The Ad5/34 Chimeric Fiber of ICVB-1042 Binds Human CD46 for Efficient Tumor Cell Entry

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INTRODUCTION

- Viral entry into target cells through interactions with surface receptor proteins is a major determinant of viral tropism.
- Adenoviruses (Ads) are well characterized viruses with broad tropism and high transduction efficiency in many human cell types. Ads engineered with mutations to enable tumor-selective cytotoxicity have been extensively utilized in oncolytic virus (OV) therapies.
  - Ads contain a trimeric fiber protein with a central shaft and globular knob that helps tether it to the cell surface receptor.
  - Ad type-5 (Ad5), previously used in OV and gene therapy, utilizes the coxsackie adenovirus receptor (CAR) for cell entry. However, CAR downregulation during cancer progression[1,2] limits the therapeutic efficacy of OVs reliant on this surface protein.
  - Group B Ads use CD46 (a ubiquitously expressed receptor frequently overexpressed in cancer)[3,4] for cell entry, thereby presenting an opportunity to equip OVs with chimeric fibers to enhance tropism to malignant cells.
- We engineered ICVB-1042, a potent, selective, and systemically available OV, with an Ad5/34 chimeric fiber to enable viral entry via CD46 instead of CAR proteins.
- We demonstrate the cell entry requirements for ICVB-1042 compared to ICVB-421, WT Ad5 with a Ypet-2a insertion in frame with the ADP ORF.

OBJECTIVES

- Examine binding of ICVB-1042 to HEK293 cells overexpressing proteins known or expected to interact with viral particles during entry.
- Confirm modified tropism of ICVB-1042, such that it binds and enters cells through CD46 and not CAR.
- Compare viral entry and cytotoxicity of ICVB-1042 and ICVB-421 in A549 cells (which endogenously express CD46) to A549 cells with a CD46 knockout (KO).
- Determine if human CD46 expression on mouse cells is sufficient to enable entry by OV with chimeric Ad5/34 fiber.

METHODS

Determination of interactions with binding partners

- Work was performed at contract research organization (CRO).
- HEK293 cells were reverse transfected with expression vectors encoding transmembrane proteins that have been reported to interact with Ad capsid components.
- ICVB-1042 was incubated with HEK293s. Cells were fixed and stained for ICVB-1042. Binding interactions were identified by observation of coincident increased viral binding with protein overexpression.

Quantification of viral entry

- A mixed population of wild-type (WT) A549 cells and A549 cells with CD46 expression knocked out were combined and incubated with ICVB-1042 or ICVB-421 at multiplicity of infection (MOI) 10 for 2 hours. Non-bound virus was washed away, and cells were incubated for 24 hours.
- Cells were harvested, stained for CD46 surface expression, and analyzed by FACS.
- Cells were grouped into CD46+ or CD46- by staining signal. Viral entry was evaluated in CD46+ and CD46- cells by quantifying the percent of cells expressing YPET, a viral genome-encoded fluorescent reporter.

Quantification of viral cytotoxicity

- Viral cytotoxicity was measured in WT(CD46+ and CAR+), CD46-, and CAR- cells by monitoring cell index, a cell viability surrogate, using the xCELLigence real-time cell analysis instrument.
  - Cell index represents the measure of cell adherence, and a decrease in cell index over time can be used to determine the kinetics of viral-mediated cytolysis.
- First, the cell index was monitored for 4 hours to establish a baseline measurement. Following infection with ICVB-1042 or ICVB-421 at MOI of 10, cell index was measured every 30 minutes for 144 hours (6 days).
- The percentage of cytolysis was quantified relative to controls (no virus).

Transduction of LL/2 cells with a vector version of ICVB-1042

- Mouse lung carcinoma LL/2 cells and a derivative cell line expressing transgenic human CD46 (LL/2-hCD46) were incubated with ICVB-1042 or a replication-independent YPET-expressing vector version of ICVB-1042 (ICVB-1042-vector).
  - Mouse LL/2 cells harboring human CD46 transgenes were generated via piggyBac transposition.
- 24 hours post-infection, YPET levels were measured using Cytation 5 cell imaging multimode reader.
- YPET fluorescence signal intensity was calculated as the average signal intensity (arbitrary units) of pixels with background fluorescence subtracted.

RESULTS

ICVB-1042 Ad5/34 Chimeric Fiber Binds CD46 and not CAR

- Protein interaction screen performed to identify transmembrane surface proteins that bind ICVB-1042.
- Three isoforms of CD46, a known receptor for group B Ads, was found to bind ICVB-1042.
- Neither isoform of CAR, the receptor for WT Ad5, interacts with the Ad5/34 fiber of ICVB-1042.

Table 1. Summary of Binding Interaction Screen with ICVB-1042

Gene	ICVB-1042	PBS
CXADR v1		
CXADR v2		
CD46 v1		
CD46 v2		
CD46 v3		
CD44		
NT5E v1		
NT5E v2		
CD151		
DSG2		
CD80		
EPCAM		
CD86		
EGFR		

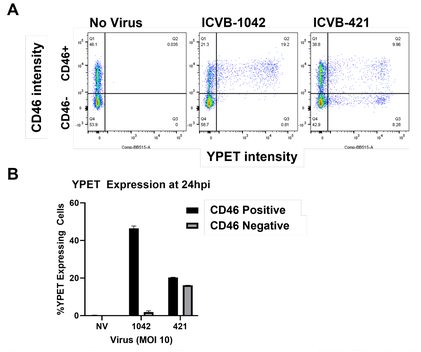
Table Legend

- No interaction
- Non-Specific Interaction
- Specific Weak Interaction
- Specific Moderate or Greater Interaction

CD46 Knockout Results in Reduced Entry by ICVB-1042

- Flow cytometry analysis of YPET expression in WT and CD46 KO cell lines showed increased YPET expression only in CD46+ cells 24 hours post-infection with ICVB-1042, suggesting loss of CD46 expression impairs ICVB-1042 cell entry (**Figure 1A**).
- Cell Entry, defined as the percent of YPET+ cells following infection with ICVB-1042 was 46.5% in CD46+ cells compared to 1.9% in CD46- cells (p=0.0005, t-test) (**Figure 1B**).
- ICVB-421 cell entry was less sensitive to loss of CD46 expression (20.3% in CD46+ versus 16.2% in CD46- cells, p=0.001, t-test) (**Figure 1B**).

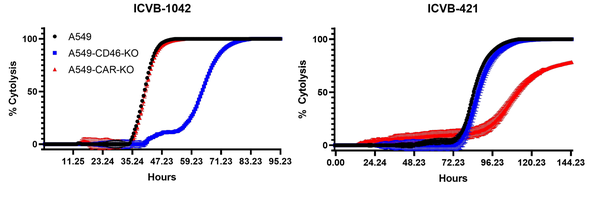
Figure 1: Flow cytometry analysis of YPET expression in CD46+ and CD46- cell lines (A) and plots showing the percentage of YPET-expressing CD46+ and CD46- cells (B)



CD46 Deficiency Induces Resistance to ICVB-1042-induced Tumor Killing

- Cell viability following infection with ICVB-1042 or ICVB-421 (MOI 10) was monitored for 6 days post infection.
- % Cytolysis was quantified as cell viability of samples divided by the viability of controls that did not receive virus. **(Figure 2)**
- ICVB-1042 infection required an additional 24 hours to mediate 100% cytolysis of A549-CD46-KO compared to WT A549 and A549-CAR-KO.
- ICVB-421 killed WT A549 and A549-CD46-KO with equal efficiencies but required additional time to completely lyse A549-CAR-KO.

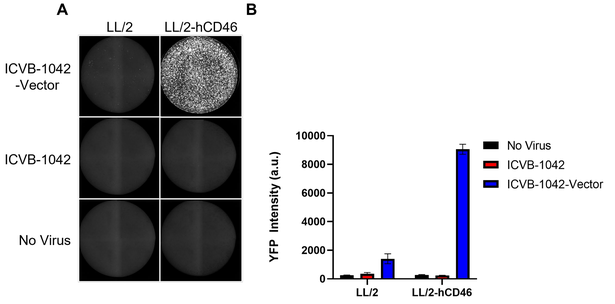
Figure 2: Viability of WT, CD46-, and CAR- cell lines infected with ICVB-1042 or ICVB-421



Human CD46 Expression is Sufficient for ICVB-1042 Entry, but not Replication, in Mouse Cells

- LL/2 and LL/2-hCD46 subline were incubated with ICVB-1042 or a ICVB-1042-vector at MOI 100 for 24 hours and YPET expression levels analyzed. (**Figure 3A,B**).
- High YPET expression observed from ICVB-1042-vector infection of LL/2-hCD46, but not WT LL/2, cells indicates hCD46 expression enables cell entry.
- No YPET expression observed from ICVB-1042 infection, indicating absence of viral replication following cell entry.

Figure 3: YPET fluorescence in mouse lung carcinoma LL/2 cells following infection with ICVB-1042 or ICVB-1042-Vector (A) and plots showing average YPET fluorescence signal intensity (B)



CONCLUSIONS

- Ad5/34 fiber of ICVB-1042 binds CD46 and not CAR
- CD46 expression is required for efficient infection by ICVB-1042.
- Time-course viral cytotoxicity measurement shows that CD46 deficiency induces resistance to ICVB-1042-induced cytotoxicity
- Expression of human CD46 on mouse cells allows viral entry, but not replication of ICVB-1042

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Conflicts of Interest and Acknowledgements

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