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Setting up clinical trials for success: applying preclinical advances in combined TGFβ/PD-L1 inhibition to ongoing clinical studies

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Abstract

Combined TGFβ/PD-L1 inhibition is currently undergoing clinical trials in multiple cancer types. The early reported clinical trials of bintrafusp alfa, a bifunctional fusion protein targeting both of these pathways, have had mixed results. Here, we briefly review recent preclinical advances that can be used to refine these ongoing clinical trials and improve their outcomes.

A major focus in cancer immunotherapy development is identifying combination therapies that can raise its low response rate in cancers with low immunogenicity. For example, pembrolizumab or nivolumab monotherapies for head and neck squamous cell carcinoma (HNSCC) have hazard ratios either at or above the efficacy boundary of 0.70,[1,2] implying that combination therapies are needed to improve their effect. TGFβ signaling has emerged as one such potential target, with research showing that it contributes to the evasion of immune checkpoint blockade by excluding cytotoxic T lymphocytes from tumors, promoting regulatory T cell differentiation, and inducing Th17 cell-mediated immune suppression in the tumor microenvironment (TME).[3–5] Therapeutically targeting TGFβ has led to promising results in preclinical research, but has not led to successful clinical trials in cancer patients. This is likely due to a combination of the toxicity associated with the complexity of TGFβ functions and off-target effects of inhibiting this pathway, as well as the need for a combination treatment that can eradicate tumor cells.[6] One such combination therapy is bintrafusp alfa, a bifunctional fusion protein targeting both TGFβ and PD-L1, which is currently in development by Merck KGaA.[7] The co-localization of TGFβ inhibition and PD-L1 blockade by a single molecule has the potential to reduce off-target effects outside of the tumor, while at the same time increasing the effect of inhibiting both pathways as seen with other targeted inhibitors in the TME.[8] Early clinical trial results of bintrafusp alfa have been exciting, with partial and complete responses seen in its phase I dosing trial (NCT00356460), and clinical activity in HPV+ solid tumors (NCT02517398, NCT03427411).[9–11] However, while many bintrafusp alfa trials are still ongoing, reports for its first phase II clinical trials have brought disappointments. It has failed to match Merck's Keytruda head to head in PD-L1-positive non-small cell lung cancer (NSCLC) patients (NCT03631706) and produced an overall response rate of just 10.1% in biliary tract cancer (BTC), ultimately leading to the termination of its phase II trial in locally advanced BTC

(NCT04066491). These mixed clinical trial results reflect the complex biology of the TME, and are limited by the cancer types and enrollment criteria in these clinical trials rather than an overall biological failure of targeting these signaling pathways.

The most critical need of any successful clinical trial is patient selection. Future bintrafusp alfa clinical trials should therefore give preference for PD-L1-positive patients with high levels of secreted TGFβ, allowing the drug to target both simultaneously. However, measuring secreted TGFβ in patient tumors is not a common clinical evaluation, and thus not a practical way to recruit patients to a large-scale clinical trial. Although TGFβ dysregulation is common across many cancer types and its role as a regulator of anti-tumor immunity is well known, some cancers may present a better target for TGFβ inhibition than others. Notably, TGFβ overexpression is associated with both tumor initiation and progression in HNSCC, with the epithelial-specific loss of Smad4 or the TGFβ receptor resulting in spontaneous carcinomas in multiple mouse models.[12] These observations are consistent with clinical data that TGFβ is overexpressed in HNSCC,[13] and its level inversely correlated with SMAD4 chromosomal copy numbers.[14] In contrast, TGFβ dysregulation occurs much later in pancreatic, colorectal, and importantly BTC, at locally advanced or metastatic stages.[12] A result of this dysregulation is increased TGFβ secretion from other stromal cells rather than the tumor cells themselves.[15,16] Because PD-L1 can be upregulated on both tumor cells and infiltrating immune cells,[17] this creates a target-rich environment for bintrafusp alfa and thus more opportunity to provide localized TGFβ inhibition in the tumor. Thus, the poor performance of bintrafusp alfa in the BTC clinical trial may not be indicative of its potential effectiveness in squamous cell carcinoma (SCC) or other cancer types driven by TGFβ dysregulation. Future bintrafusp alfa trials should continue to pursue the application of bintrafusp alfa in a diverse range of tumor types, but its overall therapeutic success may not be easily inferred from its performance in a single cancer type.

The recent phase II clinical trial disappointments of bintrafusp alfa were as a monotherapy in BTC and NSCLC. It is well established that radiotherapy or chemotherapy induces PD-L1 expression in the TME,[18,19] resulting in a synergistic effect of combined radio- or chemotherapy and PD-L1/PD-1 immune checkpoint blockade. Radiotherapy also induces TGFβ production and secretion, which in turn mediates radiotherapy-induced fibrosis that inhibits the immune response to cancer. A recent study by Lan *et al.* shows that in addition to improving the abscopal effect of radiotherapy, bintrafusp alfa mitigates this fibrotic response and restores immune function in TME.[20] The upregulation of TGFβ signaling in response to radiation is confined to tumor tissue,[21] so tumor-specific targeting of a bifunctional fusion protein may be especially potent in that context. Thus, it may be more clinically effective in concert with radio- or chemotherapy rather than as a monotherapy, and several ongoing bintrafusp alfa clinical trials are testing the potential advantage of these combinations (Table 1). The complication, however, is that different chemotherapeutic agents or different radiation regimens (dose and dose rate) differentially affect the TME, particularly TGFβ and PD-L1 expression. Therefore, without biomarker-driven patient accruals, it is difficult to predict the clinical trial outcomes. To this end, any anticancer therapeutic will have distinct responders and non-responders, and even a failed clinical trial can be used to more effectively predict responders for a follow-up clinical study. In our own preclinical study of bintrafusp alfa in a SCC model, we found that responders and

non-responders could be reliably differentiated as early as four days after the initiation of bintrafusp alfa treatment.[17] Applying these sorts of post-hoc analyses to bintrafusp alfa clinical trials may help identify characteristics that can be used to predict—and thus pre-select for—patients that will have a higher overall response rate to combined TGFβ and PD-L1 inhibition than those under their current accrual criteria.

Lastly, clinical trials for TGFβ/PD-L1 inhibition should also consider the temporal and spatial patterns of TGFβ and PD-L1 expression. For example, our recent study shows that cancer associated fibroblasts (CAFs) contribute more TGFβ to the TME than tumor cells.[16] Because PD-L1 expression on CAFs is either low or a result of increased anti-tumor immunity,[22] inhibiting TGFβ-mediated fibrosis followed by targeted TGFβ/PD-L1 inhibition to infiltrating $TGF\beta^{+}PD\text{-}L1^{+}$ myeloid cells may be more effective than concurrent dual inhibition. Therefore, preclinical studies that explore the combination therapeutic types and regimens will be essential to narrow down the optimal therapeutics before moving into clinic.

In summary, combined TGFβ/PD-L1 inhibition continues to show promise in preclinical studies, and current clinical trials should take those into account in order to maximize their chance of success. Specifically, studies should be designed around cancer types that present a rich environment for TGFβ inhibition, differences between pre- and post-chemoradiation PD-L1 scores should be taken into account for determining study eligibility, and care should be taken to identify stromal cells as potential contributors to PD-L1 and TGFβ within the TME.

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References

1. Ferris RL et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. The New England journal of medicine 375, 1856–1867, doi:10.1056/NEJMoa1602252 (2016). [PubMed: 27718784]
2. Cohen EEW et al. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. Lancet (London, England) 393, 156–167, doi:10.1016/s0140-6736(18)31999-8 (2019).
3. Mariathasan S et al. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 554, 544–548, doi:10.1038/nature25501 (2018). [PubMed: 29443960]
4. Dodagatta-Marri E et al. α-PD-1 therapy elevates Treg/Th balance and increases tumor cell pSmad3 that are both targeted by α-TGFβ antibody to promote durable rejection and immunity in squamous cell carcinomas. J Immunother Cancer 7, 62, doi:10.1186/s40425-018-0493-9 (2019). [PubMed: 30832732]
5. Ravi R et al. Bifunctional immune checkpoint-targeted antibody-ligand traps that simultaneously disable TGFβ enhance the efficacy of cancer immunotherapy. Nature Communications 9, 741, doi:10.1038/s41467-017-02696-6 (2018).

6. Teixeira AF, ten Dijke P & Zhu H-J On-Target Anti-TGF-β Therapies Are Not Succeeding in Clinical Cancer Treatments: What Are Remaining Challenges? Frontiers in Cell and Developmental Biology 8, doi:10.3389/fcell.2020.00605 (2020).
7. Knudson KM et al. M7824, a novel bifunctional anti-PD-L1/TGFβ Trap fusion protein, promotes anti-tumor efficacy as monotherapy and in combination with vaccine. Oncoimmunology 7, e1426519–e1426519, doi:10.1080/2162402X.2018.1426519 (2018). [PubMed: 29721396]
8. Wang Y et al. Co-inhibition of the TGF-β pathway and the PD-L1 checkpoint by pH-responsive clustered nanoparticles for pancreatic cancer microenvironment regulation and anti-tumor immunotherapy. Biomaterials science 8, 5121–5132, doi:10.1039/d0bm00916d (2020). [PubMed: 32820750]
9. Lind H et al. Dual targeting of TGF-β and PD-L1 via a bifunctional anti-PD-L1/TGF-βRII agent: status of preclinical and clinical advances. J Immunother Cancer 8, doi:10.1136/jitc-2019-000433 (2020).
10. Strauss J et al. Bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1, in patients with human papillomavirus-associated malignancies. J Immunother Cancer 8, doi:10.1136/jitc-2020-001395 (2020).
11. Strauss J et al. Phase I Trial of M7824 (MSB0011359C), a Bifunctional Fusion Protein Targeting PD-L1 and TGFβ, in Advanced Solid Tumors. Clinical cancer research : an official journal of the American Association for Cancer Research 24, 1287–1295, doi:10.1158/1078-0432.ccr-17-2653 (2018). [PubMed: 29298798]
12. Malkoski SP & Wang X-J Two sides of the story? Smad4 loss in pancreatic cancer versus head-and-neck cancer. Febs Letters 586, 1984–1992, doi:10.1016/j.febslet.2012.01.054 (2012). [PubMed: 22321641]
13. Lu S-L et al. Overexpression of Transforming Growth Factor β1 in Head and Neck Epithelia Results in Inflammation, Angiogenesis, and Epithelial Hyperproliferation. Cancer research 64, 4405–4410, doi:10.1158/0008-5472.can-04-1032 (2004). [PubMed: 15231647]
14. Hernandez AL et al. Inter- and intra-tumor heterogeneity of SMAD4 loss in head and neck squamous cell carcinomas. Molecular carcinogenesis 58, 666–673, doi:10.1002/mc.22958 (2019). [PubMed: 30575147]
15. Yang L et al. Abrogation of TGFβ Signaling in Mammary Carcinomas Recruits Gr-1+CD11b+ Myeloid Cells that Promote Metastasis. Cancer Cell 13, 23–35, doi:10.1016/j.ccr.2007.12.004 (2008). [PubMed: 18167337]
16. Shi X et al. Cancer-Associated Fibroblasts Facilitate Squamous Cell Carcinoma Lung Metastasis in Mice by Providing TGFβ-Mediated Cancer Stem Cell Niche. Frontiers in Cell and Developmental Biology 9, doi:10.3389/fcell.2021.668164 (2021).
17. Strait AA et al. Distinct immune microenvironment profiles of therapeutic responders emerge in combined TGFβ/PD-L1 blockade-treated squamous cell carcinoma. Communications Biology 4, 1005, doi:10.1038/s42003-021-02522-2 (2021). [PubMed: 34433873]
18. Sato H, Okonogi N & Nakano T Rationale of combination of anti-PD-1/PD-L1 antibody therapy and radiotherapy for cancer treatment. Int J Clin Oncol 25, 801–809, doi:10.1007/s10147-020-01666-1 (2020). [PubMed: 32246277]
19. Bailly C, Thuru X & Quesnel B Combined cytotoxic chemotherapy and immunotherapy of cancer: modern times. NAR Cancer 2, doi:10.1093/narcan/zcaa002 (2020).
20. Lan Y et al. Simultaneous targeting of TGF-β/PD-L1 synergizes with radiotherapy by reprogramming the tumor microenvironment to overcome immune evasion. Cancer Cell, doi:10.1016/j.ccell.2021.08.008 (2021).
21. Rodríguez-Ruiz ME et al. TGFβ Blockade Enhances Radiotherapy Abscopal Efficacy Effects in Combination with Anti-PD1 and Anti-CD137 Immunostimulatory Monoclonal Antibodies. Molecular cancer therapeutics 18, 621–631, doi:10.1158/1535-7163.mct-18-0558 (2019). [PubMed: 30683810]
22. Teramoto K et al. Clinical significance of PD-L1-positive cancer-associated fibroblasts in pN0M0 non-small cell lung cancer. Lung Cancer 137, 56–63, doi:10.1016/j.lungcan.2019.09.013 (2019). [PubMed: 31546072]

Table 1:
Active and suspended clinical trials for bintrafusp alfa (BA).

Bintrafusp alfa clinical trials are listed by their phase, with their combination treatments where applicable, cancer type, and trial status. Abbreviations: intrahepatic cholangiocarcinoma (iCCA), triple-negative breast cancer (TNBC), head and neck squamous cell carcinoma (HNSCC), human papillomavirus (HPV), non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), biliary tract cancer (BTC), esophageal squamous cell carcinoma (ESCC).

Trial ID	BA Treatment Combination	Cancer type	Phase	Trial Status
NCT04708067	Radiotherapy	iCCA	I	Recruiting
NCT04756505	Radiotherapy + IL-12	HR+/HER2− breast cancer	I	Recruiting
NCT04349280	Monotherapy	Urothelial cancer	I	Recruiting
NCT02517398	Monotherapy	Solid tumors	I	**Active, not recruiting**
NCT03579472	Chemotherapy	TNBC	I	Recruiting
NCT02699515	Monotherapy	Solid tumors	I	**Active, not recruiting**
NCT03524170	Radiation	$HER2^-$ breast cancer	I	**Active, not recruiting**
NCT03620201	Monotherapy	$HER2^+$ breast cancer	I	Recruiting
NCT04235777	Radiation + IL-12	Genitourinary cancer	I	**Suspended**
NCT04220775	Radiotherapy	HNSCC	I/II	Recruiting
NCT04789668	MEK1/2 inhibitor	Brain metastases	I/II	Recruiting
NCT04708470	IL-12 + HDAC inhibitor	Advanced solid tumors	I/II	**Suspended**
NCT04287868	IL-12	HPV^+ cancers	I/II	**Suspended**
NCT03493945	Vaccine + IDO1 inhibitor + IL-15 agonist	Prostate cancer	I/II	**Suspended**
NCT04574583	Vaccine + CXCR1/2 inhibitor	Solid tumors	I/II	**Active, not recruiting**
NCT04247282	Vaccine + CXCR1/2 inhibitor	HPV^- HNSCC	I/II	**Suspended**
NCT03840915	Chemotherapy	NSCLC	I/II	**Active, not recruiting**
NCT04633252	Chemotherapy + IL-12	Prostate cancer	I/II	Recruiting
NCT03554473	Chemotherapy	SCLC	I/II	**Suspended**
NCT04327986	Radiation + IL-12	Pancreatic cancer	I/II	**Suspended**
NCT03451773	Chemotherapy	Pancreatic cancer	I/II	**Terminated**
NCT04303117	IL-12	Karposi sarcoma	I/II	Recruiting
NCT04432597	HPV vaccine	HPV^+ cancers	I/II	Recruiting
NCT03436563	Monotherapy	Colorectal cancer	I/II	Recruiting
NCT04971187	Chemotherapy	NSCLC	II	Recruiting
NCT04396535	Chemotherapy	NSCLC	II	**Active, not recruiting**
NCT04551950	Chemoradiation	Cervical cancer	II	**Active, not recruiting**
NCT03315871	Vaccine	Prostate cancer	II	Recruiting
NCT04727541	Monotherapy	BTC	II	Recruiting
NCT04428047	Monotherapy	HNSCC	II	Recruiting
NCT04560686	Surgical resection	NSCLC	II	Recruiting
NCT04595149	Chemoradiation	ESCC	II	Recruiting

Trial ID	BA Treatment Combination	Cancer type	Phase	Trial Status
NCT05012098	Monotherapy	Neuroblastoma	II	Recruiting
NCT04491955	Vaccine + IL-12	Bowel and colorectal cancer	II	**Suspended**
NCT04246489	Chemotherapy	Cervical cancer	II	**Active, not recruiting**
NCT04489940	Monotherapy	$HMGA^+$ TNBC	II	**Active, not recruiting**
NCT03840902	Chemoradiation	NSCLC	II	**Active, not recruiting**
NCT04501094	Monotherapy	Urothelial cancer	II	**Suspended**
NCT03427411	Monotherapy	HPV^+ cancers	II	**Active, not recruiting**
NCT03833661	Monotherapy	BTC	II	**Active, not recruiting**
NCT04417660	Monotherapy	Thymic cancer	II	**Suspended**
NCT03707587	Monotherapy	Respiratory papillomatosis	II	**Active, not recruiting**
NCT04066491	Chemotherapy	BTC	II/III	**Active, not recruiting**
NCT03631706	Pembroluzimab (head to head)	NSCLC	III	**Active, not recruiting**