

Mechanism of Tumor-Selective Tropism of Seneca Valley Virus (SVV-001), A Newly Discovered Systemically Deliverable Oncolytic Picornavirus for Treatment of Human Neuroendocrine Cancers

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Abstract

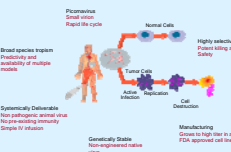
Seneca Valley Virus (SVV-001) is a recently discovered native non-pathogenic picornavirus with exquisite tumor selectivity for human cancers with neuroendocrine features and is being developed as a systemically deliverable oncolytic virus (Reddy et al., JNCI, 2007; Wadhwa et al., Cancer Res, 2007; Hales et al., Acta Crystallographica, 2008; Venkatraman et al., J Gen Virol, 2008). SVV-001 was tested in a Phase III dose-escalation study and exhibited evidence of clinical activity upon systemic delivery without dose-limiting toxicity. To understand the mechanism of selective infection and killing of neuroendocrine tumor cells, a systematic approach to identify proteins involved in viral tropism was undertaken. Real-time fluorescent microscopy using Alexa Fluor 647 and Syto-82 labeled SVV-001 was used to follow individual virus particles during infection. These experiments revealed that the majority of non-permissive cells did not bind and/or internalize SVV-001, confirming that binding and entry through a productive internalization pathway was the primary determinant of viral tropism for neuroendocrine tumor cells. Mouse monoclonal antibodies (mAbs) to cell surface proteins of permissive cells were generated to identify mAbs that block virus binding and/or internalization. One antibody (17E11) was shown to efficiently block binding, and a second antibody (8G10) was shown to specifically block internalization. Further studies demonstrated that both antibodies recognize epitopes on the surface of permissive cells. Western Blot analysis of permissive cell lines indicated that these antibodies bind distinct epitopes of different proteins. To identify protein binding targets for these mAbs, a lentiviral cDNA library was generated utilizing cDNA from the permissive SCLC cell line H446, Chinese hamster ovary (CHO-K1) cells, which do not express either of the mAb target antigens, were infected with the lentiviral cDNA library and single cell clones expressing lentivirus-encoded marker were isolated. Drug-resistant clones that expressed the epitope for 17E11 or 8G10 were isolated by fluorescence-activated cell sorting (FACS). All clones which were 8G10-positive by FACS demonstrated an identical banding pattern relative to each other and to H446 cells on 8G10 Western blots, confirming these clones and H446 expressed the same 8G10-reactive proteins. Selection and screening is ongoing with 17E11. PCR and sequence analysis is expected to lead to the identification of the cell surface antigens for the mAbs 17E11 and 8G10. These antigens would represent the first host proteins identified in the life cycle of SVV-001 and likely elucidate key steps in the mechanism of tropism, as well as lead to the development of a biomarker for selection of patients with responsive tumors in future clinical testing.

Background

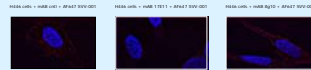
Phase I first in man study completed. 30 neuroendocrine positive patients dosed (carcinoid, small cell lung cancer, various). Patients were active progressors by RECIST. Dosing was by intravenous infusion in outpatient setting at 12 sites in US and covered 10^7 to 10^{11} vp/kg. No DLT or drug related SAEs experienced. Drug related AEs were mild transient flu-like symptoms. SCLC patients (3rd-5th line) had a 3x improvement in overall survival as compared to historical controls for 2nd line, 20% long term survivors, and replication in tumor masses observed. Carcinoid patients had a 70% response rate (PR's by PET, Near PR's CT, Stable disease) and comparable to best Phase II studies using drug combinations.

Newly discovered picornavirus (Seneca Valley Virus, NTX-010) in new genus called Senecavirus. Broad species tropism and non-pathogenic in all species tested. Swine a natural host. NTX-010 has exquisitely high selectivity for killing tumor cells having neuroendocrine properties such as SCLC, Large cell NSCLC, and nearly all pediatric solid tumors. Effective (CRs, PRs) as a single IV dose in over 32 animal models; primary transplantable, orthotopic, metastatic, chemorefractory, syngeneic, and several models demonstrated by the NCI to be predictive of clinical response. Only minimal transient toxicity observed by intravenous infusion in multiple strains of mice (up to 10^{11} vp/kg), swine (up to 3×10^{11} vp/kg), and primates (up to 10^{13} vp/kg).

The Next Generation: Seneca Valley Virus



Permissivity to SVV-001 is Determined by Cell Binding and/or Entry

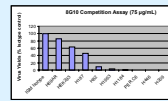
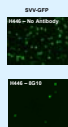


- In 17/22 non-permissive cell lines, the virus infection is blocked because virus does not bind to cells and/or internalize
- In 5/22 non-permissive cell lines, the virus gets in but doesn't replicate suggesting an internal block and/or wrong entry pathways
- Thus at least the majority of mechanism relates to virus not getting in (80%) or getting in but not replicating (20%)

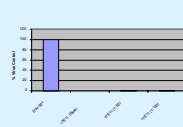
Isolation of Monoclonal Antibodies to Cell Surface Proteins

- Hybridomas generated by immunizing mice with permissive H446 or H82 cells.
- Hybridoma clones screened for ability to block SVV-001 infection.

8G10

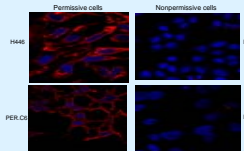


17E11

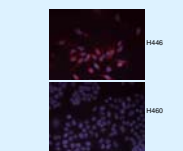


mAb Immunofluorescence

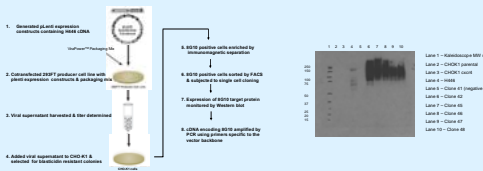
8G10



17E11

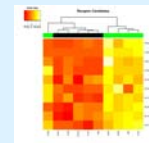


cDNA Expression Library Approach to Identify Target Proteins of 8G10 and 17E11



Microarray Analysis of Gene Expression in SCLC Cell Lines

5 permissive and 5 non-permissive SCLC lines were profiled on the Affymetrix GeneChip hgu133plus2.

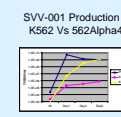
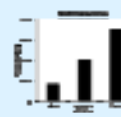


Cell Line	Gene	Log2 Fold Change	P-value	Rank
Permissive	ITGA4	1.5	0.0001	1
	ITGA4	1.5	0.0001	2
	ITGA4	1.5	0.0001	3
	ITGA4	1.5	0.0001	4
	ITGA4	1.5	0.0001	5
Non-permissive	ITGA4	1.5	0.0001	1
	ITGA4	1.5	0.0001	2
	ITGA4	1.5	0.0001	3
	ITGA4	1.5	0.0001	4
	ITGA4	1.5	0.0001	5

Integrin alpha4 associates with permissivity and shown to corelate by FACS on wide panel of permissive and non-permissive cell lines

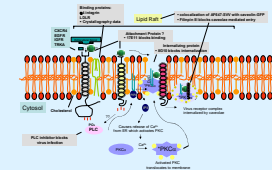
Functional gene set enrichment shows significant enrichment of several gene sets in the permissive group. Among the top ten enriched gene sets were four stem cell gene sets (ranked 1,2,3, & 7).

Binding to Integrin Alpha4 (ITGA4) Expressing CHOK1 and H446



- Integrins link the extracellular space with the inside of the cell
- Integrins serve as cellular receptors for other picornaviruses
- Motif LDV known to bind alpha1 integrin is found in a surface-exposed loop of VP2 - a capsid protein
- Virus mutated in this site could not be rescued from culture

SVV-001 Binding and Entry - Current Hypothesis



Summary

SVV-001 is a novel oncolytic picornavirus with remarkable natural selectivity towards human neuroendocrine tumors. In Phase I/IIa testing, SVV-001 exhibited an excellent safety profile with no DLT and evidence of activity. A predictive biomarker could dramatically reduce the time and cost of ongoing development by increasing patient responder populations. Towards this goal, attention was focused on discovering cell proteins directly involved in the mechanism of viral tropism. Proprietary mAbs which bind cell surface determinants important for SVV-001 infection were isolated, and attempts to identify their cell protein targets are ongoing. Microarray and FACS indicated permissivity is associated with expression of integrin alpha4. An integrin alpha4 binding motif is found on the viral capsid and preliminary evidence suggests that the presence of integrin alpha4 increases viral binding and viral production. Finally, microarray profiling also indicated a pattern of stem cell gene expression in permissive SCLC lines, suggesting SVV-001 can infect early progenitor or stem cells within tumors.