Abstract: 859

Novel oncolytic RNA viruses, IVX037 and IVX055, demonstrate potent antitumor activity.

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BACKGROUND

Oncolytic viruses have emerged as promising therapeutic agents to selectively target, infect and destroy cancer cells while synergizing with novel immunotherapies including Checkpoint inhibitors (CPI) and Chimeric Antigen Receptor (CAR) T- cells to increase anti-tumor efficacy. We developed two novel oncolytic non-enveloped RNA viruses, IVX037 and IVX055, using a proprietary receptor-focused bio-selection platform technology. IVX037 and IVX055 are small, single stranded RNA viruses, utilizing independent cell surface receptors to facilitate tumor targeting, cell entry and subsequent potent lytic replication. Intratumoral (i.t) IVX037 challenge of human microsatellite-stable colorectal cancer (MSS-CRC) xenografts increases expression of PD-L1 and mediates a widespread inflammation phenotype, suggested to allow increased migration of anti-tumor lymphocytes, elevation of cellular targets for immune checkpoint and cell-based anti-cancer therapies

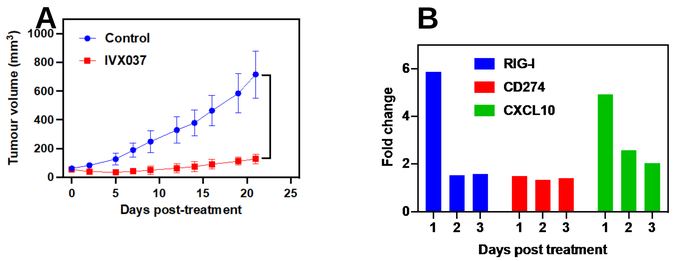

Figure 1. *(A) A single injection of IVX037 (1x10^8 TCID$_{50}$) was administered intratumorally (i.t) into WiDr tumor xenografts.* ***(B)*** *Expression of RIG-I, CD274 and CXCL10 at 1-, 2- and 3-day post treatment. In preclinical studies SCID mice were inoculated with WiDr (MSS-CRC) cell, when tumors reached to an average size (approximately 50 mm^3), mice were treated with intratumoral IVX037 (n = 8 mice) or control formulation buffer (n = 6 mice) on the day 0. Mice were sacrificed at 1, 2, and 3-day post treatment. tumors were collected and RNA was extracted. Expression of RIG-I, CD274 and CXCL10 was measured using RT-PCR and calculated as a fold change.*

METHODS

- *In vitro* monolayer cultures of human cancers were challenged with various input multiplicities of IVX037 or IVX055 with lytic infection assessed by a XTT viability assay and microscopic examination.
- For mouse models, immunocompromised mice (SCID or nu/nu) were seeded with single flank human tumor cell administrations. Following development of palpable tumors, IVX037 was administered via the intratumoral route. Calliper measurements were employed to assess tumor burden.
- Checkerboard titrations of IVX037 and a mesothelin-targeted CAR T were utilized to assess anti-tumor activity in monolayer cultures of human gastric cancer cells (NCI-N87).

RESULTS

- IVX037 demonstrated potent oncolytic *in vitro* activity in human hepatocellular cancer (HCC) cell cultures (***Figure 2***).
- Intratumoral administration of IVX037 in human tumor xenograft models using immunocompromised mice were well tolerated and displayed potent anti-tumor activity against HCC cancer xenografts (***Figure 3***).
- IVX055 induced rapid cell lysis in non-small cell lung *in vitro* cell preparations (***Figure 4***).
- Intratumoral administration of IVX055 in human tumor xenograft models using immunocompromised mice were well tolerated and displayed potent anti-tumor activity against NSCLC cancer xenografts (***Figure 5***).
- In reference, to cell-based therapies, preliminary *in vitro* combination studies with mesothelin-targeted CAR T-cells and IVX037 suggest enhanced tumor cell killing compared to that of either agent alone in a human gastric cancer cell culture model (***Figure 6***).

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RESULTS – continued

Oncolytic activity of IVX037 in HCC

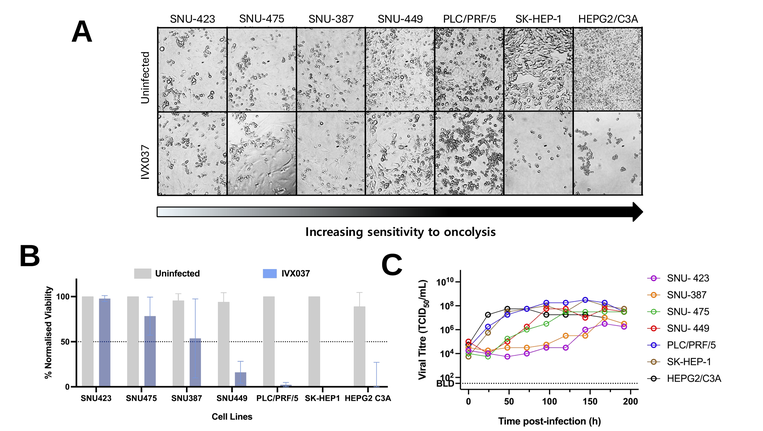

Figure 2. *(**A**) IVX037-mediated destruction in a panel of liver cancer cell lines within seven days. Cell lines were infected with IVX037 at MOI 1000 and photomicrographs (10x) were taken seven days post-infection.* ***(B)*** *IVX037-mediated oncolysis in a panel of liver cancer cells. Cells were infected with IVX037 at MOI 100 TCID$_{50}$/cell and viability was measured seven days later via the XTT viability assay. Results were expressed as mean percentages of viability, normalised to mock-infected, ± SD.* ***(C)*** *Viral growth curve of IVX037 in various liver cancer cell lines over 8 days post-infection. Cells were infected with IVX037 at MOI = 1. and viral titres were determined at 0, 24, 48, 72, 96, 120, 144, 168, and 192 hrs post-infection. BLD denotes below level of detection.*

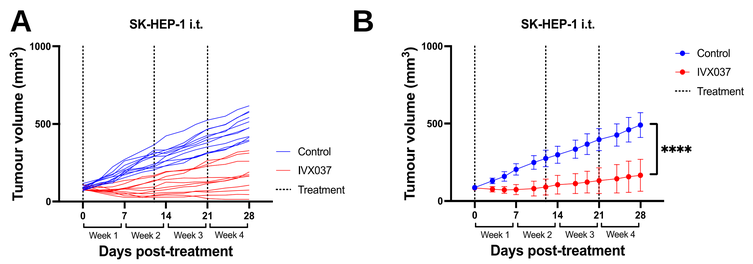

Figure 3. *IVX037 infects and replicates in human liver cancer xenograft resulting in tumor growth inhibition. Three injections of IVX037 (1x10^8 TCID$_{50}$) were administered intratumorally (i.t.) to the SK-HEP-1 tumor xenografts. (A) Individual tumors and (B) mean tumor volumes. Tumor volumes were expressed as the mean ± SD (mm^3). ****P<0.0001.*

Combination of IVX037 + mesothelin-CAR T

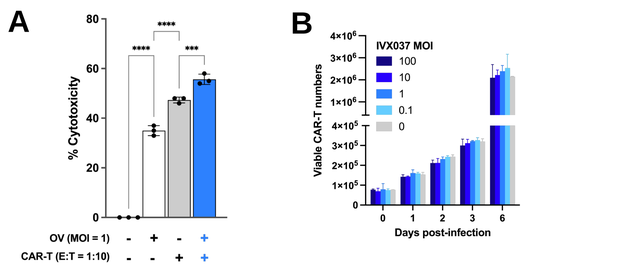

Figure 6. (A) *NCI-N87 gastric cancer cells were treated with IVX037 (MOI = 1 TCID$_{50}$/cell) in combination with meso-CAR T (E:T = 1:10). Cytotoxicity was measured 48 hours later. One-way ANOVA corrected with Tukey's method revealed significantly higher cytotoxicity in the NCI-N87 cell line when treated with the combination (***P<0.001, ****P<0.0001).* ***(B)*** *Co-culture of IVX037 with meso-CAR T over a period of 6 days. No decrease in meso-CAR T cell viability observed at any of the challenge MOIs.*

Oncolytic activity of IVX055 in NSCLC

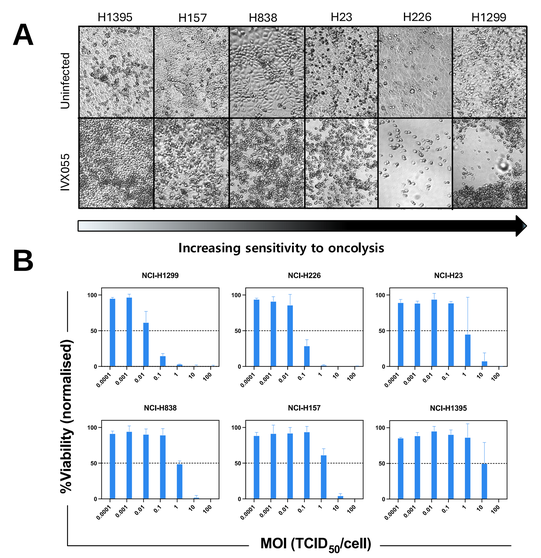

Figure 4. (A) *IVX055 drives rapid CPE in all cell lines within five days. Various non-small cell lung cancer cell lines were infected with IVX055 at MOI 0.1 in reduced-serum media (n=1). Photomicrographs were taken at five days post-infection. (**B**) IVX055 induces oncolysis across all NSCLC cell lines. Various non-small cell lung cancer cell lines were infected with IVX055 at MOI = 0.0001, 0.001, 0.01, 0.1, 1, 10, and 100 in reduced-serum media. Cell viability of the six cell lines were determined at 72h post-infection via the XTT viability assay (n=2). Results were expressed as mean percentages of viability, normalised to mock-infected, ± SD*

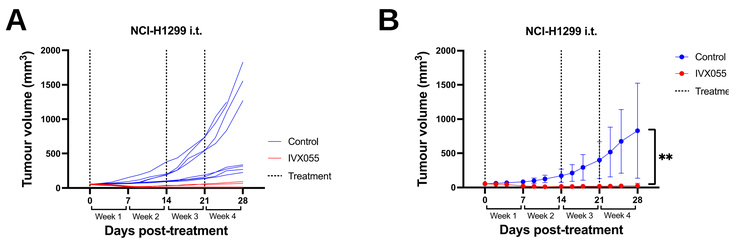

Figure 5. (A) *IVX055 infects and replicates in human non- small cell lung cancer xenograft resulting in tumor growth inhibition. Three injections of IVX055 (1x10^8 TCID$_{50}$) were administered intratumorally (i.t.) to the NCI-H1299 tumor xenografts. Tumor volumes (**A**) individual and (**B**) mean ± SD (mm3). **P=0.0017.*

CONCLUSIONS

- The promising preclinical anti-tumor activity of IVX037 and IVX055 against a wide range of human malignancies warrants further clinical evaluation in combination with novel immunotherapy strategies.
- To this end, IVX037 is currently being investigated in an ongoing Phase 1b combination trial with sintilimab (anti-PD-1) in patients with advanced MSS-CRC, gastroesophageal, ovarian and HCC cancer (NCT05427487) ***See poster CT115 (AACR 2025).***
- Future plans for IVX055 include evaluation in combination with immune checkpoint therapy in patients with advanced non-small cell lung cancer (NSCLC) bearing metastatic liver disease.

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