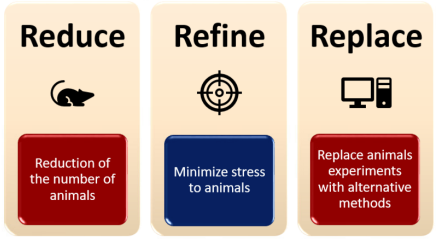
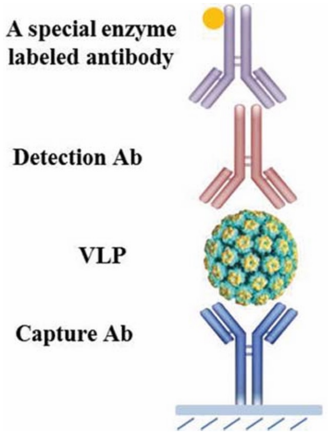


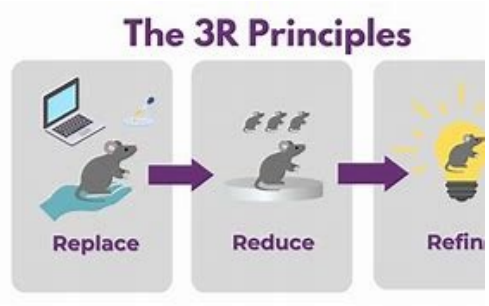
mAb-based in vitro relative potency (IVRP) tests

A case study with HPV vaccines

Qinjian Zhao
Chongqing Medical University
16 April 2025



Outline



- Why IVRP? (3Rs in animal use)
- Validation of IVRP – IVRP vs. ED50 correlation
- Key reagents – mAbs recognizing clinically relevant epitopes
- Sources for key reagents

[Human Vaccines 1:5, 191-197, September/October 2005]; ©2005 Landes Bioscience

Research Paper

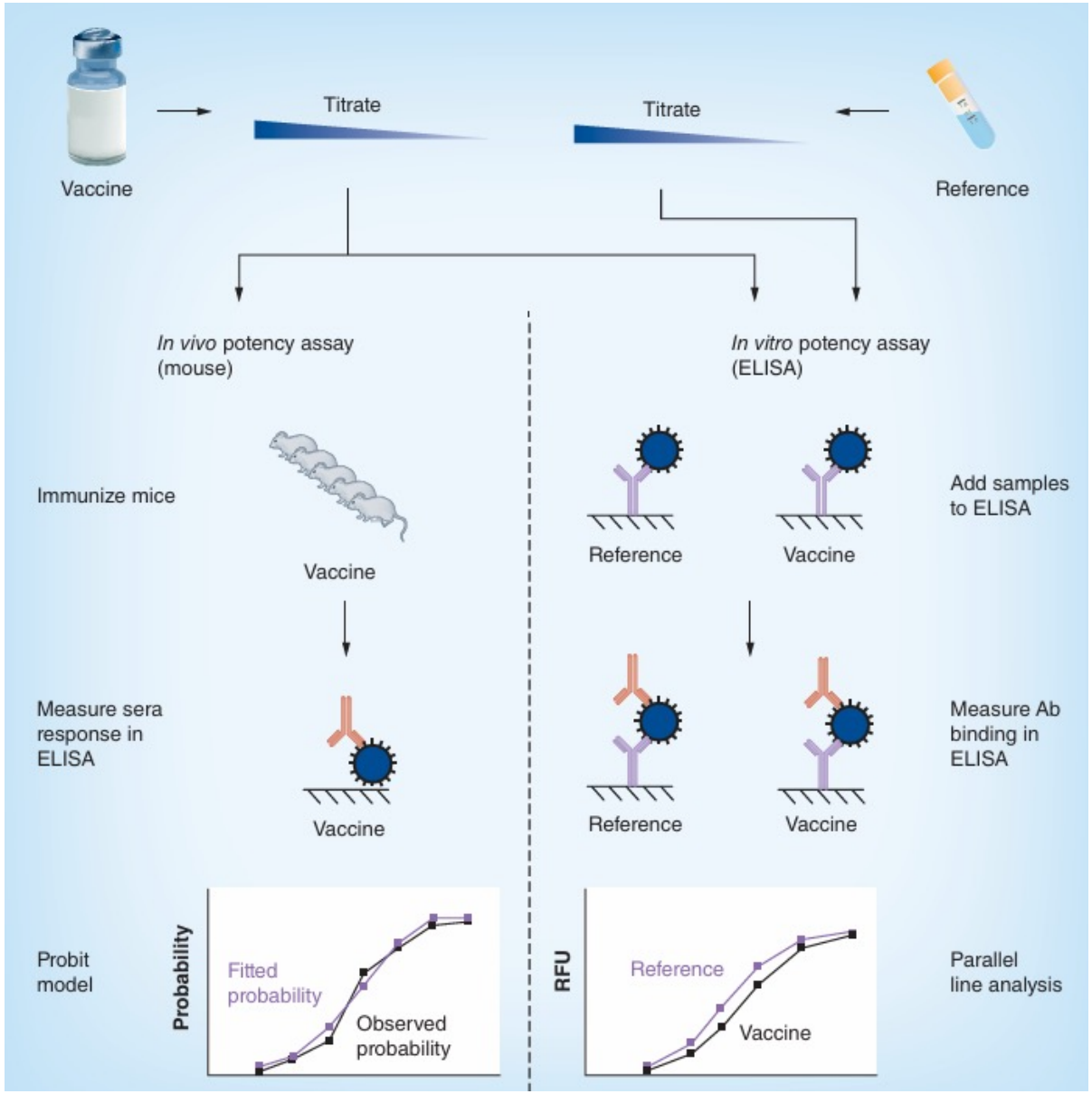
Correlation between Mouse Potency and In Vitro Relative Potency for Human Papillomavirus Type 16 Virus-Like Particles and Gardasil® Vaccine Samples

M. Shank-Retzlaff[1,*]

ABSTRACT

Parallel line analysis

- Confirming similar dilution behaviors
- Setting titration series based of Ag content



Curve-fitting (Dose-response curves)

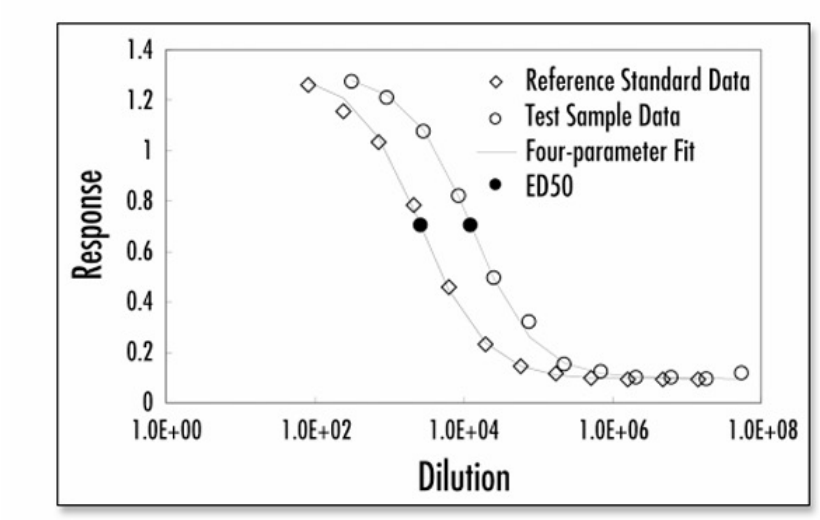
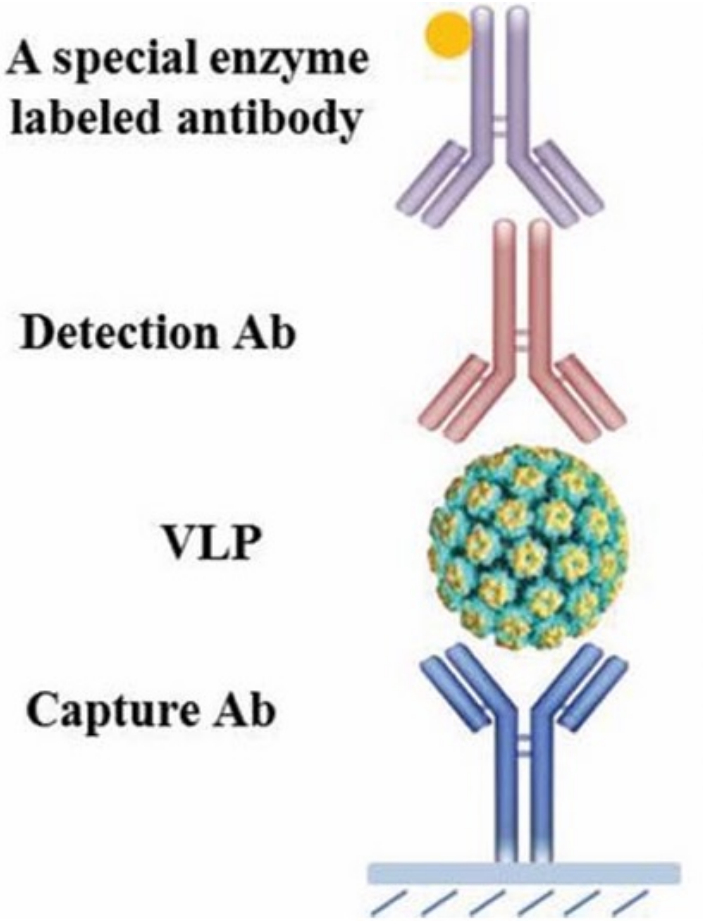


Figure 2. Example IVRP data for a reassembled sample. The test sample and reference standard were analyzed on the same 96-well plate and the resulting responses compared using a four-parameter logistic function. The sample IVRP is determined by taking the ratio of the sample and reference standard ED_{50}s, the sample dilution that produces a response equal to 50% of the maximum response, and multiplying by the IVRP of the reference standard.



Availability of products with a range of different activity

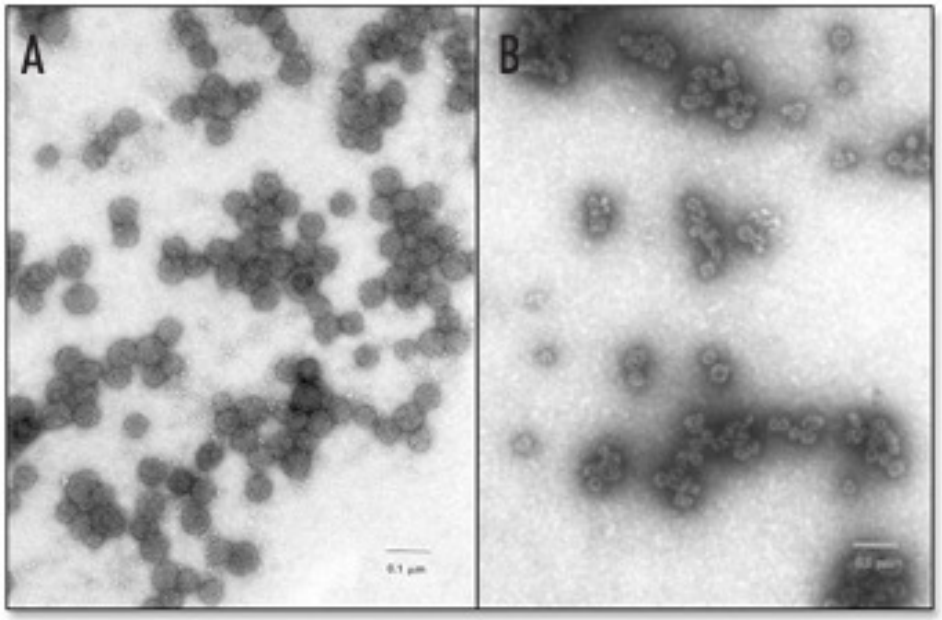


Figure 3. Transmission Electron Micrographs of HPV Type 16 VLPs. (A) Reassembled samples and (B) Non-reassembled samples.

Correlation established

- ED50 vs IVRP correlation
- Validation of the in vitro assay
- Stability-indicating assay

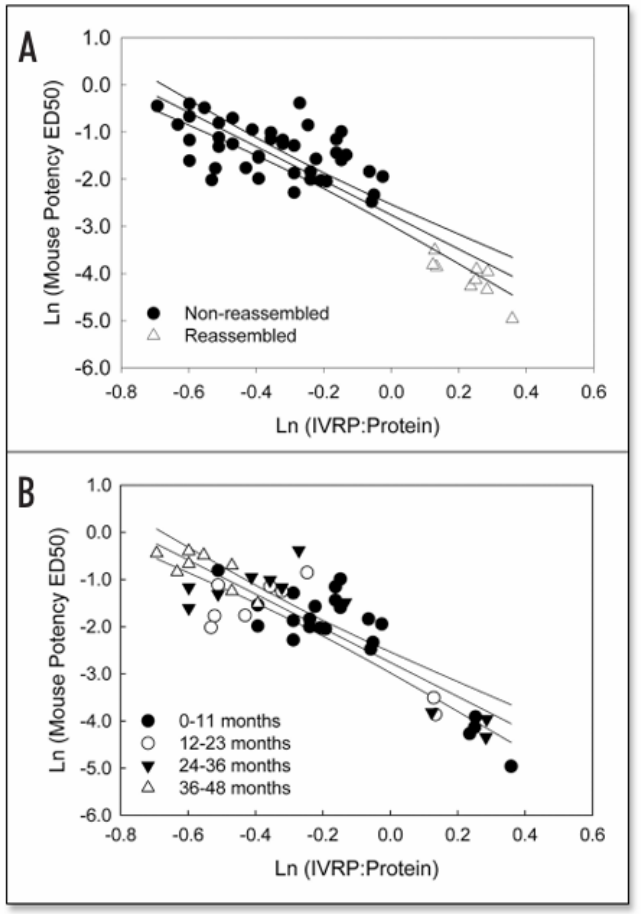


Figure 4. Correlation between the mouse potency and IVRP results for samples containing Type 16 VLPs. (A) Results plotted by sample type: ●, non- reassembled samples; ○, reassembled samples. (B) Result plotted by age of the sample at the time of testing: ●, 0–11 months; ○, 12–23 months; ▼, 24–35 months; △, 36–48 months. The solid lines indicate the results of linear regression analysis and the corresponding 95% confidence interval. All samples were stored at 2–8°C.

Expanding the type coverage – 2v/4v to 9-valent

Table 1. Basic information on the globally licensed human papillomavirus (HPV) vaccines.

	2vHPV (Cervarix®)	4vHPV (Gardasil®)	9vHPV (Gardasil 9®)
Vaccine vial image			
Manufacturer	GlaxoSmithKline	Merck and Co., Inc.	Merck and Co., Inc.
Expression system	Baculovirus (*Trichoplusia ni* insect cell)	Yeast (*Saccharomyces cerevisiae*)	Yeast (*Saccharomyces cerevisiae*)
HPV types	16/18	6/11/16/18	6/11/16/18/31/33/45/52/58
VLPs protein (μg)	20/20	20/40/40/20	30/40/60/40/20/20/20/20/20
Adjuvants	500 μg aluminum hydroxide, 50 μg 3-O-desacyl-4′ monophosphoryl lipid A	225 μg amorphous aluminum hydroxyphosphate sulfate	500 μg amorphous aluminum hydroxyphosphate sulfate
Volume per dose	0.5 mL	0.5 mL	0.5 mL
Dosing regime	0, 1, 6 month	0, 2, 6 month	0, 2, 6 month
Route of administration	Intramuscular injection	Intramuscular injection	Intramuscular injection
Approval time by FDA	October, 2009	June, 2006	December, 2014

1. The information of Cervarix® is available from http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM186981.pdf;
2. The information of Gardasil® is available from http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM111263.pdf;
3. The information of Gardasil 9® is available from http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM426457.pdf;
(Accessed on 20 April 2017)

mAb-based IVRP and serological assays

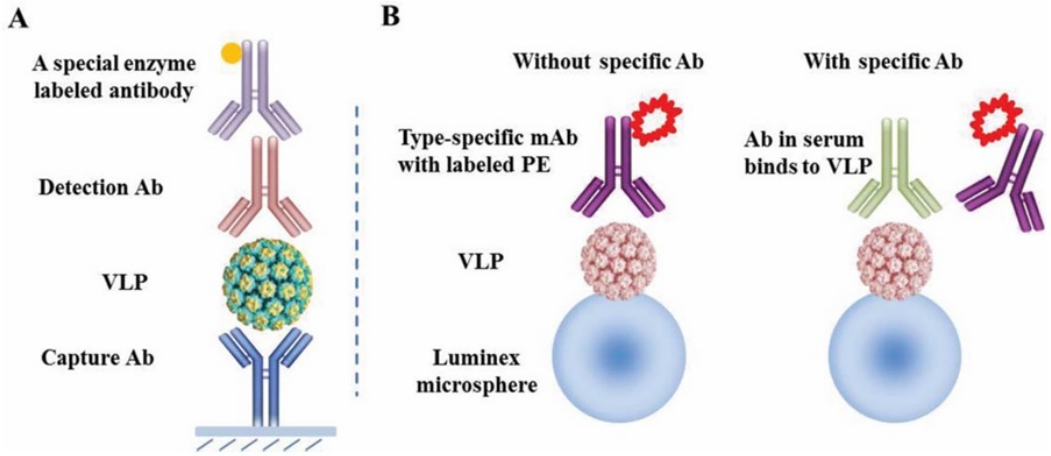


Figure 3. Schematic illustrations of IVRP for vaccine antigenicity on product quality and for serological assay for type-specific and epitope-focused determination of antibody titers elicited by vaccination. (A) IVRP for antigenicity. The IVRP assay is used for monitoring vaccine product quality during lot-release and stability testing. One monoclonal antibody is used to capture the type-specific VLP on the microplate, while the other monoclonal antibody is used for detection in a sandwich format. The final readout is performed with a horseradish peroxidase (HRP) or alkaline phosphatase (AP)-labeled secondary antibody that is specific to the subclass of the detection mAb. (B) Serological assay. The competitive Luminex immunoassay evaluates the level of functional antibody titers in vaccinees. The immunoassay quantitatively measures the ability of the type-specific antibody in serum to compete with a phycoerythrin (PE) labeled, HPV type-specific mAb for a given type (Table 3) for binding to the same epitope. The Luminex microspheres were coupled with a given VLP type via covalent bonds. Owing to the competition with the labeled detection Ab, the fluorescent signals binding to the Luminex beads decrease if there are L1-specific neutralizing antibodies in serum samples. Abbreviations: mAb, monoclonal antibody; VLP, virus like particle; PE, phycoerythrin.

What enables the development of good assays? Availability of good reagents/mAbs

Table 3. The type-specific and neutralizing monoclonal antibodies used for product potency analysis and for serological assay.

Types	mAb used in IVRP		mAb used in cLIA	Notes
	Capture	Detection		
HPV 6	H6.M48	H6.B10.5	H6.M48	
HPV 11	K11.B2	H11.B2	K11.B2	HPV11 was chosen as the calibrator in HPV-9 cLIA.
HPV 16	H16.J4	H16.V5	H16.V5	
HPV 18	H18.J4	H18.R5	H18.J4	
HPV 31	H31.5F12	H31.5D10	H31.5D10 *	H31.5D10 appeared cross reactivity with HPV58 VLP in cLIA.
HPV 33	H33.5D4	H33.6G9	H33.6G9	
HPV 45	H45.6G6	H45.10B4	H45.10B4*	H45.6G6 was shown to be cross-reactive with HPV18 VLP.
HPV 52	H52.8D11	H52.9F7	H52.9F7*	
HPV 58	H58.2C3	H58.6E11	H58.6E11*	

Abbreviations: mAb, monoclonal antibody; IVRP, *in vitro* relative potency; cLIA, competitive Luminex immunoassay; EC_{50}, half maximal effective concentration.

1. The type- specific mAbs used in cLIA were all matched with detection mAbs in the IVRP assay except H6.M48, K11.B2, H18.J5, which is capture antibody of HPV6, 11, 18 in the IVRP assay, respectively. In cLIA, H31.5D10 *, H45.10B4*, H52.9F7*, H58.6E11* were also named with suffices as H31.5D10.E4, H45.10B4.H4, H52.9F7.E1, H58.6E11.F4.
2. HPV11 was chosen as the calibrator for the additional HPV types as it had been shown to have a positive correlation between the level of HPV11 L1 VLP-specific IgG in animals immunized with HPV 11 virions and neutralization of HPV 11 in the athymic mouse xenograft model.
3. HPV31.5D10 showed cross reactivity with HPV58 VLP in the assay of the specificity evaluation of the mAbs used in cLIA.
4. H45.6G6 was shown to be cross-reactive with HPV18 VLP in the assay of quantifying the binding affinity of the mAbs to the specific HPV VLPs.
5. The information of the capture and detection mAbs of HPV6, 11, 16 and 18 used in IVRP collected from Christensen ND, et al.[37–39] The information of mAbs of HPV31, 33, 45, 52 and 58 used in IVRP was obtained from Martha J. Brown, et al.[40] The information of the type-specific mAbs used in the HPV-9 cLIA was obtained from Christine Roberts, et al; [41]

Information on mAbs – compiled here

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PRODUCT REVIEW

Expanded strain coverage for a highly successful public health tool: Prophylactic 9-valent human papillomavirus vaccine

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ABSTRACT

Human papillomavirus is considered the causative factor for cervical cancer, which accounts for approximately 5% of the global cancer burden and more than 600,000 new cases annually that are attributable to HPV infection worldwide. The first-generation prophylactic HPV vaccines, Gardasil® and Cervarix®, were licensed approximately a decade ago. Both vaccines contain the most prevalent high-risk types, HPV16 and 18, which are associated with 70% of cervical cancer. To further increase the type coverage, 5 additional oncogenic HPV types (31, 33, 45, 52 and 58) were added to the existing Gardasil-4 to develop a 9-valent HPV vaccine (9vHPV), Gardasil 9®, increasing the potential level of protection from ~70% to ~90%. The efficacy of the vaccine lies primarily in its ability to elicit type-specific and neutralizing antibodies to fend off the viral infection. Therefore, type-specific and neutralizing murine monoclonal antibodies (mAbs) were used to quantitate the antigenicity of the individual vaccine antigens and to measure the antibody levels in the serum samples from vaccinees in a type- and epitope-specific

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Efforts on HPV serology assay standardization

The HPV Serology Standardization Initiative - 2017
(Sponsored by NCI and The Bill & Melinda Gates Foundation)

HPV Serology Lab
Advancing HPV Assay Standardization

Mission:

- To work in **partnership** with the **international** HPV serology community to **promote further standardization, harmonization and proficiency of HPV serology assays** to assess vaccine immunogenicity in vaccine trials through:
  - development of **qualified assay standards**, **critical reagents** (HPV Virus-Like Particles), **multiplex assays and guidelines** available to the scientific community

Impact:

- Enable comparisons of data between different vaccines and studies
- Accelerate implementation of new vaccines and new vaccine recommendations

Partners:

Frederick National Laboratory: Ligia Pinto, Troy Kemp

NCI: Doug Lowy, John Schiller, Sean Hanlon

The Bill & Melinda Gates Foundation: Peter Dull

CDC: Elizabeth Unger

Karolinska Institute: Joakim Dillner

Public Health England: Simon Beddows

Biostat Consulting, LLC: Brian Plikaytis

https://frederick.cancer.gov/science/hpvserologylab/overview


Thank you