# ENHANCED EFFICACY OF CTX-471, A CD137 AGONIST ANTIBODY, IN MODELS OF IMMUNE CHECKPOINT FAILURE VIA SIMULTANEOUS BLOCKADE OF NEO-ANGIOGENESIS

Amy Daniel Ulumben, Diana I. Albu, Rachael Duffy, Tristan Lubinski, Jason Kong, Jessica Zolotarevsky, Melissa Brundin, Mei Su, Ruturaj Jadhav, Bing Gong, Thomas J. Schuetz, Kris F. Sachsenmeier and Alberto Visintin*

*Compass Therapeutics Inc. | 80 Guest Street | Boston MA 02135 | USA* ** alberto@compasstherapeutics.com*

**#7297**

## ABSTRACT

**CTX-471 is a monoclonal agonistic anti-CD137 antibody currently under investigation in patients with solid tumors. To increase the breadth and duration of response to CTX-471 we set out to investigate its anti-tumor efficacy in combination with anti-angiogenesis agents in murine models. CTX-009 is a bispecific antibody targeting VEGF-A and DLL4 which is undergoing clinical investigation in patients with multiple different cancers, including biliary tract and colorectal cancers. Encouraged by early findings demonstrating activity of the combination in the MC38$^{B2m}$ knockout mouse tumor model, engineered to mimic HLA loss in patients, we broadened the exploration of the combinatorial treatment in an additional B2m knockout model using a different murine genetic background (CT26$^{B2m-/-}$, Balb/c). Furthermore, we developed two novel mouse models of immunotherapy resistance without the enhanced NK cell susceptibility bias conferred by complete MHC-I loss due to B2m deletion[1]. CT26 cells with an engineered deletion of the B2m gene were passaged in tumor-experienced mice and a line of CT26$^{B2m-/-}$ cells that escaped immune rejection was established (CT26 B2m knockout escapers, CT26$^{B2m-/-E}$). For the second model, the H-2k1 MHC-I locus was knocked out in MC38 cells, resulting in targeted homozygous loss. Using this approach, the expression of the other MHC-I alleles, and therefore natural resistance to NK cells, were left intact.**

**Results: Coupling VEGF-A/DLL4 targeting with CD137 agonism markedly increased the anti-tumor efficacy of the individual treatments alone in the MC38$^{B2m-/-}$, MC38$^{H-2k1-/-}$, CT26$^{B2m-/-}$ models as well as the highly immune checkpoint inhibitor (ICI) resistant CT26$^{B2m-/-E}$ model. Tumor growth inhibition was accompanied by pharmacodynamic evidence consistent with an enhancement of cellular cytotoxic immunity. CTX-471 monotherapy was still efficacious, albeit blunted, in these ICI resistant models. The latter preclinical results mirrored data from a Phase 1 clinical trial of CTX-471, where evidence of NK activation following CTX-471 treatment was seen in both blood and tissue samples of patients with known defects in the antigen presentation machinery. The findings presented here suggest that an agonistic anti-CD137 antibody may provide enhanced clinical benefit when employed alongside an anti-angiogenesis agent, such as CTX-009, in tumors where immune checkpoint inhibitors had previously failed.**

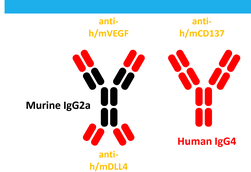
## INTRODUCTION

CTX-471, a fully human IgG4 anti-CD137 agonistic antibody developed at Compass Therapeutics[2], was designed to optimally activate T and NK cells while minimizing toxicity seen with this class of therapeutics. In an ongoing Phase 1 trial (NCT03881488) in patients with advanced cancers progressing after PD-1/PD-L1 inhibitors (post-ICI patients), CTX-471 has shown good tolerability below 1.2 mg/kg, with most adverse events being mild (Grade 1-2). Notably, one patient with small-cell lung cancer achieved a durable complete response, while partial responses were observed in patients with melanoma and mesothelioma[3]. Of 12 treated patients with pre-treatment biopsies evaluable by NGS, 2 (17%) had loss of HLA-I alleles. CTX-471 demonstrated disease control (CR or SD) in both patients with HLA defects suggesting CTX-471 might be active in this type of tumor despite defective antigen presentation. CTX-471 induced pharmacodynamic changes consistent with immune activation, including increased CD4+ and CD8+ T cells expressing CD137 and higher levels of activated NK cells[4]. Based on this, we hypothesized that combining CTX-471 with an anti-angiogenic agent could enhance therapeutic efficacy by normalizing tumor vasculature and further increasing immune infiltration, making this combination a potential treatment of choice for post-ICI patients. To provide preclinical proof of concept, we developed mouse models with MHC defects commonly seen in post-ICI patients, including those in our trial. Specifically, we modeled total MHC loss by deleting B2m in MC38 and CT26 isografts and partial loss by deleting both H2-K1 alleles in MC38 cells (which eliminates 1/3 of the MHC-I presentation potential). To counteract potential increased susceptibility to NK killing from total MHC loss, we passaged CT26$^{B2m-/-}$ cells in vaccinated mice and selected a line with increased NK resistance (CT26$^{B2m-/-E}$).

Tovecimig (CTX-009), a bispecific IgG1 antibody targeting DLL4 and VEGF-A, has demonstrated promising efficacy in solid tumors. Phase 1 trials showed activity as monotherapy and with chemotherapy, while a Phase 2 trial in biliary tract cancer reported a 37.5% response rate with paclitaxel.

Here, we present the results of testing the CTX-471 + CTX-009 combination in these novel checkpoint-refractory mouse models, designed specifically to replicate the clinical setting of post-CPI resistance.

## 1. THE DRUGS USED IN THIS STUDY



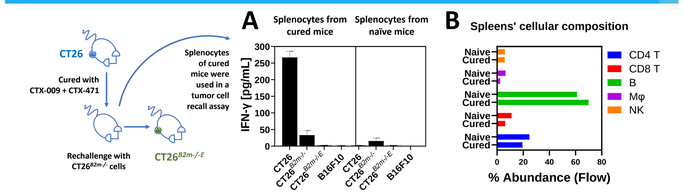

A bispecific antibody targeting murine VEGF and DLL4 was generated to model Tovecimig's activity in isogenic tumor models (mCTX-009). mCTX-471 is an affinity optimized version of CTX-471 with improved binding to murine CD137. The parental antibody CTX-471 is the anti CD137 monoclonal antibody in development at Compass Therapeutics Inc.

**Summary of *in vivo* activity**

| Cell line name | Strain | Notes | Inoculum* | Monotherapy Activity: mCTX-471 | Monotherapy Activity: mCTX-009 | Monotherapy Activity: αPD-L1 | Combo Activity**: mCTX-471 +CTX-009 | Combo Activity**: PD-L1 +CTX-009 |
|---|---|---|---|---|---|---|---|---|
| MC38 | C57BL/6 | | 0.25 | ++++ | + | ++++ | No | No |
| MC38$^{H-2k1-/-}$ | C57BL/6 | CRISPR KO H-2k1 | 0.25 | ++++ | ++ | ++ | Yes§ | No |
| CT26 | BALB/c | | 0.5 | ++++ | +++ | ++++ | Yes§ | Yes |
| CT26$^{B2m-/-}$ | BALB/c | CRISPR KO B2m | 1 | + | + | - | Yes | No |
| CT26$^{B2m-/-E}$ | BALB/c | Passaging of CT26$^{B2m-/-}$ in cured mice | 0.1 | ++ | ++ | - | Yes | Yes |

**Cells implanted subcutaneously in millions.*
***Refers to whether combination produces better tumor growth inhibition than monotherapies.*
*§Effect most notable at earlier time points.*

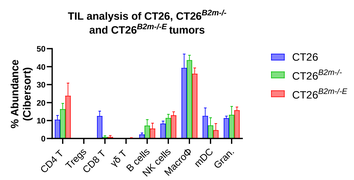
## 2. ENHANCED RECOGNITION THRESHOLD OF IO-RESISTANT CT26 CELLS BY SPLENOCYTES OF TUMOR-EXPERIENCED MICE




**Target cells**

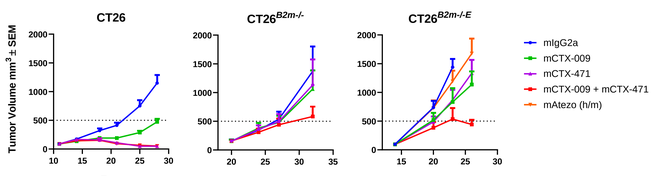
**A.** Splenocytes from mice previously challenged with CT26 tumors and cured *via* CTX-471 and CTX-009 treatment (left half of panel A) contain cells that react to CT26 cells, producing approximately 10 times more IFN-γ when exposed to the indicated target cell lines (x-axis) compared to splenocytes from naïve mice (right half of panel A). Neither cured nor naïve splenocytes recognized CT26$^{B2m-/-E}$ cells. B16F10 cells (which, unlike CT26 cells, are of the C57Bl/6 genetic background) served as negative controls. These findings support a model in which CT26$^{B2m-/-E}$ cells have evaded anti-tumor immunity by reducing their recognition by host splenocytes. Data shown corresponds to an E:T ratio of 10:1. **B.** Similar percentage of CD4, CD8, B, Mono/macs (MΦ) and NK cells were observed in the spleens from naïve vs tumor experienced mice.

## 3. BASELINE LOSS OF CD8 T AND mDCs AND INCREASE IN CD4T, B AND NK CELLS IN THE CT26$^{B2m-/-E}$ MODEL




Baseline tumor infiltrating leukocyte (TIL) characterization in the CT26 tumor series. RNA from tumors with the indicated genetic background (n = 4) was extracted, sequenced, and the relative amount of different cell types estimated by cibersort deconvolution (via the Timer2.0 portal). Both CT26$^{B2m-/-}$ and CT26$^{B2m-/-E}$ had similar relative amounts of TILs and diverged from the parental by showing an increase in CD4T, B and NK cells, whereas mDCs and CD8T cells were markedly decreased. "Gran." = other granulocytes.

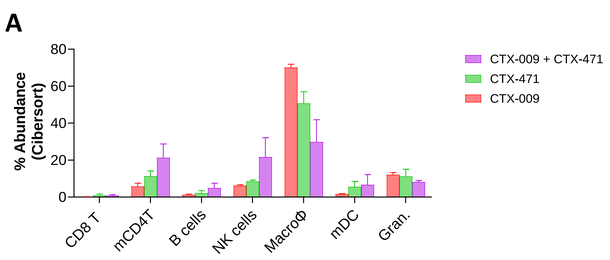
## 4. THE CTX-009 + CTX-471 COMBINATION IS EFFECTIVE IN THE CT26$^{B2m-/-E}$ MODEL



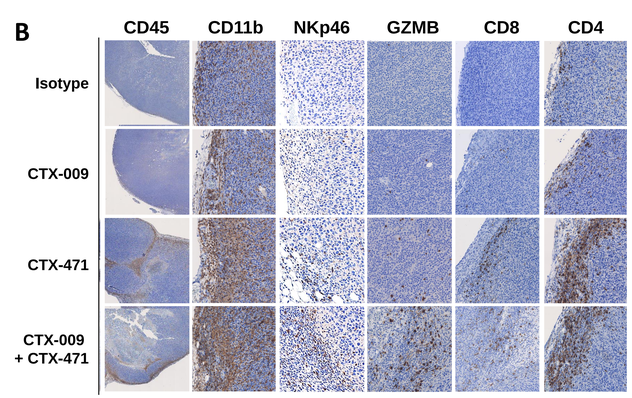

The combination of CTX-471 and CTX-009 is efficacious in CT26 models where conventional ICI (anti PD-L1) shows reduced activity (orange line). Mice bearing the indicated tumor models were treated with the specified antibodies (mCTX-009: 5 mpk, q3dx3; mCTX-471: 0.1 mpk, q7dx2; mAtezo: 3 mpk, q3dx3) when tumors reached ~100 mm$^3$. Tumor volumes (Y-axis) were recorded over time. The parental CT26 model was highly responsive to mCTX-471 and the combination of mCTX-471 + mCTX-009. In contrast, CT26$^{B2m-/-}$ and CT26$^{B2m-/-E}$ exhibited reduced sensitivity to monotherapies, while the combination treatment showed additive efficacy.

## 5. CHANGES IN TIL COMPOSITION FOLLOWING TREATMENT OF CT26$^{B2m-/-E}$ TUMORS

**A.** Tumoral RNA (n = 3) was processed as in 3. Compared to monotherapies, treatment with the CTX-009 + CTX-471 combination led to an increase in total CD4 T cells, B and NK cells, whereas it decreased the proportion of monocytes to macrophages. "Gran." = other granulocytes.

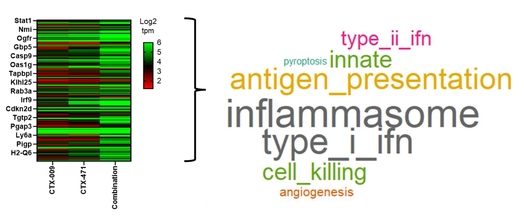



**B.** Immunohistochemical (IHC) analysis of the tumors in A and B (focused on the cortical areas of the tumors). At baseline, tumors lacked CD8$^+$ T cells and showed moderate CD4$^+$ T cell infiltration. CTX-009 and CTX-471 induced distinct infiltration patterns, with CTX-471 promoting immune cell (CD45) recruitment in the tumor cortex, which strongly correlated with tumor growth inhibition (Figure 4). Notably, the combination treatment uniquely increased the frequency of activated immune cells, as indicated by Granzyme B staining

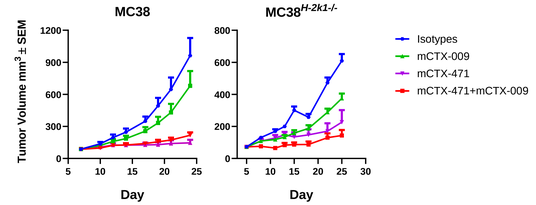



## 6. THE CTX-009 + CTX-471 COMBINATION ENHANCES THE INFLAMMASOME, CYTOLYSIS AND INTERFERON PATHWAYS

The heatmap shows the log2 TPM values for all genes showing significant (Anova, p<0.01) differences in RNA levels between tumor samples taken from mice treated with CTX-009 and CTX-417 alone or in combination. Word cloud of unsupervised GO analysis (geneontology.org) of significantly regulated genes, revealed enrichment of inflammatory and innate immune-related pathways by the combination of CTX-009 and CTX-471 in this model.

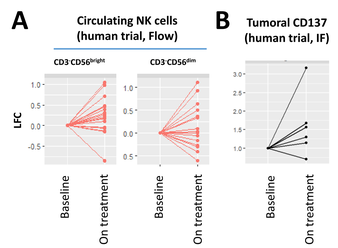



## 7. THE CTX-471 + CTX-009 COMBINATION IS EFFICACIOUS IN A NOVEL MODEL OF MHC-I LOSS




Mice bearing the indicated tumor models were treated with the indicated antibodies (mCTX-009: 5 mpk, q3dx3; mCTX-471: 0.1 mpk, q7dx2 and their combination) when tumors reached ~80 mm$^3$. Tumor volumes (Y-axis) were recorded over time. The parental MC38 model was highly responsive to mCTX-471 and the combination of mCTX-471 + mCTX-009 did not result in increased TGI). In contrast, MC38$^{H-2k-/-}$ exhibited reduced sensitivity to CTX-471 monotherapy, while the combination treatment showed additive efficacy, especially at the earlier treatment points. Data shown is representative of at least 2 biological replicate studies. Dosing and regimen as in 4.

## 8. EVIDENCE OF NK ACTIVATION IN CTX-471 TREATED PATIENTS




**A.** Increase of circulating NK cells (primarily CD56 bright) in anti CD137 treated patients. **B.** Increase in the fraction of CD137 positive cells in paired slides (pre-treatment and post-treatment) with CTX-471. Tumor upregulation of CD137 post-treatment suggests enhanced effector activity. CD137 agonism may drive a feedback loop, further activating cells and aligning with the mechanism of action of anti-CD137 antibodies. For data in panel A, levels of circulating cells with the indicated surface markers were measured by flow cytometry in blood samples collected before treatment and after 9 weeks of CTX-471 monotherapy. In panel B, the number of CD137 positive cells was measured using multi-panel immunofluorescence from tumor samples taken before and after 9 weeks of CTX-471 monotherapy treatment.

## TAKEAWAYS

- CTX471 monotherapy increases the tumoral immune infiltrate, specifically NK cells in both post-CPI patients and post-CPI models in mice;
- CTX-471 agonism is effective in several distinct murine models of immunotherapy resistance with increased translational relevance;
- The activity of CTX-471 can be further enhanced by targeting angiogenesis;
- Combining CTX-471 with CTX-009 might provide clinical benefit to patients previously treated with CPIs;
- Mechanistically, the combination of CTX-471 and CTX-009 might prominently enhance inflammasome activation, pyroptosis, and interferon-mediated signaling.

## REFERENCES

**1.** Lanier, L. L. Five decades of natural killer cell discovery. *J. Exp. Med.* 221, e20231222 (2024). **2.** Eskiocak, U. *et al.* Differentiated agonistic antibody targeting CD137 eradicates large tumors without hepatotoxicity. *JCI Insight* 5, e133647. **3.** Barve et al. ASCO 2024, **4.** Sachsenmeier et al. AACR 2024.

## ACKNOWLEDGEMENTS

The authors express their appreciation to Anna Gifford for her exceptional editorial assistance, and to the Compass' leadership team for their steadfast support. Gratitude is also extended to the numerous unnamed individuals within the company whose contributions were integral to the success of this project.

**Presented at the American Association for Cancer Research (AACR) Annual Conference 2025, April 25-30, Chicago, IL, USA & Online**