Minireview

Preclinical and clinical studies of bintrafusp alfa, a novel bifunctional anti-PD-L1/TGFβRII agent: Current status

Sofia R Gameiro[1], Julius Strauss[1], James L Gulley[2] and Jeffrey Schlom[1]

[1]Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA; [2]Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA
Corresponding author: Jeffrey Schlom. Email: schlomj@mail.nih.gov

Impact Statement

Bintrafusp alfa (anti-PD-L1/TGFβRII) is a first-in-class bifunctional agent that acts both as a checkpoint inhibitor and as a "trap" of TGFβ in the tumor microenvironment (TME). This article reviews (1) recent preclinical data interrogating the mode of action of bintrafusp alfa and the potential role of TGFβ in human papillomavirus (HPV)-associated malignancies, (2) results from extended phase I and phase II cohorts of bintrafusp alfa in a range of malignancies, and (3) monotherapy and preliminary combination therapy studies in patients with HPV-associated malignancies, in which bintrafusp alfa outperforms standard-of-care therapies in both checkpoint naïve and refractory patients. These studies demonstrate the importance of the appropriate patient population in clinical studies and the potential importance of employing multiple immune modulators, each designed to engage different aspects of the immune system and the TME.

Abstract

Bintrafusp alfa (anti-PD-L1/TGFβRII) is a first-in-class bifunctional agent designed to act both as a checkpoint inhibitor and as a "trap" for TGFβ in the tumor microenvironment (TME). This article is designed to review the preclinical studies interrogating the mode of action of bintrafusp alfa and to present a comprehensive overview of recent bintrafusp alfa clinical studies. Preclinical studies have demonstrated that bintrafusp alfa immune-mediating and antitumor activity can be enhanced by combining it with a human papillomavirus (HPV) therapeutic cancer vaccine, a tumor-targeting interleukin 12 (IL-12) immunocytokine and/or an IL-15 superagonist. The importance of TGFβ in HPV-associated malignancies is also reviewed. The clinical studies reviewed span extended phase I cohorts in patients with a spectrum of malignancies, two randomized phase II studies in lung and one in biliary tract cancers in which bintrafusp alfa did not demonstrate superiority over standard-of-care therapies, and provocative results in patients with HPV-associated malignancies, where as a monotherapy, bintrafusp alfa has shown response rates of 35%, compared to overall response rate (ORR) of 12–24% seen with other Food and Drug Administration (FDA)-approved or standard-of-care agents. This article also reviews preliminary phase II study results of patients with HPV$^+$ malignancies employing bintrafusp alfa in combination with an HPV therapeutic vaccine and a tumor-targeting IL-12 immunocytokine in which the combination therapy outperforms standard-of-care therapies in both checkpoint naïve and checkpoint refractory patients. This review thus provides an example of the importance of conducting clinical studies in an appropriate patient population – in this case, exemplified by the role of TGFβ in HPV-associated malignancies. This review also provides preclinical and preliminary clinical study results of the combined use of multiple immune-modulating agents, each designed to engage different immune components and tumor cells in the TME.

Keywords: Bintrafusp alfa, HPV$^+$ malignancies, HPV-associated malignancies, combination therapies, TGF-β, checkpoint inhibition, immunotherapy, HPV vaccine, NHS-IL12 immunocytokine, anti-PD-L1/TGFβRII

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Introduction

Targeting tumor immune suppressive networks such as programmed death 1 (PD-1) and its ligand 1 (PD-L1) has achieved unprecedented clinical responses in multiple indications.[1] The PD-1/PD-L1 axis is a key component of tumor-induced NK and T cell immunosuppression, resulting in reduced antitumor responses.[1,2]

Antibodies targeting the PD-1/PD-L1 axis have demonstrated effective and durable survival benefit across malignancies, including for subsets of patients with Merkel cell carcinoma, melanoma, and lung, urothelial, head and neck, and renal cell cancers, among others.[3] However, most patients with solid malignancies do not respond to anti–PD-1/PD-L1 targeting antibodies, underscoring the urgent need for novel therapies able to improve clinical response.[1]

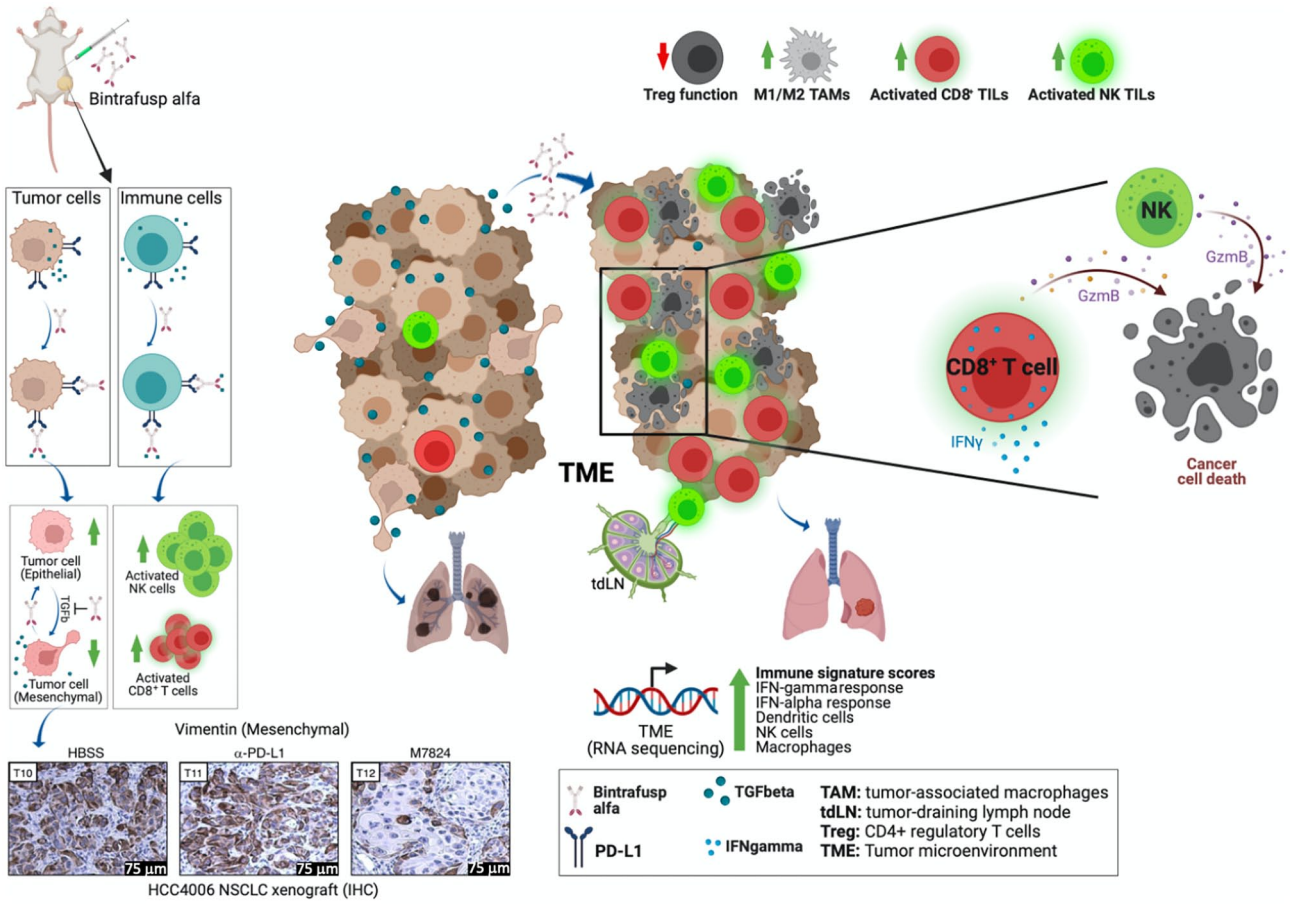

Figure 1. Bintrafusp alfa proposed mode of action from preclinical studies.
Vimentin immunohistochemistry adapted from the study by David *et al.*,[7] with permission. Scale bar, 75 µm. Figure created in *BioRender.com.*

One strategy is to simultaneously target additional immunosuppressive pathways in the tumor microenvironment (TME), such as transforming growth factor-β (TGFβ).

TGFβ is a pleiotropic cytokine with multiple cellular functions in normal physiology, including suppression of tumor initiation, and enforcement of immune homeostasis and tolerance.[4,5] Dysregulation in TGFβ signaling underlies inflammatory diseases and promotes tumor initiation. TGFβ can drive tumor progression by fostering angiogenesis, stromal modification, epithelial-to-mesenchymal transition (EMT), and suppression of innate and adaptive immunity.[4–7] In non-small cell lung cancer (NSCLC) cell lines, TGFβ was shown to induce Smad2-dependent upregulation of PD-L1 transcription and protein expression. This association between TGFβ/Smad2 signaling and PD-L1 expression was further corroborated by immunohistochemistry analysis of 72 evaluable NSCLC tumor samples.[7] TGFβ overexpression in advanced malignancies has been correlated with poor clinical outcome and resistance to immune and other therapies.[5,8] To date, clinical studies with agents targeting TGF-β including both TGF-β receptor I (TGF-βRI) small molecule inhibitors and anti-TGF-β neutralizing monoclonal antibodies (MAbs) have seen limited efficacy.[9,10]

Simultaneous targeting of PD-L1 and TGFβ constitutes a rational approach as both represent key immunosuppressive and tumor-promoting pathways with complementary functions. Thus, dual targeting of PD-L1 and TGFβ may result in increased tumor control relative to single-targeting therapeutic approaches.

Bintrafusp alfa, previously known as M7824, is a novel first-in-class bifunctional immune checkpoint inhibitor encompassing the C-terminus of an IgG1 anti-PD-L1 antibody, covalently linked to the extracellular domain of two TGF-βRII molecules.[11] Bintrafusp alfa was developed via a Collaborative Research and Development Agreement between the National Cancer Institute and EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, an affiliate of Merck KGaA.

The mode of action of bintrafusp alfa on tumor cells, and various subsets of immune cells, both in the periphery and in the TME, has been the subject of numerous preclincial studies. The fact that bintrafusp alfa binds to both human and murine PD-L1, as well as to human and murine TGFβ isoforms, has enabled studies in both human and murine *in vitro* analyses as well as *in vivo* studies in syngeneic murine models and in humanized mice bearing human tumor xenografts and reconstituted with human peripheral blood mononuclear cells (PBMCs).[7,11–19] Key preclinical findings are highlighted in Figure 1.

Bintrafusp alfa has been the subject of two prior review articles.[20,21] This article will review more recent data on the mechanism of action of bintrafusp alfa not included in those

articles, as well as recent clinical trial results. Both preclinical and clinical studies will describe the use of bintrafusp alfa as a monotherapy and in combination therapies.

Bintrafusp alfa in preclinical studies

Bintrafusp alfa alleviates tumor mesenchymal features and therapy resistance

TGFβ pathway dysregulation in human cancers denies its tumor suppressor ability in support of tumor progression, invasion, and metastatic dissemination.[22] In this context, TGFβ is a potent inducer of EMT, promoting tumor cell loss of epithelial features toward a mesenchymal state translated by altered morphology, loss of cell polarity and downregulation of cell–cell adhesions. As a result, tumor cells acquire increased migratory and invasive properties and heightened resistance to anticancer modalities, including chemotherapies, radiation, and immunotherapy.[5,23,24] In addition, transcription factors involved in EMT can promote tumor TGFβ expression and secretion resulting in a positive feedback loop supporting their mesenchymal state while inducing EMT in neighboring tumor cells.[5,24,25] However, novel agents targeting drivers of EMT can reverse these TGFβ-mediated effects and sensitize tumors to immune-mediated attack.[23,24]

Recent *in vitro* and *in vivo* studies demonstrated the ability of bintrafusp alfa to suppress TGFβ1-induced EMT and immune resistance in human NSCLC cells and xenograft tumor models.[7,20] *In vitro*, bintrafusp alfa inhibited TGFβ1-induced downregulation of epithelial proteins including E-cadherin and occludin, as well as the upregulation of mesenchymal markers such as fibronectin, vimentin, and Snail. Bintrafusp alfa–mediated sequestration of TGFβ not only alleviated but also reversed TGFβ-induced EMT and chemotherapy resistance. In these studies, TGFβ-induced PD-L1 upregulation enhanced NSCLC cell sensitivity to NK-facilitated antibody-dependent cytotoxicity (ADCC) mediated by bintrafusp alfa and PD-L1 targeting MAbs. The ability of bintrafusp alfa to mediate ADCC has also been demonstrated *in vitro* against a spectrum of additional human carcinoma cell lines, including of bladder, cervical, and triple-negative breast (TNBC) origin.[12,14] In one study, increased ADCC was also observed in cisplatin-resistant urothelial cancer cells treated with bintrafusp alfa.[12] Notably, ADCC lysis mediated by bintrafusp alfa was shown to require binding to PD-L1. Moreover, bintrafusp alfa has been shown to increase the sensitivity of human urothelial cancer cells to TRAIL and CEA-specific $CD8^+$ T cell–mediated lysis.[12] Heightened immune cell recognition and urothelial cancer cell sensitivity to immune-mediated lysis was associated with signs of immunogenic modulation elicited by bintrafusp alfa, including increased ICAM-1, CEA, PD-L1, and Fas cell-surface protein expression.[12] Transcriptome analysis further dissected the immunomodulatory effects elicited by simultaneous blockade of PD-L1 and TGFβ sequestration.[12] Relative to anti-PD-L1 exposure, bintrafusp alfa upregulated multiple genes associated with immune trafficking/chemotaxis (CCR2, CXCL11) and neutrophil degranulation (ANPEP, CFP), while dampening expression of genes associated with angiogenesis (CHRNA7, NOS3), extracellular matrix (ECM2), tumor aggressiveness, and metastases (ITGB6, PLA2G10, SOX17).[12]

Tumor targeting ability of bintrafusp alfa

Preclinical studies in syngeneic models of cancer have validated the bifunctional targeting effects of bintrafusp alfa. *In vivo*, bintrafusp alfa has been shown to sequester plasma TGFβ, localize to the TME via PD-L1 binding, decreasing TGFβ levels and SMAD2 signaling in the TME.[11,15,26] Molecular imaging of bintrafusp alfa radiolabeled with ^{89}Zr in breast tumor-bearing mice indicated ^{89}Zr-Df-bintrafusp alfa to display nanomolar affinity binding to PD-L1 *in vivo*, comparable to that of the ^{89}Zr-labeled PD-L1 targeting antibody avelumab.[26] Biodistribution analysis of ^{89}Zr-Df-bintrafusp alfa indicated a similar *in vivo* pattern to that of other anti-PD-L1 antibodies. Studies in the presence of competing non-radiolabeled antibodies suggest that the biodistribution of bintrafusp alfa is mainly determined by its PD-L1 binding. Notably, imaging analysis 2 and 7 days after ^{89}Zr-Df-bintrafusp alfa administration demonstrated significant tumor uptake and highly favorable tumor-to-blood ratios.

Antitumor efficacy and immune-mediated effects of bintrafusp alfa

The tumor-targeted ability of bintrafusp alfa demonstrated *in vivo* in syngeneic models of breast and colorectal carcinomas translated into significant antitumor efficacy, reduction of spontaneous breast metastases in the lung, and increased survival relative to the PD-L1-targeting antibody avelumab or a TGFβ sequestering agent. Moreover, bintrafusp alfa elicited long-term protective memory against tumor rechallenge.[11,15]

The PD-1/PD-L1 axis is a negative regulator of T cell and NK cell function.[22,27–29] Whereas the immunosuppressive effects of PD-1/PD-L1 interaction on T cells have been well characterized, evidence of PD-1 expression in human NK cells has recently emerged across tumor types, correlating with poor prognosis in some cases.[29–31] $PD\text{-}1^+$ NK cells display suppressed cytotoxicity and antitumor immunity.[29] High tumor PD-L1 expression inhibits both T cell and NK cell responses and has been associated with poor clinical outcome.[27,29,32]

In carcinoma patients, elevated plasma TGFβ1 has been correlated with impairment of T cell as well as NK cell function via downregulation of the NK-activating receptor NKG2D.[4,33] Murine and human NK cell exposure to TGFβ1 has been shown to negate direct NK cell cytotoxicity, ADCC function, and IL-15-induced NK activation via reduction of metabolic and proliferative activity and downregulation of multiple NK cell receptors.[14,28] *In vivo*, deletion of TGFβRII on NK cells rescued its cytotoxicity activity in response to IL-15. Furthermore, suppression of TGFβ signaling in NK cells was shown to augment the ability of NK cells to limit metastases in preclinical tumor models.[28] Recent *in vitro* studies have shown the ability of bintrafusp alfa but not anti-PD-L1 to alleviate TGFβ1-mediated lytic capacity of endogenous human NK cells and a human activated NK (haNK) cell line. Moreover, bintrafusp alfa rescued the lytic ability of human

NK cells in response to IL-15 stimulation and alleviated the immunosuppressive activity of human regulatory T cells on $CD4^+$ T cell proliferation.[14]

In non-tumor bearing syngeneic mice, PD-L1 blockade and TGFβ sequestration elicited by bintrafusp alfa induced significant activation and proliferation of splenic $CD8^+$ T cells and NK cells, with their increased localization to regional lymph nodes.[15] In syngeneic tumor models, the antitumor effects of bintrafusp alfa were shown to be dependent on both NK cells and $CD8^+$ T cells. Tumor-bearing mice treated with bintrafusp alfa displayed $CD8^+$ tumor-infiltrating lymphocytes (TILs) with increased activation, proliferation, effector/effector memory phenotype, and an increased IFNγ-producing population. Tumor transcriptome analysis further revealed increased immune signature scores suggestive of an inflamed TME, including IFNγ and IFNα responses.[11] Similarly, NK TILs displayed increased activation and cytotoxic phenotype.[11,15] Additional studies using a NSG-$\beta2m^{-/-}$ mouse strain humanized with PBMCs harboring human bladder, cervical, and TNBC tumor xenografts demonstrated signs of tumor control elicited by bintrafusp alfa (Figure 2). This was associated with significant TGFβ sequestration in both the periphery and the TME. Furthermore, analysis of bladder xenografts demonstrated tumor control to be immune-mediated, and associated with increased presence of $CD8^+$ and $CD4^+$ TILs, including IFNγ-producing T cells, marked increase in innate-like $NKG2D^+$ $CD8^+$ TILs, and higher frequency of $CD8^+$ TILs co-expressing NKG2D, IFNγ, and Granzyme B.[17] Overlapping findings were observed in the TNBC MDA-MB-231 xenograft model. Transcriptome analysis of bladder and cervical xenograft tumors indicated that bintrafusp alfa increased immune activation scores. Collectively, these preclinical findings highlight the ability of bintrafusp alfa to promote T cell and NK cell functions, promoting a pro-inflammatory TME conducive to tumor control.

Bintrafusp alfa in combination therapies

Recent preclinical studies have examined the potential of bintrafusp alfa in combination with other immunotherapies and additional treatment modalities. In a syngeneic model of orthotopic breast cancer, bintrafusp alfa elicited superior tumor control and survival relative to TGFβ- or PD-L1-targeted therapies when combined with an adenoviral therapeutic cancer vaccine targeting a transcription factor (Ad-Twist1) involved in metastatic progression.[15] Treatment of syngeneic mice harboring orthotopic murine $Her2^+$ breast tumors with bintrafusp alfa in combination with the class I HDAC inhibitor Entinostat, Ad-Twist1 vaccine, and Adotrastuzumab emtansine (T-DM1) elicited significant tumor control, superior to all single, double, and triple agent combinations. The antitumor effects of tetra therapy were associated with induction of high levels of antigen-specific T cell responses, elevated tumor $CD8^+$ T cell/regulatory $CD4^+$ T (Treg) cells ratio, and increased presence of $CD8^+$ T cells producing IFNγ and/or TNFα. Similar effects were observed in tumor $CD4^+$ effector T cells.[34]

In preclinical models of breast and lung cancer, combination therapy with bintrafusp alfa and the CXCR1/2 inhibitor SX-682 elicited synergistic tumor control. The antitumor effects of combination therapy were associated with decreased tumor mesenchymal features and increased epithelial markers such as E-cadherin while significantly increasing NK cell tumor lysis. Furthermore, combination therapy decreased the presence of suppressive granulocytic myeloid-derived suppressor cells (G-MDSCs) while promoting T cell infiltration in the TME.[13] Elegant preclinical studies have also demonstrated the synergistic antitumor effects of bintrafusp alfa in combination with chemotherapy.[11] In a recent report, combination therapy with bintrafusp alfa and radiation therapy was shown to modulate tumor stroma, and reprogram the TME to a more inflamed milieu, leading to increased survival in multiple therapy-resistant, immune-poor murine tumor models.[16]

Preliminary findings in our laboratory suggest that bintrafusp alfa can attain superior antitumor effects in combination with cytokine therapy (Figure 3). Preclinical studies with concurrent administration of bintrafusp alfa and NHS-IL12 led to complete eradication of immune-poor murine breast tumors and development of protective memory (Figure 3(A) and (B)). In addition, neoadjuvant treatment of an immune-poor orthotopic TNBC tumor model with bintrafusp alfa and the IL-15 superagonist N-803 promoted significant reduction of lung metastases burden, not observed with single agent therapy (Figure 3(C)).

Clinical applications of bintrafusp alfa

While the preclinical evaluation of bintrafusp alfa, as detailed above, demonstrated the antitumor effects as a monotherapy and more importantly in combination therapeutic regimens, the clinical evaluation of bintrafusp alfa is demonstrating mixed results; this may be due to the type of cancer being evaluated, and the trial design such as enrollment and endpoint criteria. Here we will outline the results obtained in recent and ongoing clinical trials.

The phase 1 study of bintrafusp alfa was a 3 + 3 dose escalation design in heavily pretreated patients (n = 19) with advanced solid tumors. Patients received 1, 3, 10, or 20 mg/kg every 2 weeks until confirmed progression or unacceptable toxicity. The primary endpoint was safety and maximum tolerated dose (MTD) with secondary endpoints of immunogenicity, response, and pharmacokinetics.[35] Bintrafusp alfa demonstrated saturation of peripheral PD-L1 and sequestered all TGFβ1, β2, and β3 at doses greater than 1 mg/kg throughout the dosing period. There was evidence of clinical activity at different dose levels; this included one durable complete response (CR; cervical cancer), two durable partial responses (PRs; anal, pancreatic), one near PR (cervical), and two prolonged stable disease (SD; pancreatic, carcinoid). The MTD was not exceeded in the phase 1 study. Treatment-related adverse events (TRAEs) ≥ Grade 3 occurred in 4 out of 19 patients. The clinical activity seen in the HPV-associated cancers was the rationale for phase 2 studies in that patient population and the extremely encouraging clinical responses as both a bintrafusp alfa monotherapy and in combination therapies, as will be detailed below.

Extended phase 1 cohorts were then carried out in a range of advanced malignancies. Patients with post-platinum

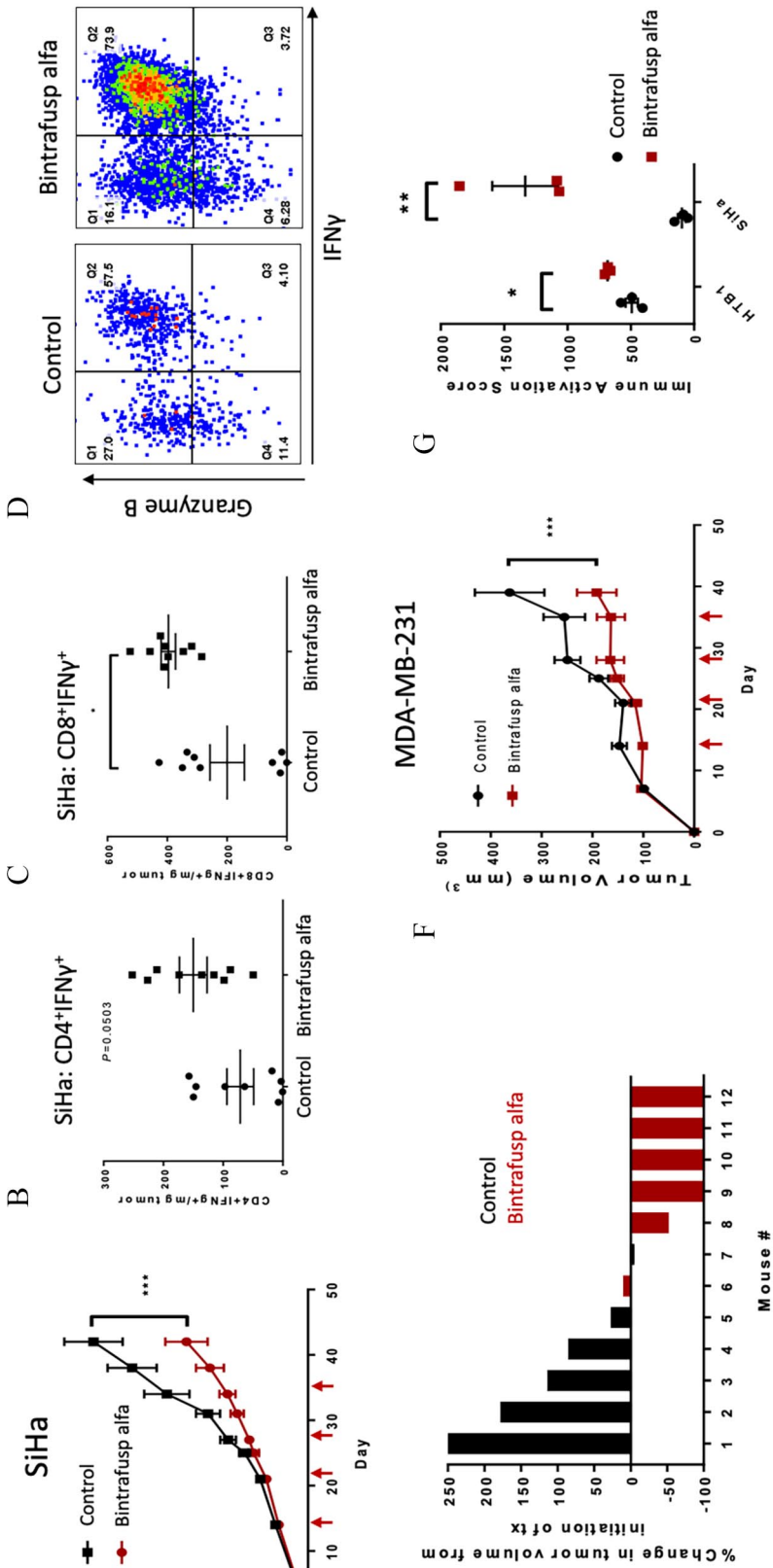

Figure 2. Analysis of bintrafusp alfa activity in humanized NSG-β2 $m^{-/-}$ mice bearing human tumor xenografts and reconstituted with human PBMC. Bintrafusp alfa elicits robust antitumor effect in the HPV^+ SiHa cervical and MDA-MB-231 triple-negative breast cancer models. (A) Average SiHa tumor volumes from control or bintrafusp alfa-treated PBMC humanized mice. (B, C) Tumor infiltrating $CD4^+$ (B) and $CD8^+$ (C) IFNγ-producing T cells per milligram tumor weight at day 21 post-initiation of treatment. (D) Representative dot plots from the tumor of control and bintrafusp alfa-treated mice bearing SiHa tumors. IFNγ is represented on the *x*-axis and Granzyme B on the *y*-axis. (E) Waterfall plot of the percentage change in SiHa tumor volume from the initiation of treatment. (F) Average MDA-MB-231 tumor volumes from control-IgG1 or bintrafusp alfa-treated PBMC humanized mice. (G) Immune activation score derived from gene expression profiles from HTB-1 and SiHa tumors from control and bintrafusp alfa-treated mice. Adapted from the study by Morillon *et al.*,[17] with permission.

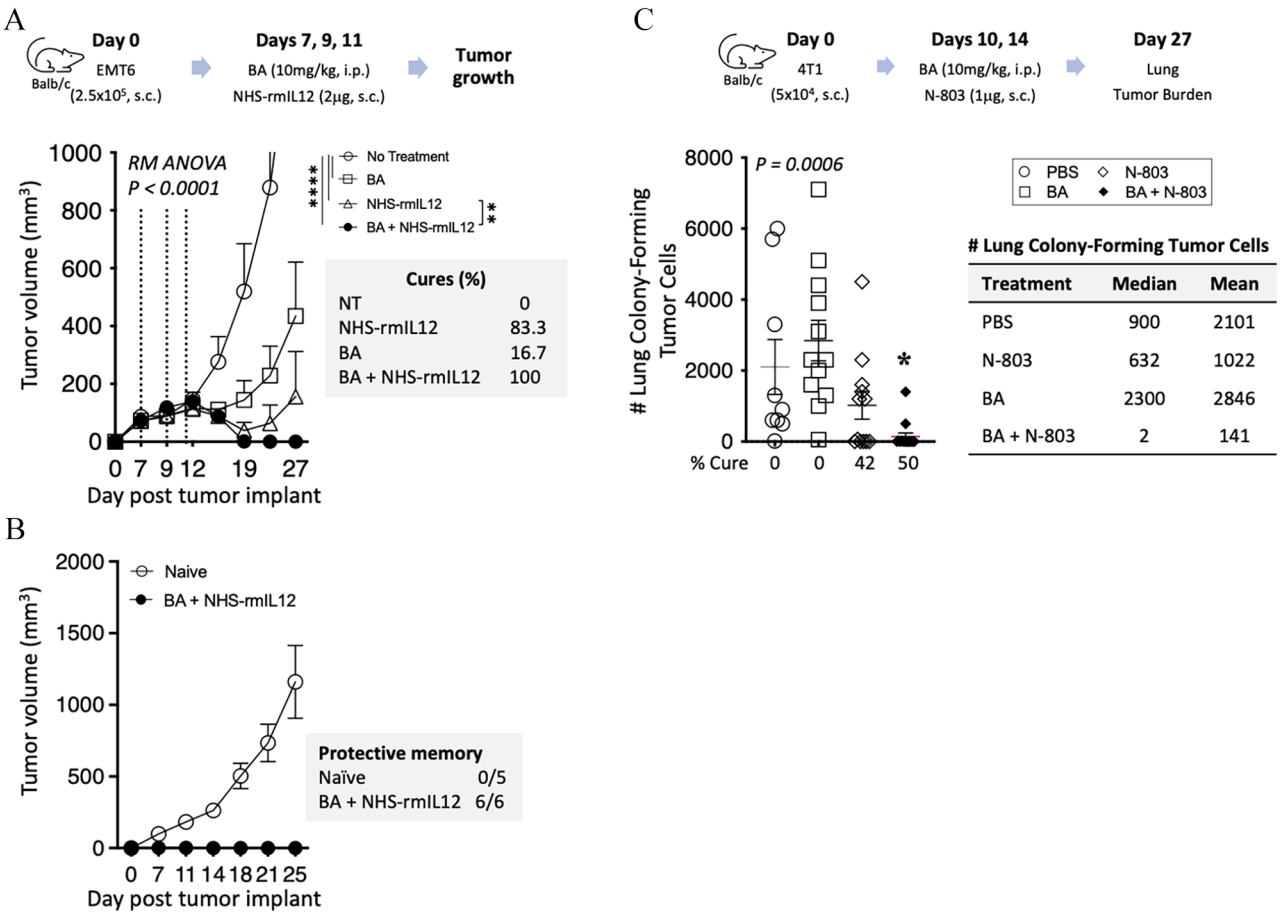

Figure 3. Bintrafusp alfa promotes synergistic antitumor effects in combination with cytokine therapy. (A) Balb/c female mice orthotopically implanted with EMT6 murine breast tumor cells were treated with bintrafusp alfa (BA), NHS-rmIL12, or both. Graph depicts tumor growth with dotted lines indicating treatment days. Table indicates percentage of cured mice. (B) At least 1 month after tumor resolution, naïve and combination therapy-cured mice were challenged with EMT6 as before. Table shows number of mice/cohort attaining complete tumor rejection. (C) Balb/c female mice orthotopically implanted with 4T1 triple-negative murine breast tumor cells were treated with bintrafusp alfa (BA), N-803, or both. Graph depicts the number of lung colony-forming 4T1 tumor cells enumerated *ex vivo*. Table shows median and mean numbers of colony-forming 4T1 cells present in murine lungs at day 27 post tumor implant. (A color version of this figure is available in the online journal.)

PD-L1 unselected esophageal adenocarcinoma received 1200 mg every 2 weeks until unacceptable toxicity or progression. The majority of patients (80%) had two or more prior anticancer regimens. The investigator-assessed and independent review–confirmed overall response rate (ORR) was 13.3% and 20%, respectively, with 83% of responses occurring in tumors with an immune-excluded phenotype.[36] Nineteen patients (63%) had TRAEs, with seven having grade 3 events. In a cohort in Asia in patients with esophageal squamous cell carcinoma investigator-assessed confirmed ORR rate and independent review, ORR was 20% and 10%, respectively.[37] Interestingly, all responses occurred in immune-excluded tumors. Most (77%) patients had two or more prior anticancer regimens; 7 out of 30 patients had grade 3/4 TRAEs. There were three grade 3 and one grade 4 TRAEs. In the cohort of Asian patients with heavily pretreated recurrent or refractory gastric cancer, there was an objective response rate of 16% and a disease control rate of 26%. Responses occurred irrespective of tumor PD-L1 expression.[38] Six patients (19%) experienced grade 3 TRAEs. In the phase 1 cohort of 32 patients with advanced squamous cell carcinoma of the head and neck (SCCHN; both HPV positive and negative), per immune RECIST criteria there were four PRs and four SDs with a control rate of 34%. Per investigator reports, there were five PRs, including two patients with delayed PRs. Reponses were in both HPV positive (33%) and HPV negative (5%) tumors. Eleven patients had grade 3 TRAEs.[39] In 35 patients with recurrent glioblastoma in the phase 1 extension, 23% experienced disease control. In patients (n = 6) with IDH-mutant glioblastoma, the disease control rate (DCR) was 66.7%. In all patients, disease control was seen regardless of PD-L1 expression. Six patients experienced TRAEs of ⩾ grade 3.[40]

Preliminary data from randomized clinical trials

There have been three randomized studies including bintrafusp alfa that were discontinued in 2021. These included two studies in lung cancer and one in biliary tract cancer (BTC). The first lung cancer study was based on data from an 80-patient expansion cohort in the phase 1 study of bintrafusp alfa. In that study, at the recommended phase 2 dose (n = 40), the ORR was 25% with 36% in PD-L1$^+$ and six of seven in PD-L1 high.[41] This led to a randomized phase 2 study of bintrafusp alfa versus pembrolizumab in patients with PD-L1 high NSCLC. While data from that study is not

publicly available (publication in preparation), the IDMC recommended discontinuation of the study because it was unlikely to meet the primary (superiority) endpoint.[42] It should be cautioned that this does not necessarily mean that this is an inactive drug, merely that it was not likely to be better than the most widely active and successful immunotherapy drug ever developed. In addition, in light of the clinical data described above in the various expansion cohorts of the phase 1 trial, one could postulate that the more appropriate patient population for bintrafusp alfa to be compared with pembrolizumab would be in patients with PD-L1 low/negative tumors. A second NSCLC study with concomitant chemoradiation in unresectable stage III NSCLC was also stopped. The estimated primary completion date was estimated to be mid-2024; however, the sponsor decided to discontinue the study due to a low likelihood the experimental arm would achieve superiority over the control arm with durvalumab.

A third randomized study in BTC was based on a small phase 1 expansion cohort in BTC.[43] This cohort enrolled 30 patients and the ORR was 20% with 6-month and 12-month PFS of 32% and 24%, respectively. This led to a randomized study of gemcitabine and cisplatin with or without bintrafusp alfa. This study was also stopped early when the IDMC recommended closing the study as it was not likely to meet the endpoint of improvement in overall survival (OS).

The three randomized studies described above were stopped early for futility, but not necessarily because the agent did not have clinical activity. In addition, the relative activity might be dependent on the underlying biology of the tumor. Indeed, TGF-β appears to restrain proliferation in the basal layer of papillomas in recurrent respiratory papillomatosis, and HPV-driven neoplastic process that is noninvasive (but can be deadly due to local growth).[44] However in HPV-driven invasive cancers, bintrafusp alfa has showed promising activity compared with standard-of-care therapies (see below). Thus understanding the biology of the tumor and developing appropriate biomarkers are key to understanding how best to use agents like bintrafusp alfa.

Bintrafusp alfa and HPV-associated malignancies

As outlined above, the phase 1 study of bintrafusp alfa showed appreciable clinical activity in patients with advanced HPV-associated cancers.[35] From the mechanistic point of view, there is indeed a rationale for employing a dual PD-L1/TGFβR1 targeting agent in this patient population. For example, it has been previously shown that the HPV E6 and E7 oncoproteins induce activation of the TGFβ promoter in cervical cancer cell lines.[45] RNAseq analyses of HPV$^+$ oropharyngeal SCC revealed an enriched TGFβ gene signature in patients with a poor prognosis.[46] Genome-wide association studies showed that HPV$^+$ SCCHN and cervical cancer are associated with the TGFβ pathway[47] and the TGFβ receptor 1 is overexpressed in both these cancers. Moreover, SCCHN patients with HPV$^+$ tumors having a specific polymorphism in TGFβ1 were shown to have a better OS compared to patients with the common genotype.[48]

A recent publication reported on 59 HPV$^+$ checkpoint naïve patients treated with bintrafusp alfa.[49] The primary endpoint was best overall response per RECIST criteria. The confirmed objective response rate by RECIST criteria was 30.5%, including five CRs. Eight patients had stable disease for a disease control rate of 44.1%. In addition, three patients had a delayed PR following initial progression, indicating a clinical response rate of 35.6%. In addition, a patient with vulvar cancer had an unconfirmed response. Sixteen patients had grade 3/4 TRAEs; there were no treatment-related deaths. It should be noted that PD1 inhibitors nivolumab and pembrolizumab evaluated in patients with HPV$^+$ malignancies have demonstrated ORRs ranging from 12–24%.[50–55] Pembrolizumab was FDA approved for PD-L1$^+$ cervical cancer with a response rate of 14.6%.[51]

The development and employment of prophylactic vaccines targeting HPV, via the induction of HPV-specific antibodies, have met with enormous success; HPV-associated malignancies, however, continue to be a major public health problem both in the United States and worldwide with 44,000 new cases annually in the United States and over 630,000 new cases of HPV-related cancers reported annually worldwide. While the field of the development of HPV therapeutic vaccines, aimed at induction and for activation of HPV-specific T cell responses, is extremely active, these vaccines have met with limited success as a monotherapy for the treatment of advanced carcinoma lesions.[56] Preclinical studies were undertaken in our program to combine bintrafusp alfa with an HPV peptide-based vaccine.[19] This DOTAP formulated vaccine, designated PDS0101, has previously been evaluated in a phase 1 trial of cervical intra-epithelial neoplasia (CIN).[57] In addition, preclinical studies also evaluated the addition of a tumor targeting IL-12 immunocytokine, designated NHS-IL12, with both the vaccine and/or bintrafusp alfa.[19] The NHS-IL12 agent was employed with the rationale of activating T cells in the TME.[58,59] This agent had also recently been evaluated in a phase 1 study.[60]

Table 1 shows the antitumor activity of these agents as monotherapies, in doublet combinations and with all three combined.[19] While the doublets increase activity, the best antitumor responses are seen with the triplet combination. T cell clonality in the TME was also evaluated for each group. As is seen in Table 1, while some agents/combinations either increase or decrease clonality, the best increase in T cell clonality in the TME is observed in the use of the triplet. These studies[19] and others provided the rationale for the use of these three agents in combination in clinical studies.

The results of an ongoing phase 2 study combining bintrafusp alfa, the HPV therapeutic vaccine designated PDS0101, and NHS-IL12 in patients (n = 24) with HPV$^+$ malignancies were recently reported (Table 2).[61] There was an ORR of 42% (tumor reduction 54%) in all patients with advanced HPV$^+$ disease. The ORR was 83% in patients who were HPV16$^+$ checkpoint naïve, and an ORR of 42% (tumor reduction 58%) in patients (n = 12) with checkpoint refractory HPV16$^+$ disease. With a median follow-up of 8 months in the ongoing phase 2 study of the triplet, 80% of responses are ongoing with 6/6 (100%) of patients with checkpoint inhibitor naïve disease alive, and 10/12 (83%) of patients with HPV16$^+$ checkpoint refractory disease still alive.[61] It should again be noted that while these results are preliminary, prior studies have reported ORR of 5–10% or less in HPV$^+$ patients with checkpoint refractory disease.[61]

Based on the findings described above, we believe future clinical studies involving bintrafusp alfa should consist of three

Table 1. Combination immunotherapy for HPV$^+$ tumors.

Treatment	No. of mice with tumor volume $< 300\,mm^3$	T cell clones per 25% of TCR repertoire (average)
PBS control	0/16	18
R-DOTAP	0/8	ND
PDS0101	3/16	12
Bintrafusp alfa	0/16	22
NHS-IL12	6/16	25
PDS0101 + bintrafusp alfa	5/16	8
PDS0101 + NHS-IL12	10/16	6
NHS-IL12 + bintrafusp alfa	8/16	18
PDS0101 + NHS-IL12 + bintrafusp alfa	13/17	3

Taken from the study by Smalley Rumfield *et al*.[19]
ND: not determined; PBS, phosphate buffered saline; R-DOTAP, (R)-1,2-dioleoyl-3-trimethylammonium-propane; PDS0101, Versamune-HPV, a liposomal HPV-126 E6/E7 multipeptide vaccine from PDS Biotech; TCR, T-cell receptor.

Table 2. Phase II trial in patients with HPV$^+$-associated malignancies (HPV therapeutic vaccine (PDS0101) plus bintrafusp alfa plus NHS-IL12).

Status of results of ongoing phase II study in patients with HPV16$^+$ disease		
	ORR (%)	Tumor reduction (%)
All patients with HPV16$^+$ disease	56	67
Checkpoint inhibitor naïve HPV16$^+$ disease	83	83
Checkpoint inhibitor refractory HPV16$^+$ disease	42	58
After a median of 8-month follow-up: 80% responses ongoing 6/6 (100%) alive in checkpoint naïve disease 10/12 (83%) alive in checkpoint refractory disease Accrual is ongoing		

Taken from ASCO 2021 abstract 2501 [study by Jackisch *et al*.[62]].
ORR: overall response rate.

paths: (1) a randomized trial employing bintrafusp alfa versus standard of care in checkpoint naïve patients with HPV-associated malignancies; (2) expansion of the ongoing trial employing the triplet of bintrafusp alfa plus NHS-IL12 plus the PDS HPV therapeutic vaccine (PDS Biotech, Florham Park, NJ) in checkpoint refractory patients with HPV-associated malignancies; and (3) studies in checkpoint refractory patients in other malignancies such as NSCLC employing bintrafusp alfa alone or in combination therapy.

Route of administration

MAbs are used in the treatment of a wide range of malignancies. Virtually all checkpoint inhibitor agents thus far approved by the FDA for a specific indication(s) or in experimental use are MAbs and are administered intravenously (i.v.). Several recent studies, however, have shown that when MAbs are administered as subcutaneous (s.c.) injection they are just as effective and safe as when given intravenously. For example, in a phase 3 randomized trial, patients with ERBB2$^+$ early breast cancer received trastuzumab either i.v. (n = 297) or s.c. (n = 294). There was comparable safety and efficacy for both routes.[62] In another example, patients with advanced solid tumor malignancies received anti-PD-L1 MAb PF-06801591 across different dose levels via either s.c. or i.v. route, with similar antitumor activity and tolerability of both routes.[63] Other examples of comparability of results with i.v. versus s.c. routes have been reported.[64–66] Since bintrafusp alfa is a bifunctional agent combining both checkpoint inhibition and anti-TGFβ activities, preclinical studies were carried out to evaluate the mode of action and antitumor activity when bintrafusp alfa was administered in murine models systemically (intraperitoneal) or via the s.c. route.[18] These studies demonstrated that when bintrafusp alfa was administered either s.c. or systemically at five different dose levels, there was comparable antitumor activity at each dose level with either route used (Figure 4). Moreover, analyses of the TME for the presence of CD8$^+$ or CD4$^+$ T cells, Tregs, or MDSCs, either monocytic or granulocytic, showed similar phenotypes employing route of bintrafusp alfa administration. Sequestration of soluble TGFβ in the periphery was also shown to be similar with either route.[18] These studies provide the rationale for the evaluation of bintrafusp alfa as a s.c. administered agent in clinical studies. There are clearly several reasons to administer any MAb, including a bifunctional agent such as bintrafusp alfa via the s.c. route: (1) convenience for the patient not dealing with an i.v. infusion, (2) convenience for the health-care provider, and for the institution in the preparation of the infusion, (3) extra cost associated with an infusion, and (4) the ability for the s.c. agent to be given by the local caregiver versus a hospital or inpatient setting. An additional point should also be considered involving administration of bintrafusp alfa. Cytokines have been shown to have different toxicities and efficacies when given s.c. versus i.v. For example, both recombinant IL-15 and the IL-15 superagonist N803 have been shown to be more efficacious and

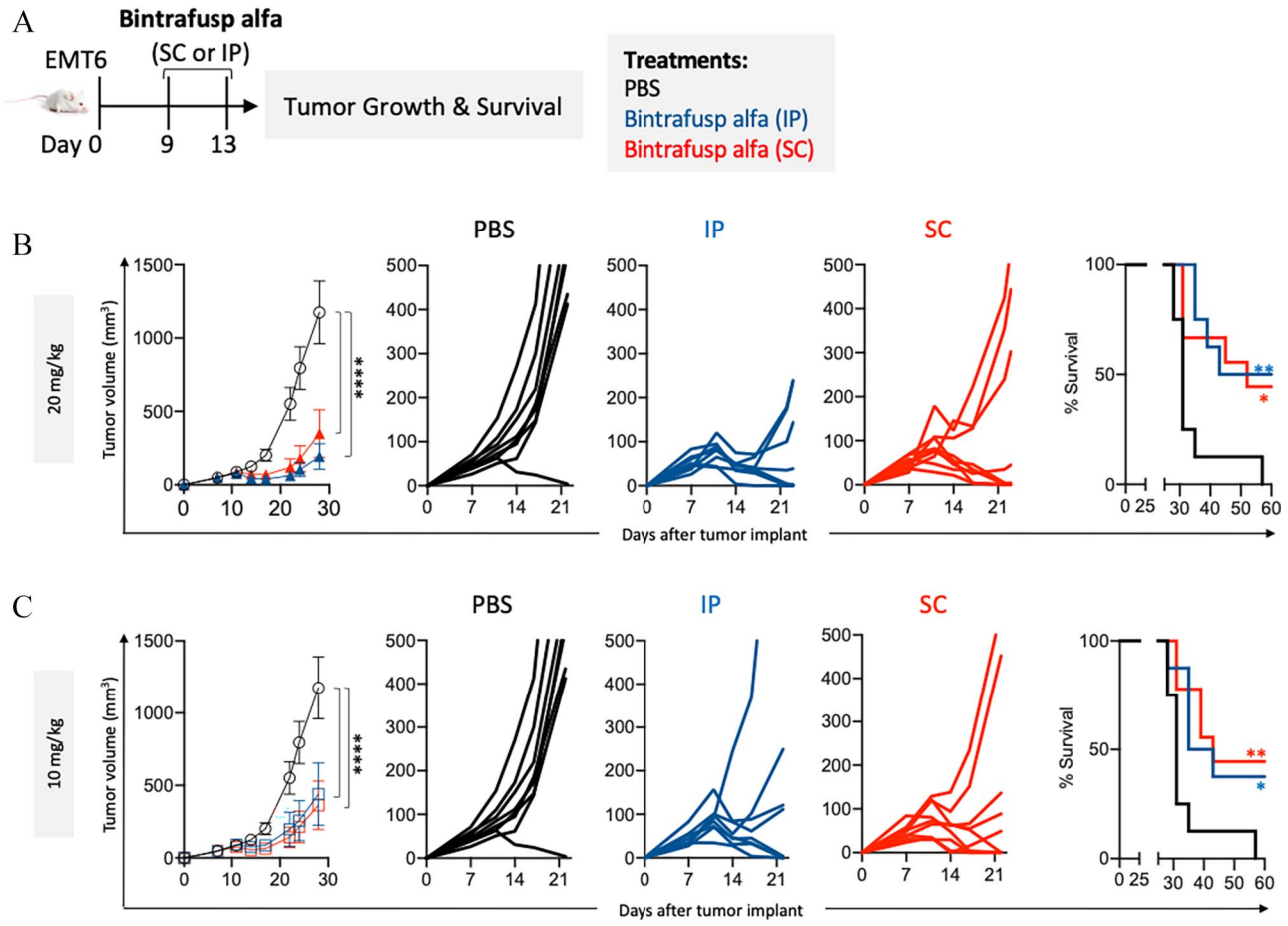

Figure 4. Systemic (intraperitoneal) or subcutaneous administration of bintrafusp alfa elicits similar and significant tumor control and increased survival in tumor-bearing mice. EMT6 murine breast carcinoma cells were implanted in the mammary fat pad of Balb/C female mice. When tumor volume reached 50–100 mm^3, mice were randomized and treated with phosphate buffered saline (PBS), or with two different doses (20 mg/kg or 10 mg/kg) of bintrafusp alfa via intraperitoneal (i.p.) or subcutaneous (s.c.) injection, as depicted in the schematic (A). Tumor growth and survival were monitored. Tumor mean (±SEM) growth curves, individual tumor growth curves, and survival of mice treated with 20 mg/kg (B) or 10 mg/kg (C). Taken from the study by Ozawa *et al.*,[18] with permission.

with lower toxicity profiles when administered s.c. versus i.v. Thus, bintrafusp alfa would be an excellent candidate for evaluation as an s.c. administered therapeutic.

AUTHORS' CONTRIBUTIONS

JSt, JLG, JSc, and SRG wrote and edited the article. SRG conducted experiments and created the graphics.

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ORCID IDS

Sofia R Gameiro https://orcid.org/0000-0002-2392-8122
Julius Strauss https://orcid.org/0000-0002-7550-4938
James L Gulley https://orcid.org/0000-0002-6569-2912
Jeffrey Schlom https://orcid.org/0000-0001-7932-4072

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