Evaluation of the anti-tumor activity of ICVB-1042, a novel E2F-tumor selective oncolytic virus, targeting tumor cells in an established human glioblastoma mouse model

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INTRODUCTION

- Glioblastoma (GBM) is the most common and most aggressive life-threatening brain tumor in adults. In 2022, ~13,000 Americans will be diagnosed with GBM. Standard of care (SOC) is surgical resection, followed by concomitant temozolomide (TMZ) and continued TMZ for 6 months.[1]
- A major challenge in oncolytic virotherapy is to engineer highly potent tumor-killing viruses that can be delivered systemically for the selective treatment of metastatic disease while leaving normal cells unharmed.
- ICVB-1042 is a novel E2 transcription factor (E2F)-dependent oncolytic adenovirus (Ad) rationally designed with genomic modifications to confer tumor selective replication, allow intravenous delivery, enhance potency and tumor tropism, and includes expression of a fluorescent reporter protein to aid in tracking viral replication in clinical samples.

OBJECTIVE

- To compare anti-tumor activity and tumor selectivity of ICVB-1042 to that of ICVB-940, an Ad5-based oncolytic virus (OV) engineered with a 24 base pair deletion in E1A and an RGD-4C motif in HI loop of the fiber knob (D24RGD). ICVB-940 is similar to another D24RGD Ad5 OV that has been evaluated in clinical trials in glioblastoma patients.[2,3]

METHODS

Viruses

- ICVB-1042 is a novel E2F-tumor selective oncolytic Ad composed of an Ad serotype 5 (Ad5) backbone with genomic modifications to confer novel specific properties. ICVB-1042 has a genetically encoded fluorescent protein YPet (**Figure 1 top**).
- ICVB-940 is an Ad5-based OV engineered with a 24 base pair deletion in E1A and an RGD-4C motif in the fiber H-loop (D24RGD). ICVB-940 is similar to a clinical stage AdOV being developed for treatment of GBM[4,5] (**Figure 1 top**).
- ICVB-2006 shares the same sequence with ICVB-940 except for the YPet reporter expression cassette inserted at the same region as ICVB-1042.

***In vitro* cell viability assays**

- The cytotoxicity of ICVB-1042 and ICVB-940 were compared in human primary astrocytes and in GBM tumor cell lines *in vitro* using WST-1 method (**Figure 1 middle**).
- The cytotoxicity of ICVB-1042 and ICVB-2006 were compared in cell culture directly dissociated from human primary GBM tumor (dissociated GBM tumor cells) using impedance-based cell viability measurement and live imaging of viral YPet expression (**Figure 1 bottom**).

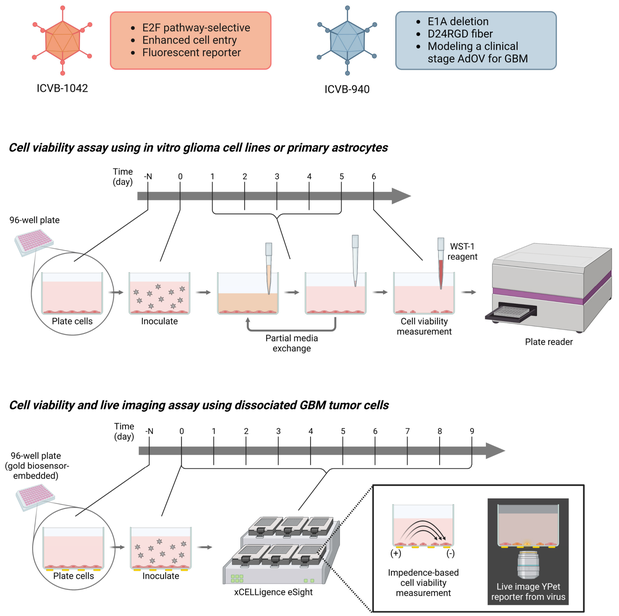
***In vivo* GBM anti-tumor activity**

- The efficacy of ICVB-1042 was evaluated against a U251-luc human GBM implanted subcutaneously in the high axilla of female NSG mice (6 mice per group).
- ICVB-1042 was administered at 3-day intervals (Days 0, 3, and 6) intratumorally (IT) for a total of 3 doses at the dose level of 1 x 10^8 pfu/injection followed by tumor volume measurement (**Figure 3 left**).

PK/BioD measurement

- ddPCR analysis was performed on DNA isolates from tissues or plasma using specific primer/probe set and normalized based on initial virus dose (tumor and tissues) or sample input volume (plasma).

Figure 1. Engineering of ICVB-1042 and ICVB-940 OVs and *in vitro* viral assay procedure



RESULTS

- ICVB-1042 demonstrated enhanced cytotoxicity over ICVB-940 in 11 of 13 human glioma tumor lines *in vitro*, whereas both viruses show similar slow cytotoxicity in human primary astrocytes *in vitro* (**Table 1**).
- ICVB-1042 demonstrated enhanced cytotoxicity over ICVB-2006 (YPet-expressing version of ICVB-940) in dissociated human GBM tumor cells *in vitro* 8 days post infection (**Figure 2**).
- ICVB-1042 showed significant decreases in tumor volume and significant increases in time to progression (TTP) between treated and control animals, whereas ICVB-940 showed no significant effects in tumor volume or TTP compared to control (**Figure 3**).
- Increased levels of ICVB-1042, but not ICVB-940, were detected at end of life compared to 72 hours post last dose.
- Over 1000-fold more viral genomes were detected in the tumor in comparison to liver and lung tissues at both 3 days post last dose and end of life (**Figure 4**).
- No deaths or group mean body weight loss was observed in the treatment window.

Table 1. ICVB-1042 shows enhanced cytotoxicity in glioma cell lines compared to ICVB-940 but similar low cytotoxicity in primary astrocytes

Glioma cell line	ICVB-1042 IC_{50} (MOI)	ICVB-940 IC_{50} (MOI)	Fold ratio of ICVB-940 IC_{50} and ICVB-1042 IC_{50}
NP8	0.0023	0.0059	2.55
H4	0.0036	0.31	84.9
KNS42	0.034	0.080	2.36
M095J	0.030	0.11	3.69
U138MG	0.069	0.29	4.29
A172	0.039	0.36	9.21
U251MG	0.093	0.067	0.73
LN229	0.070	0.36	5.19
ANGMCSS	0.062	0.20	3.28
SW1088	0.24	0.58	2.41
LN18	0.19	2.71	14.3
T98G	0.97	6.68	6.89
CCF-STTG	1.06	0.60	0.56
Primary cell type	**ICVB-1042 IC_{50} (MOI)**	**ICVB-940 IC_{50} (MOI)**	**Fold ratio of ICVB-1042 IC_{50} and ICVB-940 IC_{50}**
Astrocyte	0.49	0.28	0.58

Abbreviations: MOI = multiplicity of infection

Figure 2. ICVB-1042 shows enhanced cytotoxicity in dissociated GBM tumor cells compared to ICVB-2006 (YPet-expressing version of ICVB-940)

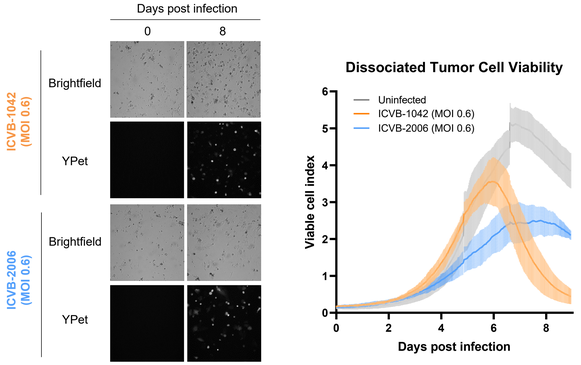
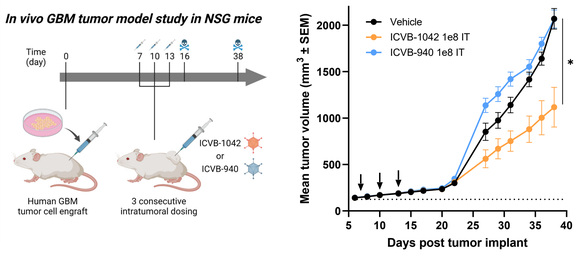
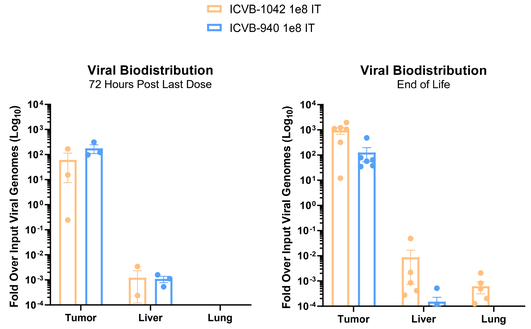


Figure 3. ICVB-1042 shows enhanced anti-tumor activity in U251-luc human GBM implanted subcutaneously in NSG mice



* $P < 0.005$ (t-test) for ICVB-1042 vs vehicle or ICVB-940 at end of life (Day 38)

Figure 4: Tumor and peripheral tissue distribution of ICVB-1042 and ICVB-940 at 72 hours post the third dose versus end of life



CONCLUSIONS

- This analysis, performed *in vitro* on a panel of glioblastoma tumor cell lines and *in vivo* in an established subcutaneous human GBM model in NSG mice, showed overall enhanced activity of ICVB-1042 compared to that of the clinically relevant ICVB-940 virus.
- ICVB-1042 showed low adverse cell killing effects on human primary astrocytes at the comparable level to ICVB-940.
- In the *in vivo* GBM tumor xenograft model, there was a significant difference in anti-cancer response observed in favor of ICVB-1042 over ICVB-940.
- ICVB-1042 showed virus genome levels increase in the injected tumor over time.
- ICVB-1042 and ICVB-940 showed undetectable or low levels of virus genome in the non-targeted tissues (liver, lung). Plasma was also assessed for both treatment groups and undetectable to very low levels of virus genome was found.
- All treatments were well-tolerated in mice.

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Conflicts of Interest and Acknowledgements

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