VEGF-DEPENDENT CLEAVAGE OF CELL SURFACE PD-1 MEDIATED BY CTX-10726, A NOVEL PD-1×VEGF-A BISPECIFIC ANTIBODY

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ABSTRACT

BACKGROUND

Dual inhibition of PD-1 and VEGF-A has demonstrated clinical benefit, yet the mechanistic interplay between these pathways remains incompletely understood. CTX-10726 is a tetravalent bispecific antibody comprising an anti-VEGF-A IgG1 with Fc-silencing mutations fused to anti-PD-1 scFvs. We investigated its mechanism of action, focusing on PD-1 receptor dynamics, tumor microenvironment (TME) modulation, and activity in combination with the CD137 agonist CTX-471.

METHODS

Binding and functional activity were assessed by ELISA, mixed lymphocyte reaction (MLR), and flow cytometry. PD-1 surface fate (internalization versus shedding) was evaluated in PD-1–overexpressing cells in the presence or absence of VEGF-A. *In vivo* PD-1 loss was assessed in two systems: (i) matrigel plug assays using JurkatPD-1–expressing cells with systemic administration of recombinant VEGF-A, and (ii) co-implantation of JurkatPD-1 cells with VEGF-A–secreting HCC827 tumor cells. Anti-tumor activity and mechanism were studied in an immune checkpoint inhibitor–resistant CT26 syngeneic subcutaneous tumor model using bulk RNA profiling and immunohistochemistry.

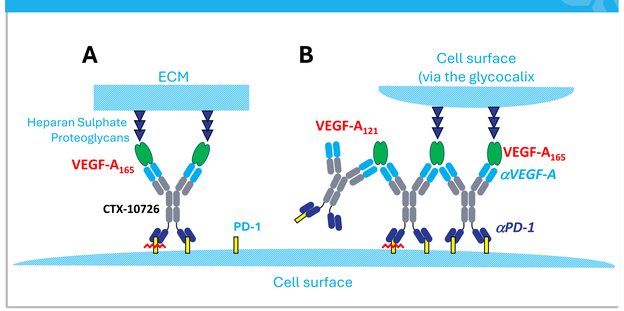
RESULTS

CTX-10726 demonstrated dual blockade activity and revealed VEGF-A-dependent regulation of PD-1 receptor fate. In the absence of VEGF-A, PD-1 was predominantly internalized, whereas VEGF-A promoted PD-1 shedding and accumulation of soluble PD-1. VEGF-A–dependent PD-1 loss was confirmed in both *in vivo* models. CTX-10726 alone primed the tumor microenvironment without inducing a fully inflamed state. In combination with CTX-471, enhanced anti-tumor activity was observed, accompanied by coordinated upregulation of chemokines, interferon-stimulated genes, and inflammatory cytokines. These changes were associated with increased immune cell recruitment and establishment of a more permissive TME.

CONCLUSIONS

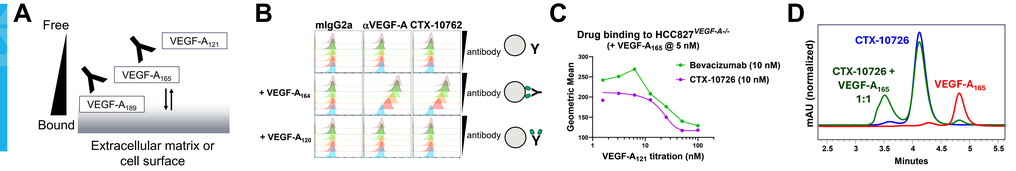
We propose a model in which VEGF-A regulates PD-1 surface expression on activated T cells within the TME, representing a novel mechanistic paradigm for this class of bispecific therapeutics. CTX-10726 exhibits a differentiated mechanism characterized by VEGF-A-dependent regulation of PD-1 fate, driving either receptor internalization or shedding and thereby linking angiogenic signaling to checkpoint control. In combination with CD137 agonism, CTX-10726 further amplifies immune activation and reshapes the TME, promoting immune cell recruitment and functional engagement. These findings position CTX-10726 as a mechanistically distinct immunotherapy with potential utility in immunotherapy-resistant settings.

MODEL



VEGF-A bridges CTX-10726 to turn PD-1 binding into stripping. Each bivalent VEGF-A dimer binds the anti–VEGF-A arms of CTX-10726 while docking onto heparan sulfate proteoglycans in the extracellular matrix **(A)** or the cell glycocalyx **(B)**, potentially forming a multivalent antibody:VEGF-A lattice that clusters PD-1 against a fixed anchor. As we previously showed for CTX-8371[1] (a PD-1✕PD-L1 bispecific antibody), such bridging strips PD-1 from the engaged cell. Here VEGF-A replaces PD-L1 as the partner, but being abundant, tumor-derived, and matrix- or cell-anchored, the TME itself supplies the anchor. Anchoring drives stripping, which, therefore, should scale in potency with VEGF-A abundance and matrix retention (Fig. 2B).

1. VEGF-A bridges anti–VEGF-A antibodies onto cell surfaces in an isoform dependent manner



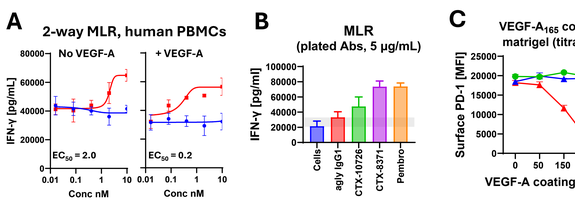
A. VEGF-A isoforms differ in affinity for the extracellular matrix and glycocalyx (mainly heparan sulfate proteoglycans): VEGF-A_{189} binds most avidly, VEGF-A_{165} intermediately, and VEGF-A_{121} remains free. In a CCLE survey, all VEGF-A–secreting lines expressed VEGF-A_{189} and VEGF-A_{121}, and ~50% also expressed VEGF-A_{165}. Anti–VEGF-A antibodies added to the ECM are therefore expected to lock matrix-bound isoforms into a lattice, stabilizing the VEGF-A gradient around the secreting cell.

B. VEGF-A–binding antibodies bind tumor cell surfaces (CT26) when complexed with VEGF-A. VEGF-A_{164} bridges this interaction efficiently; VEGF-A_{120}, lacking HSPG-binding domains, does not. CTX-10762 is a mouse surrogate of CTX-10726 used in this experiment and in all the *in vivo* studies. VEGF-A_{164} and VEGF-A_{120} are the mouse orthologs of human VEGF-A_{165} and VEGF-A_{121}.

C. Binding of antibody (anti-VEGF-A or CTX-10726):VEGF-A_{165} immunocomplexes to cells rendered unable to produce VEGF-A by CRISPR knockout (HCC827$^{VEGF\text{-}A\text{-}/\text{-}}$) is displaced dose-dependently by increasing concentrations of VEGF-A_{121}, confirming the specificity of VEGF-A bridging.

D. SEC-HPLC profiles show that adding VEGF-A_{165} to CTX-10726 generates aggregates, including a peak consistent with the (mostly dimeric) species reported for bevacizumab[2] and ivonescimab[3].

2. VEGF-A crosslinking potentiates CTX-10726 activity and drives loss of surface PD-1



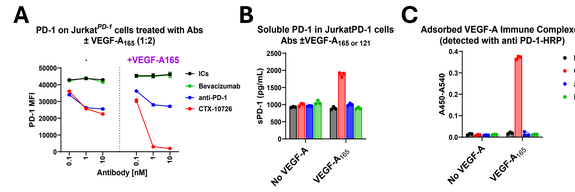
A. CTX-10726 shifts the dose-response ~10-fold (EC_{50} 2.0 → 0.2 nM) when recombinant VEGF-A is added to the reaction.

B. Plate-immobilized anti–PD-1 antibodies increase IFN-γ output in MLRs, indicating that anchoring alone is sufficient to boost signal.

C. Jurkat$^{PD\text{-}1}$ cells (a variant of Jurkat cells engineered to overexpress human PD-1) were incubated overnight on VEGF-A_{165}-coated Matrigel plates and analyzed by flow cytometry. Surface PD-1 loss was seen only in the presence of CTX-10726.

D. After removal of supernatants in C, plates were probed for PD-1, which remained linked to CTX-10726. As expected, a cleaved PD-1 product is detected bound to the plate.

3. Soluble VEGF-A drives CTX-10726–specific loss of surface PD-1 and its release as soluble PD-1 fragment

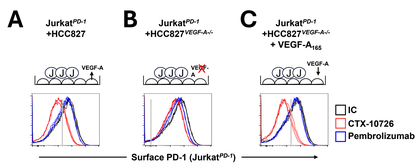


A. VEGF-A_{165} markedly enhances CTX-10726–mediated loss of surface PD-1 on Jurkat$^{PD\text{-}1}$ cells, an effect not seen with anti–PD-1, bevacizumab, or isotype control antibody; soluble VEGF-A_{121} gave comparable results (flow cytometry).

B. The lost receptor accumulates as soluble PD-1 in the supernatant, only with CTX-10726 plus VEGF-A_{165}, confirming VEGF-A–dependent shedding rather than internalization (ELISA).

C. After removal of the supernatant, plates in A were probed with an anti–PD-1–HRP antibody. CTX-10726:VEGF-A immune complexes adsorbed in extracellular matrix deposited during incubation retain the stripped PD-1, showing the shed receptor remains bound within the VEGF-A:antibody complex and that VEGF-A complexes tend to stick to ECM components.

4. Tumor cell-derived VEGF-A is required for CTX-10726–mediated stripping of surface PD-1 in co-culture

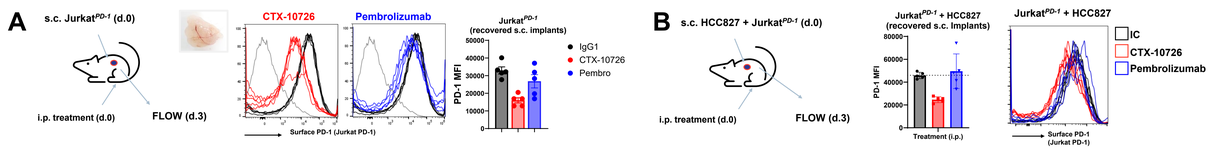


A. HCC827 cells secrete endogenous VEGF-A (not shown). Cell-derived (tumor-secreted) VEGF-A is required for CTX-10726 to strip surface PD-1 in Jurkat$^{PD\text{-}1}$/HCC827 co-cultures, indicating this can occur within VEGF-A–rich tumors *in vivo*. Pembrolizumab is inert throughout. With VEGF-A–secreting HCC827, CTX-10726 (red) lowers surface PD-1, whereas pembrolizumab and isotype control (IC) do not.

B. Knocking out VEGF-A abolishes PD-1 stripping, confirming the requirement for tumor-derived VEGF-A.

C. Adding back recombinant VEGF-A_{165} to the culture supernatants restores PD-1 stripping.

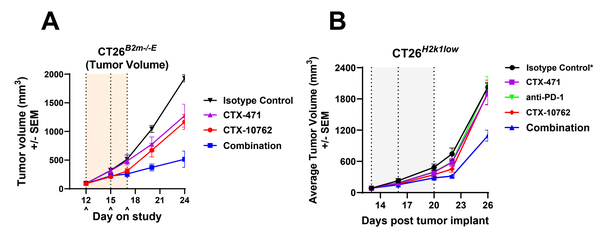
5. CTX-10726 strips surface PD-1 *in vivo*, and the effect is driven by both recombinant and tumor-derived VEGF-A



A. Matrigel plugs were seeded with Jurkat$^{PD\text{-}1}$ cells plus recombinant human VEGF-A. Mice were dosed i.p. once (3 mg/kg) and plugs were excised at day 3. CTX-10726 (red) reduces surface PD-1 whereas pembrolizumab (blue) and IgG1 do not — showing the antibody permeates the plug and promotes stripping of PD-1 *in vivo* (right, PD-1 MFI quantitation).

B. In s.c. co-implants of Jurkat$^{PD\text{-}1}$ with VEGF-A–secreting HCC827 (tumor-derived rather than recombinant human VEGF-A as in A, same readout), CTX-10726 again lowers surface PD-1, whereas pembrolizumab and IC do not.

6. The CTX-10762 + CTX-471 combination controls checkpoint-refractory tumors, even where anti–PD-1 alone is ineffective

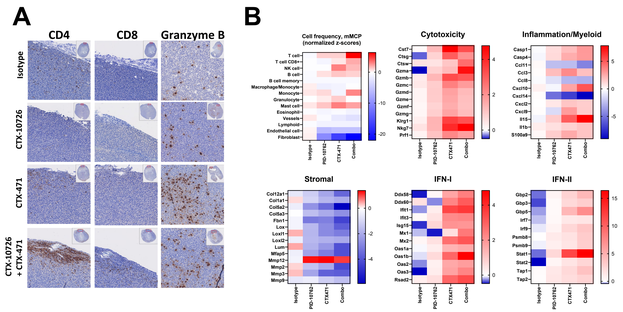


CTX-10762 is the mouse surrogate of CTX-10726. Models shown are CT26$^{B2m\text{-}/\text{-}E}$ (an IO-refractory version of the CT26 model lacking B2m and selected to possess increased resistance to NK cell killing), and CT26^{H2k1low} (a low MHC-I H2-k1 version of CT26, which no longer responds to anti–PD-1 therapy). Dotted lines indicate the day of treatment (5 mpk).

A. In CT26$^{B2m\text{-}/\text{-}E}$, the CTX-10762 + CTX-471 combination (blue) markedly slows mean tumor growth versus either single agent or isotype control.

B. In CT26^{H2k1low}, where low MHC-I renders tumors unresponsive to anti–PD-1 (green), the combination (blue) remains effective.

7. The combination reshapes the tumor microenvironment, driving effector cell infiltration with coordinated activation of interferon and cytotoxicity programs



A. IHC of CT26B2m-/-E tumors (as in Fig 6). CD4, and to a lesser extent CD8, and granzyme B staining rise with treatment and are greatest with the CTX-10762 + CTX-471 combination. The infiltrate is cortical rather than strictly excluded, and cortical recruitment tracks with tumor control.

B. mMCP deconvolution of immune-cell frequencies and differential expression of custom selections of genes representative of different pathways. The combination markedly increases cytotoxic, inflammation and type I/II IFN pathways while overall reducing the stromal/fibroblast signal.

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