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.

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. In monotherapy, these medications are sufficient to produce objective clinical responses and achieve satisfactory disease control for many cancer types. However, despite the rapid progress in immunotherapy in recent years, several cancers appear largely refractory to ICIs, particularly as monotherapy. 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. While the effects of TGFβ on tumor cells are varied and context-specific, the role for TGFβ signaling in immune evasion appears to be somewhat similar across a wide range of tumor types. TGFβ is a central mediator of immune tolerance, and its immunosuppressive effects are well-documented. 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 (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.
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%).

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. 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. 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 |
| LY734947 + anti-PD-L1         | –                  | CT26, MC38      | Improved tumor growth inhibition, increased rate of complete regressions | 8 |
| SARI359469 + anti-PD-1        | –                  | MC38            | Improved anti-tumor immunity and restrained tumor growth | 9 |
| Bintrafusp alfa              | –                  | MC38            | Reduced tumor burden and metastasis, improved survival | 10 |
| YM101                         | –                  | CT26            | Increased tumor infiltrating lymphocytes and dendritic cells, and enhanced cytokine production | 11 |
| anti-GARP:TGF-β1 + anti-PD-1 | –                  | CT26            | Increased cytokytic immune responses and improved survival | 12 |
| Bintrafusp alfa              | Oxaliplatin        | MC38            | Increased CTL activation, reduced tumor burden | 13 |
| Bintrafusp alfa              | NHS-muLiL2         | MC38            | Reduced tumor burden, improved survival | 14 |
| Pancreatic cancer             |                    |                 |         |       |            |
| Galunisertib + anti-PD-1      | –                  | KPC             | Significantly improved survival and increased T-cell infiltration | 15 |
| LY364947 + anti-PD-L1         | –                  | KPC1            | Addition of anti-PD-L1 did not improve results beyond LY364947 alone | 16 |
| Galunisertib + siPD-L1        | –                  | Panc02          | Enhanced anti-tumor immunity and restrained tumor growth | 17 |
| Galunisertib + anti-PD-1      | Gemcitabine        | KPC             | Increased anti-tumor immunity and restrained tumor growth | 18 |
| LY364947 + anti-PD-L1         | Gemcitabine        | KP16            | Increased CTL infiltration/activation, reduced tumor burden, improved survival | 19 |
| Bintrafusp alfa              | Localized Radiotherapy | Orthotopic KPC | Reduced tumor growth | 20 |
| Lung cancer                   |                    |                 |         |       |            |
| Bintrafusp alfa              | –                  | HCC4006         | Transient responses observed | 21 |
| anti-TGFβ antibodies + anti-PD-1 | Radiation Therapy | Lewis Lung Carcinoma | Reduced tumor burden and improved survival | 22 |
| YM101                         | –                  | Lung Cancer Cell Lines and 3LL Lewis Lung Carcinoma murine model | Increased complete responses and promoted immune-mediated tumor regression | 23 |
| anti-TGFβ + anti-PD-L1        | –                  | Lewis Lung Carcinoma | Increased rates of complete responses | 24 |
| Breast cancer                 |                    |                 |         |       |            |
| anti-TGFβ + anti-PD-L1        | –                  | EMT-6           | Increased rates of complete responses, enhanced CTL-mediated tumor regression | 25 |
| Bintrafusp alfa              | –                  | EMT-6           | Enhanced activation of innate and adaptive immune systems | 26 |
| Radiation Therapy            | –                  | 4T1             | Reduced tumor burden, eradicated lung metastases, improved survival | 27 |
| NHS-muLiL2                    | –                  | EMT-6, 4T1      | Restrained tumor growth, improved survival | 28 |
| YM101                         | –                  | EMT-6           | Reduced tumor burden, restored anti-tumor immune responses | 29 |
| SRK-181-mIgG + anti-PD-L1    | –                  | EMT-6           | Restored anti-tumor immune responses, improved Survival | 30 |

(Table 1 continues on next page)
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.

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

| Anti-TGFβ and ICI Medications | Additional therapy | Model system(s) | Results | Notes | References |
|--------------------------------|-------------------|-----------------|---------|-------|------------|
| anti-CTLA-4-TGFβR2            | –                 | Partially humanized mice w/HLA-matched MDA-MB231 xenografts | Reduced tumor growth, restored anti-tumor immune responses |       | 26         |
| anti-PD-L1-TGFβR2             | –                 | 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      | –                 | SQ1             | Reduced tumor burden, improved survival |       | 25         |
| anti-PD-L1-TGFβR2             | –                 | 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 ΔExon 7 + 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         |
| LY2109561 + anti-PD-L1        | –                 | A223            | Improved efficacy in combination | Protected from tumor rechallenge | 30         |
| Bintrafus alfa                | –                 | A223            | Enhanced CD8-mediated tumor regression |       | 30         |
| LY215299 + anti-CTLA-4        | –                 | Braf ΔExon 7 + Pten−/− Melanoma | Reduced primary tumor growth, inhibited distant metastases |       | 31         |
| anti-CTLA-4-TGFβR2            | –                 | Partially Humanized Mice Bearing HLA-matched Melanoma Xenografts | Improved efficacy compared to CTLA-4 monotherapy |       | 26         |
| Vactosertib + anti-CTLA-4     | –                 | Braf ΔExon 7 + Pten−/− Melanoma | Improved efficacy compared to CTLA-4 monotherapy |       | 28         |
| **Urothelial cancer**         |                     |                 |         |       |            |
| SRK-181-mIgG + anti-PD-1      | –                 | M8T-2           | Reduced tumor burden | Protected from tumor rechallenge | 25         |
| Bintrafus 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**   |                     |                 |         |       |            |
| Bintrafus 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).
| Anti-TGFβ and ICI Medications | Additional therapy | NCT identifier | Phase | Sample size | Response rate | Median OS (%) | Median OS (Months) | Notes |
|-------------------------------|-------------------|---------------|-------|-------------|---------------|---------------|-------------------|-------|
| Colorectal cancer             |                   |               |       |             |               |               |                   |       |
| Vactosertib + Pembrolizumab   | –                 | NCT0324851    | 2     | 33          | 15.2%         | NR            | NR                | Pre-treated, ICI-naive, MSS, metastatic CRC |
| NIS793 + Spartalizumab        | –                 | NCT0294165    | 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          | 3.12%         | 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% | Advanced, platinum-treated NSCLC |
| Cervical cancer               |                   |               |       |             |               |               |                   |       |
| Bintrafusp alfa               | –                 | NCT02517398   | 1     | 39          | 30.77%        | NR            | 13.4              | Pre-treated, recurrent/metastatic cervical cancer |
|                              | –                 | NCT03424141   | 2     | 39          | –             |               |                   |       |
|                              | –                 | NCT02424282   | 1     | 14          | 86%           | NR            | NR                | Previously untreated, surgically resectable HPV-unrelated HNSCC |
| Head and neck cancer          |                   |               |       |             |               |               |                   |       |
| Bintrafusp alfa               | –                 | NCT02517398   | 1     | 32          | 12.5%         | 18-month: 44.0% | 24-month: 36.0% | 36-month: 24.0% | Heavily pre-treated, advanced HNSCC |
|                              | –                 | NCT04242822   | 1     | 14          | 86%           | NR            | NR                | Previously untreated, surgically resectable HPV-unrelated HNSCC |
| HPV-associated cancers        |                   |               |       |             |               |               |                   |       |
| Bintrafusp alfa               | –                 | NCT02517398   | 1     | 39          | 32%           | 12-month: 59.7% | 18-month: 51.5% | NR               | Advanced, pre-treated HPV-associated cancers |
|                              | NHS-IL12          | NCT03427411   | 2     | 14          | 71%           | NR            | NR                | HPV-positive, relapsed or refractory advanced cancers |
|                              | PDS0101           | NCT04287868   | 2     | 14          | –             |               |                   |       |
| Neurologic malignancies       |                   |               |       |             |               |               |                   |       |
| Bintrafusp alfa               | –                 | NCT02517398   | 1     | 35          | 5.71%         | –              | 5.3               | Recurrent GBM following radiation and Temozolomide treatment |
| Multi-cancer studies          |                   |               |       |             |               |               |                   |       |
| NIS793 + Spartalizumab        | –                 | NCT0294165    | 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). *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. 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. 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.

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.

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. 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. 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.97–99 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.100

As with lung cancer and melanoma, ICIs have rapidly advanced in the treatment of head and neck squamous cell carcinoma (HNSCC).101 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%.102 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.102 Interestingly, responses were observed largely independent of PD-L1 status, but more commonly in HPV-positive tumors.102

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 2020,103 long-term survival data has now been posted. The combined cohort included 39 cervical cancer patients, 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 39.7% and 51.5%, respectively. Bintrafusp alfa was well tolerated, with five patients (6.7%) developing grade 3 anemia.104

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.105 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.67 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.35-37 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.39-42 As discussed, TGFβ signaling is emerging as a central means of immune evasion in several cancers,43-46 Accordingly, several anti-TGFβ therapies are emerging in clinical trials,47 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       | NCT04166491    | 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 | 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 N803 NHS-IL12 | NCT04491955   | 2     | Locally advanced or metastatic MSS small intestine or colorectal adenocarcinoma ICI-naive |
| **Pancreatic cancer**         |                   |               |       |       |
| SHR-17011                    | Gemcitabine/Albumin-Paclitaxel/nab-Paclitaxel/Gemcitabine | NCT04624217   | 1b/2  | Advanced PDAC |
| NIS/93 + Spartalizumab       |                   | 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     | Paclitaxel        | NCT04835896   | 1b/2  | Second-line treatment for recurrent/metastatic disease |
| Bintrafusp alfa              | Paclitaxel        | NCT04595149/  | 2     | Nonresectable esophageal or gastro-esophageal junction SCC |
|                              | Carboplatin       | NCT04481256   |       |       |
|                              | Carboplatin/Paclitaxel |            |       |       |
|                              | Carboplatin/Pemetrexed/Intensity Modulated Radiation Therapy | | | |
|                              | Carboplatin/Pemetrexed/Intensity Modulated Radiation Therapy | | | |
|                              | 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 | 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 |
| **Primary lung cancers**      |                   |               |       |       |
| Vactosertib + Durvalumab     |                   | NCT03722274   | 1b/2a | Advanced, platinum-treated NSCLC |
| Vactosertib + Pembrolizumab  |                   | NCT04515979   | 2     | Advanced, untreated, PD-L1-expressing NSCLC |
| Bintrafusp alfa              | Cisplatin/Etoposide Carboplatin/Paclitaxel Carboplatin/Pemetrexed | NCT03840902   | 2     | Nonresectable Stage III NSCLC |
|                              | Cisplatin/Pemetrexed/Intensity Modulated Radiation Therapy | | | |
|                              | 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 | 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              | Cisplatin Carboplatin Paclitaxel Bevacizumab Radiation Therapy | NCT04246489/NCT04551050 | 2/1   | Non-resectable, platinum-treated advanced cervical cancer Locally advanced/advanced disease |
|                              | Carboplatin Paclitaxel | NCT05145569   | 1     | Advanced ovarian cancer |
### Anti-TGFβ and ICI medications

| Additional therapy | NCT identifier | Phase | Notes |
|--------------------|----------------|-------|-------|
| **HNSCC** | | | |
| Galunisertib | NCT04605562 | 2 | High-risk, locoregionally advanced nasopharyngeal carcinoma |
| + Unspecified anti-PD-1 antibodies | | | |
| Bintrafusp alfa | NCT04428047 | 2 | Untreated, resectable HNSCC |
| | NCT04247282 | 1/2 | Untreated, resectable, non-HPV-associated HNSCC |
| - TriAd vaccine N-803 Stereotactic Body Radiation Therapy | NCT04220775 | 1/2 | Recurrent or second primary HNSCC |
| | NCT04196886 | 2 | Previously treated, recurrent or metastatic non-keratinizing nasopharyngeal carcinoma |

| **Other HPV-associated cancers** | | | |
| Bintrafusp alfa | PRGN-2009 | 1/2 | Locally advanced or metastatic HPV-positive cancers |

| **Urologic cancers** | | | |
| Vactosertib + Durvalumab | | | |
| Bintrafusp alfa | NCT0464190 | 2 | Urothelial cancer refractory to anti-PD-1/PD-L1 |
| | NCT0449280 | 1b | Locally advanced or metastatic, platinum-treated urothelial cancer |
| | | | |
| - NHS-IL12 Stereotactic Body Radiation Therapy | NCT04878250 | 2 | Neoadjuvant treatment for resectable urothelial carcinoma |
| - NHS-IL12 Docetaxel | NCT0433252 | 1 | Metastatic, non-prostate genitourinary malignancies |
| | | | Metastatic prostate cancer |
| O'SORI PROSTVAC-V PROSTVAC-F | NCT03315871 | 2 | Recurrent prostate cancer |
| Bintrafusp alfa | NCT04432597 | 1/2 | Castration-resistant prostate cancer |

| **Neurologic malignancies** | | | |
| Bintrafusp alfa | NCT05012098 | 2 | Previously treated, recurrent or metastatic olfactory neuroblastoma |
| | 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 | NCT04874311 | 2 | Advanced soft-tissue sarcoma |
| | 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 |
| | NCT04574583 | 1/2 | Locally advanced or metastatic solid tumors |
| - SX-682 CV301 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 |
| | 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. However, PD-L1 expression may not be a universal biomarker given data from biliary cancer or HNSCC patients, where responses to Bintrafusp alfa were unrelated to PD-L1 expression. 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. 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 neo-adjuvant Bintrafusp alfa in which the objective pathologic response rate was 86%. Interestingly, in this cohort, the detection of neoeptitope-specific tumor T cell responses correlated with the development of a pathologic response. Additionally, neoeptitope-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. 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.

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, LRRRC15-expressing CAFs, which predict poor responses to anti-PD-L1 antibodies across over 600 patients across six different tumor types. 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. However, it should be noted that SMAD2/3 phosphorylation is not unique to TGFβ signaling and overlaps with the Activin signaling network, and non-canonical TGFβ signaling overlaps significantly with MAP Kinase and Receptor Tyrosine Kinase signaling pathways.

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. 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 combined with the inherent risks of ICI-based immunotherapy. Hence, even in patients likely to respond, these potentially overlapping issues are a barrier to efficacy and should be further evaluated.

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.

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. Given the synergy between Galunisertib and chemotherapy in pancreatic and colorectal cancer patients, 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. Hence, as concomitant TGFβ and PD-1/PD-L1 inhibition continues to advance in clinical trials, the addition of the appropriate chemotherapy 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 be a meaningless 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, 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. 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, Google Scholar, 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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