Session 9: Introduction to Sieve Analysis of Pathogen Sequences, for Assessing How VE Depends on Pathogen Genomics– Part I

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Outline of Module 16: Evaluating Vaccine Efficacy

Session 1 (Gabriel)	Introduction to Study Designs for Evaluating VE
Session 2 (Follmann)	Introduction to Vaccinology Assays and Immune Response
Session 3 (Gilbert)	Introduction to Frameworks for Assessing Surrogate Endpoints/Immunological Correlates of VE
Session 4 (Follmann)	Additional Study Designs for Evaluating VE
Session 5 (Gilbert)	Methods for Assessing Immunological Correlates of Risk and Optimal Surrogate Endpoints
Session 6 (Gilbert)	Effect Modifier Methods for Assessing Immunological Correlates of VE (Part I)
Session 7 (Gabriel)	Effect Modifier Methods for Assessing Immunological Correlates of VE (Part II)
Session 8 (Sachs)	Tutorial for the R Package *pseval* for Effect Modifier Methods for Assessing Immunological Correlates of VE
Session 9 (Gilbert)	**Introduction to Sieve Analysis of Pathogen Sequences, for Assessing How VE Depends on Pathogen Genomics**
Session 10 (Follmann)	Methods for VE and Sieve Analysis Accounting for Multiple Founders

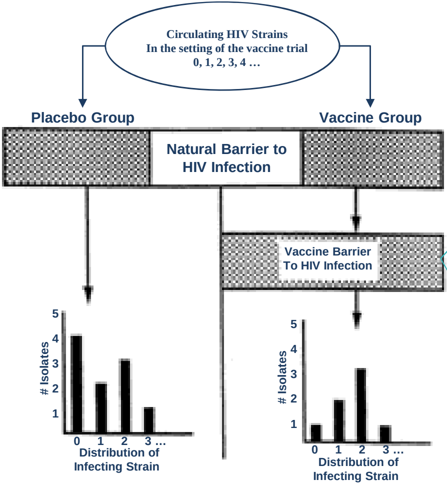
Circulating HIV Strains
In the setting of the vaccine trial
0, 1, 2, 3, 4 …
Placebo Group
Vaccine Group
Natural Barrier to HIV Infection
Vaccine Barrier To HIV Infection
# Isolates
5
4
3
2
1
0 1 2 3 …
Distribution of Infecting Strain
# Isolates
5
4
3
2
1
0 1 2 3 …
Distribution of Infecting Strain

Figure 1 from Gilbert, Self, Ashby (1998, *Biometrics*)

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1. Sieve Analysis Via Cumulative and Instantaneous VE Parameters
2. Cumulative VE Approach: NPMLE and TMLE
3. Mark-Specific Proportional Hazards Model
4. Example 1: RV144 HIV-1 Vaccine Efficacy Trial
5. Example 2: RTS,S Malaria Vaccine Efficacy Trial

Cumulative Genotype-Specific VE

- T = time from study entry (or post immunization series) until study endpoint through to time τ_1 (e.g., HIV-1 infection)
- t = fixed time point of interest $t < \tau_1$

- **Discrete** genotype-specific cumulative VE

$$VE^{\text{cml/disc}}(t,j) = \left[1 - \frac{P(T \leq t, J = j|\text{Vaccine})}{P(T \leq t, J = j|\text{Placebo})}\right] \times 100\%, \;\; t \in [0, \tau_1]$$

- **Continuous** genetic distance-specific cumulative VE

$$VE^{\text{cml/cont}}(t,v) = \left[1 - \frac{P(T \leq t, V = v|\text{Vaccine})}{P(T \leq t, V = v|\text{Placebo})}\right] \times 100\%, \;\; t \in [0, \tau_1]$$

- J = discrete genotype subgroup such as binary, unordered categorical, ordered categorical
- V = (approximately) continuous genetic distance to a vaccine sequence

Cumulative *VE* Sieve Effect Tests

Fix t at the primary time point of interest

- $VE^{\mathrm{cml/disc}}(t, j)$:

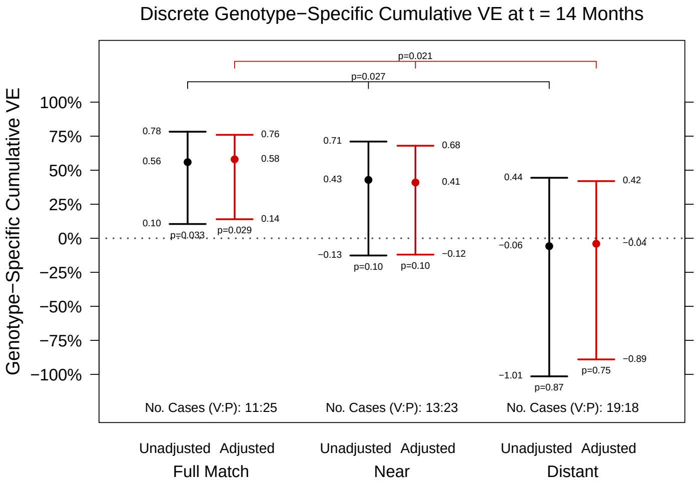
$$\begin{aligned} H_0 &: VE^{\mathrm{cml/disc}}(t,j) \ \text{ constant in } \ j \\ H_1^{mon} &: VE^{\mathrm{cml/disc}}(t,j) \ \text{ decreases in } \ j \\ H_1^{any} &: VE^{\mathrm{cml/disc}}(t,j) \ \text{ has some differences in } \ j \end{aligned}$$

- $VE^{\mathrm{cml/cont}}(t, v)$:

$$\begin{aligned} H_0 &: VE^{\mathrm{cml/cont}}(t,v) \ \text{ constant in } \ v \\ H_1^{mon} &: VE^{\mathrm{cml/cont}}(t,v) \ \text{ decreases in } \ v \\ H_1^{any} &: VE^{\mathrm{cml/cont}}(t,v) \ \text{ has some differences in } \ v \end{aligned}$$

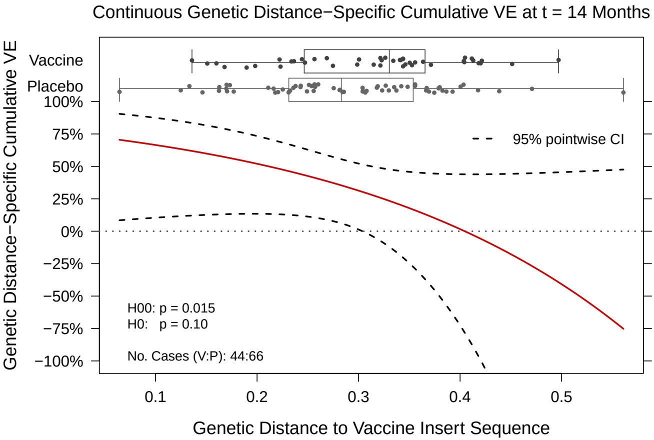
A "sieve effect" is defined by H_1^{mon} or H_1^{any} being true (i.e., differential VE by pathogen genotype)

Illustration: Cumulative $VE^{cml/disc}(t = 14, j)$ for 3-Level J^*



[*] Aalen-Johansen (1978, *Scand J Stat*) nonparametric MLE (Aalen, 1978, *Ann Stat*; Johansen, 1978, *SJS*); test for differential *VE* by Neafsey, Juraska et al. (2015, *NEJM*)

Illustration: Cumulative $VE^{cml/cont}(t = 14, v)$ for Continuous Distance V^{*}



[*] Aalen-Johansen (1978, *Scand J Stat*) nonparametric MLE (Aalen, 1978, *Ann Stat*; Johansen, 1978, *SJS*); test for differential *VE* by Neafsey, Juraska et al. (2015, *NEJM*)

Estimation of Cumulative *VE* Parameters: Approach Without Covariates

- **Nonparametric maximum likelihood estimation and testing**

Assumptions Required for Consistent Inference

- **No interference:** Whether a subject experiences the malaria endpoint does not depend on the treatment assignments of other subjects
- **A randomized trial**
- **Random dropout:** Whether a subject drops out by time t does not depend on observed or unobserved subject characteristics
- **MCAR genotypes:** Endpoint cases with missing pathogen genomes have missingness mechanism Missing Completely at Random (MCAR)

Estimation of Cumulative *VE* Parameters: With Covariates

- **Targeted minimum loss-based estimation (tMLE) and testing**

Assumptions Required for Consistent Inference

- **No interference**
- **A randomized trial**
- **Correct modeling of dropout**
- **Missing at Random genotypes**

Advantages of approach with covariates

- Correct for bias due to covariate-dependent dropout
- Increase precision via covariates predicting the endpoint and/or dropout
- Correct for bias from covariate-dependent missing genotypes (e.g., pathogen load-dependent)
- Increase precision by predicting missing genotypes (the best predictors would be based on pathogen sequences of later-sampled pathogens)

Instantaneous Genotype-Specific *VE* Parameters

- $h(t, j)$ = Hazard of the malaria endpoint with discrete genotype j
- $\lambda(t, v)$ = Hazard of the malaria endpoint with continuous genetic distance v

- **Discrete** genotype-specific instantaneous vaccine efficacy

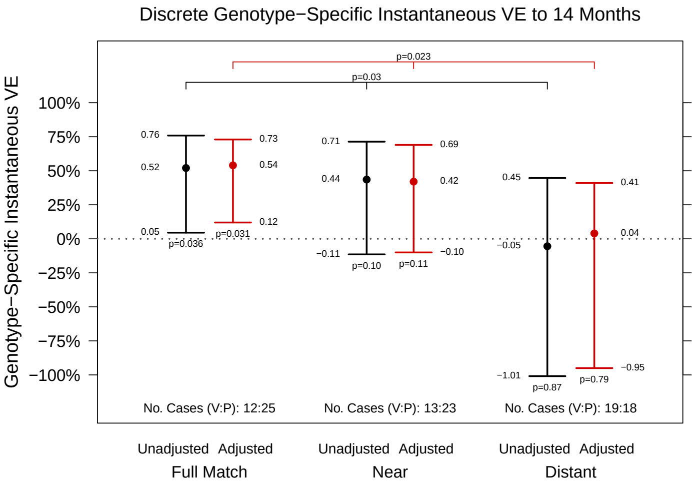
$$VE^{\mathrm{haz/disc}}(t, j) = \left[1 - \frac{h(t, j|\text{Vaccine})}{h(t, j|\text{Placebo})}\right] \times 100\%$$

- **Continuous** genetic distance-specific instantaneous vaccine efficacy

$$VE^{\mathrm{haz/cont}}(t, v) = \left[1 - \frac{\lambda(t, v|\text{Vaccine})}{\lambda(t, v|\text{Placebo})}\right] \times 100\%$$

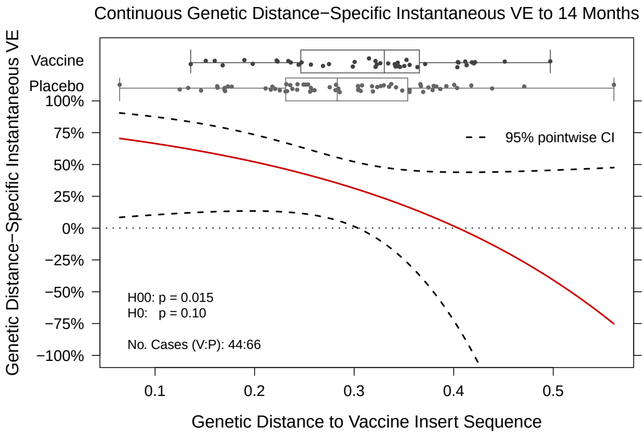
- Proportional hazards assumption: $VE^{haz/disc}(t, j) = VE^{haz/disc}(j)$ and $VE^{haz/cont}(t, v) = VE^{haz/cont}(v)$ for all $t \in [0, \tau_1]$

Illustration: Instantaneous $VE^{haz/disc}(j)$ for 3-Level J^*



[*] Gilbert (2000, *Stat Med*): genotype-specific Cox model

Illustration: Instantaneous $VE^{haz/cont}(v)$ for Continuous Distance V^*



*Juraska and Gilbert (2013, *Biometrics*): overall endpoint Cox model + semiparametric biased sampling model

Discussion of Instantaneous vs. Cumulative VE Approaches

- **Disadvantages:**
  - The instantaneous approach requires the extra assumption of proportional hazards (typically fails because of waning *VE*)
  - The *VE* parameters are hard to interpret under violation of proportional hazards
  - With currently available methods, cannot adjust for covariates without changing the target parameter to one that is not of main interest
    - Must rely on a random dropout assumption (cannot allow dropout to depend on covariates)
    - Cannot increase statistical power and precision by leveraging covariates, nor flexibly correct for accidental confounding
- **Advantages:**
  - If proportional hazards holds, the *VE* parameter is interpretable in terms of leaky genotype-specific vaccine efficacy
  - If proportional hazards approximately holds, may be reasonably interpretable and have increased efficiency by aggregating the vaccine efficacy over all time points

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Cumulative Genotype-Specific *VE*: Aalen-Johansen NPMLE

Discrete genotype-specific cumulative *VE*

$$VE^{\mathrm{cml/disc}}(t,j) = \left[1 - \frac{P(T \le t, J = j|\text{Vaccine})}{P(T \le t, J = j|\text{Placebo})}\right] \times 100\%, \;\; t \in [0, \tau_1]$$

- Observe $\tilde{T} \equiv \min(T, C)$ and $\Delta J \equiv I(\tilde{T} = T)J$
- With independent censoring, identify $P(T \le t, J = j|Z = z)$ via hazards:

$$\begin{aligned} \bar{Q}^z_j(t) &\equiv P(\tilde{T} = t, \Delta J = j|Z = z, \tilde{T} > t-1) \\ \bar{Q}^z_\cdot(t) &\equiv \sum_{i=1}^{K} \bar{Q}^z_i(t) \end{aligned}$$

$$P(T \le t, J = j|Z = z) = \sum_{t'=1}^{t} \left[\bar{Q}^z_j(t') \prod_{s=1}^{t'-1} \{1 - \bar{Q}^z_\cdot(s)\}\right]$$

Cumulative Genotype-Specific *VE*: Aalen-Johansen NPMLE

- Aalen-Johansen estimator plugs in empirical estimates

$$\begin{aligned}
\bar{Q}^{z}_{j,n}(t) &= \frac{\text{No. type j events at t in group z}}{\text{No. at risk at t-1 in group z}} \\
\widehat{P}(T \leq t, J = j | Z = z) &= \sum_{t'=1}^{t} \left[\bar{Q}^{z}_{j,n}(t') \prod_{s=1}^{t'-1} \{1 - \bar{Q}^{z}_{\cdot,n}(s)\} \right]
\end{aligned}$$

Limitations

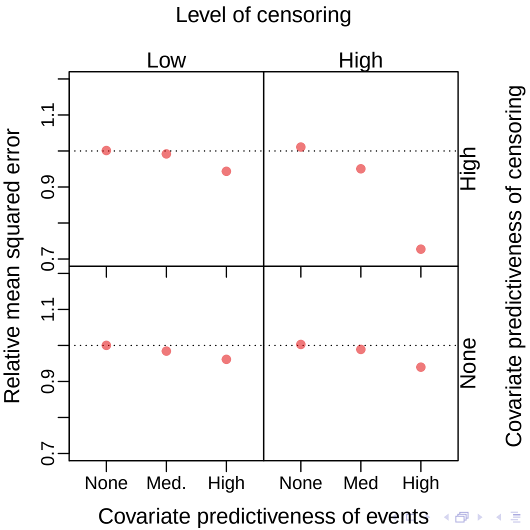
- For consistency need random censoring (cannot depend on covariates)
- Efficient if no prognostic factors

Incorporating Covariates: TMLE

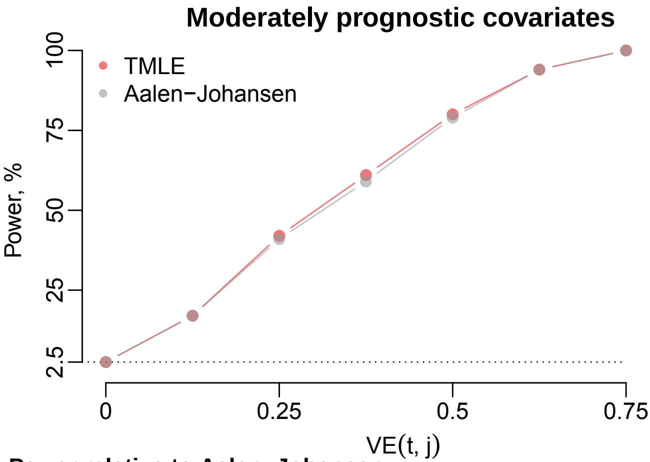
$$\begin{aligned} P(T \leq t, J = j|Z = z) &= E_W\left[P(T \leq t, J = j|Z = z, W)\right] \\ &= \sum_w P(T \leq t, J = j|Z = z, W = w)P(W = w|Z = z) \end{aligned}$$

- TMLE optimizes bias-variance trade-off for estimating $P(T \leq t, J = j|Z = z)$
- Incorporates flexible models of $P(T \leq t, J = j|Z = z, W)$ and of $P(C \leq t|Z = z, W)$
- TMLEs are doubly robust and asymptotically normal
  - Also asymptotically efficient if both $P(T \leq t, J = j|Z = z, W)$ and $P(C \leq t|Z = z, W)$ are estimated consistently
- Benkeser, Carone and Gilbert (2017) developed this TMLE, with R code

Mean Squared Error TMLE vs. Aalen-Johansen



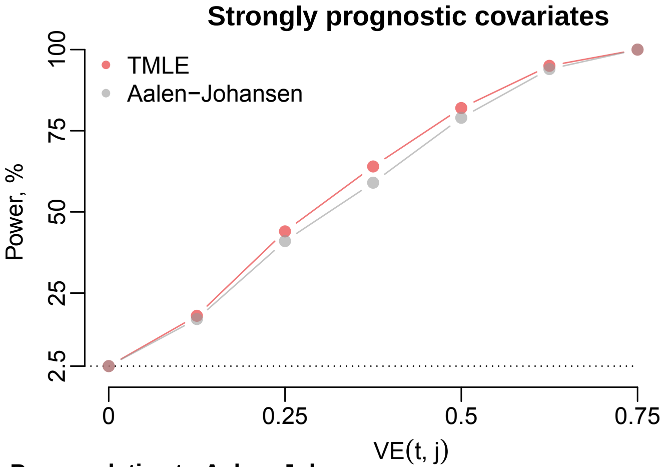
Power of Wald Tests TMLE vs. Aalen-Johansen



Power relative to Aalen−Johansen

TMLE	1.00	1.02	1.03	1.01	1.00	1.00

Power of Wald Tests TMLE vs. Aalen-Johansen



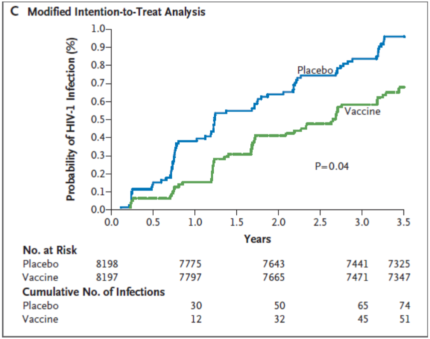
Power relative to Aalen−Johansen

TMLE	1.06	1.07	1.08	1.04	1.01	1.00

Sieve Analysis of RV144 Thai Trial

Background on Thai Trial

- Conducted 2004–2009 in the general population of Thailand
- 16,403 randomized 1:1 vaccine:placebo, primary endpoint HIV-1 infection by 3.5 years
- $\widehat{VE} = 31\%$, 95% CI 1% to 51%, $p = 0.04$ (Rerks-Ngarm et al., 2009, *NEJM*)



C Modified Intention-to-Treat Analysis

No. at Risk					
Placebo	8198	7775	7643	7441	7325
Vaccine	8197	7797	7665	7471	7347
Cumulative No. of Infections					
Placebo		30	50	65	74
Vaccine		12	32	45	51

Sieve Analysis of RV144 Thai Trial

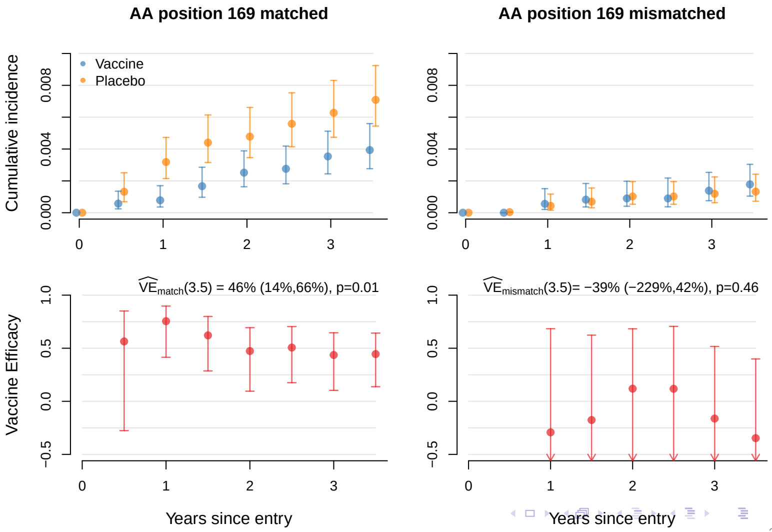
- Cox model (Lunn and McNeil, 1995, *Biometrics*) and Aalen-Johansen (1978) sieve analysis yielded the inference

$$VE^{cml/disc}(3.5, v = 0) > VE^{cml/disc}(3.5, v = 1)$$

with V defined by match ($v = 0$) vs. mismatch ($v = 1$) of the infecting HIV-1 with the vaccine sequences at position 169 of HIV-1 Env V2

- TMLE adjusting for rish behaviors, gender, age, gave a similar result with increased precision (Benkeser, Carone, Gilbert, 2017); next slide

TMLE Cumulative VE Sieve Results: RV144 Thai Trial



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Mark-Specific Proportional Hazards Approach with Missing Pathogen Sequences

- Sun and Gilbert (2012, *Scand J Stat*)
- Gilbert and Sun (2015, *JRSS-B*)

- These methods pose a continuous mark-specific proportional hazards model and use inverse probability weighting (IPW) or augmented IPW

Competing Risks Model in Vaccine Efficacy Trials

- Conditional mark-specific hazard rate function:

$$\lambda(t, v|z) = \lim_{h_1, h_2 \to 0} \frac{P\{T \in [t, t + h_1), V \in [v, v + h_2) | T \geq t, Z = z\}}{h_1 h_2}$$

- Covariate-adjusted mark-specific vaccine VE:

$$\mathrm{VE}(t, v|z) = 1 - \frac{\lambda_v(t, v|z)}{\lambda_p(t, v|z)},$$

where $\lambda_v(t, v|z)$ and $\lambda_p(t, v|z)$ are the conditional mark-specific hazard functions for the vaccine and placebo groups, respectively

Mark-Specific Proportional Hazards Models

- Stratified mark-specific proportional hazards model:

$$\lambda_k(t, v|z_{ki}(t)) = \lambda_{0k}(t, v)\exp\left\{\beta(v)^T z_{ki}(t)\right\}, k = 1, \ldots, K$$

  where $\lambda_{0k}(t, v)$ is an unspecified baseline function and $\beta(v)$ is p-dimensional regression coefficient functions

- $z = (z_1, z_2)$; $z_1 =$ vaccine group indicator; z_2 other covariates; $\beta_1(v) =$ coefficient corresponding to z_1

  Mark-specific vaccine efficacy:

$$VE(v) = 1 - \exp(\beta_1(v))$$

Completely Observed Competing Risks Data

Completely observed competing risks data:

$$(Z_{ki}, X_{ki}, \delta_{ki}, \delta_{ki} V_{ki}), \qquad i = 1, \cdots, n_k, k = 1, \ldots, K,$$

where $X_{ki} = \min\{T_{ki}, C_{ki}\}$, $\delta_{ki} = I(T_{ki} \leq C_{ki})$

When the failure time T_{ki} is observed, $\delta_{ki} = 1$ and the mark V_{ki} is also observed, whereas if T_{ki} is censored, the mark V_{ki} is unknown

Assume C_{ki} is independent of T_{ki} and V_{ki} conditional on Z_{ki}

Missing Marks in HIV Vaccine Efficacy Trials

Observed data

$$O_{ki} = \{X_{ki}, Z_{ki}, \delta_{ki}, R_{ki}, R_{ki}\delta_{ki}V_{ki}, \delta_{ki}A_{ki}\}, i = 1 \ldots, n_k, k = 1, \ldots, K,$$

R_{ki} = complete-case indicator; $R_{ki} = 1$ if V_{ki} is known or if T_{ki} is censored and $R_{ki} = 0$ otherwise

- Auxiliary variables A_{ki} can be used to predict whether the mark is missing and to predict the missing marks
  - E.g., A_{ki} = sequence information from a later sampled virus
- Model the relationship between A_{ki} and V_{ki} to predict V_{ki}

Inverse Probability Weighted Complete-Case Estimator

- $r_k(W_{ki}, \psi_k) =$ parametric model for the probability of complete-case, where ψ_k is a q-dimensional parameter
- The IPW estimator $\hat{\beta}^{ipw}(v)$ solves the estimating equation for β:

$$U_{ipw}(v, \beta, \hat{\psi}) = \sum_{k=1}^{K} \sum_{i=1}^{n_k} \int_0^1 \int_0^\tau K_h(u - v)\big(Z_{ki}(t) - \tilde{Z}_k(t, \beta, \hat{\psi}_k)\big) \frac{R_{ki}}{\pi_k(Q_{ki}, \hat{\psi}_k)} N_{ki}(dt, du),$$

where

$$\begin{aligned} \tilde{Z}_k(t, \beta, \psi_k) &= \tilde{S}_k^{(1)}(t, \beta, \psi_k)/\tilde{S}_k^{(0)}(t, \beta, \psi_k), \\ \tilde{S}_k^{(j)}(t, \beta, \psi_k) &= n_k^{-1} \sum_{i=1}^{n_k} R_{ki}(\pi_k(Q_{ki}, \psi_k))^{-1} Y_{ki}(t) \exp\{\beta^T Z_{ki}(t)\} Z_{ki}(t)^{\otimes j} \end{aligned}$$

Augmented IPW Complete-Case Estimator

- $W_{ki} = (T_{ki}, Z_{ki}, A_{ki})$ and $w = (t, z, a)$
  More efficient estimation can be achieved by incorporating the knowledge of the conditional mark distribution:

$$\begin{aligned}\rho_k(w, v) &= P(V_{ki} \leq v | \delta_{ki} = 1, W_{ki} = w) \\ &= \frac{\int_0^v \lambda_k(t, u|z) g_k(a|t, u, z)\, du}{\int_0^1 \lambda_k(t, u|z) g_k(a|t, u, z)\, du},\end{aligned}$$

  where $g_k(a|t, v, z) = P(A_{ki} = a | T_{ki} = t, V_{ki} = v, Z_{ki} = z, \delta_{ki} = 1)$

- Let $\hat{g}_k(a|t, u, z)$ be a parametric / semiparametric estimator of $g_k(a|t, u, z)$; then $\rho_k(w, v)$ can be estimated by

$$\hat{\rho}_k^{ipw}(w, v) = \frac{\int_0^v \hat{\lambda}_k^{ipw}(t, u|z) \hat{g}_k(a|t, u, z)\, du}{\int_0^1 \hat{\lambda}_k^{ipw}(t, u|z) \hat{g}_k(a|t, u, z)\, du}$$

Analysis of the RV144 Thai Trial

- Assessed how VE against subtype CRF01_AE HIV-1 infection depends on a weighted Hamming distance (Nickle et al., 2007, *PLoS One*) of breakthrough HIV-1 sequences to the A244 reference sequence contained in the vaccine
  - Include published gp120 AA sites in contact with broadly neutralizing monoclonal antibodies
- $T =$ time to HIV-1 infection diagnosis with subtype CRF01_ HIV-1
  - Infection with subtype B or unknown subtype treated as right-censoring
- 106 HIV-1 subtype CRF01_AE infected participants (42 vaccine, 64 placebo); 94 (37 vaccine, 57 placebo) with an observed mark
  - Between 2 and 13 HIV-1 sequences (total 1030 sequences) per infected participant
  - $V =$ participant-specific median distance

HIV-1 Sequence Distances to the Vaccine Sequence A244

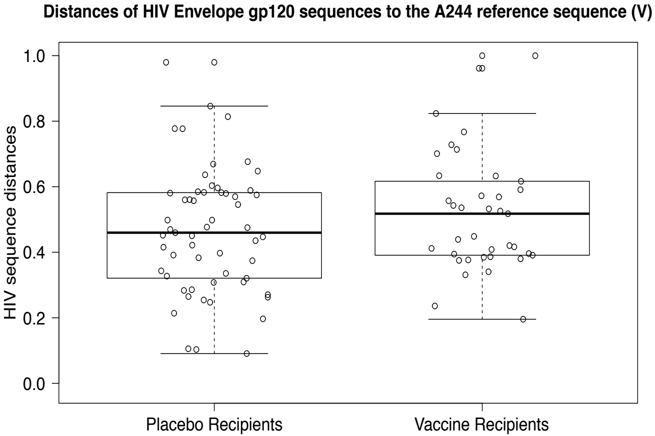

Figure: Boxplots of the marks/distances V for the 94 HIV-1 CRF01_AE infected subjects in the Thai trial with an observed mark

Vaccine Efficacy by gp120 HIV-1 Sequence Distance

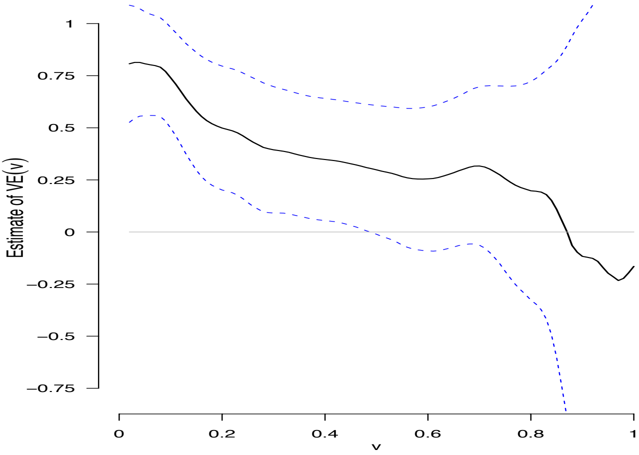

Figure: IPW point and 95% interval estimates of $VE(v)$ for the Thai trial with bandwidths $h_1 = 0.5$, $h_2 = h = 0.3$

Selected Literature on Sieve Analysis Methods

1. Proportional hazards *VE* for a discrete genotype (Gilbert, 2000, 2001, *Stat Med*, Cox model)
2. Extension of 1. accounting for missing data on genotypes (Hyun, Lee, and Sun, 2012, *J Stat Plan Inference*, AIPW)
3. Cumulative incidence *VE* for a discrete genotype (Gilbert, 2000, 2001, *Stat Med*, Aalen-Johansen NPMLE)
4. Extension of 3. for covariate-adjustment and modeling dropout (Benkeser, Carone, Gilbert, 2017, in press, tMLE)
5. Cumulative incidence *VE* for a continuous mark genotype (Gilbert, Sun, and McKeague, 2008, *Biostatistics*)
6. Proportional hazards *VE* for a continuous mark genotype (Sun, Gilbert, and McKeague, 2009, *Ann Stat*; local partial likelihood and kernel smoothing)
7. Extension of 6. for multivariate continuous mark genotypes (Sun and Gilbert, 2013, *Biostatistics*, local partial likelihood and kernel smoothing; Juraska and Gilbert, 2013, Biometrics, Cox model + semiparametric biased sampling model)
8. Extension of 6. allowing missing data on genotypes (Sun and Gilbert, 2012, *Scand J Stat*, Gilbert and Sun, 2012, *JRSS-B*, add AIPW; Juraska and Gilbert, 2015, *LIDA*, add IPW)

Ongoing Sieve Analysis Statistical Methods Research

- Replace augmented IPW with TMLE (Benkeser, Carone, and Gilbert, 2017)
  - Unbiased under weaker assumptions; more efficient
- The missing data methods assume a validation set– a subgroup of cases where the founding pathogen genotype(s) is known with certainty
  - For pathogens that evolve very quickly post-infection (e.g., HIV-1), there may be no validation set!
  - Replace with measurement error methods, incorporating models predicting (imperfectly) founder HIV genotypes
- Targeted learning approaches with **data adaptive genotype-specific VE target parameters** that combine inference with model selection on the marks/genotypes