

Asthma & Allergic Diseases Cooperative Research Centers

AADCRC-UWI-02

A First-in-Human Safety and Dose- Finding Study of New Type-16 Human Rhinovirus (RG-HRV16) Inoculum in Healthy Volunteers

Principal Investigator:

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Objectives

- To assess the **safety** of inoculation of human subjects with RG-HRV16 produced by reverse genetics
- To **determine the dose** of inoculum that induces at least moderate cold symptoms in $\geq 75\%$ of individuals. (*mean cold symptom score of ≥ 7 on the standardized Jackson Criteria scoring system*).

Background (1)

Utilization of HRV Inoculation

- **HRV infections are a major cause of exacerbations of asthma**
- Experimental inoculation with human rhinovirus type 16 (HRV16) administered intranasally has been used for over 30 years.
- Use of the model:
 - to evaluate inflammatory mechanisms
 - to test the efficacy of treatments for the common cold
 - **to study how rhinoviruses affect the lower airways and induce asthma exacerbations.**

Background (2)

Development of HRV 16 through reverse genetics

- Inoculum has historically been developed from nasal secretions as the “seed virus”.
- It is impossible however to ensure that the secretions contain only the pathogen of interest.
- This problem is minimized through the use of virus derived from a cDNA clone produced in *E. coli*.
- The cDNA clone, reproduced by the much more accurate *E. coli* DNA polymerase (compared to the viral RNA polymerase), provides a stable source of virus sequence for production of future inocula.
- Production of RG-HRV16 in a GMP facility

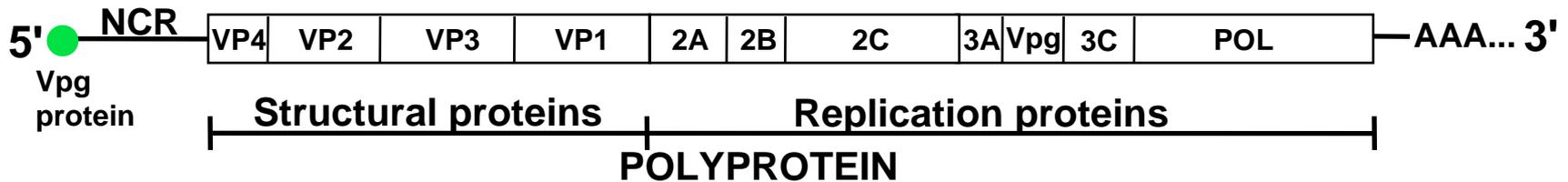
History of the RG-HRV16 Human Inoculation Virus

- 1972** HRV16 isolated from a 7-year-old with a cold in Madison, WI
Cultured in WI-38 lung fibroblasts X2 (SA212, GLP)
- 1984-5** Inoculate donors, naturally transmitted to recipients
Nasal lavage from a recipient with a cold
Passed in WI-38 cells X2 (KC939, GLP)
- 1990** Inoculate donors, naturally transmitted to recipients
Nasal lavage from a recipient with a cold
- 2000** Passed in Wis.L cells X2 (WIS1088, GLP)
Current GLP inoculum, produces moderate cold symptoms
- 2007-8** KC939 was sequenced
Cloned onto plasmid

Viral Production from HRV16 cDNA

- **HRV virion has a positive strand genome.**

- *Similar to mRNA*
- *Native viral RNA → protein*



- **HRV-16, like other strains, exists as a “quasi-species”**

- *Mixture of genomes*
- *Different isolates of the same strain can vary in sequence by 3-4%*

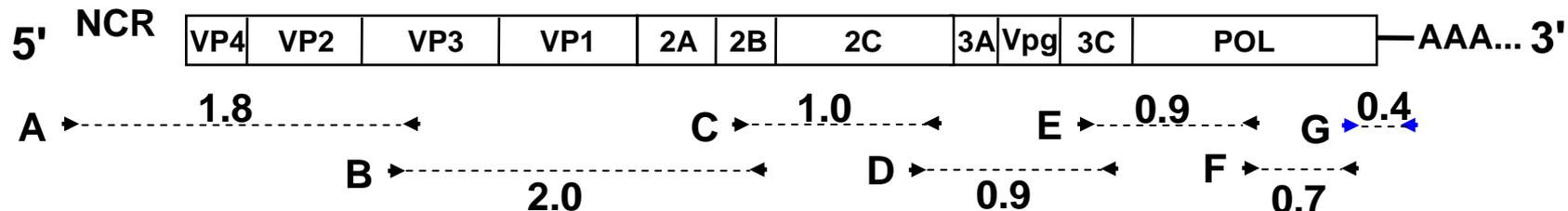
- **Naked virion RNAs of HRV are infectious.**

- *Transfect into cells → viral replication*
- *Infectivity of in vitro transcripts is similar to that of virion RNA (Lee, J Virol 77, 6235)*

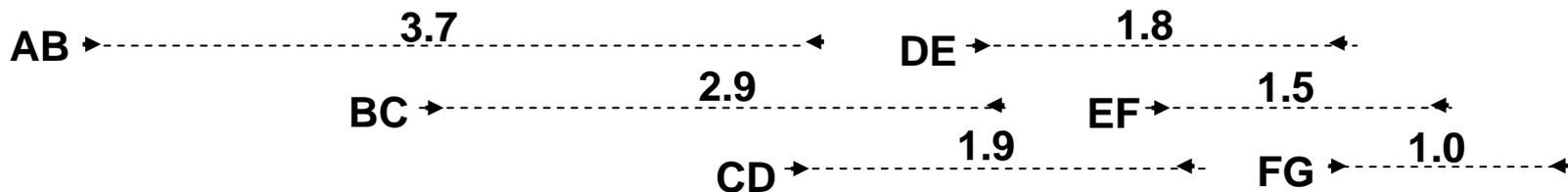
Cloning HRV16: Overview

- **The 1985 human inoculum virus stock (KC939) was used for cloning.**
 - Closest to the original isolate that was still available.
 - Extensive experience with this virus in experimental inoculation studies
- **Cloning strategy:**
 1. Based on the sequence of HRV lab strain (Lee, Virus Genes, 9, 177).
 2. The 7.2-Kb genome was divided into 7 fragments for RT-PCR to promote efficient PCR amplification.
 3. ~12 clones of each PCR fragment were isolated and sequenced.
 4. Fragments with the consensus sequence were selected.
 5. The consensus fragments was assembled into a full-length clone.
 6. The RNA transcripts of the full-length clones were tested for infectivity.
 7. The entire plasmid containing the infectious clone was sequenced.

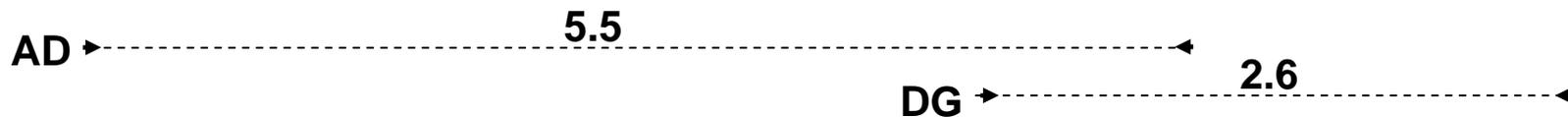
Clone Assembly From PCR Fragments



Step 1



Step 2

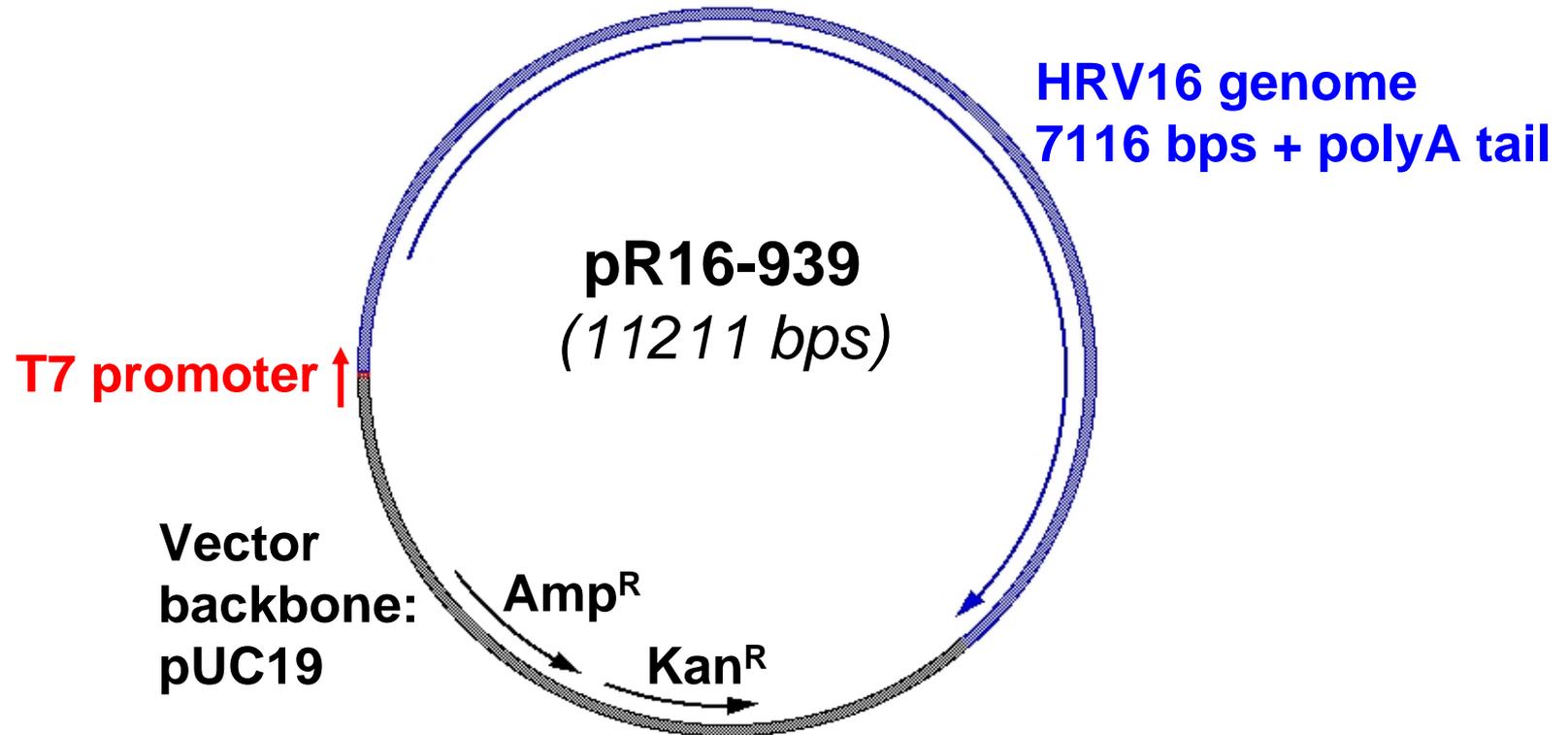


Step 3



Final product: 7.1 Kbp HRV16 cDNA clone

Plasmid Structure and Sequence Verification



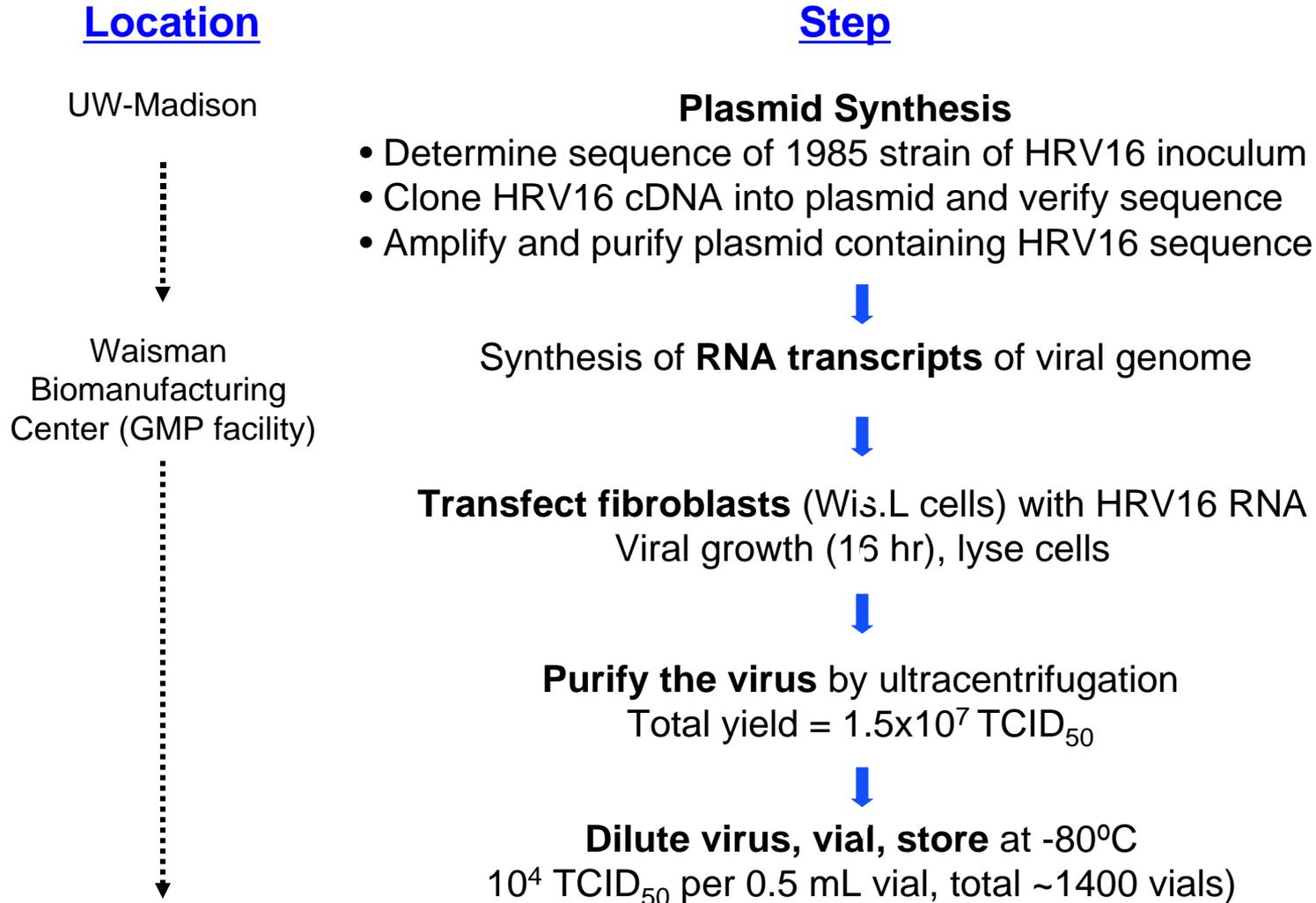
Selection of Wis.L Cells for Producing HRV16 Inoculum

- 1. Human diploid embryonic lung fibroblast cells, isolated at the Wisconsin State Lab of Hygiene in 1968.**
- 2. Normal 46XX karyotype at passage #31**
- 3. In 2003, a master cell bank from passage#1 cells was created under cGMP conditions at the Waisman Biomanufacturing Facility**
- 4. The master cells have passed all the FDA-required tests for producing biologicals for human use (pathogen-free).**

Testing of Wis.L Master Cell Bank

Test	Target Specification
Morphology	Normal Human Fibroblast
Isoenzyme Characterization	Human Phenotype
Endotoxin	≤ 20 EU/ml
Sterility Test by Direct Transfer	No bacteria/fungi detected
Bacteriostatic / Fungistatic Activity of Test Article	No bacteriostatic or fungistatic activity
Mycoplasma	None detected
In Vitro Adventitious Virus	None detected
In Vivo Adventitious Virus	None detected
Transmission EM for Viruses and Retroviruses	None observed
PERT Assay of Retrovirus	None detected
PCR Assay of Human Polyoma Virus BKV and JCV	None detected
PCR Assay for HIV 1 & 2	None detected
PCR Assay for Hepatitis B	None detected
RT- PCR Assay for Hepatitis C	None detected
PCR Assay for Epstein Barr Virus	None detected
PCR Assay for Cytomegalovirus	None detected
PCR Assay for Human Herpes Virus 6	None detected
PCR Assay for Human Herpes Virus 7	None detected
PCR Assay for Human Herpes Virus 8	None detected
PCR Assay for HTLV 1 & 2	None detected
PCR Assay for SV 40	None detected
In Vitro Assay for Bovine Viruses	None detected
In Vitro Assay for Porcine Viruses	None detected
Tumorigenicity	None observed

Production of RG-HRV16



Study Design (1)

- Single blind, 5+5 design for dose finding with dose de-escalation.
- Up to 40 healthy, HRV16 seronegative adult subjects will be inoculated intranasally with one of 3 doses of RG-HRV16 (100, 1000, 10,000 TCID₅₀,) or placebo in groups of 10 subjects/dose (5 subjects in each of 2 cohorts).
- The initial 5 subjects will receive the 100 TCID₅₀ dose of RG-HRV16 one subject at a time with a window of at least 7 days between each of the first 5 subjects
- Total of 8 visits – Screening, Inoculation and 5 Follow-Up visits

Study Design (2)

■ Inclusion criteria

- 18 and 65 years
- Provide informed consent (in English)
- Be able to cooperate with study procedures and requirements including willingness to avoid cold symptom medications during the study.
- Absence of HRV16 neutralizing antibody

Safety labs

- Normal CBC with differential
- AST/ALT < 2.5X Upper Limit of Normal (ULN),
- BUN \leq 26 mg/dL, creatinine \leq 1.7 mg/dL
- Normal immunoglobulins and lymphocyte count*

Study Design (3)

■ Exclusion Criteria

- A chronic medical condition, which may increase risk or interfere with the conduct of the study
 - includes, but not limited to heart disease, Type I or II diabetes mellitus, asthma, COPD, other chronic lung disease, perennial rhinitis and chronic rhinosinusitis
- Subjects with household contacts; with chronic lung disease, who are children under the age of 2 years and who are adults over the age of 65 years.
- Seasonal allergies will be excluded only if allergy symptoms are present at baseline, or are anticipated to occur during the study period.
- Upper respiratory infection (URI or sinusitis) in the past 4 weeks.
- Pregnant or breastfeeding women. Unwillingness to use adequate birth control methods during the course of the study.
- Smoking within the past 6 months.
- Immunosuppressive treatment within 12 months prior to study entry*
- History of reaction to or intolerance to a previous attenuated live virus vaccine

Study Design (4)

Endpoints

■ Primary Endpoints:

- The number of subjects per dosing group (n=10) who developed colds of at least moderate intensity (**weekly peak symptom** score ≥ 7 out of 39) during the first week after inoculation
- Safety as determined by adverse event reporting

■ Secondary Endpoints:

- The **Mean Cold Symptom Score** per RG-HRV16 dose
- Infection rate per RG-HRV16 dose as measured by the percentage of individuals in the dosing cohort with detectable virus
- Mean Cold Symptom Scores for each RG-HRV16 dose vs placebo.

Study Design (5)

	Screen Visit A Within 6w of V1	Screen Visit B Within 2w of V1	Visit 1 Day of RG-HRV16	Visit 1a 24h after RG-HRV16 First 5 participants	Visit 2 48 hrs after RG-HRV16	Visit 3 72 hrs after RG-HRV16	Visit 4 96 hrs after RG-HRV16	Visit 5 7-10d after RG-HRV16	Visit 6 21-28d after RG-HRV16
Consent	X								
Medical History	X								
Blood draw HRV16 Ab	X		X						X
Urine Pregnancy Test		X	X						
Nasal lavage			X		X	X	X	X	X
Cold symptom diary		X	X	X	X	X	X	X	
Physical exam		X						(X) first 5 participants	X
HRV16 inoculation			X						
AE assessments				X	X	X	X	X	X
Safety labs		X							X

Study Design (6)

Cold Symptom Scores (Jackson Criteria)

■ Symptom Scoring

- **Daily Peak Symptom Score:** For each day, this represents the sum of the highest score (the AM or the PM score) obtained for each symptom; this score is obtained per subject. (*Used to build dosing decision*)
- **Weekly Peak Symptom Score:** This represents the highest of the Daily Peak Symptom Scores for the 7-day evaluation period; this score is obtained per subject. (*Used to make dosing decision and address Primary endpoint*)
- **Mean Cold Symptom Score:** This represents the group average of the Weekly Peak Symptom Scores. (*Secondary endpoint*)

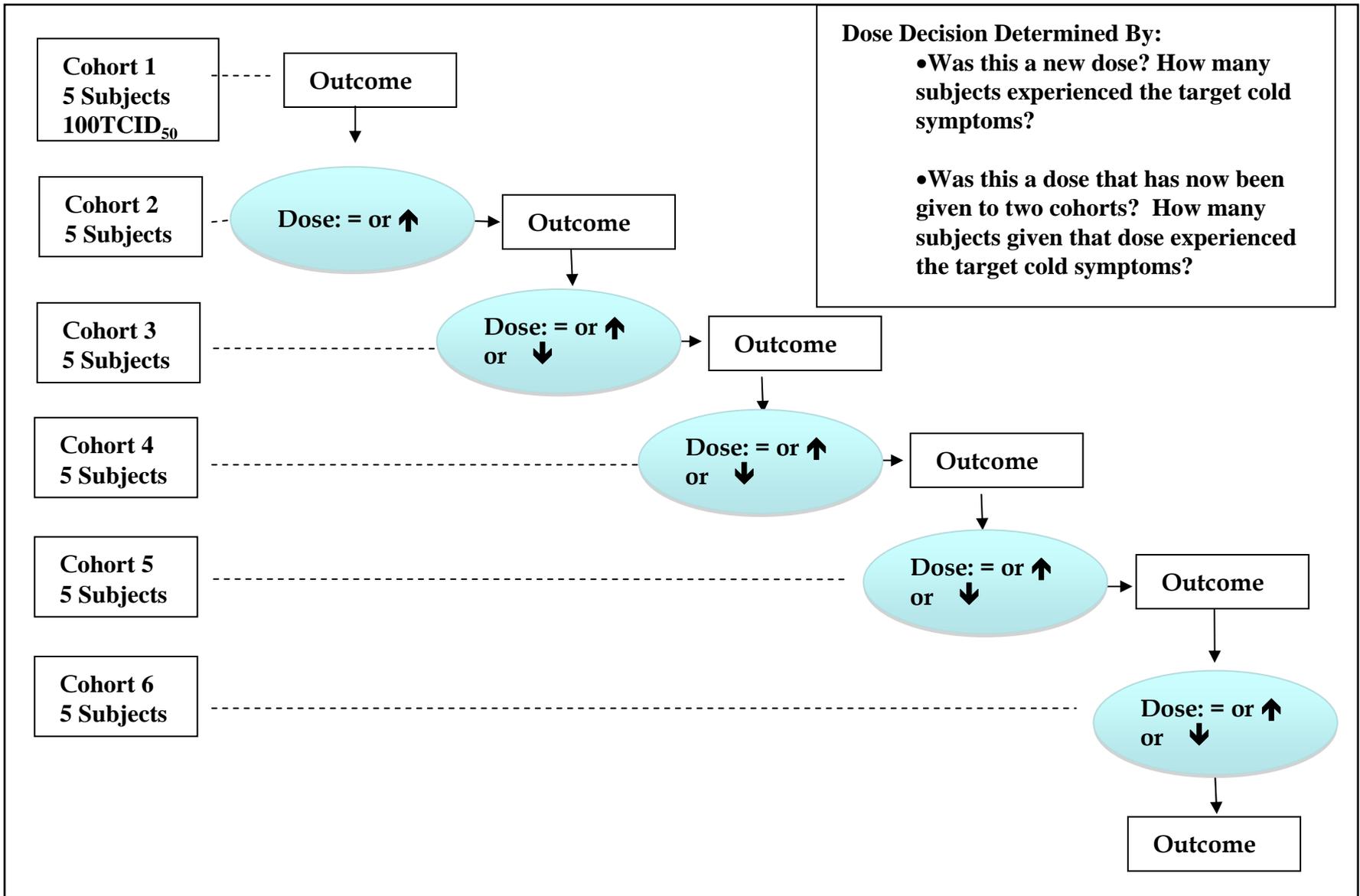
- The severity of the induced cold for a study participant is defined on the basis of the **Weekly Peak Symptom Score** and will be categorized as mild (score <7), moderate (score 7-12) or severe (score >12).

Study Design (7)

Inoculation Dose per Cohort

- Start first cohort (5 subjects) with lowest dose (100 TCID₅₀)
- The dose for the next cohort (i.e. whether to maintain or to increase the dose) is dependent upon how many subjects in the just-completed cohort experienced a moderate-to-severe cold (**Weekly Peak Symptom Score** of ≥ 7)
- The dose for subsequent cohorts (i.e. whether to maintain, increase or decrease the dose) is dependent upon how many subjects in the just-completed cohort (n=5) experienced a moderate-to severe cold **AND** whether the just completed dose was used for the first or second time

Study Design (8)



Safety Considerations (1)

Expected Clinical Findings

- Usual, expected common cold symptoms of nasal congestion, rhinorrhea, sore throat, cough and sneezing (> Gr 3 will be recorded on the Adverse Event CRF)
- Other symptoms associated with HRV-16 inoculation include headaches, muscle aches, chilliness and low-grade fevers which may occur 10-20% of the time will be captured as an adverse event and graded according to the provided in the FDA Toxicity Scale for Healthy Adults in Vaccine Trials.
- Rarely nausea is reported as a result of excess mucous production and post nasal drip and will be captured as an adverse event graded according to the Toxicity Scale.
- Complications such as vomiting and diarrhea or secondary bacterial infection including pneumonia, acute bacterial sinusitis, ear infection may occur rarely (<5% of the time). (*All events are recorded as an AE*)

Safety Considerations (2)

First Cohort (5 subjects)

- One subject to be inoculated per week
- Each of the subjects will be tested for viremia at the one-week post inoculation study visit*
- A questionnaire monitoring all household members for the first 2 weeks after inoculation of a specific study subject will be utilized*
- Independent Medical Monitor review and written approval prior to moving to the next subject. These assessments will be done at the 7-day post-inoculation visit for each subject.
- NIAID Medical Officer review and written approval after completion of the first cohort
- DSMB to perform interim review of safety data after the first 5 subjects have completed the study, or sooner if an SAE occurs*

Safety Considerations (3)

Study Stopping Rules

■ Individual Stopping Rules

- Appearance of a secondary infection related to virus inoculation, including but not limited to, a sinus infection, ear infection or pneumonia occurs during the first seven days.

■ Study Stopping Rules

- Any subject develops a study-related serious adverse event
- 4 subjects develop a secondary infection related to viral inoculation
- 2 of 5 subjects in cohort 1 continue to report cold symptoms at Visit 6 (21-28d post inoculation)
- The on-site, independent medical monitor may halt a dosing cohort dependent upon review of the safety assessments, as in the case of the first 5 subjects in cohort 1, or after review of aggregate adverse event reports.