Nonclinical Characterization of ICVB-1042, a Novel E2F-tumor Selective Oncolytic Virus with Potential to Treat Solid Tumors

Jessica Field, Valentino Gantz, Nathaniel Rice, Joshua Messinger, Matthew Lanahan, Alex Kato, Michael Pokrass, and Heba Nowyhed • IconOVir Bio, San Diego, CA

All authors are employees of IconOVir Bio and may own stock or stock options.

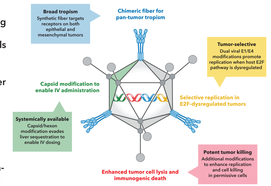
INTRODUCTION

- Oncolytic viruses (OVs) represent a class of cancer therapeutics based on the selective replication of viruses in cancer cells and their lysis and subsequent spreading within a tumor without causing damage to normal tissue.[1,2]
- Adenovirus (Ad)-based vectors are regarded as highly promising for cancer therapies as they do not integrate into host DNA, can be produced to high titers using established protocols, and have proven safety in human gene therapy and cancer applications.[3]
- ICVB-1042 (**Figure 1**) is a novel Ad5/Ad34 chimeric OV that is potent, selective, and systemically available with the potential to treat a broad range of solid tumors.
- Since ICVB-1042 carries a modified fiber with an Ad34 knob, its tropism is predicted to be different from other Ad5-based OVs. Since the Ad34 knob on ICVB-1042 allows the virus to enter cells expressing human CD46, we evaluated its cell-killing ability in mouse, rat, rabbit, cotton rat, hamster, and pig cells and did not observe cytotoxicity even at the highest multiplicity of infection (MOI).
- Since these preliminary data suggested that ICVB-1042 does not replicate in these animal models, we decided to evaluate the infectious efficacy, toxicology, and safety profile of ICVB-1042 in tumor-bearing NSG, CD-1 and huCD46tg mice.

This nonclinical characterization of ICVB-1042 includes:

- *In vitro* evaluation of replication, tumor-cell-killing activity, and selectivity for tumor cells over healthy cells
- *In vivo* evaluation of anti-tumor activity in NSG mice in bladder and breast cancer models
- *In vivo* evaluation of safety and toxicology of intravenous (IV) administration in CD-1 mice
- *In vivo* evaluation of immunotoxicity of IV administration in mice expressing the human CD46 receptor

Figure 1. Key features of ICVB-1042



METHODS

Cell Viability Assay: Tumor cell killing was measured with a colorimetric/metabolic cell viability assay (WST-1); absorbance values were reported for each virus and MOI condition and reported as a relative value to the signal read from uninfected wells. An interpolated viability curve was calculated, and (half maximal inhibitor concentration (IC_{50}) was determined.

Evaluation of ICVB-1042 Anti-Tumor Activity in a Subcutaneous Model of Bladder Carcinoma in NSG Mice: The anti-tumor activity of ICVB-1042 was evaluated in NSG mice implanted subcutaneously (SC) with SW780 bladder carcinoma cells in the high axilla. ICVB-1042 was administered (IV or intratumorally [IT]) on Days 0, 3, and 6 at 2 dose levels (2.00E8 plaque forming units [PFU] or 1.00E9 PFU). Efficacy was evaluated by tumor volume measurements. ICVB-1042 biodistribution plasma concentrations were assessed using digital droplet polymerase chain reaction (ddPCR). Plasma and tumors were collected 72 hours (hr) post dosing (3 animals/group). Plasma, tumor, liver, spleen, and lung samples were collected at end of life (6 animals per group).

Evaluation of ICVB-1042 Anti-Tumor Activity in an Orthotopic Model of Human Breast Carcinoma in NSG Mice: Human MDA-MB-231 breast carcinoma cells (1.00E6 cells) were injected into the fourth mammary fat pad of female NSG mice. When the tumors were ~123 mm^3 mice were randomized into 14 groups based on tumor burden: pharmacokinetics (PK) and biodistribution were assessed in Groups 1 to 7 (15 animals/group); efficacy was assessed in Groups 8 to 14 (5 animals/group). Vehicle, wildtype (Wt) Ad5, or ICVB-1042 were administered on Treatment Days 25, 28, and 31.

Safety and Toxicology of IV ICVB-1042: ICVB-1042 (ICVB-1042 at 8.00E10 viral particles [VP] per animal IV; ICVB-1042 at 2.00E10 VP per animal IV; ICVB-1042 at 2.00E11 VP per animal IV)] or vehicle was administered IV to female CD-1 mice on days 0, 3, and 6. The animals were monitored for clinical observations, body weight, viral shedding, clinical pathology, immunogenicity, ddPCR, and cytokines. At 1 and 24 hrs and 15, 30, 60, and 90 days post first ICVB-1042 administration, 3 animals per group were euthanized for complete postmortem examination, tissue collection for histopathology, and analysis of the presence of viral genomes by ddPCR analysis.

Immunotoxicity of IV-Delivered ICVB-1042 in Mice Expressing the Human CD46 Receptor: ICVB-1042 or Wt Ad5 at 1.00E11 VP per animal were administered to C57BL/6-huCD46 mice or their C57BL/6 littermates (N=36/group) on days 0, 3, and 6. Survival bleeds were taken on days -1, 7, 15, and 30 post first dose. Cytokine levels were measured using a 10-plex proinflammatory cytokine Meso Scale Discovery kit.

RESULTS

EXPERIMENT 1

Broad Tropism of ICVB-1042 Tumor Cell Killing *In Vitro*

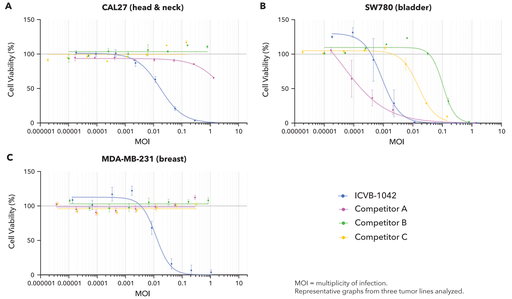
Objective 1: Evaluate ICVB-1042 replication and tumor cell killing in a panel of 70 solid tumor human cancer cell lines

Objective 2: Compare ICVB-1042 to other Ad OVs currently being evaluated in clinical trials

In addition to ICVB-1042, other viruses tested (engineered to parallel the performance of clinical competitors) included:

- **Competitor A:** Ad11/Ad3 chimeric virus; infects cancer cells via the CD46 receptor; currently in clinical trials for a wide range of tumors
- **Competitor B:** Ad5-based virus; infects cancer cells via the coxsackievirus–adenovirus receptor (CAR) receptor; currently in clinical trials for bladder tumors
- **Competitor C:** Ad5-based virus; infects cancer cells via the CAR receptor and the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins; currently in clinical trials for glioblastoma

Figure 2. *In Vitro* Tumor Cell Killing



MOI = multiplicity of infection.
Representative graphs from three tumor lines analyzed.

Table 1: ICVB-1042 IC_{50} Values in Human Solid Tumor Cell Lines

Cell lines	Tissue	Viruses (indication)			
		ICVB-1042	Competitor A (Broad therapeutic)	Competitor B (Bladder)	Competitor C (Glioblastoma)
A549	Lung	0.003	0.0009	0.1	0.04
H4	Brain	0.002	2	0.7	0.07
LN229		0.03	> 5 *	1	0.1
HT1197		0.01	2	2	0.02
SW780	Bladder	0.0009	0.0001	0.1	0.02
UMUC3		0.4	> 5 *	0.3	0.04
CAL27	Head & Neck	0.02	4	> 5 *	> 5 *
FADU		0.07	0.2	1	0.03
PC3	Prostate	0.02	0.002	> 5 *	> 5 *
LNCAP		0.002	0.02	0.002	0.0005
MDA-MB-415	Breast	0.04	0.01	0.2	0.05
MDA-MB-231		0.01	> 5 *	> 5 *	> 5 *

IC_{50} = corresponds to the MOI value required to get 50% cell killing within a well; MOI = "multiplicity of infection": is the number of virions divided by the number of cells within a well. * = in these experimental conditions the IC_{50}value was higher than the highest MOI value tested (MOI = 5).

Results

- ICVB-1042 efficiently replicated in 67/70 solid tumor lines.
- ICVB-1042 exhibited consistent and potent tumor cell-killing activity and broad tropism in a panel of 12 cell lines and overall outperformed 3 competitor viruses currently in clinical trials.

EXPERIMENT 2

ICVB-1042 is Highly Selective for Tumor Cells Over Healthy Cells *in vitro*

Objective:
Evaluate ICVB-1042 selectivity for tumor cells over healthy cells in a panel of 20 human primary cell lines

Results

- ICVB-1042 was >100-fold less cytotoxic in 18/20 human primary cell lines than A549 tumor cells.
- In 2 cell lines (umbilical vein endothelial cells and renal epithelial cells), ICVB-1042 was ~69- and 1.72-fold less cytotoxic, respectively, than to A549. These 2 cell lines were not fully quiescent, suggesting that the lytic activity may have been due in part to higher levels of cell proliferation and may have been an artifact of the *in vitro* cell-culture system. These results help identify tissues that may be affected and will need to be monitored during clinical trials.

Table 2: ICVB-1042 IC_{50} Values in a Panel of 20 Healthy Human Primary Cells

Cell Type	IC_{50} values
A549 Tumor Cells	0.0003
Aortic Smooth Muscle	2
Astrocytes	0.3
Bladder Epithelial Cells	61
Bladder Smooth Muscle	> 10
Bronchial Epithelial	0.1
Cardiac Fibroblasts	> 2
Cardiac Microvascular Endothelial	0.4
Hepatocytes	0.3 *
Intestinal Epithelial	0.3
Keratinocytes	> 6
Lung Fibroblasts	0.03
Mammary Epithelial	0.3
Melanocytes	> 10
Ovarian Epithelial	> 10
Prostate Epithelial	5
Prostate Stromal	0.3
Renal Cortical Epithelial	1
Renal Epithelial	0.001
Small Airway Epithelial	0.06
Umbilical Vein Endothelial	0.02

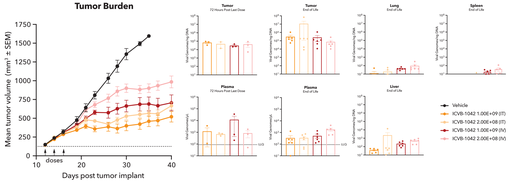
IC_{50} = corresponds to the MOI value required to get 50% cell killing within a well;
MOI = "multiplicity of infection": is the number of virions divided by the number of cells within a well;
* = the IC_{50} value was estimated from the selectivity ratio obtained by using ddPCR performed on cell extracts detecting virus DNA.

EXPERIMENT 3

ICVB-1042 Administered IT or IV Results in Tumor Growth Inhibition and Similar Viral Genome Levels in the Tumor in a Subcutaneous Model of Human Bladder Carcinoma

Objective: Evaluate ICVB-1042 anti-tumor activity, plasma and tissue distribution in immunodeficient NSG mice bearing established SW780 Human bladder carcinoma tumors

Figure 3. Tumor Burden and Biodistribution of ICVB-1042 in NSG Mice Bearing SW780 Human Bladder Carcinoma Tumors



Data presented as mean ± SEM. Animals excluded when 50% or less of a group remain on study.
IT = intra-tumoral; IV = intravenous; LLQ = lower limit of quantitation; SEM = standard error of the mean.

Results

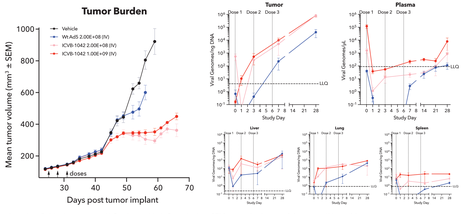
- ICVB-1042 administered either IV or IT was well tolerated and resulted in significant tumor growth inhibition compared to the vehicle-treated group.
- This study shows that ICVB-1042 administered IV requires only a 5-fold higher dose to achieve equal tumor control to IT injection, supporting its potential for systemic delivery
- All ICVB-1042-treated groups exhibited 100% survival
- Viral genomes increased over the course of the study indicating sustained viral replication in the presence of permissive human tumor cells. At study end, viral genomes were detected in the tumors at the highest levels and at much lower concentrations in the liver, lung, and spleen as expected
- There were no significant differences in viral genomes between the different dosing groups in the plasma or in the tumor, again indicating no relative differences in direct versus systemic delivery of ICVB-1042

EXPERIMENT 4

Multiple Doses of ICVB-1042 (IV) Reduced Tumor Burden and Outperformed Wt Ad5 in an Orthotopic Model of Human Breast Carcinoma in NSG Mice

Objective: Evaluate ICVB-1042 tumor burden, plasma and tissue distribution in female NSG mice engrafted with human MDA-MB-231 mammary carcinoma cells

Figure 4. Tumor Burden and Plasma and Tissue Distribution of ICVB-1042 compared to Wt Ad5 in NSG mice bearing an orthotopic human breast carcinoma



Ad5 = adenovirus 5; IV = intravenous; LLQ = Lower Limit Quantitation; SEM = standard error of the mean; Wt = wild type.

Results

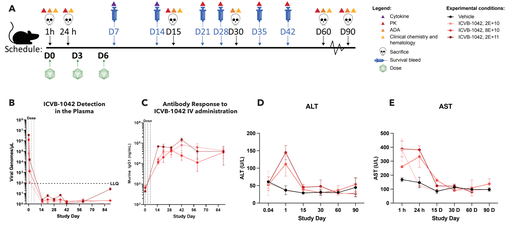
- ICVB-1042 (IV) resulted in significant tumor growth inhibition in an orthotopic model of human breast carcinoma in female NSG mice.
- Administered in comparable doses, ICVB-1042 outperformed the progenitor Wt Ad5 virus in tumor growth inhibition in this model.
- ICVB-1042 viral genomes increased in the tumor and were consistently higher than Wt Ad5.
- Rapid clearing of Wt Ad5 was observed in plasma, and to some degree in other tissues, while ICVB-1042 remained high in circulation.
- ICVB-1042 was well tolerated with no deaths, and minimal body weight changes during the treatment window.

EXPERIMENT 5

IV Administration of ICVB-1042 was Safe and Well-Tolerated in CD-1 Mice

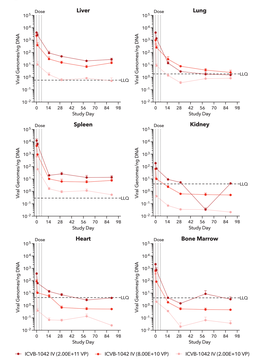
Objective: Evaluate safety and toxicity (clinical pathology, immunogenicity, PCR, cytokine, and histopathology) of ICVB-1042 following repeated IV administration in CD-1 mice

Figure 5. Toxicological Analysis of IV ICVB-1042



ADA = antidrug antibodies; ALT = alanine aminotransferase; AST = aspartate aminotransferase; D = day; IgG = immunoglobulin; IV = intravenous; LLQ = lower limit of quantification; PK = pharmacokinetics.
Black arrows are scheduled sacrifice time points; blue arrows are weekly blood draws; triangles indicate collection time points (purple = cytokine, red = PK, yellow = clinical chemistry and hematology, orange = ADA).

Figure 6. Distribution of ICVB-1042

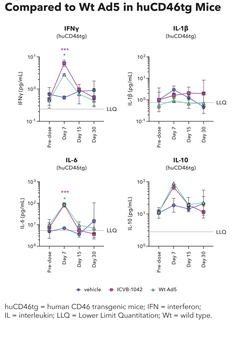


IV = intravenous; LLQ = Lower Limit Quantitation; VP = viral particles.
The level of viral copies in tissue was determined using ddPCR; means and SEM were calculated for each timepoint.

Results

- Post the first administration of ICVB-1042, aspartate aminotransferase (AST) increased at 1 and 24 hrs, and alanine aminotransferase (ALT) increased at 24 hrs. ALT and AST were similar to vehicle control animals from day ≥15.
- Microscopic findings were observed in liver, bone marrow, and spleen in a dose dependent manner. Findings included hepatocyte hypertrophy and minimum to mild apoptosis in bone marrow and spleen. Findings were present starting 24 hours after the first administration and were either not present or of minimal severity at day 90, demonstrating resolution or trending towards recovery.

Figure 7. Immune Activation of ICVB-1042 Compared to Wt Ad5 in huCD46tg Mice



huCD46tg = human CD46 transgenic mice; IFN = interferon; IL = interleukin; LLQ = Lower Limit Quantitation; Wt = wild type.

Results

- Acute immune activation was observed on day 7 after administration of the first ICVB-1042 dose and resolved to baseline levels by day 15.
- These observations are consistent with an immune response following IV administration of a virus and clearance in the following weeks.
- Immune activation triggered by ICVB-1042 in huCD46tg mice, is comparable to Wt Ad5 (which instead uses the CAR receptor for murine cell entry). The elevated cytokines were observed at day 7 and back to vehicle levels on day 15 and 30.

CONCLUSIONS

- ICVB-1042 is a potent OV with broad tropism which supports its potential to treat a wide range of tumors (**Table 1**).
- ICVB-1042 is >100-fold more cytotoxic in A549 than in most normal primary cells tested, supporting high tumor selectivity and a low likelihood that primary tissues will be targeted by ICVB-1042 (**Table 2**).
- ICVB-1042 administered IV or IT was effective in reducing tumor burden in a human bladder tumor mouse model and administered IV was effective at inhibiting tumor growth in a human breast cancer mouse model. These findings support ICVB-1042 effectiveness in targeting human tumors and its potential to be delivered IV (**Figure 3**, **Figure 4**).
- ICVB-1042 is detected 24 hours post third-dose in both plasma and tumor, with ~100-fold increase over the following three weeks indicating viral replication and continuous therapeutic activity (**Figure 4**).
- A transient inflammatory cytokine response was observed after ICVB-1042 administration and it resolved 1 week after the third dose (**Figure 7**).
- There were no differences in cytokine response in huCD46tg animals versus non-carrier littermates and no difference in response to ICVB-1042 administration versus Wt Ad5, suggesting that ICVB-1042 is likely to have a comparable immune reaction to previous Ad5-based therapies (**Figure 7**).
- In all studies, ICVB-1042 was well tolerated, minimally impacting the health of the dosed animals, strongly supporting safety of the drug for systemic (IV) delivery.

References

1. Cantwell et al. 1996 *Blood*. 88: 4676-4683.
2. Hemminki et al. 2020 *J Hematol Oncol*. 13: 84.
3. Wold et al. 2013 *Curr Gene Ther*. 13: 421-433.

ICONOVIR BIO