

Abstract

Background & Objectives: Clinical response to immunotherapies in patients with biliary tract cancer (BTC) has supported approval of these therapies^{1,2}. However, a proportion of patients have tumors which never respond or subsequently become non-responsive. For these patients, clinically actionable data is needed to inform treatment decisions. For example, a readily measurable biological assay with results linked to a potentially successful treatment option could enable use of that option in patients with BTC after treatment with immunotherapies.

Methods and Results: We report preclinical evidence showing activity in a mouse treated with a bispecific antibody targeting both mouse DLL4 and VEGF-A in models of checkpoint inhibitor resistance. Specifically, a mouse cross-reactive surrogate of CTX-009, an anti-DLL4/VEGFA bispecific antibody currently in clinical trials, shows remarkable tumor growth inhibition in a mouse model lacking both class I MHC as well as the tumor suppressor CDKN2A – both well-documented tumor escape mechanisms from immunotherapy³⁻⁹. A cohort of patient tumors evaluated using the Tempus xT NGS assay showed evidence of genomic alterations consistent with immune checkpoint escape. Specifically, of 345 evaluable samples, approximately 50% of the tumors showed loss of heterozygosity at the HLA locus and an additional 62% showed deletion of CDKN2A/B. 37 (~11%) tumors showed deletions in both of these loci. Interestingly, 23 (~10%) of the CDKN2A/B deleted tumors showed concurrent loss of MLLT3, suggesting co-deletion of type 1 IFN genes immediately adjacent to CDKN2A/B on chr9p21⁷ and between CDKN2A/B and MLLT3. These observations are consistent with known immune checkpoint therapy escape mechanisms and provide a context for anticipated analyses of tumors from patients treated with CTX-009. That is, it will be important to learn whether tumor responses to CTX-009 in the clinic show the same independence with respect to these two resistance mechanisms as has been observed in mouse preclinical models.

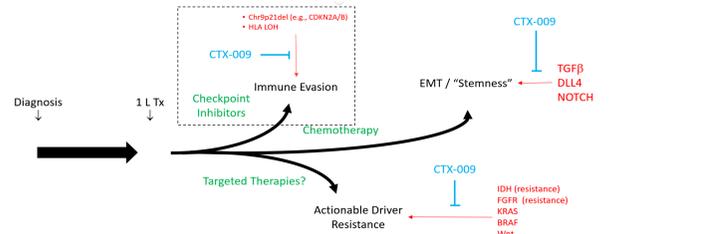
Conclusions: In summary, these data support the testing of CTX-009 in patients with BTC whose tumors have biomarkers identifiable with standard approved genomic sequencing assays.

Introduction

BTC tumors can become resistant to immune checkpoint inhibitors. If resistance can be detected using a biomarker during or before treatment, chances of successful subsequent treatment are enhanced. To help with this challenge, we studied the frequency of known potential immune checkpoint inhibitor resistance biomarkers. We focused on DNA biomarkers detectable using commercially available and clinically-validated next generation sequencing assay(s).

Given the prevalence of two important immune checkpoint inhibitor resistance biomarkers in large BTC patient datasets, we constructed a mouse model for resistance. These studies suggest potential activity of CTX-009 independent of resistance biomarkers.

BTC Drug Resistance Mechanisms Linked With CTX-009



BTC tumors can escape from drug therapy through many different pathways. Escape from immune checkpoint inhibitors such as anti-PD1/L1 include changes in tumor DNA such as loss of pieces of chromosome 9p21 and / or loss of human leukocyte antigen (HLA LOH).

Why is this important? By targeting resistance mechanisms linked with first line BTC therapy, CTX-009 may offer an option for patients with tumors resistant to immune checkpoint inhibitors.

Methods

Real-World Data: Data for human BTC tumors was obtained from the published literature as cited in the References section. De-identified real-world BTC data (n=345) was evaluated using the Tempus database (Tempus AI, Inc., Chicago, IL).

Mouse Studies: B2m knockout cell lines were generated and tested in MC38 mouse colorectal tumor cells as described in the figure legends. Tumor growth inhibition studies were carried out as described in the legends.

Results

Figure 1. Characterization of CTX-009

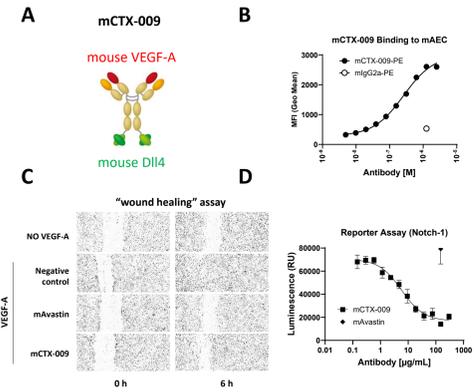


Figure 1. A bispecific antibody targeting murine VEGF-A and DLL4 was generated to model CTX-009 activity in syngeneic tumor models (Panel A, mCTX-009). The Kd for binding of mCTX-009 to VEGF-A and DLL4 were 2.22 pM and 13.1 nM respectively, which are both comparable to CTX-009. mCTX-009 bound to mouse aortic endothelial cells (mAEC, Panel B), delayed the VEGF-dependent reconstitution of a cellular monolayer in the mAEC scratch assay (Panel C) as well as blocked DLL4-induced NOTCH1 activity in an assay where in which plastic bound DLL4 activates a NOTCH1 responsive Luciferase reporter construct (Panel D).

Why is this important? These data show that CTX-009 is functionally active in biochemical and cellular experiments and supports subsequent studies in animal models.

Figure 2. Immune Checkpoint Inhibitor Resistance Biomarkers in BTC

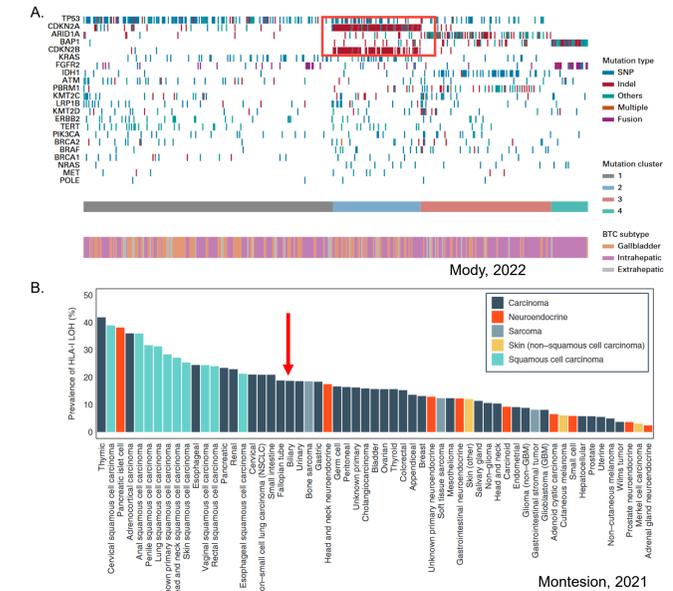


Figure 2. Two DNA biomarkers for checkpoint resistance have been shown to occur in BTC. Panel A. Doctor Kabir Mody¹⁰ and others, worked with a sequencing provider, TEMPUS, to study the DNA of over 450 BTC samples¹¹. They found that deletions in parts of chromosome 9 – specifically a tumor suppressor “CDKN2A/B” – occurred in approximately 1 in 5 tumors. Panel B. Another study of DNA sequencing across many tumor types⁸ showed that loss of HLA was detected in about 20% of BTC tumors.

Why is this important? These data show that biomarkers of resistance to anti-PD1/L1 therapy can be found in BTC tumors.

Figure 3. Prevalence of Two Resistance Biomarkers in Real World Data

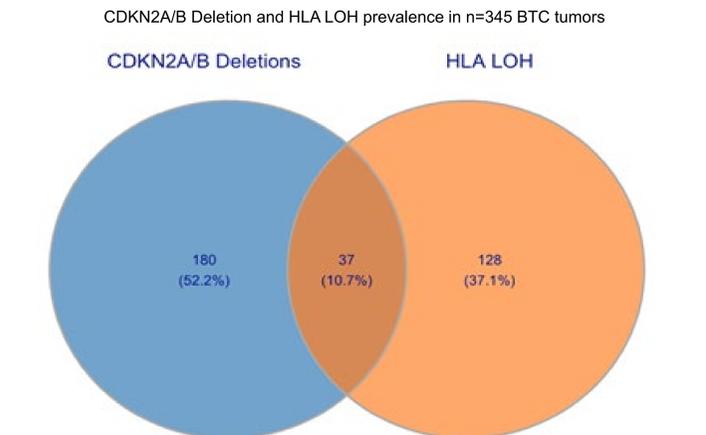


Figure 3. As part of the larger Tempus collection of real world sequencing data, 345 samples were evaluable for both CDKN2A/B deletion and HLA LOH. Approximately 50% of the tumors showed loss of heterozygosity at the HLA locus and an additional 62% showed deletion of CDKN2A/B. The co-occurrence of both resistance biomarkers through loss of both HLA and CDKN2A/B was ~11% (37 tumors). Interestingly, 23 (~10%) of the CDKN2A/B deleted tumors showed concurrent loss of a neighboring gene MLLT3, suggesting co-deletion of type 1 interferon genes immediately adjacent to CDKN2A/B on chr9p21 and between CDKN2A/B and MLLT3. This pattern of gene deletion is consistent with known immune checkpoint therapy escape mechanisms.

Why is this important? These data show that biomarkers of two therapy resistance pathways are not uncommon and provide a context for anticipated analyses of tumors from patients treated with CTX-009.

Figure 4. Shared Biomarkers of Mouse and Human Tumors Relevant to Immunotherapy Resistance

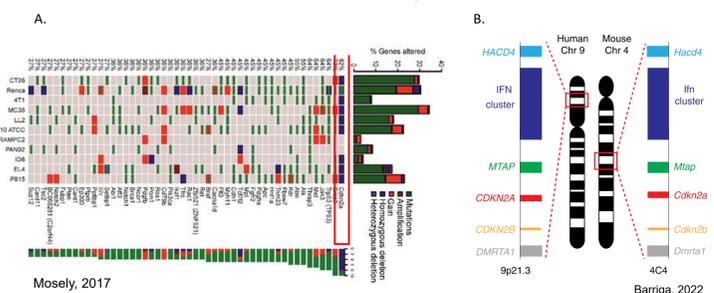


Figure 4. Panel A. Mouse cancer models, including MC38, show deletions in the Cdkn2a region of their DNA¹¹. Panel B. In both the mouse and human genomes, Cdkn2a/CDKN2A genes are located immediately next to the type 1 interferon gene cluster¹² which plays a key role in anti-tumor immunity.

Why is this important? Similarity between mouse and human tumors enables testing of drug response and resistance ideas in mouse models before testing in human tumors.

Figure 5. Mouse Models for Immunotherapy Resistance

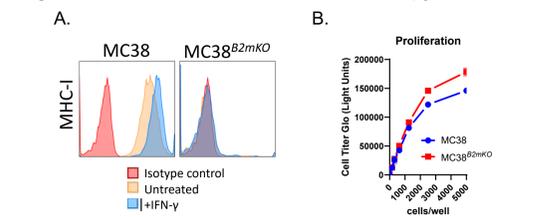
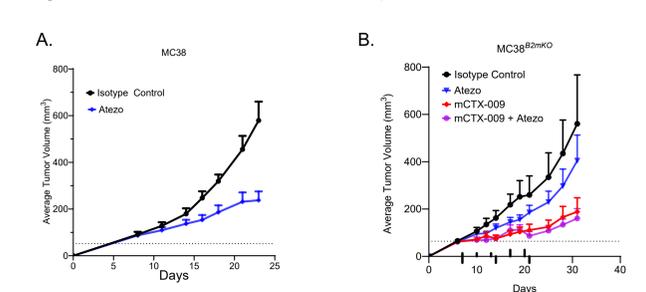


Figure 5. Deletion of the B2m gene in mouse cancer cell line MC38 imitates HLA LOH in humans. Panel A. Note that normal MC38 cells express MHC-I (the mouse version of HLA) which can be further increased by treatment with IFN-γ unlike B2mKO cells, where IFN-γ fails to rescue MHC-I expression. Panel B. As a test for other effects of B2m deletion, no significant effects upon cell proliferation were observed.

Why is this important? These data show that the mouse model behaves as expected for studying human resistance to immune checkpoint inhibitors.

Figure 6. CTX-009 Tumor Control Despite Resistance Biomarkers



MC38 cells implanted subcutaneously in mice are sensitive to anti-PDL1 antibody atezolizumab (Panel A). In MC38 cells that were rendered MHC-I negative by B2m deletion, anti-PDL1 tumor growth control was reduced as expected. In contrast, mCTX-009 treatment was more effective at controlling MC38 tumor growth than anti-PDL1. A combination of both CTX-009 and anti-PDL1 also did not reduce mCTX-009 activity (Panel B).

Why is this important? If CTX-009 controls mouse tumor growth, there is a chance that this will translate to human trials testing CTX-009.

Patient-Friendly Summary

The Challenge:

1. BTC tumors can become resistant to immunotherapies, such as anti-PD1/L1.
2. Resistance is linked with specific biomarkers that can be measured in a biopsy or blood sample.

The Strategy:

1. We surveyed large databases of human genomic data to determine how often resistance biomarkers can be found in BTC tumors.
2. We created a mouse tumor model that is similar to immunotherapy-resistant tumors in humans.
3. We tested a mouse version of our drug CTX-009 in those mice with a tumor resistant to immunotherapy.

The Results:

1. From the database analysis, we concluded that resistance biomarkers were found in about half of the human BTC tumors.
2. The mouse model replicated key aspects of the human BTC tumors resistant to immunotherapy. This allows us to study aspects of human BTC tumors using mouse models.
3. The mouse version of CTX-009 controlled tumor growth in the resistant model.

The Conclusions:

- Mouse data provide reason to believe that CTX-009 may show activity in human BTC despite the presence of resistance biomarkers.

Next Steps:

- Proceed to study CTX-009 in patients for whom immunotherapy has failed.

References

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