Bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1, in patients with human papillomavirus-associated malignancies

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ABSTRACT

Background Bintrafusp alfa is a first-in-class bifunctional fusion protein composed of the extracellular domain of transforming growth factor (TGF)-βRII (a TGF-β ‘trap’) fused to a human IgG1 mAb blocking programmed cell death ligand 1. This is the largest analysis of patients with advanced, pretreated human papillomavirus (HPV)-associated malignancies treated with bintrafusp alfa.

Methods In these phase 1 (NCT02517398) and phase 2 trials (NCT03427411), 59 patients with advanced, pretreated checkpoint inhibitor-naïve HPV-associated cancers received bintrafusp alfa intravenously every 2 weeks until progressive disease, unacceptable toxicity, or withdrawal. Primary endpoint was best overall response per Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1; other endpoints included safety.

Results As of April 17, 2019 (phase 1), and October 4, 2019 (phase 2), the confirmed objective response rate per RECIST V.1.1 in the checkpoint inhibitor-naïve, full-analysis population was 30.5% (95% CI, 19.2% to 43.9%; five complete responses); eight patients had stable disease (disease control rate, 44.1% (95% CI, 31.2% to 57.6%)). In addition, three patients experienced a delayed partial response after initial disease progression, for a total clinical response rate of 35.6% (95% CI, 23.6% to 49.1%). An additional patient with vulvar cancer had an unconfirmed response. Forty-nine patients (83.1%) experienced treatment-related adverse events, which were grade 3/4 in 16 patients (27.1%). No treatment-related deaths occurred.

Conclusion Bintrafusp alfa showed clinical activity and manageable safety and is a promising treatment in HPV-associated cancers. These findings support further investigation of bintrafusp alfa in patients with advanced, pretreated HPV-associated cancers.

BACKGROUND

Human papillomavirus (HPV) causes almost all cervical cancers and a large proportion of anogenital and oropharyngeal cancers. Worldwide, approximately 630,000 new cases of HPV-associated malignancies are reported annually. Advanced HPV-associated cancers are often incurable and poorly palliated by traditional chemotherapies.

Host immunity impacts HPV infection and progression to cancer, and several immune-related pathways are linked to HPV-associated cancers. Transforming growth factor β (TGF-β), a pleiotropic cytokine that suppresses tumor growth and inhibits tumor-promoting inflammation in the premalignant state, is associated with tumor growth, evasion of immune surveillance, invasion, and metastasis in the advanced cancer state. Genome-wide association studies showed that the TGF-β pathway is associated with cervical cancer and HPV-positive squamous cell carcinoma of the head and neck (SCCHN), and TGF-β receptor I is significantly overexpressed in these cancers compared with benign tissue. Another study found a positive correlation between HPV infection and TGF-β levels in saliva and serum of patients with oral squamous cell carcinoma (SCC). E6 and E7 oncoproteins induce activation of the TGF-β promoter in cervical cancer cell lines, and RNAseq analysis of HPV-positive oropharyngeal SCC showed that patients with poor survival were enriched for a TGF-β gene signature and had elevated levels of HPV-E6 protein expression. A recent study found that patients with HPV-positive SCCHN with a specific polymorphism in TGFBI had significantly better overall and disease-specific survival compared with patients with the common genotype and that a similar benefit was not seen in patients with HPV-negative
SCCHN cancers.\textsuperscript{12} Hence, dysregulation of the TGF-β pathway may play a critical role in HPV-mediated carcinogenesis, and this pathway may be a potential therapeutic target.

Results from two phase 1b, three phase 2 (including one basket trial), and one randomized phase 3 study showed objective response rates of 12%-24% for single-agent programmed cell death 1 (PD-1) inhibitors (nivolumab and pembrolizumab) in HPV-associated anal, cervical, and head and neck cancers.\textsuperscript{13-18} Studies in murine SCC models showed that anti-PD-1 therapy rarely led to complete regression, but adding anti-TGF-β synergistically enhanced antitumor responses.\textsuperscript{19} The synergy was partly driven by anti-TGF-β-mediated suppression of anti-PD-1 resistance and by attenuating epithelial-mesenchymal transition and stimulating immunosurveillance.

Bintrafusp alfa (M7824) is a first-in-class bifunctional fusion protein composed of the extracellular domain of the human TGF-β receptor II (TGF-βRII or TGF-β trap) fused via a flexible linker to the C-terminus of each heavy chain of an IgG1 antibody blocking programmed cell death ligand 1 (anti-PD-L1). In preclinical studies, compared with TGF-β sequestration or anti-PD-L1 antibody alone, bintrafusp alfa extended survival, conferred long-term protective immunity, decreased regulatory T-cell function,\textsuperscript{20} substantially increased CD8\textsuperscript{T} T-cell and natural killer cell infiltration, and decreased myeloid-derived suppressor cell infiltration within tumors.\textsuperscript{21-23} In a phase 1 clinical trial, bintrafusp alfa efficiently sequestered plasma TGF-β1, TGF-β2, and TGF-β3 and bound to and saturated peripheral PD-L1.\textsuperscript{24} Treatment was well tolerated and clinically active, producing durable responses in several solid tumor types. Here, we report pooled safety and efficacy data from the subset of patients with checkpoint inhibitor-naive HPV-associated cancers from the phase 1 (study 001) and 2 (study 012) trials of bintrafusp alfa.

**METHODS**

**Study design and subjects**

This is a post hoc analysis of an ongoing global, phase 1, open-label trial of bintrafusp alfa in patients with heavily pretreated advanced solid tumors and a phase 2 single-center trial of patients with advanced HPV-associated cancers. All patients with HPV-associated cancers from study 001 were from prospectively defined cohorts (cervical, SCCHN) or from the prospectively defined dose-escalation cohort, and the HPV population of study 012 was also prospectively planned. The primary results of the dose-escalation part (which included three patients from this analysis; online supplemental table S1) have been previously reported.\textsuperscript{24} Full inclusion and exclusion criteria for both studies are listed in the online supplemental file.

The studies were conducted in accordance with all applicable regulatory requirements, and the protocols were approved by the institutional review boards of the participating institutions. International standards of Good Clinical Practice and the Declaration of Helsinki were followed. Each patient provided written informed consent before study enrollment. A full list of investigators and sites is listed in online supplemental table S2.

**Procedures**

**Clinical procedure and assessments**

Patients received bintrafusp alfa via 1-hour intravenous infusion every 2 weeks at doses of 0.3–30 mg/kg in the dose-escalation part of the phase 1 trial or at the recommended phase 2 dose of 1200 mg in the expansion part and the phase 2 trial.

The planned treatment duration was 1 year (for the phase 1 trial) or until progressive disease, unacceptable toxicity, or study withdrawal. Longer treatment and treatment past progression were permitted if clinically justified.

The primary objective of this post hoc analysis is to evaluate the efficacy of bintrafusp alfa monotherapy in checkpoint inhibitor-naive HPV-associated cancers. An exploratory analysis in checkpoint inhibitor-refractory HPV-associated cancers is also reported. Patients in both studies underwent tumor assessment scans every 6 weeks for the first 12 months and then every 12 weeks unless clinical symptoms warranted earlier imaging. Radiographic response was assessed by the investigator using Response Evaluation Criteria in Solid Tumors V.1.1 (RECIST V.1.1) and reviewed by an independent radiologist at the investigational site for the dose-escalation part of the phase 1 study and the phase 2 study. A central facility reviewed radiographic responses for patients in the expansion part of the phase 1 study. Responses were confirmed by repeat assessment after a minimum of 4 weeks. Total clinical response rate was defined as the number of patients with best overall response (BOR) of complete response (CR) or partial response (PR) per RECIST V.1.1, or who experienced delayed response following initial pseudoprogression. The duration of response was defined as the time from initial response to the time of disease progression or death. Safety was evaluated according to the Common Terminology Criteria for Adverse Events versions 4.03 and 5 in the phase 1 and 2 studies, respectively.

**HPV status**

For determination of HPV-positive disease, prior documentation of tumor sample HPV status was accepted. For patients without documentation in the dose-escalation cohort of the phase 1 or phase 2 study, HPV status was determined by PCR, when fresh or archived tissue was available, using the cobas 4800 HPV Test (Roche Molecular Systems) or BD Onclarity HPV Assay (Becton Dickinson). In the expansion cohort of the phase 1 study, HPV status was determined by RNA sequencing using formalin-fixed paraffin-embedded (FFPE) tissue samples according to standard protocols, with HPV content in each sample assessed as the fraction of reads mapping to any papillomavirus genome.
Laboratory correlates

Immune responses to HPV were analyzed in patients from the dose-escalation cohort of the phase 1 study and the phase 2 study as previously described. Briefly, HPV-specific T-cell responses were assessed in cryopreserved peripheral blood mononuclear cells (PBMCs) isolated before and 2 weeks after one and/or three cycles of bintrafusp alfa, by intracellular cytokine staining following in vitro stimulation with a mixture of overlapping 15-mer peptide pools encoding HPV-16 E6 and E7. Peptide pools encoding human leukocyte antigen and CEFT (a mixture of peptides of cytomegalovirus, Epstein-Barr virus, influenza, and tetanus toxin) served as negative and positive controls, respectively. The absolute number of viable CD4+ or CD8+ T lymphocytes producing cytokine or positive for the degranulation marker CD107a at the end of expansion was calculated per 1×10^6 cells plated at the start of the stimulation assay. This calculation takes into account not only the percentage but also the total number of viable antigen-specific CD4+ and CD8+ T cells expanded in the stimulation assay.

Finally, PD-L1 expression was detected by immunohistochemistry staining of FFPE tumor tissue using an anti-PD-L1 antibody clone 73-10 (Dako PD-L1 IHC 73-10 pharmDx; Dako, Carpinteria, California, USA). PD-L1 expression was measured on tumor cells and on cells of the tumor microenvironment (TME). Data herein are reported based on the percentage of tumor cells expressing PD-L1. A threshold of 1% was used to characterize tumors as either PD-L1 positive (≥1%) or negative (<1%).

Outcomes

The primary endpoint of the dose-escalation part of the phase 1 trial was safety. The primary endpoint of the expansion part of the phase 1 trial and the phase 2 trial was the BOR according to RECIST V1.1, and the secondary endpoint was safety. Progression-free survival (PFS), overall survival (OS), duration of response, and the relationship of immune responses to clinical responses were exploratory endpoints for the phase 1 trial and secondary endpoints for the phase 2 trial (except for immune response).

Statistical analysis

The sample size for the dose-escalation component of the trial followed a 3+3 design for dose-finding studies. Enrollment into multiple expansion cohorts was opened after the recommended phase 2 dose of bintrafusp alfa had been established (1200mg intravenously every 2 weeks). All patients with HPV-associated cancers who received bintrafusp alfa were included in the safety and full analysis sets described here. Safety and tolerability were analyzed using descriptive statistics. The durations of PFS, response, and OS were analyzed using the Kaplan-Meier method.

RESULTS

From January 26, 2016, to August 21, 2017, 17 patients with advanced HPV-associated cancer (cervical (n=10), anal (n=4), p16+ SCCHN (n=3)) were enrolled in the dose-escalation cohort, and 26 patients with advanced cervical cancer (n=15) or HPV-positive SCCHN (n=11) were enrolled into the expansion part of the phase 1 study. Overall, 14 patients with SCCHN and confirmed HPV-positive status from the phase 1 study are included in this analysis. The results for the overall SCCHN cohort are reported in a separate manuscript. HPV status for all patients was determined post hoc and not required for enrollment. Thirty-six patients were enrolled in the phase 2 study from February 27, 2018, to July 16, 2019, including 20 patients with checkpoint inhibitor-refractory disease.

Fifty-nine patients, including 43 from the phase 1 trial and 16 from the phase 2 trial, with checkpoint inhibitor-naive disease were included in this post hoc analysis. At the phase 1 analysis cutoff of April 17, 2019, and phase 2 analysis cutoff of October 4, 2019, the median duration of bintrafusp alfa treatment among all patients in this post hoc analysis was 3.9 months (range, 0.5–29.9 months) and 3.0 months (range, 0.5–7.8 months), respectively. Treatment was ongoing in 7 of 59 checkpoint inhibitor-naive patients (11.9%). The primary reasons for treatment discontinuation were disease progression (n=35), adverse events (n=8), non-treatment-related death (n=1), withdrew consent (n=5), investigator decision (n=1), lack of clinical benefit/patient decision (n=1), and completion of treatment (n=1).

Baseline demographic data and disease characteristics are summarized in table 1. Fifty-two patients (88.1%) had confirmed HPV-positive tumors, three patients (5.1%, all with cervical cancer) had HPV-negative disease (by RNA sequencing), and HPV status was missing or not available for four patients (6.8%, all with cervical cancer). Although the phase 2 study primarily enrolled female patients from a single center in the USA (National Cancer Institute) and enrolled patients with many more different tumor types than in the phase 1 study, age and Eastern Cooperative Oncology Group performance status were similar in both studies.

Between the two studies, 5 patients (8.5%) with checkpoint inhibitor-naive disease had a confirmed CR and 13 patients (22%) had a confirmed PR, as determined by investigator-assessed RECIST V1.1 (table 2, figure 1A, online supplemental figure S1). The confirmed objective response rate was 30.5% (95% CI, 19.2 to 43.9) in the full analysis set. Patients with confirmed CRs had cervical (n=2), anal (n=1), vaginal (n=1), and rectal SCC (n=1) cancers; the confirmed PRs occurred in four patients with SCCHN, eight with cervical cancer (including one patient with neuroendocrine cervical cancer), and one with anal cancer (figure 1B, online supplemental figure S2). Treatment responses occurred irrespective of PD-L1 expression in the phase 1 study (online supplemental figure S3). The response durations ranged from 2.8+ to 30.4 months (median, 19.1 months (95% CI, 9.6 to 27.4)); as of the data cut-off, 5 responses have lasted >18 months, and 11 responses (including one delayed response) were ongoing.
In addition, three patients with checkpoint inhibitor-naive disease (cervical (n=1) and SCCHN (n=2)) had delayed PRs after initial disease progression that lasted 14.6, 6.1, and 15.9+ months, respectively (figure 1A, online supplemental figure S1), resulting in a total clinical response rate of 35.6% in the full analysis set (table 2). Additionally, one patient with checkpoint inhibitor-naive disease (vulvar cancer) had an unconfirmed CR but died of an unrelated medical illness (osteooporotic hip fracture with resulting sequela) prior to confirmation of response. The total clinical response rates were ≥30% for most HPV-associated tumor types, including cervical cancer (10/33 (30%)), anal cancer (2/6 (33%)), and SCCHN (6/15 (40%)). In addition, confirmed responses were seen in

### Table 1 Baseline patient characteristics

|                          | Study 001 (n=43) | Study 012 (n=16) | Full analysis set (N=59) |
|--------------------------|------------------|------------------|--------------------------|
| **Sex**                  |                  |                  |                          |
| Male                     | 14 (32.6)        | 1 (6.3)          | 15 (25.4)                |
| Female                   | 29 (67.4)        | 15 (93.8)        | 44 (74.6)                |
| **Age, median (IQR), years** |                 |                  |                          |
| <65                      | 33 (76.7)        | 13 (81.3)        | 46 (78.0)                |
| ≥65                      | 10 (23.3)        | 3 (18.8)         | 13 (22.0)                |
| **Geographic region**    |                  |                  |                          |
| North America            | 23 (53.5)        | 16 (100)         | 39 (66.1)                |
| Europe                   | 13 (30.2)        | 0                | 13 (22.0)                |
| Asia Pacific             | 7 (16.3)         | 0                | 7 (11.9)                 |
| **Time since first diagnosis, median (range), months** | 34.2 (5.4–125.5) | 31.5 (9.4–80.5) | 34.2 (5.4–125.5) |
| **No of prior anti-cancer therapies** |              |                  |                          |
| 1                        | 14 (32.6)        | 6 (37.5)         | 20 (33.9)                |
| 2                        | 13 (30.2)        | 2 (12.5)         | 15 (25.4)                |
| ≥3                       | 16 (37.2)        | 8 (50.0)         | 24 (40.7)                |
| **Type of previous anti-cancer therapy for metastatic or locally advanced disease** |              |                  |                          |
| Cytotoxic therapy        | 43 (100)         | 16 (100)         | 59 (100)                 |
| Monoclonal antibodies    | 27 (62.8)        | 6 (37.5)         | 33 (55.9)                |
| Immunotherapy other than anti-PD-(L)1* | 3 (7.0)     | 1 (6.3)          | 4 (6.8)                  |
| **ECOG performance status** |              |                  |                          |
| 0                        | 21 (48.8)        | 8 (50)           | 29 (49.2)                |
| 1                        | 22 (51.2)        | 8 (50)           | 30 (50.8)                |
| **Primary tumor type**   |                  |                  |                          |
| Cervical                 | 25 (58.1)        | 8 (50.0)         | 33 (55.9)                |
| SCCHN                    | 14 (32.6)        | 1 (6.3)          | 15 (25.4)                |
| Anal                     | 4 (9.3)          | 2 (12.5)         | 6 (10.2)                 |
| Rectal SCC               | 0                | 2 (12.5)         | 2 (3.4)                  |
| Vaginal                  | 0                | 1 (6.3)          | 1 (1.7)                  |
| Vulvar                   | 0                | 1 (6.3)          | 1 (1.7)                  |
| Neuroendocrine cervical  | 0                | 1 (6.3)          | 1 (1.7)                  |
| **Primary HPV status at screening†** |               |                  |                          |
| Positive                 | 36 (83.7)        | 16 (100)         | 52 (88.1)                |
| Negative                 | 3 (7.0)          | 0                | 3 (5.1)                  |
| Unknown                  | 4 (9.3)          | 0                | 4 (6.8)                  |

*All four patients received adoptive T-cell transfer.
†In the dose-escalation cohort, when tissue was available, HPV status was determined by PCR using the cobas 4800 HPV test (Roche Molecular Systems). In the dose-expansion cohort, HPV status was determined by RNA sequencing or the investigators.

ECOG, Eastern Cooperative Oncology Group; HPV, human papillomavirus; IQR, interquartile range; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; SCC, squamous cell carcinoma; SCCHN, SCC of the head and neck.
3 of 5 (60%) rare tumor types, including vaginal (1/1 (100%)), rectal SCC (1/2 (50%)), and neuroendocrine cervical (1/1 (100%)) cancer. More than half (31/59 (53%)) of all patients had reduction in tumor diameters with bintrafusp alfa treatment, and 23 patients (39%) had tumor diameter reductions of >30% (figure 1B, online supplemental figure S2). The disease control rate according to RECIST V.1.1 was 44.1% in the full analysis set (table 2).

The median PFS was 2.8 months in the full analysis set (95% CI, 1.4 to 5.5 months; table 2, figure 2A). The median OS was not reached (95% CI, 8.6 months to not reached) in the full analysis set (table 2, figure 2B). Kaplan-Meier estimated proportions of patients with PFS and OS at different time points from baseline are shown in table 2. Of 59 patients in the full analysis set, 34 (58%) were alive at the cutoff, after a median follow-up of 9.2 months.

Twenty patients refractory to immune checkpoint inhibitors were also enrolled and were not part of the full analysis set. The confirmed objective response rate for this group was 10% (95% CI, 1.2% to 31.7%; 1 CR (anal) and 1 PR (SCCHN)), with both responses ongoing and with durations of 1.4+ and 3.7+ months. Neither patient had received checkpoint inhibitor therapy for several months prior to enrolling, suggesting that these responses were not due to prior checkpoint therapy. The median PFS and OS for patients with checkpoint inhibitor-refractory disease was 1.4 months (95% CI, 1.3 to 3.3 months) and 3.4 months (2.3 months to not reached), respectively.

An evaluation of immune responses to HPV-16 in patients who had a BOR to bintrafusp alfa therapy of

| Table 2 | Summary of tumor response and survival data |
|---------|-----------------------------------------------|
|          | Study 001 (n=43) | Study 012 (n=16) | Full analysis set (N=59) |
| Confirmed BOR, n (%) | | | |
| CR | 3 (7.0) | 2 (12.5) | 5 (8.5) |
| PR | 9 (20.9) | 4 (25.0) | 13 (22.0) |
| SD | 6 (14.0) | 2 (12.5) | 8 (13.6) |
| PD | 20 (46.5) | 7 (43.8) | 27 (45.8) |
| Not evaluable | 5 (11.6) | 1 (6.3) | 6 (10.2) |

| Confirmed ORR, n (%; 95% CI) | 12 (27.9; 15.3 to 43.7) | 6 (37.5; 15.2 to 64.6) | 18 (30.5; 19.2 to 43.9) |
| Disease control, n (%; 95% CI)† | 18 (41.8; 27.0 to 57.9) | 8 (50.0; 24.7 to 75.3) | 26 (44.1; 31.2 to 57.6) |
| Total clinical response rate, n (%; 95% CI)‡ | 15 (34.9; 21.0 to 50.9) | 6 (37.5; 15.2 to 64.6)§ | 21 (35.6; 23.6 to 49.1)§ |

| Duration of response, median, months (95% CI) | 19.1 (4.2 to 27.4) | NR (4.2 to NR) | 19.1 (9.6 to 27.4) |
| KM-estimated PFS, median, months (95% CI) | 2.8 (1.4 to 4.6) | 3.3 (1.4 to NR) | 2.8 (1.4 to 5.5) |
| KM-estimated PFS rate, % (95% CI) | | | |
| 6 months | 31.0 (17.8 to 45.0) | 43.8 (19.8 to 65.6) | 34.2 (22.4 to 46.4) |
| 12 months | 26.2 (14.1 to 40.0) | 29.2 (9.6 to 52.3) | 27.0 (16.3 to 38.9) |
| 18 months | 23.3 (11.8 to 37.0) | – | 24.3 (13.8 to 36.4) |
| KM-estimated OS, median, months (95% CI) | 16.2 (7.1 to NR) | NR (3.7 to NR) | NR (8.6 to NR) |
| KM-estimated OS rate, % (95% CI) | | | |
| 6 months | 73.7 (57.5 to 84.5) | 72.1 (41.5 to 88.6) | 73.1 (59.4 to 82.9) |
| 12 months | 56.5 (40.1 to 70.0) | 72.1 (41.5 to 88.6) | 58.8 (44.3 to 70.8) |
| 18 months | 48.8 (32.8 to 63.0) | – | 51.4 (36.5 to 64.3) |

Data are according to investigator-assessed RECIST V.1.1.  
†Due to confirmed PD before onset of response, these patients did not meet response criteria by RECIST V.1.1.  
‡ORR per RECIST V.1.1 plus delayed PR after initial disease progression.  
§One additional patient with a vulvar tumor had an unconfirmed CR.  
BOR, best overall response; CR, complete response; HPV, human papillomavirus; KM, Kaplan-Meier; NR, not reached; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.
stability disease or better versus patients who had a BOR of progressive disease was performed in the dose-escalation cohort and phase 2 study patients after one and/or three cycles of bintrafusp alfa. Sufficient PBMCs to test for HPV-16-specific T cells before and after treatment were available from 33 patients; 31 patients were evaluated before and after cycle 1, and 23 patients were evaluated before and after cycle 3 according to PBMC availability (online supplemental table S3). HPV-16-specific T cells were calculated as the absolute number of CD4+ or CD8+ T cells producing cytokine or positive for CD107a (lysosome-associated membrane protein 1, a functional marker of T-cell and natural killer cell activity) after expansion per 1x10^6 PBMCs plated at the start of the stimulation assay, which takes into account not only the percentage of positive lymphocytes, but also the number of total antigen-specific T cells that are expanded. In this analysis, after cycle 1, 9 of 14 patients (64.3%) with stable disease or better developed HPV-16-specific T cells versus 4 of 17 (23.5%) with progressive disease (p=0.03; online supplemental table S3). After cycle 3, 9 of 12 patients (75%) with stable disease or better developed HPV-16-specific T cells versus 6 of 11 (54.5%) with progressive disease (p=0.40; online supplemental table S3). Patients who had a BOR of stable disease or better had, on average, sixfold more HPV-16-specific T cells that produced cytokines or were positive for CD107a after cycle 1 (p=0.04) and cycle 3 (p<0.001) than patients who had a BOR of progressive disease (online supplemental figure S4). Trends in differences between responders and non-responders were also noted when HPV-16-specific T cells were quantified as a percentage of viable lymphocytes; using a twofold change as a cutoff, 11 of 14 patients (78.6%) with a BOR of stable disease or better had, on average, sixfold more HPV-16-specific T cells that produced cytokines or were positive for CD107a after cycle 1 (p=0.04) and cycle 3 (p<0.001) than patients who had a BOR of progressive disease (online supplemental figure S4). Trends in differences between responders and non-responders were also noted when HPV-16-specific T cells were quantified as a percentage of viable lymphocytes; using a twofold change as a cutoff, 11 of 14 patients (78.6%) with a BOR of stable disease or better had, on average, sixfold more HPV-16-specific T cells that produced cytokines or were positive for CD107a after cycle 1 (p=0.04) and cycle 3 (p<0.001) than patients who had a BOR of progressive disease (online supplemental figure S4). 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Table 3  TRAEs occurring at any grade in ≥5% of patients or grade ≥3 in any patient and any AEs of special interest (AESIs) from the full analysis set

| Study 001 (n=43) | Study 012 (n=16) | Full analysis set (N=59) |
|------------------|------------------|-------------------------|
|                  | Any grade        | Grade 3                 | Any grade        | Grade 3                 | Any grade        | Grade 3                 |
| Patients with any TRAE | 35 (81.4) | 11 (25.6) | 14 (87.5) | 5 (31.3) | 49 (83.1) | 16 (27.1) |
| Pruritus          | 10 (23.3) | 0   | 5 (31.3) | 0   | 15 (25.4) | 0   |
| Dermatitis acneiform | 7 (16.3) | 0   | 5 (31.3) | 0   | 12 (20.3) | 0   |
| Keratoacanthoma   | 9 (20.9) | 2 (4.7) | 0   | 0   | 9 (15.3) | 2 (3.4) |
| Hypothyroidism    | 7 (16.3) | 1 (2.3) | 2 (12.5) | 0   | 9 (15.3) | 1 (1.7) |
| Rash maculopapular | 6 (14.0) | 0   | 3 (18.8) | 0   | 9 (15.3) | 0   |
| Anemia            | 4 (9.3) | 1 (2.3) | 5 (31.3) | 3 (18.8) | 9 (15.3) | 4 (6.8) |
| Fatigue           | 2 (4.7) | 0   | 5 (31.3) | 1 (6.3) | 7 (11.9) | 1 (1.7) |
| Stomatitis        | 3 (7.0) | 0   | 2 (12.5) | 0   | 5 (8.5) | 0   |
| Rash macular      | 3 (7.0) | 1 (2.3) | 0   | 0   | 3 (5.1) | 1 (1.7) |
| Alanine aminotransferase increased | 2 (4.7) | 0   | 1 (6.3) | 0   | 3 (5.1) | 0   |
| Aspartate aminotransferase increased | 2 (4.7) | 0   | 1 (6.3) | 0   | 3 (5.1) | 0   |
| Asthenia          | 3 (7.0) | 0   | 0   | 0   | 3 (5.1) | 0   |
| Diarrhea          | 2 (4.7) | 0   | 1 (6.3) | 0   | 3 (5.1) | 0   |
| Epistaxis         | 2 (4.7) | 0   | 1 (6.3) | 0   | 3 (5.1) | 0   |
| Decreased appetite| 3 (7.0) | 0   | 0   | 0   | 3 (5.1) | 0   |
| Influenza-like illness | 1 (2.3) | 0   | 2 (12.5) | 0   | 3 (5.1) | 0   |
| Infusion-related reaction | 2 (4.7) | 0   | 1 (6.3) | 0   | 3 (5.1) | 0   |
| Mouth hemorrhage (mucosal bleeding) | 0   | 0   | 3 (18.8) | 0   | 3 (5.1) | 0   |
| Nausea            | 3 (7.0) | 0   | 0   | 0   | 3 (5.1) | 0   |
| Colitis           | 1 (2.3) | 1 (2.3) | 1 (6.3) | 0   | 2 (3.4) | 1 (1.7) |
| Pneumonitis       | 2 (4.7) | 1 (2.3) | 0   | 0   | 2 (3.4) | 1 (1.7) |
| Hypokalemia       | 1 (2.3) | 1 (2.3)* | 0   | 0   | 1 (1.7) | 1 (1.7)* |
| Squamous cell carcinoma of skin | 1 (2.3) | 1 (2.3) | 0   | 0   | 1 (1.7) | 1 (1.7) |
| γ-glutamyltransferase increased | 1 (2.3) | 1 (2.3) | 0   | 0   | 1 (1.7) | 1 (1.7) |
| Diabetic ketoacidosis | 1 (2.3) | 1 (2.3) | 0   | 0   | 1 (1.7) | 1 (1.7) |
| Neutrophil count decreased | 0   | 0   | 1 (6.3) | 1 (6.3) | 1 (1.7) | 1 (1.7) |
| Hyperglycemia     | 0   | 0   | 1 (6.3) | 1 (6.3) | 1 (1.7) | 1 (1.7) |
| Cystitis non-infective | 1 (2.3) | 1 (2.3) | 0   | 0   | 1 (1.7) | 1 (1.7) |
| Impaired gastric emptying | 1 (2.3) | 1 (2.3) | 0   | 0   | 1 (1.7) | 1 (1.7) |
| Pleural effusion   | 1 (2.3) | 1 (2.3) | 0   | 0   | 1 (1.7) | 1 (1.7) |
| Upper gastrointestinal hemorrhage | 0   | 0   | 1 (6.3) | 1 (6.3) | 1 (1.7) | 1 (1.7) |
| Hyperkeratosis follicularis et parafollicularis | 1 (2.3) | 1 (2.3) | 0   | 0   | 1 (1.7) | 1 (1.7) |
| Any AESIs          | 12 (27.9) | 4 (9.3) | 0   | 0   | 12 (20.3) | 4 (6.8) |

Data are n (%) of the safety set.
*Grade 3 hypokalemia progressed to grade 4.
†Includes MedDRA V2.0.0 and 21.1 preferred terms squamous cell carcinoma of skin, basal cell carcinoma, keratoacanthoma, hyperkeratosis, actinic keratosis, lip squamous cell carcinoma, and Bowen’s disease. Not included in the table were five patients (8%) in study 012 who were noted by the MedDRA System Organ Class of Neoplasms benign, malignant, and unspecified (including cysts and polyps), but the MedDRA preferred term was not captured (although it was deemed to be related to keratoacanthoma).

AESI, adverse event of special interest; MedDRA, Medical Dictionary for Regulatory Activities; SCC, squamous cell carcinoma; TRAE, treatment-related adverse event.
TRAE was pruritus, which occurred in 15 patients (25.4%). Grade 3 TRAEs occurred in 16 patients (27.1%); the most common was anemia, which occurred in four patients (6.8%). A patient who had grade 3 gastroparesis developed asymptomatic grade 3 hypokalemia, which worsened to grade 4 (one grade 4 event (1.7%)) and led to permanent study treatment discontinuation. This was medically managed without corticosteroids, gradually improved, and resolved completely within 2 months. No treatment-related deaths occurred.

Seven patients (11.9%) discontinued bintrafusp alfa due to TRAEs (colitis, gastroparesis (described above), infusion-related reaction, non-infective cystitis, pneumonia, acneiform dermatitis, and psoriasiform dermatitis). Treatment-related infusion-related reactions occurred in three patients (5.1%). Adverse events of special interest, including potential TGF-β-related skin lesions, which included Medical Dictionary for Regulatory Activities (DMARD) terms of keratoacanthoma, SCC of skin, basal cell carcinoma, hyperkeratosis, actinic keratosis, lip SCC, and Bowen’s disease, occurred in 12 patients (27.9%) (table 3, online supplemental table S4). These skin lesions were well managed with observation or local therapy (cryotherapy or excision) and did not require any patient to discontinue treatment. Thirty-eight patients (64.4%) in the full analysis set experienced treatment-emergent bleeding; nine patients (15.3%) had grade 3 bleeding events, and no patients had grade 4 or 5 events.

DISCUSSION

Safety and efficacy data are presented from a post hoc combined analysis of 59 patients with advanced pretreated, checkpoint inhibitor-naive HPV-associated cancers who were enrolled in global phase 1 and 2 studies of bintrafusp alfa. Responses to bintrafusp alfa occurred in patients with several different types of HPV-associated cancers (SCCHN, cervical (including neuroendocrine), anal, vaginal, vulvar, rectal SCC). These responses were durable, ranging from 2.8+ to 30.4 months. While responses were observed irrespective of PD-L1 expression, given that PD-L1 expression was determined from archival samples, the age of the sample or previous therapy may have influenced the results.

Historical data observed with PD-1 inhibitors pembrolizumab and nivolumab in patients with HPV-associated cancers demonstrated objective response rates of 12%–24% and median OS of 7.5–11.5 months. Based on safety and efficacy data from these phase 1 and phase 2 studies, bintrafusp alfa in patients with advanced HPV-associated malignancies compares favorably with historical data of these PD-1 inhibitors. Survival also seems to be longer, with a median OS not reached after 18 months of follow-up; however, data from these studies cannot be compared directly due to differences in study design, eligibility criteria, and patient characteristics. To increase the response rates in HPV-associated cancers and other solid tumors, checkpoint inhibitors are being evaluated in combination with other novel immunotherapies, and our findings may support TGF-β as a therapeutic target in HPV-associated cancers.

The safety profile of bintrafusp alfa was consistent with historically observed safety profiles of bintrafusp alfa in other tumor types. The severity and type of immune-related adverse events observed with bintrafusp alfa were also comparable to those observed with PD-(L)1 inhibitors. Additional toxicities that were seen with bintrafusp alfa that have not been described with PD-(L)1 inhibitors included keratoacanthomas and low-grade mucosal bleeding (eg, epistaxis, gingival bleeding). Study limitations from this combined analysis include the post hoc nature of this analysis and absence of a comparator treatment arm. Patients were selected for this analysis based on HPV-associated disease. Therefore, this analysis does not provide any conclusions about whether HPV-positive status is an independent biomarker predictive of response in all HPV-associated cancers; however, in the SCCHN expansion cohort, response rates in those with HPV-positive disease (determined by viral RNA detected in tumor samples) were 33% (3 of 9 patients) compared with 5% (1 of 22) in those without evidence of HPV infection. Finally, the small numbers of patients with rare tumors (cervical neuroendocrine, anal, vaginal, vulvar, rectal SCC) limits conclusions for safety and efficacy in these tumors.

In conclusion, targeting TGF-β and PD-L1 bifunctionally with bintrafusp alfa is a promising therapeutic approach for patients with HPV-associated cancers. Bintrafusp alfa had a manageable safety profile and resulted in an objective response rate of 30.5% in patients with pretreated checkpoint inhibitor-naive HPV-associated cancers, with clinical activity observed in patients who were refractory to PD-(L)1 treatment. Bintrafusp alfa continues in a range of phase 2 studies, including studies of patients with HPV-associated malignancies.

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Contents
Inclusion Criteria for Dose-Escalation Part of Study 001 ................................................. 2
Inclusion Criteria for Expansion Cohorts of Study 001 .................................................... 2
Exclusion Criteria (applicable to all patients, Including all expansion cohorts) of Study 001 .................................................................................................................................. 3
Inclusion Criteria for Study 012 ....................................................................................... 5
Exclusion Criteria for Study 012 ...................................................................................... 6
Supplemental Methods: Lab Correlates ........................................................................7
Table S1. Prior Report of Data on Patients With HPV-Associated Malignancies Enrolled in the Phase 1 Trial of Bintrafusp Alfa ............................................................................. 8
Table S2. Investigator Sites ............................................................................................ 9
Figure S1. Clinical Response to Bintrafusp Alfa ............................................................ 10
Figure S2. Best Percentage Change in Target Lesions From Baseline by Cancer Type as Assessed by Investigators .................................................................................. 11
Figure S3. Tumor Response by PD-L1 Expression According to RECIST 1.1 as Assessed by Investigator in Study 001 .................................................................................. 11
Table S3. Best Overall Response to Bintrafusp Alfa in Patients in the Dose-Escalation Cohort and Phase 2 Study ......................................................................................... 13
Figure S4. Immune Responses in the Dose-Escalation Cohort and Phase 2 Study ..... 15
Table S4. Patients reporting any skin lesions* ................................................................16
Full Inclusion/Exclusion Criteria

For inclusion in the trial, all of the following inclusion criteria must be fulfilled.

Inclusion Criteria for Dose-Escalation Part of Study 001

1. Ability to understand the purpose of the study, to provide signed and dated informed consent, and to comply with all procedures.
2. Male or female patients aged ≥ 18 years.
3. Histologically or cytologically proven metastatic or locally advanced solid tumors, for which no effective standard therapy exists or standard therapy has failed.
4. Life expectancy of ≥ 12 weeks, as judged by the Investigator.
5. Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 to 1 at trial entry.
6. Evaluable or measurable disease at baseline.
7. Adequate hematologic function defined by white blood cell count of ≥ 3 × 10^9/L with absolute neutrophil count (ANC) of ≥ 1.5 × 10^9/L, lymphocyte count of ≥ 0.5 × 10^9/L, platelet count of ≥ 120 × 10^9/L, and hemoglobin of ≥ 9 g/dL (in absence of blood transfusion).
8. Adequate hepatic function defined by total bilirubin level of ≤ 1.5 × upper limit of normal (ULN), aspartate aminotransferase (AST) level of ≤ 2.5 × ULN, and alanine aminotransferase (ALT) level of ≤ 2.5 × ULN. For patients with liver involvement in the tumor, AST of ≤ 5.0 × ULN, ALT of ≤ 5.0 × ULN, and bilirubin level of ≤ 3.0 × ULN are acceptable.
9. Adequate renal function defined by an estimated creatinine clearance of > 50 mL/min according to the Cockcroft-Gault formula or by measure of creatinine clearance from 24-hour urine collection.
10. Highly effective contraception (ie, methods with a failure rate of < 1% per year) for both male and female patients if the risk of conception exists. (Note: The effects of the trial treatment on the developing human fetus are unknown; thus, women of childbearing potential and men must agree to use highly effective contraception, defined in the study protocol or as stipulated in national or local guidelines.) Highly effective contraception must be used 30 days prior to first trial treatment administration, for the duration of trial treatment, and for ≥ 4 months after stopping trial treatment. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this trial, the treating physician should be informed immediately.

Inclusion Criteria for Expansion Cohorts of Study 001

1. Ability to understand the purpose of the study, to provide signed and dated informed consent, and to comply with all procedures.
2. Male or female patients aged ≥ 18 years.
3. Availability of fresh tumor biopsies (excluding bone biopsies) is mandatory for eligibility in the non-small cell lung cancer (NSCLC) biomarker expansion, NSCLC anti–PD-1/PD-L1 failure, and melanoma anti–PD-1/PD-L1 failure cohorts (it is preferable to not biopsy a target lesion; however, if only 1 lesion is amenable for biopsy and it is the only target lesion, the medical monitor should be consulted for patient eligibility). The biopsy or surgical specimen must have been collected within 28 days prior to the first bintrafusp alfa administration. For other expansion cohorts, availability of either tumor archival material or fresh biopsies within 28 days is acceptable (excluding bone biopsies), with one of these being mandatory (when possible, fresh biopsies are preferred). If no archival material is available and only 1 lesion is amenable for biopsy and it is the only target lesion, the medical monitor should be consulted for patient eligibility. Tumor biopsies and tumor archival material must be suitable for biomarker assessment as described in the laboratory flowchart.
4. Life expectancy of ≥ 12 weeks, as judged by the Investigator.
5. Patients must have 1 of the following:
**SCCHN, second line or greater**
Histologically confirmed recurrent or metastatic SCCHN (oral cavity, pharynx, larynx), stage III/IV, and not amenable to local therapy with curative intent (surgery or radiation therapy with or without chemotherapy)

- Tumor progression or recurrence within 6 months of last dose of platinum therapy in the adjuvant (ie, with radiation after surgery), primary (ie, with radiation), recurrent, or metastatic setting
- Patients may have received prior cetuximab
- HPV tumor testing must be reported if known

**Cervical cancer, second line or greater:**
Histologically confirmed recurrent or persistent SCC, adenosquamous carcinoma, or adenocarcinoma of the cervix following standard of care treatment with systemic therapy for advanced disease (typically doublet cytotoxic chemotherapy and bevacizumab, where approved)

- HPV tumor testing must be reported if known

6. ECOG PS of 0 to 1 at trial entry.
7. Disease must be measurable, with ≥ 1 unidimensionally measurable lesion by RECIST 1.1.
8. Adequate hematologic function defined by white blood cell count of ≥ 3 × 10⁹/L with ANC of ≥ 1.5 × 10⁹/L, lymphocyte count of ≥ 0.5 × 10⁹/L, platelet count of ≥ 120 × 10⁹/L, and hemoglobin of ≥ 9 g/dL (in absence of blood transfusion).
9. Adequate hepatic function defined by total bilirubin level of ≤ 1.5 × ULN, AST level of ≤ 2.5 × ULN, and ALT level of ≤ 5.0 × ULN. For patients with liver involvement in the tumor, AST level of ≤ 5.0 × ULN, ALT level of ≤ 5.0 × ULN, and bilirubin level of ≤ 3.0 is acceptable.
10. Adequate renal function defined by an estimated creatinine clearance of > 50 mL/min according to the Cockcroft-Gault formula or by measure of creatinine clearance from 24-hour urine collection.
11. Highly effective contraception (ie, methods with a failure rate of < 1% per year) for both male and female patients if the risk of conception exists. (Note: The effects of the trial treatment on the developing human fetus are unknown; thus, women of childbearing potential and men must agree to use highly effective contraception, defined in study protocol or as stipulated in national or local guidelines.) Highly effective contraception must be used 30 days prior to first trial treatment administration, for the duration of trial treatment, and for ≥ 4 months after stopping trial treatment. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this trial, the treating physician should be informed immediately.

**Exclusion Criteria (applicable to all patients, including all expansion cohorts) of Study 001**

Patients are not eligible for this trial if they fulfill any of the following exclusion criteria.

1. Concurrent treatment with nonpermitted drugs and other interventions.
2. Except for the anti–PD-1/anti–PD-L1–experienced NSCLC expansion and melanoma expansion cohorts, prior therapy with any antibody/drug targeting T-cell coregulatory proteins (immune checkpoints) such as anti–PD-1, anti–PD-L1, or anti–CTLA-4 antibody (consult medical monitor if necessary) is not allowed (consult with medical monitor as needed), inclusive of intrahepatic, localized administration of such agents.
3. Anticancer treatment within 28 days before the start of trial treatment (eg, cytoreductive therapy, radiotherapy [with the exception of palliative radiotherapy delivered in a normal organ-sparing technique], immunotherapy, or cytokine therapy [with the exception of sorafenib for patients with hepatocellular carcinoma (HCC), which must have been stopped within 14 days]).
4. Major surgery within 28 days before the start of trial treatment (prior diagnostic biopsy is permitted).
5. Systemic therapy with immunosuppressive agents within 7 days before the start of trial treatment or use of any investigational drug within 28 days before the start of trial treatment. (Note: For patients with glioblastoma, steroid use is allowed according to standard of care and local guidelines.)

6. Previous malignant disease (other than the target malignancy to be investigated in this trial) within the last 3 years. Patients with a history of cervical carcinoma in situ, superficial or no-invasive bladder cancer, or basal cell or SCC in situ previously treated with curative intent are not excluded. Patients with other localized malignancies treated with curative intent need to be discussed with the medical monitor.

7. Rapidly progressive disease which, in the opinion of the Investigator, may predispose to inability to tolerate treatment or trial procedures.

8. Patients with active central nervous system (CNS) metastases causing clinical symptoms or metastases that require therapeutic intervention are excluded. Patients with a history of treated CNS metastases (by surgery or radiation therapy) are not eligible unless they have fully recovered from treatment, demonstrated no progression for ≥ 2 months, and do not require continued steroid therapy. Patients with CNS metastases incidentally detected during screening which do not cause clinical symptoms and for which standard of care suggests no therapeutic intervention is indicated, should be discussed with the sponsor medical responsible.

9. Receipt of any organ transplant, including allogeneic stem cell transplant, but with the exception of transplants that do not require immunosuppression (eg, corneal transplant, hair transplant).

10. Significant acute or chronic infections including, among others:
   - Known history of testing positive test for HIV or known acquired immunodeficiency syndrome
   - Except for the HCC cohort, hepatitis B virus (HBV) or hepatitis C virus (HCV) infection (HBV surface antigen positive and HBV core antibody positive with reflex to positive HBV DNA or HBV core antibody positive alone with reflex to positive HBV DNA or positive HCV antibody with reflex to positive HCV RNA).
   - Patients with active tuberculosis (history of exposure or history of positive tuberculosis test plus presence of clinical symptoms and physical or radiographic findings).

11. Active autoimmune disease that might deteriorate when patient is receiving an immunostimulatory agent.
   - Patients with diabetes type I, vitiligo, alopecia, psoriasis, hypo- or hyperthyroid disease not requiring immunosuppressive treatment are eligible
   - Patients requiring hormone replacement with corticosteroids are eligible if the steroids are administered only for the purpose of hormonal replacement and at doses ≤ 10 mg of prednisone or equivalent per day
   - Administration of steroids for other conditions through a route known to result in a minimal systemic exposure (topical, intranasal, intraocular, or inhalation) is acceptable

12. Known severe hypersensitivity reactions to monoclonal antibodies (grade ≥ 3 National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE] v4.03), any history of anaphylaxis, or recent (within 5 months) history of uncontrolled asthma.

13. Persisting toxicity (except alopecia and vitiligo) related to prior therapy of grade > 1 NCI-CTCAE v4.03; however, sensory neuropathy of grade ≤ 2 is acceptable.

14. Pregnant or currently lactating.

15. Known alcohol or drug abuse.

16. Clinically significant cardiovascular/cerebrovascular disease as follows: cerebral vascular accident/stroke (< 6 months prior to enrollment), myocardial infarction (< 6 months prior to enrollment), unstable angina, congestive heart failure (New York Heart Association class ≥ II), or serious cardiac arrhythmia.
17. Clinically relevant diseases (eg, inflammatory bowel disease) and/or uncontrolled medical conditions, which, in the opinion of the Investigator, might impair the patient's tolerance or ability to participate in the trial.
18. Any psychiatric condition that would prohibit the understanding or rendering of informed consent.
19. Legal incapacity or limited legal capacity.
20. Vaccine administration within 4 weeks of bintrafusp alfa administration. Vaccination with live vaccines while on trial is prohibited. Administration of inactivated vaccines is allowed (eg, inactivated influenza vaccines).

Additional exclusion criteria for patients in the HCC cohort include:

21. Clinical ascites (ie, not per radiological assessment only) within past 6 months not adequately controlled with medical therapy, history of variceal bleeding within past 3 months, history of uncontrolled hepatic encephalopathy in the past 3 months, or history of obstructive jaundice not amenable to stenting in the past 3 months.
22. Hepatitis D virus co-infection with HBV; if HBV surface antigen or HBV DNA positivity at screening then must check for hepatitis D status).
23. Chemoembolization or radioembolization within 28 days prior to bintrafusp alfa administration.

Inclusion Criteria for Study 012

1. Age ≥ 18 years.
2. Ability of patient to understand and willingness to sign a written informed consent document.
3. Patients with cytologically or histologically confirmed locally advanced or metastatic HPV-associated malignancies, including the following:
   - Non-neuroendocrine cervical cancers
   - P16⁺ oropharyngeal cancers
   - Anal cancers
   - Vulvar, vaginal, penile, squamous cell rectal, and neuroendocrine cervical cancers
   - Other locally advanced or metastatic solid tumors (eg, lung, esophagus) that are known to be HPV-positive
4. Patients must have disease that is not amenable to potentially curative resection.
5. Patients must have measurable disease.
6. ECOG PS of < 1.
7. Adequate hematologic function at screening, as follows:
   - ANC of ≥ 1 × 10⁹/L
   - Hemoglobin of ≥ 9 g/dL
   - Platelet count of ≥ 75,000/μL
8. Adequate renal and hepatic function at screening, as follows:
   - Serum creatinine of ≤ 1.5 × ULN or creatinine clearance of ≥ 40 mL/min according to the Cockcroft-Gault formula
   - Bilirubin level of ≤ 1.5 × ULN or in patients with Gilbert syndrome, a total bilirubin level of ≤ 3.0 × ULN
   - ALT and AST levels of ≤ 2.5 × ULN, unless liver metastases are present, then values must be ≤ 3 × ULN
9. The effects of bintrafusp alfa on the developing human fetus are unknown; thus, women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry for the duration of study participation and up
to 120 days after the last dose of the drug. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

10. Patients serologically positive for HIV, HBV, or HCV are eligible as long as viral loads are undetectable by quantitative polymerase chain reaction. HIV-positive patients must have CD4 counts of ≥ 300 cells/mm$^3$ at enrollment, be receiving stable antiretroviral therapy, and have no reported opportunistic infections within 12 months prior to enrollment.

Exclusion Criteria for Study 012

1. Pregnant women are excluded from this study because this drug has not been tested in pregnant women and there is potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with bintrafusp alfa, breastfeeding should be discontinued if the mother is treated with bintrafusp alfa.

2. Patients with prior investigational drug, chemotherapy, immunotherapy, or any prior radiotherapy (except for palliative bone-directed therapy) within the past 28 days prior to the first drug administration, except if the investigator has assessed that all residual treatment-related toxicities have resolved or are minimal and believe that the patient is otherwise suitable for enrollment.

3. Major surgery within 28 days prior to the first drug administration (minimally invasive procedures such as diagnostic biopsies are permitted).

4. Known intolerance to or life-threatening side effects resulting from prior checkpoint inhibitor therapy.

5. Known active brain or CNS metastasis (< 2 months after definitive radiotherapy or surgery), seizures requiring anticonvulsant treatment (< 3 months), or clinically significant cerebrovascular accident (< 3 months). To be eligible, patients must have repeat CNS imaging ≥ 2 months after definitive treatment showing stable CNS disease. Patients with evidence of intratumoral or peritumoral hemorrhage on baseline imaging are also excluded unless the hemorrhage is grade ≤ 1 and has been shown to be stable on 2 consecutive imaging scans.

6. Active autoimmune disease that might deteriorate when a patient is receiving an immunostimulatory agent, with the exception of the following:
   - Diabetes type I, eczema, vitiligo, alopecia, psoriasis, hypo- or hyperthyroid disease, or other mild autoimmune disorders not requiring immunosuppressive treatment
   - Patients requiring hormone replacement with corticosteroids are eligible if the steroids are administered only for the purpose of hormonal replacement and at doses ≤ 10 mg of prednisone or equivalent per day
   - Administration of steroids for other conditions through a route known to result in a minimal systemic exposure (topical, intranasal, intraocular, or inhalation) is acceptable
   - Patients receiving systemic intravenous or oral corticosteroid therapy, with the exception of physiological doses of corticosteroids (≤ the equivalent of prednisone 10 mg/day) or other immunosuppressives such as azathioprine or cyclosporine A, are excluded on the basis of potential immune suppression. For these patients, these excluded treatments must be discontinued ≥ 1 week prior to enrollment for recent short-course use (≤ 14 days) or discontinued ≤ 4 weeks prior to enrollment for long-term use (> 14 days). In addition, the use of corticosteroids as premedication for contrast-enhanced studies is allowed prior to enrollment and on study.

7. Patients with a history of serious intercurrent chronic or acute illness, such as cardiac or pulmonary disease, hepatic disease, or other illness considered by the investigator as high risk for investigational drug treatment.
8. History of second malignancy within 3 years of enrollment, except for the following: adequately treated nonmelanoma skin cancer, cervical carcinoma in situ, superficial bladder cancer, or other localized malignancy that has been adequately treated.

9. Known severe hypersensitivity reactions to monoclonal antibodies (grade ≥ 3 NCI-CTCAE v5.0).

10. Receipt of any organ transplant requiring ongoing immunosuppression.

11. Patients with vulvar cancer originating from differentiated vulvar intraepithelial neoplasia (d-VIN), as opposed to vulvar intraepithelial neoplasia of usual type, are excluded. Vulvar SCC originating from differentiated VIN (d-VIN) is HPV negative; however, rare cases of HPV-positive d-VIN can occur. Patients are not excluded if the tumor has tested positive for HPV or there is no documentation of prior VIN type.

Supplemental Methods: Lab Correlates

Immune responses to HPV were analyzed in patients from the dose-escalation cohort of the phase 1 study and the phase 2 study as previously described. Briefly, HPV-specific T-cell responses were assessed in cryopreserved PBMCs isolated before and 2 weeks after 1 and/or 3 cycles of bintrafusp alfa, by intracellular cytokine staining following in vitro stimulation with a mixture of overlapping 15-mer peptide pools encoding HPV-16 E6 and E7. Peptide pools encoding human leukocyte antigen (HLA) and CEFT (a mixture of peptides of cytomegalovirus, Epstein-Barr virus, influenza, and tetanus toxin) served as negative and positive controls, respectively. Prior to the stimulation assay PBMCs were counted and assessed for cell viability with trypan blue exclusion; median cell viability was 87.1% (80.6 – 90.8% interquartile range). For analysis of intracellular cytokine staining, 3 x 10^6 events in the live gate were acquired using a BD Fortessa flow cytometer equipped with a UV, violet, blue, red, and yellow/green laser. FCS files were analyzed with FlowJo v9.7 For Macintosh (TreeStar). Nonviable cells were excluded, and fluorescence minus one controls were used for gating. The absolute number of viable CD4^+ or CD8^+ T lymphocytes producing cytokine or positive for the degranulation marker CD107a at the end of expansion was calculated per 1 x 10^6 cells plated at the start of the stimulation assay. The background signal (obtained with the HLA peptide pool) and any value obtained prior to therapy were subtracted from those obtained after therapy ([post-TAA – post-HLA] – [pre-TAA – pre-HLA]). A response to each TAA was scored as positive if a patient had more than 250 CD4+ or CD8+ viable T cells that produced IFN-g, TNF-a, IL-2, or were positive for CD107a at the end of expansion per 1 x 10^6 cells that were plated at the start of the assay. Calculation of the absolute number of CD4^+ or CD8^+ T cells that produce cytokine or are positive for CD107a at the at the end of expansion per 1 x 10^6 cells plated at the start of the in vitro stimulation assay takes into account not only the percentage, but also the total number, of viable antigen specific CD4^+ and CD8^+ T cells that were expanded in the stimulation assay. In addition, due to the large number of PBMC samples analyzed in this study, samples were run in batches with internal controls included, and importantly, the pre-PBMC and post-PBMC values from a given patient were always run simultaneously to reduce assay variability.

Heery CR, Singh BH, Rauckhorst M, et al. Phase I trial of a yeast-based therapeutic cancer vaccine (GI-6301) targeting the transcription factor brachyury. Cancer Immunol Res 2015;3:1248–56.
SUPPLEMENTARY TABLES AND FIGURES

Table S1. Prior Report of Data on Patients With HPV-Associated Malignancies Enrolled in the Phase 1 Trial of Bintrafusp Alfa

| Number of patients described in previously published report (Strauss et al., 2018) | Patients with HPV-associated cancers enrolled in dose-escalation cohort of NCT02517398 described in publication (n = 17) |
|---|---|
| 3\textsuperscript{a} |  |
| Number of patients not previously reported | 14 |

HPV, human papillomavirus. \textsuperscript{a} The 79 patients with HPV-associated malignancies who are reported in this manuscript include updated clinical data on 3 previously reported patients from the dose-escalation cohort to provide a more complete assessment of the clinical data from patients with HPV-associated malignancies.
Table S2. Investigator Sites

| Site number | Principal investigator          | Site/institution                                                                 | Patients treated in 001 (n = 43) | Patients treated in 012 (n = 36) |
|-------------|--------------------------------|-----------------------------------------------------------------------------------|---------------------------------|---------------------------------|
| 101         | Gulley, James                  | National Cancer Institute, United States                                         | 17                              | 36                              |
|             | Strauss, Julius                |                                                                                   |                                 |                                 |
| 616         | Hill, Andrew                   | Tasman Oncology Research Ltd, Australia                                           | 4                               |                                 |
| 115         | McClay, Edward                 | Pacific Oncology Associates, United States                                        | 2                               |                                 |
| 509         | Barlesi, Fabrice               | Hospital de la Timone, France                                                      | 2                               |                                 |
| 515         | Ravaud, Alain                  | CHU Bordeaux—Hôpital Saint André, France                                          | 2                               |                                 |
| 134         | Edenfield, William             | Greenville Hospital System University Medical Center (ITOR), United States        | 1                               |                                 |
| 135         | Braiteh, Fadi                  | Comprehensive Cancer Centers of Nevada, United States                              | 1                               |                                 |
| 137         | Spira, Alexander               | Virginia Cancer Specialists, United States                                       | 1                               |                                 |
| 143         | Kelly, Karen                   | University of California Davis Health System, United States                       | 1                               |                                 |
| 305         | Park-Simon, Tjoung-Won         | Medizinische Hochschule Hannover, Germany                                         | 1                               |                                 |
| 506         | Rottey, Sylvie                 | Universitair Ziekenhuis Gent, Belgium                                            | 1                               |                                 |
| 508         | Penel, Nicolas                 | Centre Oscar Lambret, France                                                      | 1                               |                                 |
| 524         | Longo Muñoz, Federico          | Hospital Universitario Ramon y Cajal, Spain                                      | 1                               |                                 |
| 530         | Cervantes Ruiperez, Andres     | Hospital Clinico Universitario de Valencia, Spain                                | 1                               |                                 |
| 548         | Paz-Ares Rodriguez, Luis       | Hospital Universitario 12 de Octubre, Spain                                      | 1                               |                                 |
| 549         | Calvo Aller, Emilano           | Centro Integral Oncologico Clara Campal, Spain                                    | 1                               |                                 |
| 550         | Rasschaert, Marika             | Universitair Ziekenhuis Antwerpen, Belgium                                        | 1                               |                                 |
| 557         | Borel, Christian               | Centre Paul Strauss, France                                                       | 1                               |                                 |
| 605         | Roberts-Thomson, Rachel        | The Queen Elizabeth Hospital, United Kingdom                                      | 1                               |                                 |
| 614         | Gedye, Craig                   | Calvary Mater Newcastle, Australia                                                | 1                               |                                 |
| 805         | Cho, Byoung Chul               | Severance Hospital, Yonsei University, South Korea                                | 1                               |                                 |
Figure S1. Clinical Response to Bintrafusp Alfa in Study 001 (A) and Study 012 (B).

A.

Study 001 (n = 43)

B.

Study 012 (n = 16)

a Patients with SCC rectal tumor.

b Patient with vulvar tumor.

c Patient with neuroendocrine cervical tumor.

d Patient with vaginal tumor.
Figure S2. Best Percentage Change in Target Lesions From Baseline by Cancer Type as Assessed by Investigators in Study 001 (A) and Study 012 (B).

A.

Study 001 (n = 43)

- Delayed PR. Due to confirmed PD before onset of response, these patients did not meet response criteria by RECIST 1.1.
- Patient had a PR of target lesions, but progression of a nontarget lesion requiring radiotherapy to the isolated nontarget lesion (best response of PD by RECIST 1.1).
- Patients had disease limited to lymph nodes that shrunk to < 1 cm in the short axis and did not completely disappear (best response of CR by RECIST 1.1).
- Patient had a CR but died of an unrelated medical illness (osteoartritic hip fracture with resulting sequela) prior to confirmation of response by investigator.

B.

Study 012 (n = 16)

Figure S3. Tumor Response by PD-L1 Expression According to RECIST 1.1 as Assessed by Investigator in Study 001
PD-L1 data are available only for patients in the expansion cohort.

PD-L1 expression on tumor cells was detected by Immunohistochemistry using a proprietary assay (Dako PD-L1 IHC 73-10 pharmDx). PD-L1 positivity was defined by a threshold level of ≥ 1% positive tumor cells of any intensity.
### Table S3. Best Overall Response to Bintrafusp Alpha in Patients in the Dose-Escalation Cohort and Phase 2 Study

| Post-Cycle Bintrafusp Alpha (vs pre) | CD4 CD107a | CD4 IL-2 | CD4 TNF-α | CD8 CD107a | CD8 IFN-γ | CD8 IL-2 | CD8 TNF-α | Summary of Immune Response |
|-------------------------------------|------------|---------|-----------|------------|-----------|---------|-----------|---------------------------|
| CR                                 | 582        | 4148    | 1379      | 4863       | 2207      | 0       | 0         | 1351                      |
| 3                                  | 0          | 0       | 271       | 0          | 0         | 0       | 0         | 386                       |
|                                    | 1          | 36      | 4203      | 819        | 5488      | 398     | 0         | 0                         |
|                                    | 3          | 185     | 5706      | 2323       | 7457      | 5243    | 464       | 2858                      |
|                                    | 1          | 0       | 45        | 0          | 0         | 0       | 0         | 0                         |
|                                    | 3          |         |           |            |           |         |           |                            |
| PR                                 | 1          | 161     | 0         | 0          | 0         | 0       | 0         | 0                         |
| 3                                  | 0          | 0       | 93        | 0          | 0         | 0       | 0         | 0                         |
|                                    | 1          | 0       | 1278      | 0          | 1391      | 0       | 1379      | 0                         |
|                                    | 3          | 0       | 0         | 0          | 0         | 0       | 181       | 171                       |
|                                    | 1          | 1439    | 0         | 66         | 0         | 0       | 391       | 0                         |
|                                    | 3          | 149     | 1767      | 0          | 171       | 0       | 2466      | 0                         |
|                                    | 1          | 0       | 0         | 0          | 0         | 0       | 112       | 0                         |
|                                    | 3          |         |           |            |           |         |           |                            |
| PR                                 | 1          | 156     | 0         | 0          | 0         | 733     | 0         | 2057                      |
| 3                                  | 0          | 0       | 0         | 0          | 0         | 0       | 0         |                            |
|                                    | 3          |         |           |            |           |         |           |                            |
| PR                                 | 1          | 101     | 1844      | 454        | 5060      | 0       | 0         | 1468                      |
| 3                                  | 477        | 0       | 249       | 1357       | 194       | 0       | 0         | 672                       |
|                                    | 0          | 0       | 0         | 0          | 0         | 0       | 0         | 1086                      |
|                                    | 1          | 2837    | 21926     | 559        | 26539     | 35621   | 54587     | 25412                     |
| 3                                  | 12117      | 19165   | 0         | 25118      | 2958      | 25347   | 231       | 10758                     |
|                                    | 1          |         |           |            |           |         |           |                            |
| MR                                 | 1          | 0       | 0         | 0          | 0         | 0       | 0         | 391                       |
| 3                                  | 641        | 0       | 0         | 2203       | 2484     | 0       | 0         | 0                         |
|                                    | 1          | 0       | 0         | 0          | 0         | 0       | 0         | 0                         |
|                                    | 3          | 501     | 0         | 0         | 8768      | 3507    | 0         | 1491                      |
|                                    | 1          | 0       | 0         | 0          | 0         | 0       | 0         | 1674                      |
|                                    | 3          | 0       | 52        | 0          | 0         | 0       | 0         | 0                         |
|                                   |           |         |           |            |           |         |           |                            |
| PD                                 | 1          | 48      | 54        | 1576       | 672       | 0       | 0         | 0                         |
| 3                                  | 0          | 0       | 0         | 0         | 110       | 243     | 0         | 0                         |
|                                    | 1          |         |           |            |           |         |           |                            |
|                                   |           |         |           |            |           |         |           |                            |
| PD                                 | 1          | 0       | 0         | 0          | 0         | 0       | 0         | 0                         |
| 3                                  | 0          | 0       | 0         | 0          | 0         | 0       | 0         | 0                         |
|                                    | 1          | 0       | 0         | 0          | 0         | 0       | 0         | 0                         |
|                                    | 3          | 99      | 1716      | 0          | 0         | 0       | 33        | 0                         |
|                                    | 1          | 57      | 0         | 0         | 0         | 0       | 66        | 0                         |
|                                    | 3          | 0       | 0         | 0         | 0         | 0       | 0         | 0                         |
|                                    | 3          |         |           |            |           |         |           |                            |
| PD                                 | 1          | 95      | 134       | 0          | 0         | 0       | 0         | 0                         |
| 3                                  | 145        | 727     | 0         | 0         | 0         | 76      | 0         | 0                         |
|                                    | 1          | 748     | 186       | 0         | 1080      | 5913    | 240       | 84                       |
|                                    | 3          | 0       | 0         | 0         | 0         | 0       | 0         | 0                         |
|                                    | 1          | 8483    | 0         | 0         | 1881      | 0       | 0         | 0                         |
|                                    | 3          | 186     | 0         | 0         | 0         | 0       | 0         | 0                         |
|                                    | 1          | 0       | 1482      | 5211      | 5726      | 400     | 311       | 870                       |
|                                    | 3          | 0       | 0         | 0         | 1035      | 0       | 0         | 0                         |
|                                   |           |         |           |            |           |         |           |                            |
| PD                                 | 1          | 0       | 0         | 0         | 0         | 0       | 0         | 0                         |
| 3                                  | 0          | 0       | 3378      | 1440       | 0         | 285     | 0         | 0                         |
|                                    | 1          | 233     | 0         | 0         | 0         | 0       | 0         | 0                         |
|                                    | 3          | 198     | 0         | 0         | 0         | 0       | 0         | 0                         |
|                                    | 1          | 30      | 0         | 0         | 4090      | 0       | 135       | 0                         |
|                                    | 3          | 2293    | 2085      | 682       | 0         | 2644    | 0         | 0                         |
|                                    | 1          | 0       | 0         | 0         | 0         | 0       | 0         | 0                         |

*Summary of Immune Response:*

- **Cycle 1**: 9/14, 9/12, 12/16 (64%) vs pre
- **Cycle 3**: 9/12, 12/16 (75%) vs pre
- **Cycle 1 or 3**: 12/16 (75%) vs pre

- **PD**: 4/17, 6/11, 10/17 (23%) vs pre
- **PD**: 5/17, 6/11, 10/17 (54%) vs pre
- **PD**: 5/17, 6/11, 10/17 (58%) vs pre

**Note:** 001395. doi: 10.1136/jitc-2020-001395.
Absolute number of CD4$^+$ or CD8$^+$ T cells that produced cytokines or were positive for CD107a per $1 \times 10^6$ cells plated at start of in vitro stimulation. Numbers in bold are positive responses after vs before bintrafusp alfa therapy and following subtraction of background: (post-HPV − post-HLA) − (pre-HPV − pre-HLA) > 250. Grey rows indicate time points at which PBMCs were not available for analysis. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.
Immune responses in patients who had clinical responses to bintrafusp alfa therapy (BOR of SD, mixed response [MR], PR, or CR) vs nonresponders (BOR of PD). A response to HPV-16 E6/E7 after therapy was scored as positive if a patient had > 250 CD4⁺ or CD8⁺ T cells that produced IFN-γ, TNF-α, or IL-2 or were positive for CD107a at the end of the stimulation assay per 1 x 10⁶ cells that were plated at the start of the assay. Each point in the graph indicates the magnitude of a single cytokine/CD107a measure, with 8 measures assessed per patient (CD8⁺IFN-γ⁺, CD8⁺TNF-α⁺, CD8⁺IL2⁺, CD8⁺CD107a⁺, CD4⁺IFN-γ⁺, CD4⁺TNF-α⁺, CD4⁺IL2⁺, CD4⁺CD107a⁺). The frequency of positive measures (>250 CD4⁺ and CD8⁺ T cells producing cytokine and/or positive for CD107a) is indicated in the graph.
Table S4. Patients Reporting Any Skin Lesions

|                         | Study 001 (n = 43) | Study 012 (n = 16) | Full analysis set (N = 59) |
|-------------------------|--------------------|--------------------|----------------------------|
|                         | Any grade | Grade 3 | Any grade | Grade 3 | Any grade | Grade 3 |
| Any skin lesions        |           |        |           |        |           |        |
| Keratoacanthoma         | 12 (27.9) | 4 (9.3) | 0         | 0       | 12 (20.3) | 4 (6.8) |
| Basal cell carcinoma    | 10 (23.3) | 3 (7.0) | 0         | 0       | 10 (16.9) | 3 (5.1) |
| Actinic keratosis       | 3 (7.0)   | 0       | 0         | 0       | 3 (5.1)   | 0       |
| Squamous cell           | 2 (4.7)   | 0       | 1 (2.3)   | 0       | 2 (3.4)   | 1 (1.7) |
| carcinoma of the skin   | 1 (2.3)   | 1 (2.3) | 0         | 0       | 1 (1.7)   | 0       |
| Hyperkeratosis          | 1 (2.3)   | 0       | 0         | 0       | 1 (1.7)   | 0       |
| Lip squamous cell       | 1 (2.3)   | 0       | 0         | 0       | 1 (1.7)   | 0       |
| carcinoma               |           |        |           |        |           |        |

*Not included in the table were 5 patients (8%) in study 012 who were noted by the MedDRA System Organ Class of Neoplasms benign, malignant and unspecified (including cysts and polyps), but the MedDRA preferred term was not captured (although it was deemed to be related to keratoacanthoma).