Development and validation of a clinical nAb assay for a replicating oncolytic virus

VSV-GP Platform nAb Assay

Richard Dambra[1], Ashlee Bell-Cohn[1], Mary Sanville[1], Andrew Brown[2], Kim Zanotti[2], Xun Wang[2], Michael Franti[1], Gurleen Saini[1], Joseph Ashour[1]

1. Drug Metabolism & Pharmacokinetics, Boehringer Ingelheim Pharmaceuticals Inc. (Ridgefield, CT) 2. QPS Holdings LLC (Newark, DE)

Abstract

Viral vectors, like other biologics, require immunogenicity evaluation in the clinic to help inform their efficacy and safety profiles. Neutralizing antibody (nAb) responses are particularly important for live virus-based therapeutics given their inherent immunogenicity, which may significantly impact pharmacological effects.

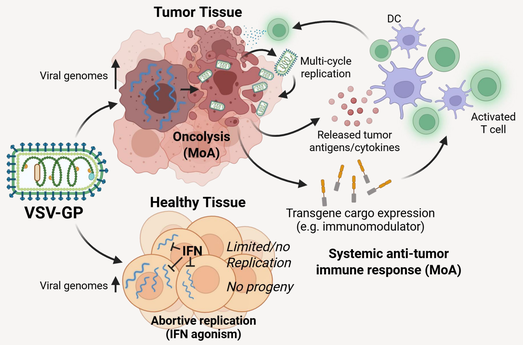
Here, we developed a robust cell-based method to detect and titer nAb responses in patient sera to support clinical development of an oncolytic rhabdovirus and cancer vaccine platform based on the VSV-GP virus. This method incorporates a tool reporter version of the virus and measures antibody-based inhibition of its protein expression and/or replication in permissive cells. The final optimized assay was successfully validated and implemented for clinical bioanalysis in support of VSV-GP trials.

Objectives

- ✓ Develop a robust assay to detect and titer VSV-GP nAbs in patient serum.
- ✓ Use a platform approach so the assay can be applied to alternative VSV-GP-cargo variants.
- ✓ Validate the assay for clinical sample analysis.

Introduction

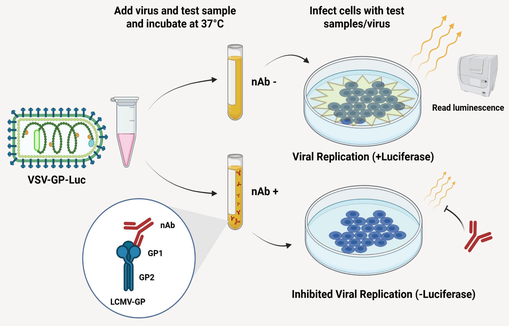
- **VSV-GP** is a replication competent oncolytic virus (OV) and cancer vaccine platform[1].



- Viral **replication** drives OV **mechanisms of action**.
- Viruses such as VSV-GP are highly immunogenic and can elicit **antibody responses which may influence efficacy and/or safety profiles**.
- VSV-GP is intended for repeat administration[1].

Approach

Micro-neutralization assay in 96-well format:

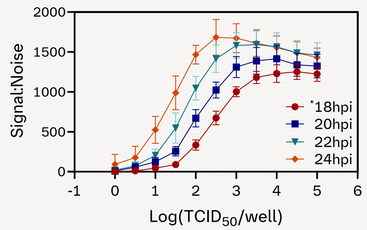


Method Parameters	
Matrix	Serum
PC (nAb)	KL25 (anti-GP mouse mAb)
Tool virus	VSV-GP-Luciferase
Cells	BHK-21 (ready-to-assay)
QCs	LPC, MPC, HPC, NC, No-Virus (NVC)
Infection	1 day (22-24h)
MRD	1:16 (6.25% serum in-well)
Sample Pre-Treatment	Heat inactivation (56°C), fiberglass filtration
Readout	Luminescence (virus replication-driven luciferase expression)

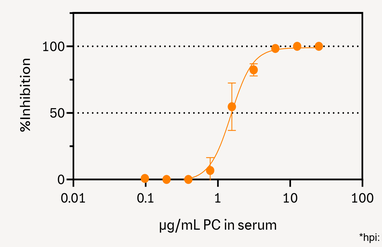
Method Development

Kinetics experiments were used to find baseline assay conditions and identify appropriate PC:

Tool virus expression kinetics



Tool virus Neutralization

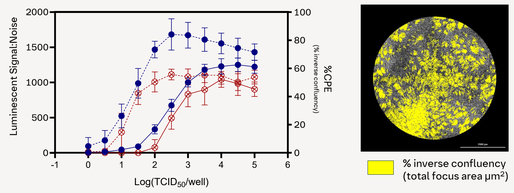


*hpi: hours post infection

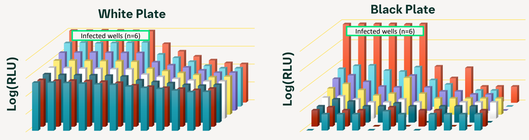
Assay Optimization

Assay was optimized to use ready-to-assay cells and improve performance.

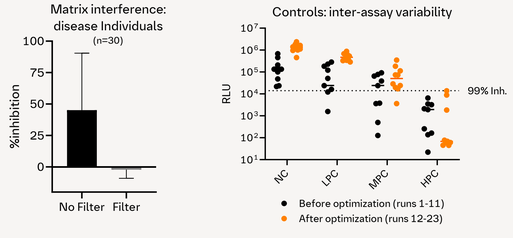
Imaging to optimize expression and cell death:



Black plates to reduce cross-talk:



Filtration to reduce disease matrix interference:



Assay Validation

- Validation runs were performed at the CRO.
- Screening cut points were determined in normal matrix and disease (solid tumor) matrix with at least 30 individuals.
- Validation screening cut point was compared to an in-study cut point calculated using pre-dose samples which was not significantly different.

Validation Results	
Sensitivity	**557 ng/mL** (LPC @ 1.3µg/mL)
Selectivity	10/10 (100%) screen pos @ HPC/LPC. 18/18 (100%) screen pos @ HPC/LPC in disease serum. 9/10 (90%) screen neg in neat normal human serum. 25/31 (80.6%) screen neg in neat disease serum*
Intra-assay Precision	NC (**10.5%**), LPC (**31.3%**), MPC (**19.7%**), HPC (**56.5%**)**
Inter-assay Precision	NC (**26.5%**), LPC (**21.3%**), MPC (**32.1%**), HPC (**38.5%**)**
Titer Precision	%CV ≤ 20.7 per dilution
Screening Cut Point	**0.83** (99% CI based on ≥ 30 donors)
Titer Cut point	**0.73** (99.9% upper bound CI)

*initial selectivity runs in unspiked samples failed to pass acceptance criteria (≥80%) prior to removal of two presumed biological outliers, which may indicate pre-existing immune response. Nonetheless, in-study cut point analysis with pre-dose samples confirmed acceptable selectivity and false positive rate, with an in-study false positive rate of 3.1%, based 98 datapoints for n=29 individuals.
**high variability of the HPC is compounded by the log-based nature of the signal responses. The expected trend of PC inhibition magnitude (LPC<MPC<HPC) was consistently observed.

Assay Highlights

2 Days to data	**150µL** Sample volume
0.83 Screening cut point	**557 ng/mL** Screening Sensitivity

Conclusions

- Replicating viral vectors require unique considerations for nAb assay development. As such, tailored tools may be needed (e.g., reporter viruses).
- Optimization and method refinement were necessary to address the unique aspects of replication and viral matrix interference.
- Our assay was successfully validated and met performance standards consistent with relevant health authority guidance for protein-based therapeutics[2].
- Analysis of initial phase-I clinical samples has been successfully completed and will continue as global VSV-GP trials progress.

Acknowledgements
QPS LLC, B2S Life Sciences, Kaitlynn Graca, Tingting Chen, Stephen Cafiero, Arti Mathur, Steve Anderlot, Stephanie Kostuk, Alvaro Maceira, and Adam Vigil

References
1. Phase I study of VSV-GP (BI 1831169) as monotherapy or combined with ezabenlimab in advanced and refractory solid tumors. *Future Oncology*. 2022. PMID: 35699077.
2. Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection. Guidance for Industry. US FDA (CDER/CBER). 2019.

QPS CUSTOM-BUILT RESEARCH™ Boehringer Ingelheim