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REVIEW ARTICLE

Tumor in the Crossfire: Inhibiting TGF-β to Enhance Cancer Immunotherapy

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Abstract

Cancer immunotherapy using monoclonal antibodies targeting immune checkpoints has undoubtedly revolutionized the cancer treatment landscape in the last decade. Immune checkpoint inhibitors can elicit long-lasting, previously unheard-of responses in a number of tumor entities. Yet, even in such tumors as metastatic melanoma and non-small cell-lung cancer, in which immune checkpoint inhibition has become the first-line treatment of choice, only a minority of patients will benefit considerably from these treatments. This has been attributed to a number of factors, including an immune-suppressive tumor microenvironment (TME). Using different modalities to break these barriers is of utmost importance to expand the population of patients that benefit from immune checkpoint inhibition. The multifunctional cytokine transforming growth factor-β (TGF-β) has long been recognized as an immune-suppressive factor in the TME. A considerable number of drugs have been developed to target TGF-β, yet most of these have since been discontinued. The combination of anti–TGF-β agents with immune checkpoint inhibitors now has the potential to revive this target as a viable immunomodulatory therapeutic approach. Currently, a limited number of small molecular inhibitor and monoclonal antibody candidates that target TGF-β are in clinical development in combination with the following immune checkpoint inhibitors: SRK 181, an antibody inhibiting the activation of latent TGF-β1; NIS 793, a monoclonal antibody targeting TGF-β; and SHR 1701, a fusion protein consisting of an anti-PD-L1 monoclonal antibody fused with the extracellular domain of human TGF-β receptor II. Several small molecular inhibitors are also in development and are briefly reviewed: LY364947, a pyrazole-based small molecular inhibitor of the serine-threonine kinase activity of TGFβRI; SB-431542, an inhibitor targeting several TGF-β superfamily Type I activin receptor-like kinases as well as TGF-β1-induced nuclear Smad3 localization; and galunisertib, an oral small molecular inhibitor of the TGFβRI kinase. One of the most advanced agents in this area is bintrafusp alfa, a bifunctional fusion protein composed of the extracellular domain of TGF-β receptor II fused to a human IgG1 mAb blocking PD-L1. Bintrafusp alfa is currently in advanced clinical development and as an agent in this space with the most clinical experience, is a focused highlight of this review.

Key Points

Simultaneous targeting of the PD-1/PD-L1 pathway and TGF-β can be done with maturing evidence of clinical activity.

Targeting the PD-1/PD-L1 pathway and TGF-β can be accomplished without prohibitive safety concerns.

Biomarker-driven approaches under development may help ascertain which patient population will derive maximal benefit from dual PD-1/PD-L1 pathway and TGF-β blockade.

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1 Background

1.1 Importance of the PD-1/PD-L1 Pathway

Over the past two decades, tumor immunobiologists have learned how the up-regulation of inhibitory receptor axes, such as cytotoxic T lymphocyte antigen 4 (CTLA-4)–CD28 and programmed cell death 1 (PD-1)–programmed cell death 1 ligand 1 (PD-L1), is an integral component of tumor immune escape, chemotherapy resistance, and disease progression [1]. Clinically, it is no secret that these discoveries have been revolutionary for the treatment of cancer and our understanding of intrinsic immune regulation. Following the approval of ipilimumab in March 2011 for metastatic melanoma [2], the landscape in which we have managed patients with advanced cancer has forever shifted. The magnitude of this paradigm change was punctuated by the 2018 Nobel Prize in Physiology or Medicine, awarded to James P. Allison and Tasuku Honjo for their discoveries leading to cancer treatments by way of suppressing negative immunomodulation [1]. In the years since the first PD-[L]1 inhibitor approval on September 4, 2014, there have been over 70 Biologic Licensing Applications (BLAs) for anti-PD-1- and anti-PD-L1-blocking antibodies approved [3]. The growing relevance of checkpoint inhibitors cannot be understated as they continue to change clinical practice and lead to the unprecedented extension of patient survival [4]. However, this story is far from over. As additional cancer and treatment-line indications are evaluated, it has become clear these agents have limits, often hampered by a variety of resistance mechanisms, including insufficient tumor immunogenicity, MHC dysfunction, T-cell exhaustion, resistance to secondary cytokines such as interferon (IFN)-γ signaling, and barriers on entering the immunosuppressive tumor microenvironment (TME) [5, 6].

1.2 Importance of the Transforming Growth Factor β (TGF-β) Pathway

A central factor underpinning tumor immune resistance is local immunosuppressive cytokines. A primary target in this space is transforming growth factor β (TGF-β). TGF-β is a 25-kDa dimeric protein [7], composed of two subunits, and is a multifunctional cytokine belonging to the transforming growth factor superfamily. This large superfamily of proteins include a substantial variety of protein families, such as bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), glial-derived neurotrophic factors (GDNFs), activins, inhibins, etc. In addition to this wide network are three different mammalian isoforms of TGF-β (TGF-β1, TGF-β2, TGF-β3), all of which function through the same receptor signaling pathways. Polypeptides from the TGF-β family were first isolated in the 1970s by de Larco and Todaro and were initially named as the sarcoma growth factor (SGF) as they could provoke the malignant transformation of rat kidney fibroblasts [8]. By the 1980s, Roberts and Sporn further described TGF-β as capable of inducing fibroblast growth and collagen production. Other groups around this time also identified TGF-β as having a dual role in its ability to inhibit cell proliferation as well [9]. Over the subsequent decades, we have learned of the numerous cellular and biological functions of the TGF-β superfamily, including regulation of cell proliferation, apoptosis, differentiation, and migration; embryonic patterning; stem cell maintenance; immune regulation; bone formation; and tissue remodeling and repair [10–14].

TGF-β1, a primary focus of this review, is composed of a latency-associated peptide (LAP) and a mature TGF-β1, which form homodimers via disulfide bonds. These homodimers then noncovalently associate as the small latent TGF-β1 complex (SLC). This secreted complex then covalently associates with a latent TGF-β binding protein (LTBP), thus creating a tripartite complex known as the large latent complex (LLC). The LLC is then sequestered within the extracellular matrix (ECM), which in turn functions as an ECM reservoir of TGF-β. Sequestration of latent TGF-β in the ECM is crucial for proper mobilization of the latent cytokine and its activation [15–18] (Fig. 1).

A growing body of evidence reveals that TGF-β1 can be activated by a variety of factors within the extracellular compartment, including plasmin, matrix metalloproteinases (MMPs), thrombospondin-1, lowered pH, and reactive oxygen species. Notably, TGF-β can also be activated by specific integrins that bind the Arg-Gly-Asp (RGD) sequence of LAPs. The integrin-RGD binding in turn results in a contractile-force-dependent conformational change of the latent complex, which releases a now-activated TGF-β. Furthermore, in proximity to the new, active TGF-β are a number of soluble extracellular agonists and antagonists that further complicate the temporal and spatial access of the ligands to receptors [17, 19–24].

TGF-β signaling involves three parallel pathways (BMP, TGF-β, and activin pathways), which converge through the canonical SMAD pathway that controls the expression of hundreds of genes, and several noncanonical pathways that regulate cell polarity, the cytoskeleton, and microRNA maturation [25]. Under normal homeostasis, TGF-β functions as a tumor suppressor, which can both induce apoptosis in premalignant cells and inhibit proliferation of cancerous cells. Under specific circumstances in which a tumor has inactivated the tumor-suppressive effects of TGF-β, either by a loss of specific downstream pathway signaling or a rewiring of this signaling, TGF-β can become a factor driving tumor progression. This co-option of TGF-β can be further skewed, wherein tumor-derived TGF-β can induce tumorigenic and

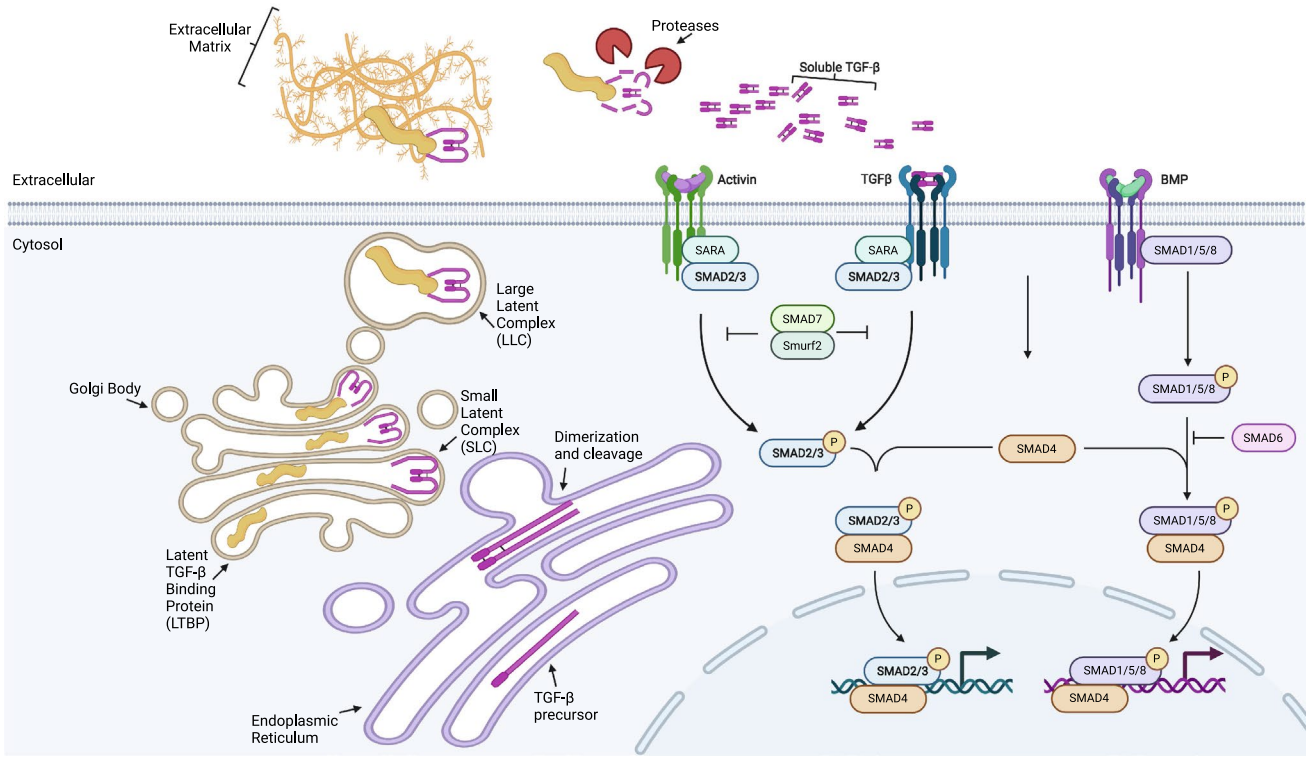

Fig. 1 TGF-β precursor within the endoplasmic reticulum undergoes dimerization via disulfide bonds. These homodimers are cleaved by furin proteases into the small latent complex (SLC). This complex then associates with the latent TGF-β binding protein (LTBP) forming the large latent complex (LLC) before being secreted into the local extracellular environment. The LLC is then sequestered within the local extracellular matrix (ECM) where it acts as a reservoir for mature TGF-β. The sequestered mature TGF-β is released by proteases resulting in signaling through the TGF-β receptor inducing SMAD2/3 phosphorylation, followed by association with SMAD4 into a transcription complex. Furin cleavage and the non-covalent nature of the SLC are not visually represented

pro-metastatic responses in cancer cells as well as the surrounding stroma, including the formation of an immune-suppressive TME [10, 26–28]. These biological changes can result clinically in more aggressive tumors, wherein reduced TGF-βRII expression in human non-small-cell lung cancer (NSCLC) leads to increased peri-tumor inflammation at least partially mediated by increased TGF-β1 expression [29]. We are also aware that TGF-β can induce chemoresistance by way of tumor quiescence, and this effect can be reversed or prevented by way of TGF-β inhibition [30].

As a result of the role TGF-β plays in both tumor propagation and metastasis, there has been interest in combining TGF-β blockade with additional immunotherapeutic approaches. This includes a significant interest in concurrent CTLA-4 or PD-1/PD-L1 blockade [31–33], as well as combinations with immunocytokines [34], cytokine/chemokine blockade [35, 36], oncolytic viruses [37], autologous tumor vaccination [38], and adoptive cell therapy [39]. The majority of these are outside the scope of this focused review.

1.3 Concentration of TGF-β Sequestration in Tumor Microenvironment (TME)

As tumors progress, they will typically generate and secrete their own TGF-β in an autocrine fashion. The TGF-β produced is sequestered as the LLC, which binds to local proteins within the ECM, predominantly fibrillin and fibronectin [16]. This ECM deposition serves as an abundant TGF-β reservoir impacting not only the tumor itself, but the local TME—inhibiting cell adhesion, inducing immunosuppression as well as angiogenesis, and lastly completing the cycle wherein further tumor-mediated or tumor-associated cell mediated degradation of the local ECM releases sequestered TGF-β and propagates the metastatic process. The latent TGF-β complex also binds glycoprotein A repetitions predominant (GARP), which is a transmembrane protein abundantly expressed on regulatory T cells and platelets [40]. GARP has been shown to play a central role in peripheral tolerance of T regulatory cells, as well as a source for ample TGF-β in the local microenvironment. This tumor-derived TGF-β not only drives the formation of cancer-associated fibroblasts [41], inhibits natural killer

(NK) cells and dendritic (DC) cells [42, 43], but also serves to polarize macrophages into tumor-associated macrophages (TAMs) [44]. In addition, TGF-β is capable of impairing adaptive antitumor immunity through the direct inhibition of clonal expansion and cytotoxicity of CD8+ cytotoxic T cells [45, 46]. Lastly, TGF-β can induce the expression of Foxp3, which confers a regulatory and immunosuppressive phenotype [47]. Compounding this cycle, the GARP promoter has a binding site for FoxP3, which could in turn lead to further GARP expression and TGF-β sequestration to the local TME [16, 40, 48].

Previous studies have suggested that pan-inhibition of TGF-β may help overcome resistance to immune checkpoint blockade, but inhibitors blocking all three isoforms proved to be either too toxic for clinical use—often hindered by dose-limiting cardiotoxicities—or failed to show significant clinical activity despite promising preclinical evidence [49–53]. Several animal models and studies on loss-of-function mutations in humans of TGF-β2 and TGF-β3 isoforms suggest these isoforms may play vital homeostatic roles in cardiac function [51, 54–57]. This has led to dedicated interest in blocking the TGF-β1 isoform, as this appears to be the driver of immune resistance within the TME [58].

2 Preclinical and Early Phase Data

Several agents targeting TGF-β have been evaluated with mixed success, including several approaches using neutralizing antibodies, ligand traps, small-molecule inhibitors, and antisense oligonucleotides. Herein, we highlight eight agents that have shown promising activity.

2.1 SRK-181

The agent SRK-181 is a high-affinity, fully humanized monoclonal antibody that inhibits latent TGF-β1 activation. Preclinical work has displayed little to no binding to latent TGF-β2 and TGF-β3 isoforms or to active TGF-β growth factors [59]. In mouse tumor models (bladder, melanoma, and breast cancer), SRK-181 (in combination with anti-PD1 therapy) overcame primary anti-PD-1 resistance and showed survival benefit [58]. This has led to an ongoing multicenter, open-label, phase I trial of SRK-181 (DRAGON trial, ClinicalTrials.gov identifier NCT04291079), which evaluates SRK-181 alone or in combination with anti-PD-L1 inhibition in patients with locally advanced or metastatic solid tumors. One arm of this study involves assessing patients who have had prior anti-PD-1/PD-L1 therapy and are considered 'nonresponders' to assess whether adding SKR-181 can overcome primary anti-PD-1 resistance [60].

2.2 NIS 793

NIS793 (formerly XPA-42-068) is a pan anti-TGF-β-neutralizing antibody that has shown preclinical activity in xenograft models of pharyngeal carcinoma and squamous cell carcinoma [61, 62]. NIS793 was initially accessed across 120 participants in a phase I/Ib study (NCT02947165) in combination with spartalizumab (PDR001, an anti-PD-1 antibody) in patients with locally advanced or metastatic solid tumors. Interim results showed the agent was well tolerated, with 11% of patients experiencing a treatment-related adverse event (TRAE), the most common being rash (3%). Some clinical activity was noted, with two microsatellite-stable colorectal cancer patients achieving a partial response (PR) [63]. The antibody is currently being tested in a phase II clinical trial for patients with metastatic pancreatic ductal adenocarcinoma in combination with gemcitabine/nab-paclitaxel chemotherapy, as well as a separate arm including spartalizumab (NCT04390763) [64].

2.3 SHR 1701

An agent largely investigated in China is SHR-1701; this bispecific antibody is an anti-PD-L1 monoclonal antibody fused with the N-terminal-truncated extracellular domain of TGF-β receptor II (TGFβRII) [65]. This agent is biologically similar to another agent, bintrafusp alfa, discussed later in this review. The fused TGFβRII component functions as a TGF-β 'trap,' binding TGF-β within the TME. SHR-1701 is being investigated in 19 different phase I and phase II clinical trials (registered on ClinicalTrials.gov as of September 16, 2021) across a number of locally advanced and metastatic solid tumors. Of the data reported, the agent appears to be well tolerated with rare dose-limiting toxicity (DLT), including an incident of immune-mediated pneumonitis in a NSCLC expansion cohort [66], as well as a 46.9% reported incidence of immune-related adverse events across 49 patients with varying tumor types [67].

2.4 LY364947

LY364947 is a pyrazole-based small molecular inhibitor capable of inhibiting the serine-threonine kinase activity of TGFβRI. In several preclinical models, LY364947 decreased the resistance of glioblastoma-initiating cells [68], the MDA-MB-231 breast cancer cell line [69], and several non-small lung cancer cell lines (NCI-H1299, A549 and murine Lewis lung cancer cells) to radiotherapy [70, 71]. This observation is suggested to be in part mediated through attenuation of the DNA damage response pathway by TGFβRI inhibition. While there appears to be some promising preclinical data, no active trials are currently underway.

2.5 SB-431542

Another small molecular inhibitor, SB-431542, targets several TGF-β superfamily type I activin receptor-like kinases, including ALK4, ALK5, and ALK7, as well as subsequent TGFβ1–induced nuclear Smad3 localization. When tested with in vitro models, SB-431542 suppressed TGFβ-induced growth stimulation of MG63 osteosarcoma cells. While no active clinical trials exist for this inhibitor, SB-431542 has found renewed utility in preclinical stem cell differentiation protocols [71].

2.6 Galunisertib (LY2157299)

An agent with substantial pre-clinical evaluation is galunisertib, an oral small molecular inhibitor of the TGFβRI kinase which downregulates the phosphorylation of SMAD2. This agent has been studied in several disease states, including myelodysplastic syndrome where galunisertib decreased anemia in a TGF-β overexpressing transgenic mouse model of bone marrow failure [72, 73]. Galunisertib has also displayed antitumor activity across several xenograft models of breast, colon, lung, and hepatocellular carcinoma [71]. This preclinical activity led to a first-in-human dose-finding study in 65 patients with progressive malignancies [74]. This study included two arms, one for dose escalation and then a second that evaluated galunisertib in combination with standard clinical doses of lomustine. As a monotherapy, 16.6% (5/30) of evaluable galunisertib-treated patients experienced either a complete or partial response (CR or PR). Safety was assessed using Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 and galunisertib was evaluated as safe, with no cardiac adverse events and only three patients (7.7%) of the monotherapy arm experiencing grade 3 or 4 toxicities that were considered possibly drug related. One possible DLT was noted for grade 4 thrombocytopenia. A subsequent randomized phase II study of galunisertib involving 158 patients was completed; this involved three arms: galunisertib monotherapy ($n = 39$), galunisertib and lomustine ($n = 79$), or lomustine and placebo ($n = 40$) [75]. This too was a negative study where the combination of galunisertib and lomustine failed to demonstrate an improvement in overall survival (OS) relative to lomustine + placebo, with similar efficacy outcomes across all three arms. Another study from 2019 evaluated galunisertib in the second-line for patients with hepatocellular carcinoma [76]. Notably, OS was longer in AFP responders (> 20% decrease from baseline) compared with non-responders (21.5 months vs 6.8 months), and longer in TGF-β1 responders (> 20% decrease from baseline) compared with non-responders. The most common grade 3/4 TRAE were neutropenia ($n = 4$), as well as fatigue, anemia, hyperbilirubinemia, hypoalbuminemia, and embolism (each, $n = 2$). Most recently, a two-part, single-arm, multinational, phase Ib study was conducted of galunisertib co-administered with the anti-PD-L1 mAb, durvalumab, in patients with recurrent/refractory metastatic pancreatic cancer. No DLTs were recorded. Among 32 patients treated with galunisertib, one patient had PR, seven had stable disease (SD), 15 had objective progressive disease (PD), and nine were not evaluable. Disease control rate was 25.0%. Median OS and progression-free survival (PFS) were 5.72 months (95% CI 4.01–8.38) and 1.87 months (95% CI 1.58–3.09), respectively [77].

2.7 Vactosertib (TEW-7197)

Vactosertib (TEW-7197) is another selective small molecule inhibitor. This agent targets the adenosine-5-triphosphate binding site of TGFβR1, in turn inhibiting phosphorylation of the Smad2 and Smad3 proteins, the key mediators in TGF-β downstream signaling. Vactosertib safety, efficacy, and association with TGF-β response signatures were evaluated in patients with advanced solid tumors, identifying a response signature associated with poor prognosis. In a phase I modified 3 + 3 dose-escalating study of vactosertib, patients ($n = 17$) who received ≥ 140 mg achieved SD (35.3%) and had higher TGF-β response signatures than those with PD. Vactosertib was safe and well tolerated, and maximum tolerated dose was not determined. The most common TRAE was fatigue, while abdominal pain, AST elevation, and pulmonary edema occurred in one patient.

3 Bintrafusp alfa

Bintrafusp alfa (formerly GSK-4045154, M7824, and MSB0011359C) is a first-in-class investigational bifunctional fusion protein designed to block TGF-β and PD-L1. The protein is composed of the extracellular domain of the TGF-βRII receptor, functioning here as a TGF-β 'trap.' This TGF-β trap is fused via a linker to the C-terminus of each heavy chain of an IgG1 antibody blocking PD-L1 (anti-PD-L1). As a result, bintrafusp alfa is designed to target tumors via first localization of the target drug, by way of anti-PD-L1 inhibition, with the simultaneous inhibition of two key mechanisms of immunosuppression in the TME [78–81] (Fig. 2). This proposed mechanism of action and drug localization was assessed by radiolabeling bintrafusp alfa with zirconium-89 (^{89}Zr) and evaluating this radiolabeled conjugate in a PD-L1/TGF-β-positive murine breast cancer model (EMT-6). In this study, nanomolar affinities for PD-L1 were achieved with ^{89}Zr-Df-bintrafusp alfa, suggesting the in vivo distribution patterns of bintrafusp alfa are driven by its PD-L1 binding arm [82].

In preclinical mouse tumor models, bintrafusp alfa showed greater antitumor activity versus anti–PD-L1 or

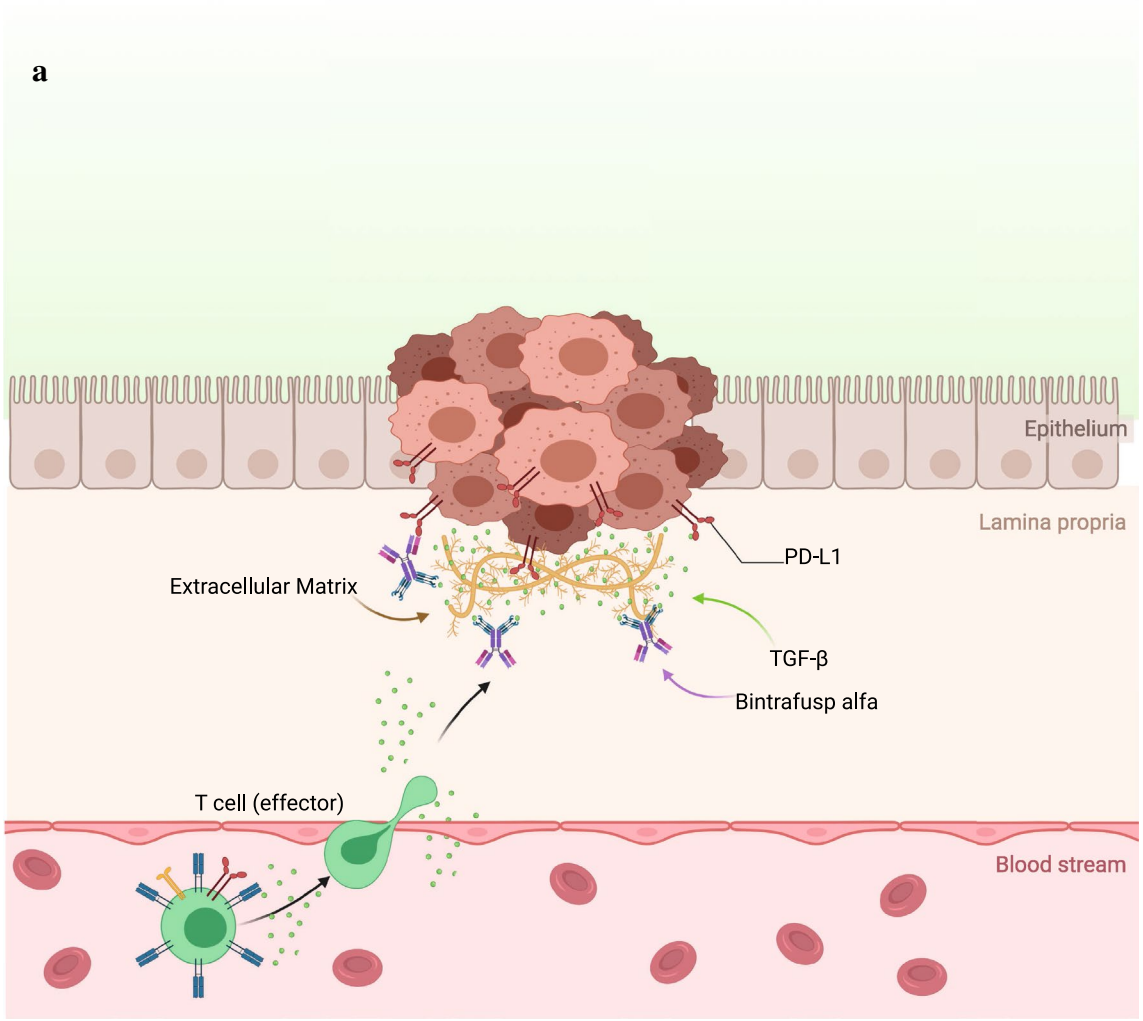

Fig. 2 **a** T effector cell trafficking to tumor site, which harbors heightened amounts of TGF-β within the local microenvironment. **b** Bintrafusp alfa binding and sequestering TGF-β within the tumor microenvironment while concurrently binding PD-L1, fostering T effector cell engagement with malignant cells

anti–TGF-β treatment alone, supporting the biodistribution noted in radiolabeling studies. Treatment with bintrafusp alfa resulted in superior tumor regression at day 24 compared with treatment with either anti–PD-L1 or the trap control (both of which also showed partial antitumor activity). They also noted improved antitumor activity in mouse models of other solid tumors, including orthotopic breast models, colorectal cancer, and subcutaneous tumors. In addition, treatment with bintrafusp alfa resulted in significantly reduced cancer-associated fibroblast activity with reduced α-SMA expression relative to isotype control or anti–PD-L1 monotherapy and was shown to also reduce fibrosis. This suggests that with the use of bintrafusp alfa and the reduction in peri-tumor fibrosis, we may be able to help revert local drug resistance, increase antitumor activity, and improve the potential for synergy with combination therapies otherwise impeded by the TME. This was subsequently evaluated: bintrafusp alfa was combined with radiation therapy, which showed enhanced antitumor activity in preclinical mouse tumor models, whereas the combination of bintrafusp alfa with radiotherapy resulted in significantly reduced tumor volume and tumor weight relative to bintrafusp alfa or radiotherapy alone as well as a significantly increased frequency of IFN-γ-producing CD8+ T cells and the reduction in gene expression of epithelial-mesenchymal transition (EMT), vascular endothelial growth factor (VEGF) pathway, and radiation therapy (RT)–induced fibrosis gene-signatures [79].

Paralleling this work, Knudson et al. demonstrated that bintrafusp alfa sequesters murine TGF-β1 in vitro and in vivo. In addition, bintrafusp alfa can both prevent the initiation of, and significantly decrease existing TGF-β signaling, particularly in the TME [83]. They demonstrated that

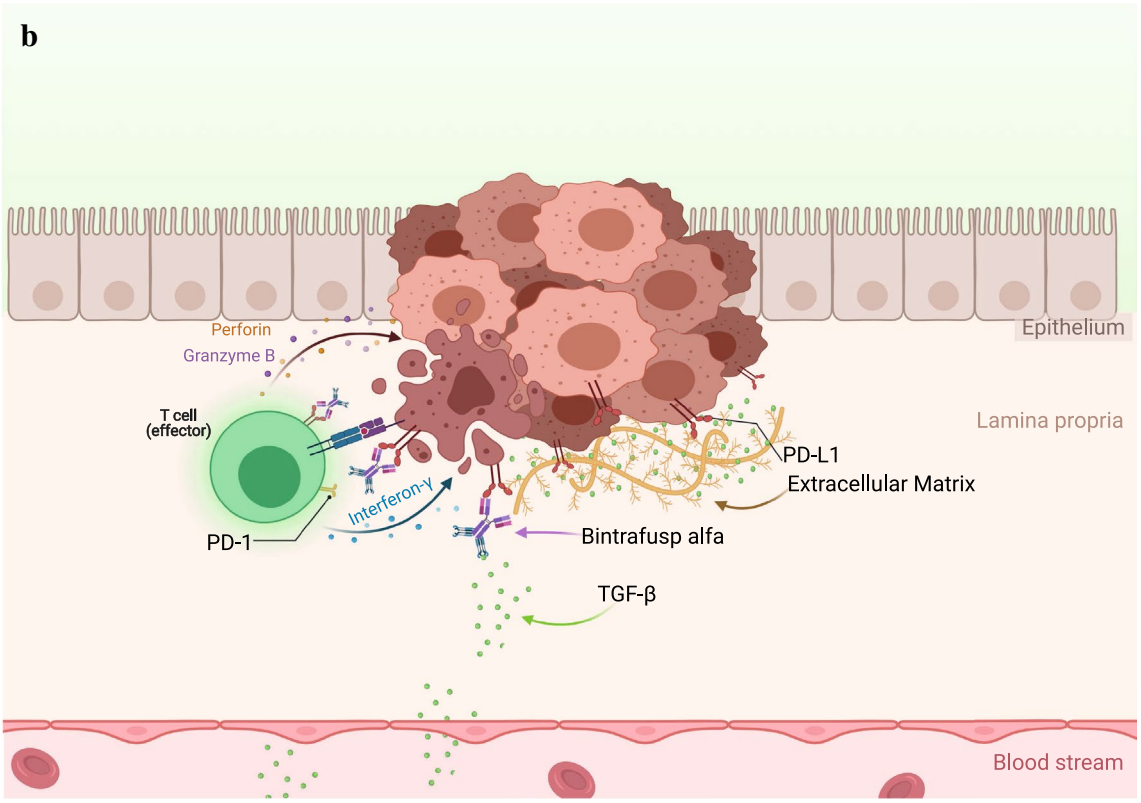

Fig. 2 (continued)

bintrafusp alfa reduces plasma TGF-β1, binds to PD-L1 in the tumor, and decreases TGF-β-induced signaling in the TME in mice. In murine breast and colon carcinoma models, bintrafusp alfa decreased both tumor burden and increased overall survival when compared with TGF-β neutralization alone. Bintrafusp alfa treatment promoted CD8+ T cell and NK cell activation, and both of these immune populations were required for optimal bintrafusp alfa-mediated tumor control. Bintrafusp alfa was superior to TGF-β- or PD-L1-targeted therapies when in combination with a therapeutic cancer vaccine. These findings demonstrate the value of using bintrafusp alfa to simultaneously target TGF-β and PD-L1/PD-1 immunosuppressive pathways to promote antitumor responses and efficacy. The studies also support the potential clinical use of bintrafusp alfa as a monotherapy or in combination with other immunotherapies, such as therapeutic cancer vaccines, including for patients who have progressed on PD-L1/PD-1 checkpoint blockade therapies [83].

Extending the potential synergy of therapeutic vaccines, Rumfield et al. investigated bintrafusp alfa in combination with a liposomal-based human papillomavirus (HPV) therapeutic vaccine consisting of an immune-activating cationic lipid (R-DOTAP) and HLA-unrestricted HPV16 peptides [84]. This study tested a syngeneic mouse model of a murine lung carcinoma cell line (TC-1) expressing HPV16 E6 and E7, devoid of PD-L1 expression to mimic a PD-L1 low patient population, with a combination of vaccine, bintrafusp alfa, and NHS-IL12 (an immunocytokine composed of two IL-12 heterodimers). HPV vaccine monotherapy generated HPV-specific T cells and antitumor activity in mice bearing TC-1 lung carcinomas, whereas bintrafusp alfa did not elicit antitumor effects or any increase in T cells in the TME. However, when combined with NHS-IL12, the three-agent therapy significantly reduced the rate of tumor growth and when compared with either therapy as a monotherapy, resulted in the lowest average tumor weight at the end of study. These results were then correlated with increases in T cells and T-cell clonality in the TME [84].

3.1 Clinical Data

Following promising preclinical data, early phase trials of bintrafusp alfa have started to reveal where it may be used alongside other agents in the burgeoning immunotherapy armamentarium to achieve antitumor synergy [80] (Table 1). Strauss et al. first evaluated bintrafusp alfa in a 3 + 3 dose-escalation phase I study to determine the safety and maximum tolerated dose (MTD). Nineteen heavily pretreated patients with ECOG 0–1 received bintrafusp alfa. Grade ≥ 3 TRAEs occurred in four patients (skin infection secondary

Table 1 Completed bintrafusp alfa studies

Trial name	NCT	Phase	Enrollment	Bintrafusp alfa Dose	Status	Target population	Primary tumor type	Median age (range)	Prior lines of therapy (% received ≥ 2 prior anti-cancer therapies)	Median follow-up time	Median duration of treatment	Grade ≥ 3 treatment-related AEs	Confirmed best overall response	References
MSB0011359C (M7824) in Metastatic or Locally Advanced Solid Tumors	NCT02517398	I	19	1 ($n = 3$), 3 ($n = 3$), 10 ($n = 3$), or 20 ($n = 7$) mg/kg IV Q2W or a 0.3 mg/kg dose followed by a 10 mg/kg dose ($n = 3$)	Completed	Metastatic or locally advanced solid tumors	Adenoid cystic carcinoma ($n = 2$), anal ($n = 2$), appendiceal ($n = 1$), bronchopulmonary carcinoid ($n = 1$), cervix uteri ($n = 4$), chordoma ($n = 1$), colorectal ($n = 2$), pancreatic ($n = 5$), small bowel ($n = 1$)	56 (33–78)	84	N/A	11.9 wk (range 4.0–41.9)	4/19 (21.1%)	CR 5% ($n = 1$), PR 10% ($n = 2$), SD 31% ($n = 6$), PD 47% ($n = 9$), NE 5% ($n = 1$)	[80]

Table 1 (continued)

Trial name	NCT	Phase	Enrollment	Bintrafusp alfa Dose	Status	Target population	Primary tumor type	Median age (range)	Prior lines of therapy (% received ≥ 2 prior anti-cancer therapies)	Median follow-up time	Median duration of treatment	Grade ≥ 3 treatment-related AEs	Confirmed best overall response	References
MSB0011359C (M7824) in Metastatic or Locally Advanced Solid Tumors	NCT02517398	I	80	500 mg or 1200 mg IV Q2W	Completed	Advanced NSCLC that progressed after platinum doublet therapy, platinum-based adjuvant or neo-adjuvant treatment, and those who also have not received previous immunotherapy	NSCLC (squamous, $n = 16$ and nonsquamous, $n = 64$)	64 (38–85)	21.3	51.9 wk (IQR 19.6–74.0)	11.9 wk (IQR 5.6–31.9)	23/80 (28.8%)	PR 23.1% ($n = 17$), SD 16.3% ($n = 13$), PD 48.8% ($n = 39$), NE ($n = 9$)	[85]

Table 1 (continued)

Trial name	NCT	Phase	Enrollment	Bintrafusp alfa Dose	Status	Target population	Primary tumor type	Median age (range)	Prior lines of therapy (% received ≥ 2 prior anti-cancer therapies)	Median follow-up time	Median duration of treatment	Grade ≥ 3 treatment-related AEs	Confirmed best overall response	References
MSB0011359C (M7824) in Metastatic or Locally Advanced Solid Tumors	NCT02517398	I	32	1200 mg IV Q2W	Completed	Advanced SCCHN not amenable to curative therapy that progressed/recurred after platinum therapy in the recurrent/metastatic setting, or < 6 mo after platinum therapy in the locally advanced setting	Advanced SCCHN	60 (53–65)	75	86.4 wk; range 2–97	12.1 wk (range 2–96)	11/32 (34%)	PR 16% ($n = 5$), SD 19% ($n = 6$), PD 56% ($n = 18$), NE 9% ($n = 3$)	[109]

Table 1 (continued)

Trial name	NCT	Phase	Enrollment	Bintrafusp alfa Dose	Status	Target population	Primary tumor type	Median age (range)	Prior lines of therapy (% received ≥ 2 prior anti-cancer therapies)	Median follow-up time	Median duration of treatment	Grade ≥ 3 treatment-related AEs	Confirmed best overall response	References
MSB0011359C (M7824) in Metastatic or Locally Advanced Solid Tumors, and M7824 in Subjects With HPV Associated Malignancies	NCT02517398, and NCT03427411	I and II	59	0.3–30 mg/kg during dose-escalation or 1200 mg IV Q2W	Completed	Advanced, pretreated, checkpoint inhibitor-naive HPV-associated cancers	Cervical ($n = 33$), SCCHN ($n = 15$), anal ($n = 6$), rectal SCC ($n = 2$), vaginal ($n = 1$), vulvar ($n = 1$), neuroendocrine ($n = 1$)	56 (48–64)	66	9.2 mo	3.9 mo (range 0.5–29.9)	16/59 (27.1%)	CR 8.5% ($n = 5$), PR 22% ($n = 13$), SD 13.6% ($n = 8$), PD 45.8% ($n = 27$), NE 10.2% ($n = 6$)	[93]

Table 1 (continued)

Trial name	NCT	Phase	Enrollment	Bintrafusp alfa Dose	Status	Target population	Primary tumor type	Median age (range)	Prior lines of therapy (% received ≥ 2 prior anti-cancer therapies)	Median follow-up time	Median duration of treatment	Grade ≥ 3 treatment-related AEs	Confirmed best overall response	References
MSB0011359C (M7824) in Subjects With Metastatic or Locally Advanced Solid Tumors	NCT02699515	I	30	1200 mg IV Q2W	Completed	Asian patients with BTC whose disease progressed after first-line chemotherapy	Gallbladder cancer ($n = 12$), intrahepatic cholangiocarcinoma ($n = 10$), extrahepatic cholangiocarcinoma ($n = 7$), ampullary cancer ($n = 1$)	67 (58–69)	13	15.3 mo	8.9 wk (IQR 5.7–32.1)	11/30 (37%)	CR 3% ($n = 1$), PR 20% ($n = 6$), SD 13% ($n = 4$), PD 57% ($n = 17$), NE 7% ($n = 2$)	[88]
MSB0011359C (M7824) in Metastatic or Locally Advanced Solid Tumors	NCT02517398	I	35	1200 mg IV Q2W	Completed	Recurrent glioblastoma that progressed after radiotherapy plus temozolomide	IDH mutant ($n = 6$), 15 patients (42.9%) had a prior gross total resection	57 (28–75)	8	19.7 mo (0.8–20.5)	1.8 mo (range 0.5–20.7)	6/35 (17.1%)	PR 5.7% ($n = 2$), SD 11.4% ($n = 4$), PD 71.4% ($n = 25$), NE 5.7% ($n = 2$)	[81]

Table 1 (continued)

Trial name	NCT	Phase	Enrollment	Bintrafusp alfa Dose	Status	Target population	Primary tumor type	Median age (range)	Prior lines of therapy (% received ≥ 2 prior anti-cancer therapies)	Median follow-up time	Median duration of treatment	Grade ≥ 3 treatment-related AEs	Confirmed best overall response	References
MSB0011359C (M7824) in Metastatic or Locally Advanced Solid Tumors	NCT02517398	I	30	1200 mg IV Q2W	Completed	Post-platinum, PD-L1-unselected esophageal adenocarcinoma	Adenocarcinoma ($n = 30$)	61 (30–80)	80	86.1 wk (2.0–55.7)	N/A	7/30 (23.3%)	PR 13.3% ($n = 4$), SD 23.3% ($n = 7$), PD 40% ($n = 12$), NE 23.3% ($n = 7$)	[110]
MSB0011359C (M7824) in Subjects With Metastatic or Locally Advanced Solid Tumors	NCT02699515	I	9	3 mg/kg ($n = 3$), 10 mg/kg ($n = 6$) IV Q2W	Completed	Asian patients with metastatic or locally advanced hepatocellular carcinoma	Hepatocellular carcinoma ($n = 9$)	63 (39–71)	33.3	N/A	5.9 wk (range 2–122)	2/9 (22%)	SD 11.1% ($n = 1$), PD or NE 88.9% ($n = 8$)	[105]

M7824 = Bintrafusp alfa

AEs adverse events, *BTC* biliary tract cancer, *CR* complete response, *HPV* human papillomavirus, *IDH* isocitrate dehydrogenase, *IQR* interquartile range, *IV* intravenously, *N/A* data not available or reported, *NE* not evaluable, *NSCLC* non-small-cell lung cancer, *PD-L1* programmed cell death 1 ligand 1, *PD* progressive disease, *PR* progressive response, *Q2W* once every 2 weeks, *SCCHN* squamous cell carcinoma of the head and neck, *SD* stable disease

to localized bullous pemphigoid, asymptomatic lipase increase, colitis with associated anemia, and gastroparesis with hypokalemia). In this study, MTD was not reached, and pharmacokinetic/pharmacodynamic studies revealed peripheral PD-L1 was saturated with > 80% occupancy throughout the dosing period. In addition, all released plasma TGF-β1, -β2, and -β3 isoforms were sequestered following bintrafusp alfa administration in a dose-dependent manner, with complete sequestration of all three isoforms found for the entire dosing period at doses > 1 mg/kg. At time of publication, the study reported efficacy across all dose levels, with a recommended phase II dose (RP2D) of 1200 mg every 2 weeks, including one ongoing confirmed CR (cervical cancer), two durable confirmed PRs (pancreatic cancer; anal cancer), one near-PR (cervical cancer), and two cases of prolonged SD (pancreatic cancer, carcinoid) [80].

Bintrafusp alfa was also studied in a separate phase I, open-label trial of advanced NSCLC that had progressed following platinum-based doublet therapy or platinum-based neoadjuvant or adjuvant treatment, as well as those who had not received prior immunotherapy [85]. Here, 80 patients were randomized at a one-to-one ratio to receive bintrafusp alfa at either 500 mg or at the RP2D of 1200 mg every 2 weeks. The median follow-up in this study was 51.9 weeks, with an overall response rate (ORR) of 25.0% in the RP2D cohort (10/40 patients). Notably, at the RP2D, patients with PD-L1-positive and PD-L1-high (≥ 80% expression on tumor cells) disease had ORRs of 36.0% (10/27 patients) and 85.7% (6/7 patients), respectively. We note in this study, given the patients receipt of prior therapy, it is unclear if the increase in PD-L1-positive responses seen were in part conditional on T-cell responses elicited following their prior systemic therapy. In this study, PD-L1 status was obtained from fresh tumor biopsies within 28 days prior to first drug administration, and all patients were required to have been free of prior systemic treatment for a minimum of 28 days. The treatment was tolerated with 68.8% (55/80 patients) experiencing a TRAE (500 mg, 27/40; 1200 mg, 28/40 patients), of which the most common (experienced by ≥ 10% of patients) were pruritis (21.3%), maculopapular rash (18.8%), decreased appetite (12.5%), and asthenia (11.3%). By study close, 10% (8/80 patients) had a TRAE that led to treatment discontinuation, with no treatment-related deaths during the study [85]. This initial study in NSCLC led to a head-to-head trial of bintrafusp alfa versus pembrolizumab, named INTR@PID lung 037, as first-line treatment in patients with advanced NSCLC [86]. However, this latter trial was discontinued in January 2020 after review by an independent data monitoring committee, which showed the study was unlikely to meet its coprimary endpoints of PFS and OS. Several criticisms have risen with respect to the trial design, including it being an unblinded study and that the clinical investigators may have been largely unfamiliar with the side effect profile of bintrafusp alfa, potentially leading to early discontinuation [87].

Highlighting the broad potential for bintrafusp alfa across epithelial cancers, a separate phase I study evaluated bintrafusp alfa in Asian patients with biliary tract cancers (BTCs) who had progressed despite prior adjuvant or neoadjuvant chemotherapy [88]. In this study, bintrafusp alfa was administered at 1200 mg every 2 weeks until either confirmed PD, unacceptable toxicity, or trial withdrawal. Median follow-up time was 15.3 months, with a median duration of therapy of 8.9 months, and three patients who remained on active treatment for > 59.7 weeks. The ORR was 20%, with 7% (2/30 patients) experiencing a CR lasting > 12.5 months, 13% (4/30 patients) experiencing PRs, and 20% (6/30 patients) with SD. Similar to prior trials, the agent was generally well tolerated, with 37% (11/30 patients) experiencing a grade 3 or greater TRAE, with the most common (experienced by ≥ 10% of patients) being rash in 13% (4/30 patients) and elevated lipase in 10% (3/30 patients). However, the study did report three patient deaths possibly related to treatment: one septic shock event due to bacteremia, which led to death, as well as two cases of interstitial pneumonitis (ILD), which led to death—one of which occurred 6 months after the last bintrafusp alfa dose. The authors note these were the only cases of ILD across their entire phase I program evaluating bintrafusp alfa (NCT02699515 and NCT02517398; combined $n = 689$ as of August 24, 2019) [88]. A subsequent phase II trial (INTR@PID BTC 047, NCT03833661) for BTCs went on to evaluate bintrafusp alfa as second-line monotherapy for patients with locally advanced or metastatic biliary tract cancers who were ineligible for or for whom first-line platinum-based chemotherapy has failed. Final results showed signs of efficacy with a 10.1% ORR at 9 months of follow up, nearly double the 5.8% ORR of pembrolizumab monotherapy in a similar patient population [89, 90]. However, although single-agent activity was noted, this study did not meet its predefined endpoint. Until August 2021, bintrafusp alfa remained under investigation for BTCs as part of the phase II/III INTR@PID BTC 055 (NCT04066491) trial, evaluating front-line use of bintrafusp alfa in combination with gemcitabine and cisplatin [91]. However, this study was discontinued early following recommendations by the trial's independent data monitoring committee, who concluded the trial was unlikely to meet its primary end point of OS [92].

More recently, bintrafusp alfa has been evaluated in HPV-associated malignancies [93]. These malignancies are viewed as those with a higher yield of response, as genome-wide association studies noted a relationship between the TGF-β pathway and cervical cancer as well as HPV-positive squamous cell carcinoma of the head and neck (SCCHN) [94]. Furthermore, TGF-β receptor I is significantly overexpressed in these cancers compared with benign tissue,

and dysregulated TGF-β signaling has been associated with malignant progression of HPV-positive cervical dysplasia, as well as evidence HPV can mediate promotion of cervical cancer by attenuating TGF-β*R1* signaling required for epithelial homeostasis at early stages of viral infection [94–96]. To assess whether this population of HPV+ malignancies may be uniquely susceptible to the tandem effects of bintrafusp alfa, a post-hoc analysis of bintrafusp alfa across a combined HPV+ population was performed. This analysis included those patients treated on a phase I, open-label trial of bintrafusp alfa with heavily pretreated advanced solid tumors ($n = 43$) as well as a phase II, single-center trial of patients with advanced HPV-associated cancers ($n = 16$). Those patients within the phase I dose-escalation trial received bintrafusp alfa once every 2 weeks at doses of 0.3–30 mg/kg, whereas those on the RP2D received bintrafusp alfa at 1200 mg every 2 weeks, for a combined population of 75 patients. Across this combined cohort of heavily pretreated patients with a median follow-up of 33 months, investigators found a confirmed ORR of 28.0% ($n = 21$, 4 CRs and 17 PRs), with three additional patients achieving a delayed PR, leading to a clinical response rate of 32.0% and the suggestion further studies in this population of HPV+ malignancies may be warranted. Notably, the median duration of response was 17.3 months, and the median OS was 21.3 months, with a 12-month OS rate of 59.7%. The TRAEs were similar to prior trials, with the most common being grade 1 pruritis in 25.3% of patients and grade 1 dermatitis acneiform. No treatment-related deaths occurred [93, 97].

A third trial evaluating bintrafusp alfa in 14 patients with HPV16+ relapsed or refractory advanced cancer has also been reported. This trial incorporated a triple combination of 1200 mg bintrafusp alfa every 2 weeks with M9241, an immunocytokine composed of IL-12 heterodimers fused to a monoclonal antibody targeting free DNA proximal to necrotic tumors [98], and PDS0101 (Versamune-HPV), a liposomal multipeptide therapeutic vaccine targeting HPV 16 E6/E7 [99]. With a median follow-up of 5 months, investigators reported one CR, and nine PRs with nine out of ten responses ongoing at time of data cut off. They noted of the 14 patients, six were checkpoint naive and eight had checkpoint refractory disease. Of those with checkpoint-naive disease, five of six (83%) experienced an objective response, whereas five of the eight patients (63%) with checkpoint refractory disease experienced an objective response. The treatment combination was largely well tolerated, with no treatment-related deaths and four grade 3 TRAEs (hematuria in two patients with cervical cancer and prior pelvic radiation as well as two patients with AST/ALT elevations).

These data highlight the potential applicability of bintrafusp alfa in a focal patient population as well as the novel toxicities seen as a result of TGF-β sequestration.

3.2 Side Effects

3.2.1 Overview

Several toxicities have been identified in TGF-β inhibitors, including bintrafusp alfa. A combined cohort of 606 patients across the phase I INTR@PID 001 and 008 studies in heavily pretreated solid tumors was presented at the 2021 ESMO annual meeting [100]. TRAEs of any grade occurred in 68.3% of patients ($n = 414$), with grade ≥3 TRAEs in 22.3% of patients ($n = 135$). Out of the 606 patients, 8.7% permanently discontinued ($n = 53$) treatment because of TRAEs. The most common adverse events included TGF-β inhibition-mediated skin adverse events (any grade: 11.9%, grade ≥3: 2.6%), immune-related adverse events (any grade: 23.3%, grade ≥3: 8.9%), anemia (any grade: 30.5%, grade ≥3: 18.0%), bleeding events (any grade: 39.3%, grade ≥3: 10.2%), and infusion-related reactions (any grade: 6.3%, grade ≥3: 0.2%). Notably, the most common skin adverse events were keratoacanthomas (KAs), typically in older, light-skinned patients with a history of sun-damage, and the most common bleeding event was epistaxis. In these trials, the eligibility criteria included an exclusion for bleeding diathesis or recent major bleeding. As the majority of reported bleeding events were mild to moderate mucosal bleeding; these were clinically manageable and resolved without the need for bintrafusp alfa discontinuation. One important difference in toxicity profile noted with bintrafusp alfa is the distinct lack of significant cardiac toxicity, a concern noted with prior pan-TGF-β inhibitors [53].

3.2.2 Bleeding

Although most of the reported bleeding events were low-grade mucosal bleeding (e.g., epistaxis, gingival bleeding), there are episodes of significant and at times life-threatening bleeding (e.g., gastrointestinal hemorrhage). Bleeding from TGF-β inhibitors was identified in early studies of fresolimumab, an engineered human monoclonal Ig that neutralizes the three major isoforms of TGF-β. Studies in fifteen patients with systemic sclerosis identified two cases of clinically significant gastrointestinal bleeding from gastric antral vascular ectasia, as well as three cases of gingival bleeding and/or epistaxis with two others reporting subconjunctival hemorrhage [101]. Three patients in a separate study of fresolimumab in patients with steroid-resistant primary focal segmental glomerulosclerosis developed grade ≥3 gingival bleeding [102].

In a phase I expansion cohort of patients with recurrent glioblastoma, six patients (17.1%) experienced gingival bleeding, whereas five patients (14.3%) experienced intratumoral or intracranial bleeding events in the setting of progressive disease. The intratumoral and intracranial bleeding

events occurred between 2 and 17 days after their last dose of bintrafusp alfa, with two of the five patients concurrently receiving anticoagulation (for deep vein thrombosis prophylaxis and as maintenance following prior pulmonary embolism). Notably, all of these events occurred in new lesions attributed to progressive disease, and this rate of intracranial hemorrhage was similar to reported rates in patients with primary brain tumors receiving disease-directed treatment who are not on anticoagulation (2.6–13.6%) and are on anticoagulation (15.5–28.1%) [103, 104]. Of note, one of these intratumoral hemorrhage events did lead to a patient death and was assessed by investigators as treatment-related in conjunction with disease progression [81].

In a phase I study of bintrafusp alfa in Asian patients with advanced solid tumors, one patient with a pituitary gland tumor developed intralesional bleeding, which was attributed as probably related to treatment. Two other patients developed grade 3 upper gastrointestinal hemorrhage and pulmonary hemorrhage, respectively, although both were attributed as unrelated to treatment [105].

In an evaluation of bintrafusp alfa from phase I and II trials in cervical cancer, there were single reports of grade 3 treatment-related upper-gastrointestinal hemorrhage as well as hematuria [106]. This cohort was assessed in a larger data set of HPV-related malignancies, and across 59 patients, 38 patients (64.4%) experienced treatment-emergent bleeding, with nine patients (15.3%) experiencing grade 3 bleeding events [93].

A poster summarizing the safety profile of bintrafusp alfa (from the INTR@PID LUNG 024 study evaluating bintrafusp alfa in combination with chemotherapy) noted epistaxis in ~30 to 44% of patients experiencing treatment-emergent adverse events, depending on cohort reviewed, as well as ~33% of patients experiencing hemoptysis, with one noted as grade ≥3 [107].

At present, it remains unclear what the mechanism of toxicity is when TGF-β is inhibited. We know TGF-β does play a vital role in the homeostasis of the adult microvasculature as well as maintaining vascular barrier function and survival [13]. Similarly, we know TGF-β1 plays a key role in enhancing platelet aggregation through the activation and maintenance of the α_{11b}/β_3 fibrinogen receptor [108]. This would imply the possibility of a Glanzmann thrombasthenia–like bleeding phenomenon; however, platelet studies on patients with bleeding have not displayed marked deficits in function (internal data, includes samples from NCT02517398, pending publication). As TGF-β inhibitors move forward in their clinical application, it will be equally important to investigate the pathology of TGF-β-inhibitor–related bleeding adverse events.

3.2.3 Skin Changes

A separate but disruptive side effect noted with TGF-β inhibitors is skin toxicity, such as KAs, at times leading to drug discontinuation. In a phase I study of bintrafusp alfa monotherapy in Asian patients with BTCs, 2 of 30 patients developed KAs [88]. In a separate analysis of bintrafusp alfa monotherapy, dosed every 2 weeks, in HPV-related malignancies across two studies (NCT02517398 and NCT03427411), 12 patients (20.3%) experienced treatment-related skin lesions, of which ten (16.9%) were KAs, and another eight reported events of basal cell carcinoma, squamous cell carcinoma of the skin or lip, hyperkeratosis, and actinic keratosis. Notably, across these reported skin lesions, only four were reported as grade 3 in severity [93].

4 Ongoing Trials and Future Directions

As of September 2021, there are 42 active, ongoing, or completing trials evaluating bintrafusp alfa across a wide array of malignancies and in combination with a multitude of cancer-directed therapies, from traditional chemotherapeutics to radiation therapy to additional checkpoint inhibitors, cytokines, and vaccines (Table 2). Each of the trial experiences with bintrafusp alfa have revealed a subset of patients who experience durable clinical benefit with noted CR and PRs among each cohort. These experiences were seen across malignancies and irrespective of PD-L1 status, suggesting an opportunity to identify a predictive biomarker signature of response. Furthermore, as bintrafusp alfa has been well tolerated across studies, it remains as a readily available agent to include in combination trials—many of which are underway.

Clinical studies have demonstrated the safety and activity of therapeutic approaches simultaneously targeting the PD-1/PD-L1 pathway and TGF-β. Although several initial phase II studies of bintrafusp alfa have not met their prespecified primary endpoint or were deemed not likely to meet them, the future for combined targeting of these two pathways remains solid. Data with bintrafusp alfa in HPV-associated malignancies remain very promising. Additional understanding of the clinical implications for the complex biology of TGF-β in the TME, and which patients might benefit most, are being pursued by multiple groups.

Table 2 Active bintrafusp alfa studies

Trial name	NCT	Phase	Estimated enrollment	Bintrafusp alfa dose	Combined with	Status	Target population	Location(s)
Bintrafusp Alfa in Previously Treated Patients With Recurrent and Metastatic (R/M) Non-keratinizing Nasopharyngeal Carcinoma (NPC)	NCT04396886	II	37	N/A	N/A	Recruiting	Recurrent and metastatic non-keratinizing nasopharyngeal carcinoma	Hong Kong
Study of the Efficacy and Safety of the Bintrafusp Alfa in Previously Treated Advanced Malignant Pleural Mesothelioma (BIMES)	NCT05005429	II	47	1200 mg Q2W	N/A	Not yet recruiting	Mesothelioma, lung	Spain
A Study to Evaluate the Efficacy and Safety of Bintrafusp Alfa (M7824) Monotherapy in Metastatic or Locally Advanced Urothelial Cancer	NCT04349280	I	40	1200 mg Q2W	N/A	Recruiting	Metastatic or locally advanced urothelial cancer	USA, Canada, France, Netherlands, Spain, United Kingdom
Neoadjuvant Bintrafusp Alfa in Patients With Resectable Biliary Tract Cancer (NEO-BIL)	NCT04727541	II	24	1200 mg Q2W	Surgery	Recruiting	Biliary tract cancer, cholangiocarcinoma	Germany
Evaluation of Bintrafusp Alfa in Operable and Untreated Head and Neck Squamous Cell Carcinoma (ICING)	NCT04428047	II	59	1200 mg Q2W	N/A	Recruiting	Squamous cell carcinoma of head and neck	France
Docetaxel With or Without Bintrafusp Alfa for the Treatment of Advanced Non-small Cell Lung Cancer	NCT04396535	II	80	N/A	Docetaxel	Recruiting	Advanced non-small cell lung carcinoma	USA

Table 2 (continued)

Trial name	NCT	Phase	Estimated enrollment	Bintrafusp alfa dose	Combined with	Status	Target population	Location(s)
Bintrafusp Alfa and Doxorubicin Hydrochloride in Treating Patients With Advanced Sarcoma (TRUST)	NCT04874311	II	80	2400 mg Q3W	Doxorubicin	Not yet recruiting	Soft-tissue sarcoma	France
Bintrafusp Alfa in High Mobility Group AT-Hook 2 (HMGA2) Expressing Triple Negative Breast Cancer	NCT04489940	II	29	1200 mg Q2W	N/A	Recruiting	HMGA2-expressing triple negative breast cancer	USA, Belgium, France, Italy, Russian Federation, Spain
Bintrafusp Alfa Monotherapy in Platinum-Experienced Cervical Cancer	NCT04246489	II	146	1200 mg Q2W	N/A	Active, not recruiting	Uterine and cervical neoplasms	USA, Argentina, Australia, Belgium, Brazil, China, France, Hungary, Japan, Republic of Korea, Russian Federation, Spain
Preoperative Bintrafusp Alfa in Operable Urothelial Carcinoma of the Bladder (PEBBLE)	NCT04878250	II	49	1200 mg Q2W	N/A	Not yet recruiting	Bladder cancer	United Kingdom
Aerosolized Azacytidine as Epigenetic Priming for Bintrafusp Alfa-Mediated Immune Checkpoint Blockade in Patients With Unresectable Pulmonary Metastases From Sarcomas, Germ Cell Tumors, or Epithelial Malignancies	NCT04648826	I, II	42	2400 mg Q3W	Azacytidine (aerosolized)	Not yet recruiting	Unresectable pulmonary metastases from sarcomas, germ cell tumors, or epithelial malignancies	USA

Table 2 (continued)

Trial name	NCT	Phase	Estimated enrollment	Bintrafusp alfa dose	Combined with	Status	Target population	Location(s)
Bintrafusp Alfa Before Surgery for the Treatment of Untreated Resectable Non-small Cell Lung Cancer	NCT04560686	II	23	N/A	Surgery	Recruiting	Resectable non-small cell lung carcinoma	USA
Phase 2 Study of Bintrafusp Alfa in Recurrent/Metastatic Olfactory Neuroblastoma (BARON)	NCT05012098	II	32	1200 mg Q2W	N/A	Not yet recruiting	Olfactory neuroblastoma	USA
Phase I/II Trial of the Combination of Bintrafusp Alfa (M7824), Entinostat and NHS-IL12 (M9241) in Patients With Advanced Cancer	NCT04708470	I, II	70	1200 mg Q2W	Entinostat and NHS-IL12 (M9241)	Recruiting	Checkpoint refractory HPV associated malignancies and MSS small bowel or colorectal cancer	USA
Bintrafusp Alfa and Pimasertib for the Treatment of Patients With Brain Metastases	NCT04789668	I, II	36	N/A	Pimasertib	Recruiting	Intracranial metastases	USA
A Phase II Study of Bintrafusp Alfa (M7824) in Checkpoint Inhibitor Naive and Refractory Subjects With Urothelial Carcinoma	NCT04501094	II	75	1200 mg Q2W	N/A	Recruiting	Urothelial cancer	USA
Hypofractionated Radiation Therapy and Bintrafusp Alfa for the Treatment of Advanced Intrahepatic Cholangiocarcinoma	NCT04708067	I	15	N/A	Hypofractionated radiation	Not yet recruiting	Intrahepatic cholangiocarcinoma	USA

Table 2 (continued)

Trial name	NCT	Phase	Estimated enrollment	Bintrafusp alfa dose	Combined with	Status	Target population	Location(s)
Bintrafusp Alfa Combination Therapy in Participants With Cervical Cancer (INTR@PID 046)	NCT04551950	I	25	N/A	Cisplatin/carboplatin, paclitaxel, bevacizumab	Active, not recruiting	Cervical cancer	USA, Japan, Spain
Bintrafusp Alfa With Pemetrexed and Platinum-Based Chemotherapy for the Treatment of Locally Advanced or Metastatic Tyrosine Kinase Inhibitor-Resistant EGFR-Mutant Non-small Cell Lung Cancer	NCT04971187	II	40	N/A	Cisplatin/carboplatin, pemetrexed	Recruiting	Non-squamous EGFR-mutant non-small cell lung carcinoma	USA
Bintrafusp Alfa (M7824) in Subjects With Thymoma and Thymic Carcinoma	NCT04417660	II	38	1200 mg Q2W	N/A	Recruiting	Thymoma and thymic carcinoma	USA
Tapestry: Addition of TGF-β and PDL-1 Inhibition to Definitive Chemoradiation in Esophageal Squamous Cell Carcinoma (TAPESTRY)	NCT04595149	II	52	2400 mg Q3W	XBRT, paclitaxel, and carboplatin	Recruiting	Esophageal squamous cell carcinoma	Netherlands
Gemcitabine Plus Cisplatin With or Without Bintrafusp Alfa (M7824) in Participants With 1L Biliary Tract Cancer (BTC)	NCT04066491[a]	II, III	512	2400 mg Q3W	Gemcitabine, cisplatin	Recruiting[a]	Locally advanced or metastatic biliary tract cancer	USA, Argentina, Australia, Belgium, Brazil, Chile, China, France, Germany, Italy, Japan, Republic of Korea, Poland, Spain, Taiwan, United Kingdom
First in Human Study of M6223	NCT04457778	I	35	N/A	M6223 (TIGIT inhibitor)	Recruiting	Metastatic or locally advanced solid unresectable tumors	USA, Canada

Table 2 (continued)

Trial name	NCT	Phase	Estimated enrollment	Bintrafusp alfa dose	Combined with	Status	Target population	Location(s)
TGF-β And PDL-1 Inhibition in Esophageal Squamous Cell Carcinoma Combined With Chemoradiation Therapy (TAPESTRY)	NCT04481256	Non-randomized feasibility study	49	2400 mg Q3W	Carboplatin, paclitaxel, radiation	Recruiting	Squamous cell carcinoma of the esophagus or gastroesophageal junction	Netherlands
Immunotherapy (NHS-IL12 & Bintrafusp Alfa) and Radiation Therapy for the Treatment of Hormone Receptor Positive, HER2 Negative Metastatic Breast Cancer, the REINA Trial	NCT04756505	I	20	N/A	Immunocytokine NHS-IL12, radiation	Not yet recruiting	HR+/HER2- metastatic breast cancer	USA
Bintrafusp Alfa and Stereotactic Body Radiation Therapy for the Treatment of Recurrent or Second Primary Head and Neck Squamous Cell Cancer	NCT04220775	I, II	21	N/A	SBRT	Recruiting	Recurrent head and neck squamous cell carcinoma	USA
Phase I/II Trial Investigating the Safety, Tolerability, Pharmacokinetics, Immune and Clinical Activity of SX-682 in Combination With BinTrafusp Alfa (M7824 or TGF-beta "Trap"/PD-L1) With CV301 TRICOM in Advanced Solid Tumors (STAT)	NCT04574583	I, II	105	1200 mg Q2W	SX-682 (CXCR1/2 inhibitor), BN-CV301 TRICOM (CEA/MUC1) vaccines	Recruiting	Advanced solid tumors	USA

Table 2 (continued)

Trial name	NCT	Phase	Estimated enrollment	Bintrafusp alfa dose	Combined with	Status	Target population	Location(s)
Bintrafusp Alfa (M7824) and M9241 in Combination With Docetaxel in Adults With Metastatic Castration Sensitive and Castration Resistant Prostate Cancer	NCT04633252	I, II	86	2400 mg Q3W	Immunocytokine NHS-IL12, Docetaxel	Recruiting	Metastatic castration sensitive and castration resistant prostate cancer	USA
Bintrafusp Alfa (M7824) and NHS-IL12 (M9241) Alone and in Combination With Stereotactic Body Radiation Therapy (SBRT) in Adults With Metastatic Non-Prostate Genitourinary Malignancies	NCT04235777	I	66	1200 mg Q2W	Immunocytokine NHS-IL12, SBRT	Recruiting	Metastatic non-prostate genitourinary malignancies	USA
M7824 and Eribulin Mesylate in Treating Patients With Metastatic Triple Negative Breast Cancer	NCT03579472	I	20	N/A	Eribulin mesylate (microtubule-targeting agent)	Recruiting	Metastatic triple negative breast cancer	USA
M7824 Versus Pembrolizumab as a First-line (1L) Treatment in Participants With Programmed Death-ligand 1 (PD-L1) Expressing Advanced Non-small Cell Lung Cancer (NSCLC)	NCT03631706	III	584	1200 mg Q2W	N/A	Discontinued, closed after DSMB assessment unlikely to hit co-primary endpoint: PFS	PD-L1-expressing advanced non-small cell lung cancer	USA, Argentina, Belgium, Brazil, Canada, China, France, Germany, Greece, Hong Kong, Italy, Japan, Republic of Korea, Netherlands, Spain, Taiwan, Turkey, Ukraine
M7824 in Treating Patients With Stage II-III HER2 Positive Breast Cancer	NCT03620201	I	20	N/A	Neoadjuvant chemotherapy	Recruiting	Stage II-III HER2 positive breast cancer	USA

Table 2 (continued)

Trial name	NCT	Phase	Estimated enrollment	Bintrafusp alfa dose	Combined with	Status	Target population	Location(s)
M7824 Monotherapy in Locally Advanced or Metastatic Second Line (2L) Biliary Tract Cancer (Cholangiocarcinoma and Gallbladder Cancer)	NCT03833661	II	159	1200 mg Q2W	N/A	Active, not recruiting	Advanced or metastatic biliary tract cancer	USA, China, France, Italy, Japan, Republic of Korea, Spain, Taiwan, United Kingdom
Dose Escalation and Expansion Study of GSK3359609 in Participants With Selected Advanced Solid Tumors (INDUCE-1)	NCT02723955	I	828	N/A	Feladilimab (Inducible T cell Co-Stimulator [ICOS] receptor agonist)	Active, not recruiting	Advanced solid tumors	USA, Australia, Canada, China, France, Italy, Japan, Netherlands, Spain
Bioimaging Study of 89Zr-M7824 in NSCLC	NCT04297748	I, II	12	1200 mg Q2W	^{89}Zirconium-M7824	Recruiting	Non-small cell lung cancer	Australia
Study of M7824 and Paclitaxel Combination as a Second-line Treatment in Patients With Recurrent/Metastatic Gastric Cancer	NCT04835896	I, II	49	1200 mg Q3W	Paclitaxel	Not yet recruiting	Metastatic or locally advanced HER2 negative gastric cancer	Republic of Korea
Radiation Therapy and M7824 in Treating Patients With Metastatic Hormone Receptor Positive, HER2 Negative Breast Cancer	NCT03524170	I	24	N/A	Radiation	Active, not recruiting	HR+/HER2- metastatic breast cancer	USA
BN-Brachyury, Entinostat, Adotrastuzumab Emtansine and M7824 in Advanced Stage Breast Cancer (BrEAsT)	NCT04296942	I	65	1800 mg Q3W	MVA-BN-Brachyury (vaccine), TRICOM, ado-trastuzumab emtansine, entinostat	Recruiting	Triple negative breast cancer or ER-/PR-/HER2+ breast cancer	USA

Table 2 (continued)

Trial name	NCT	Phase	Estimated enrollment	Bintrafusp alfa dose	Combined with	Status	Target population	Location(s)
M7824 in Patients With Metastatic Colorectal Cancer or With Advanced Solid Tumors With Microsatellite Instability	NCT03436563	I, II	74	N/A	N/A	Recruiting	Colorectal cancer (or other solid tumors with microsatellite instability)	USA
MSB0011359C (M7824) in Metastatic or Locally Advanced Solid Tumors	NCT02517398	I	600	N/A	N/A	Active, not recruiting	Metastatic or locally advanced solid tumors	USA, Australia, Belgium, Canada, France, Germany, Italy, Japan, Republic of Korea, Spain, Taiwan, United Kingdom
M7824 in Combination With Chemotherapy in Stage IV Non-small Cell Lung Cancer (NSCLC)	NCT03840915[a]	I, II	70	2400 mg Q3W	Cisplatin, carboplatin, pemetrexed, paclitaxel or Nab-paclitaxel, gemcitabine, docetaxel	Active, not recruiting[a]	Stage IV non-small cell lung cancer	USA, Belgium, France
M7824 With cCRT in Unresectable Stage III Non-small Cell Lung Cancer (NSCLC)	NCT03840902[b]	II	350	1200 mg Q2W	Cisplatin, carboplatin, pemetrexed, paclitaxel, etoposide, durvalumab	Recruiting[b]	Unresectable stage III non-small cell lung cancer	USA, Argentina, Australia, Belgium, Brazil, Canada, China, Czechia, France, Germany, Japan, Republic of Korea, Netherlands, Spain, Taiwan

M7824 = Bintrafusp alfa

DSMB Data Safety Monitoring Board, *HPV* human papillomavirus, *MSS* microsatellite stable, *N/A* not applicable, *PFS* progression-free survival, *Q2W* every 2 weeks, *Q3W* every 3 weeks

[a]Study discontinued by sponsor as unlikely to meet primary endpoint as reviewed by an independent data monitoring committee (study status is as listed on ClinicalTrials.gov as of October 1, 2021)

[b]Sponsor discontinued as a low likelihood that the experimental arm would achieve superiority in efficacy versus standard of care treatment (study status is as listed on ClinicalTrials.gov as of October 1, 2021)

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Declarations

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Conflict of interest N.T. and J.G. are employees of the National Cancer Institute, National Institutes of Health. J.G. is a senior investigator on clinical studies using bintrafusp alfa. The National Cancer Institute has a cooperative research and development agreement with EMD Serono. N.T. and J.G. have no other conflicts of interest to declare that might be relevant to the contents of this manuscript.

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