary, thus sparing these patients the potential adverse effects of these medications, particularly given their narrow

therapeutic window[72–74] combined with the inherent risks of ICI-based immunotherapy.[75] Hence, even in patients likely to respond, these potentially overlapping issues are a barrier to efficacy and should be further evaluated.[76]

Beyond identifying distinct immune subtypes and patient populations that may benefit from combined TGFβ and PD-1/PD-L1 inhibition, a recent study has provided additional insight into potential resistance mechanisms with possible application to patient stratification. In this study, the authors demonstrated that while the combination of LY2109761 and an anti-PD-L1 antibody was effective in the A223 model of HNSCC, treatment with Bintrafusp alfa created distinct responder and non-responder phenotypes. Subsequent analysis determined that responders had a more immune-permissive tumor microenvironment, associated with increased T-cell activation, enhanced expression of MHC Class I and II, and increased local levels of CXCR3 ligands. Accordingly, responses to Bintrafusp alfa were ameliorated by CXCR3 inhibition.[30]

This is consistent with a study in a poorly immunogenic model of PDAC, in which long-term treatment with Gemcitabine enhanced antigen presentation, PD-L1 expression, and the synthesis of several CCL- and CXCL-family chemokines. Accordingly, Gemcitabine synergizes with Galunisertib and an anti-PD-1 antibody, standardizing the highly variable responses observed in this model.[19] Given the synergy between Galunisertib and chemotherapy in pancreatic and colorectal cancer patients,[77,78] mechanism-driven strategies combining chemotherapy with dual TGFβ and PD-1/PD-L1 inhibition should be considered for future trials. In addition to cytotoxic chemotherapy, several mechanistic studies also support the combination of TGFβ and PD-1/PD-L1 inhibition with radiation, as described in detail above. Several studies suggest that radiation can lead to extensive reprogramming of the tumor microenvironment, altering the tumor peptidome, enhancing MHC Class I expression, and cooperating with select immunotherapy regimens.[79,80] Hence, as concomitant TGFβ and PD-1/PD-L1 inhibition continues to advance in clinical trials, the addition of the appropriate chemo- and/or radiation therapies warrants consideration and may be the most promising future direction for combined TGFβ and PD-1/PD-L1 inhibition.

Finally, though TGFβ is widely accepted as a common means of immune evasion in human cancer, one potential interpretation of these trials is that TGFβ signaling may simply not be a meaningful means of resistance to ICIs. Should future trials incorporating highly potent and specific TGFβ inhibitors still demonstrate negative results, this possibility may need to be considered. Alternatively, another potential reason for the relative lack of success in clinical trials is that immune suppressive TGFβ signaling may be more nuanced than previously realized, and additional factors must be considered when designing anti-TGFβ therapies. For example, the effects of TGFβ signaling are both localized and rapid,[4] raising important questions regarding the dose and frequency of administration to support the sustained inhibition of the TGFβ receptors and prevent the reactivation of TGFβ signals in the tumor microenvironment. This may also present a challenge when using serum levels of TGFβ as a biomarker, as some have suggested. This is further complicated by the fact that TGFβ ligands are often sequestered in fibrillin-rich microfibrils within the extracellular matrix. There, latent TGFβ is stored, where it remains biologically unavailable until its activation.[4] Thus, there may be a significant difference between local and serum TGFβ concentrations. Similarly, the bioavailability of TGFβ may be influenced by stromal remodeling induced by other therapies, potentially requiring careful adjustments of the dose and frequency of TGFβ inhibitors. Hence, these and other factors warrant consideration as anti-TGFβ therapies continue to advance in clinical testing.

Outstanding questions

The immunosuppressive effects of TGFβ signaling are well documented, and therapies targeting the TGFβ pathway have long been proposed as a means of augmenting responses to ICI-based immunotherapy. This approach has been extensively evaluated in preclinical studies, showing almost uniformly positive results across a wide range of tumor types. However, as dual TGFβ and PD-L1/PD-1 inhibition has entered clinical evaluation, results have been more nebulous. Though some studies have shown encouraging results, others have been resoundingly negative. Importantly, the many issues described in this article suggest that the disappointment of several trials exploring combined TGFβ and PD-1/PD-L1 inhibition may be rooted in a lack of patient selection and/or mechanism-driven design. This highlights not only the difficulty in translating results from preclinical to clinical studies but also the need to re-evaluate the design of these trials to incorporate novel biomarkers to identify the patients most likely to derive clinical benefit, as well as mechanism-driven combination strategies to further potentiate drug responses.

Contributors

AEM and DRP drafted the manuscript and assembled figures/tables. HGM critically edited the manuscript. All authors read and approved the final version of the manuscript.

Data availability

Not applicable.

Search strategy and selection criteria

Studies selected for this review were identified using PubMed, GoogleScholar, and/or ClinicalTrials.gov. Large-scale, advanced phase trials were prioritized, particularly those reporting high quality outcomes and/or safety data that have been published in the last five years.

Originality
All figures and tables presented in this article are original and have not been published elsewhere.

Declaration of interests
The authors have no potential conflicts to declare.

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