A Path to an HIV Vaccine: GSID Consortium Activities

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GSID GLOBAL SOLUTIONS FOR INFECTIOUS DISEASES

Project Goals

- Acquire and disseminate information that will contribute to the development of a safe and effective HIV vaccine
- Establish a consortium to characterize and evaluate antigenic variation of viruses that mediate new infections

Project Objectives

- Establish an AIDSVAX® (VAX003 and VAX004) Phase III clinical specimen repository
- Establish a web-accessible clinical and sequence database from the AIDSVAX® Phase III clinical trials
- Analyze sequence, structure, and phylogeny of gp120 from VAX003 and VAX004

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Project Objectives

- Isolate and characterize broadly neutralizing antibodies (bNAbs) from HIV+ plasma (GSID neutralization cohort)
- Characterize and evaluate antigenic variation of viruses that mediate new infections, and identify epitopes on envelope proteins recognized by bNAbs.
- Engineer antigens able to elicit antibodies to the most common polymorphisms at each neutralization site on the envelope glycoprotein

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GSID Consortium

GENOMA

- Sequence Analysis
- Phylogenetics
- Alignments
  - PI: K. Crandall

UC SANTA CRUZ

- Bioinformatics
  - PI: J. Kent
- Protein expression and epitope mapping
  - PI: P. Berman

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- Consortium management
- HIV Data Browser
- Specimen repository
  - PI: F. Sinangil

PharmaStat℠ drug development consulting and services

- Biostatistics and clinical data
  - PI: E. Li

monogram biosciences

- Pseudotype virus construction
- Evaluation of neutralization sensitivity
  - PI: B. Schweighardt and T. Wrin

AIDSVAX® (VAX003 and VAX004) Phase III clinical specimen repository has been established

- Specimen repository contains plasma and serum samples, and envelope DNA clones from AIDSVAX® VAX003 and VAX004 Phase III clinical trials
- All specimens are available to the HIV vaccine research community

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Specimen Repository

Specimen inventory	VAX004 Collection Time Points	VAX004 Sample # (tubes)	VAX003 Collection Time Points	VAX003 Sample # (tubes)	Total Sample # (tubes)
Pre-Infection					
Serum	73,137	135,042	34,484	68,966	**204,008**
Post-infection					
Plasma	2,527	29,282	1,792	15,320	**44,602**
Serum	2,852	2,852	2,171	4,255	**7,107**
Plasmid DNA library					
Full length gp120 plasmids (3 clones per individual sample)		1,047 (349 subjects)		600 (200 subjects)	**1,647**

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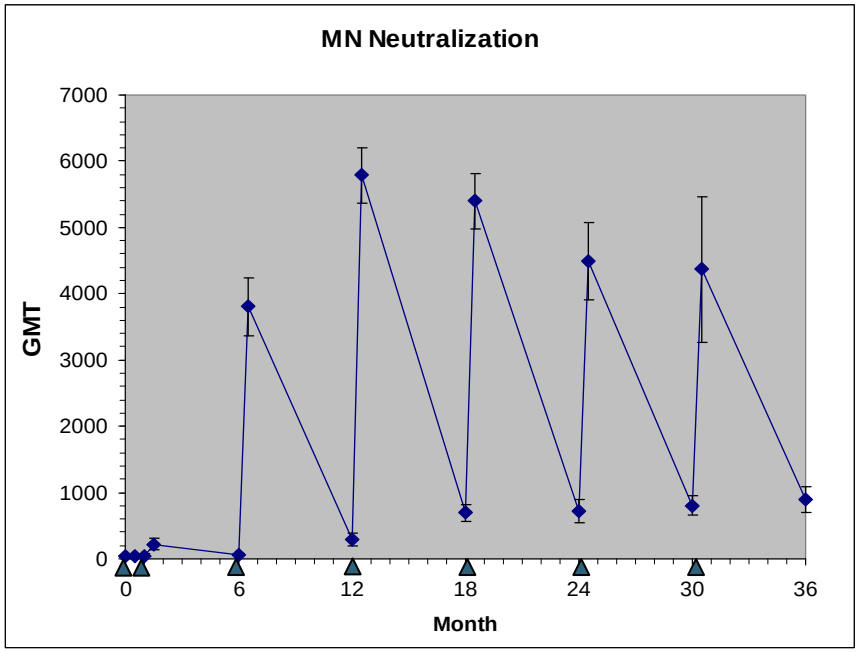
VAX003 (AIDSVAX® B/E) Specimens and Immunogenicity Data



- Uninfected random cohort – 125 subjects
  - 116 vaccinees, 9 placebo recipients
  - Immunogenicity tested at all time points (15): Month **0**, 0.5, **1.0**, 1.5, **6.0**, 6.5, **12.0**, 12.5, **18**, 18.5, **24**, 24.5, **30**, 30.5 and 36 (**immunization time points**)
- Infected subjects – 211 subjects
  - 106 vaccinees, 105 placebo recipients
  - Tested at last peak (LP) and last trough (LP) time points before infection
- All subjects tested in 5 immunogenicity assays
  - **anti-MN/A244gp120**, A244 V2, A244 V3, **MN Neutralization**, **A244gp120 CD4 Blocking** (**in GSID HIV Data Browser**)

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VAX003 (AIDSVAX® B/E) Serum Sample Inventory

Treatment Group	Uninfected Subjects	Infected Subjects
Vaccine	1162	106
Placebo	1155	105
Total	2317	211

- Comparison of immune responses in AIDSVAX® B/E and RV135 (D. Montefiori, PI)
  - Magnitude and breadth of HIV-1 neutralization (TZM-bl and PBMC assay)
  - Titer and/or affinity of peak vaccine-elicited env-specific binding antibodies (Luminex assay and BIAcore)
  - Longevity of binding and NAb response

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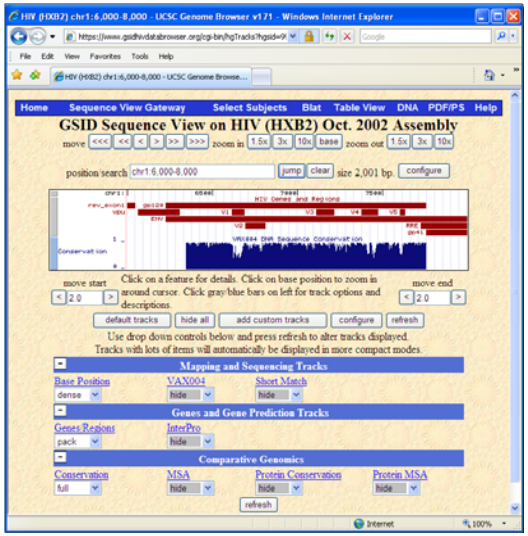
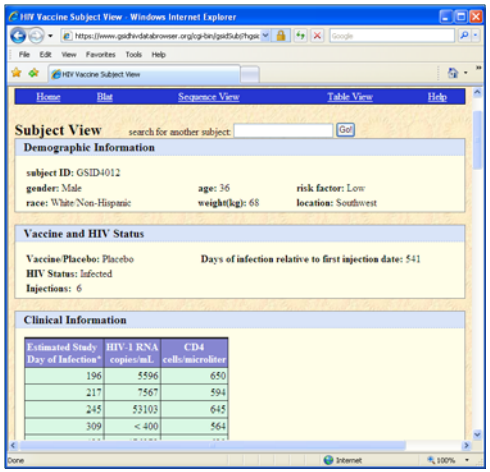
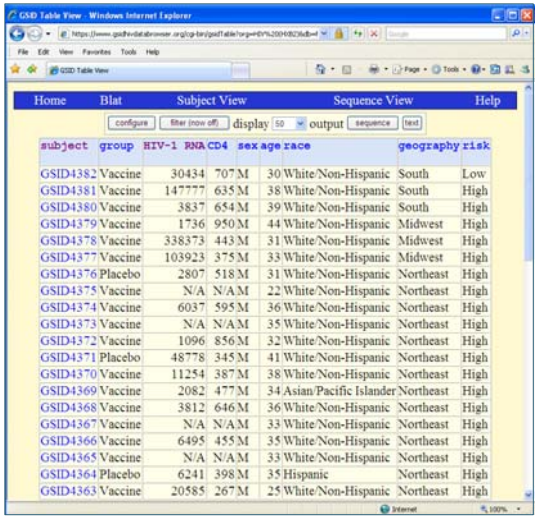
A web-accessible database has been established

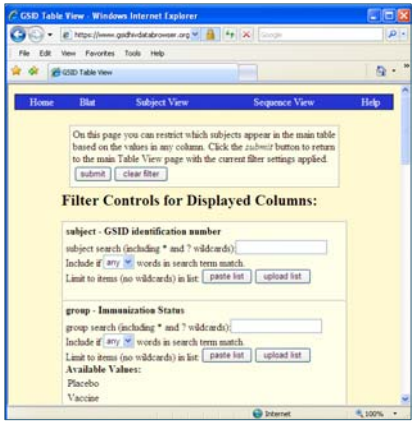
GSID HIV Data Browser

A Unique Research Tool Providing Access to AIDSVAX Trial Data and Specimens









gsidhivdatabrowser.org

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Properties of the GSID HIV Data Browser

- Relational database encompassing significant AIDSVAX® clinical trial data
- Contains largest collection of full-length gp120 sequences from current time period
- Fully annotated with demographic and clinical information
- Tools developed to analyze sequence data include Protein View, comparative genomics and positive selection
- Immunogenicity data for infected and subset of uninfected subjects in AIDSVAX® trials (VAX003 and VAX004) to be available by Q1 2010

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Sequence, structure and phylogeny analyses of gp120 from VAX003 and VAX004 completed

- Pérez-Losada M, Posada D, Arenas M, Jobes DV, Sinangil F, Berman PW, Crandall KA. Ethnic differences in the adaptation rate of HIV gp120 from a vaccine trial. **Retrovirology. 2009 Jul 15;6:67**.
- Pérez-Losada M, Jobes DV, Sinangil F, Crandall KA, Posada D, Berman PW. Phylodynamics of HIV-1 from a Phase III AIDS vaccine trial in North America. **Mol Biol Evol. 2009 Oct 28.** [Epub ahead of print]
- Pérez-Losada M, Jobes DV, Crandall KA, Posada D, Sinangil F, Berman PW. Dynamics and phylogenetic analysis of viruses mediating new infections in VAX003, a phase 3 HIV vaccine trial in Thailand. [in preparation]

GSID Consortium Strategy for Development of an Effective HIV Vaccine

- Define the sequences and range of virus variation among viruses responsible for new infections
- Assess the sensitivity/resistance of viruses to neutralization by broadly neutralizing antibodies (bNAbs) in HIV+ patient sera
- Identify epitopes on envelope proteins recognized by bNAbs in HIV+ sera
- Identify the naturally occurring polymorphisms that occur at these epitopes and their effect on virus neutralization
- Purify populations of broadly neutralizing antibodies from HIV+ plasma and define mechanism of action (specificity, affinity/avidity)
- Engineer antigens and multivalent vaccines able to elicit antibodies to the most common polymorphisms at each neutralizing site on the envelope glycoprotein

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Isolation and characterization of broadly neutralizing antibodies (bNAbs) from HIV+ plasma: GSID neutralization cohort

- Inclusion criteria
  - HIV-positive for at least one year prior to screening
  - Has never received ART
- Screening
  - Started May 12, 2009
  - 17 subjects screened for bNAbs against a panel of 24 viruses (Tier 1, 2, and 3)

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Isolation and characterization of bNAbs from HIV+ sera (GSID neutralization cohort)

	GSID 1	GSID 2	GSID 3	GSID 4	GSID 5	GSID 6	GSID 7	GSID 8	GSID 9	GSID 10	GSID 11	GSID 12	GSID 13	GSID 14	GSID 15	GSID 16	GSID 17	Z1679	Z1641	N16	Z1653	Z23
SF162	5611	11276	3019	9369	76894	5529	51234	1620	1266	2487	1,429	9,483	56,771	2,780	18,938	9,275	14,287	4099	3338	4720	137	22535
1196	3404	256	90	126	161	141	265	189	63	59	169	105	550	86	626	136	927	70	41	142	25	413
TRO	2556	81	109	46	49	89	167	89	51	54	89	119	80	61	369	44	129	215	<20	63	42	297
JRFL	4761	72	145	31	40	56	122	974	67	32	47	47	122	876	335	55	180	1011	<20	34	<20	514
BG1168	224	43	44	24	38	37	47	37	58	<20	35	30	<20	69	170	26	136	76	<20	21	21	120
QHO692	720	51	35	26	27	60	34	39	38	30	42	35	<20	36	147	48	90	36	<20	24	31	137
REJO	1206	137	145	66	59	126	369	148	224	39	34	88	770	302	1,029	87	196	64	25	62	34	515
M-SC-B-006	382	27	25	21	<20	34	39	81	<20	21	32	59	59	45	128	<20	109	26	<20	<20	<20	<100
APV-16	1101	68	56	36	46	66	100	61	45	56	87	112	144	39	80	34	223	97	38	90	51	572
M-Chronic-B-013	381	42	45	42	34	57	92	51	56	43	49	49	75	40	639	35	75	32	28	23	21	189
PVO	2328	51	42	37	72	65	84	77	32	64	50	95	69	78	332	27	125	126	32	28	27	439
M-A-002	291	23	47	29	24	32	72	55	35	28	59	42	88	46	35	28	83	<20	<20	22	<20	181
M-A-006	952	28	27	24	22	38	54	40	45	42	60	131	46	82	105	60	104	52	<20	<20	22	230
M-C-003	40	24	24	25	<20	31	32	28	21	22	47	98	38	41	46	38	111	75	23	23	<20	<100
M-C-020	1075	45	34	32	<20	43	72	39	37	31	66	111	54	71	228	61	99	<20	<20	<20	35	<100
M-D-006	2504	70	37	28	35	50	60	43	31	<20	38	20	20	37	314	20	78	102	<20	<20	21	238
M-D-009	1465	224	315	278	126	222	712	382	206	140	124	83	895	144	905	131	209	135	39	135	52	476
94UG103	785	48	34	32	36	69	81	57	51	31	70	43	72	61	433	56	135	<20	<20	30	31	121
92BR020	1241	107	103	121	219	111	267	108	69	44	140	194	603	67	292	165	185	194	<20	65	<20	272
93IN905	986	109	449	78	116	124	153	85	104	141	361	658	119	122	239	133	287	301	31	91	27	326
M-C-026	507	43	54	55	66	91	219	72	175	38	113	300	430	331	316	21	112	36	40	55	41	277
92TH021	408	69	127	53	44	109	95	55	80	44	41	449	70	103	1,406	50	138	47	<20	57	31	255
JRCSF	5933	272	65	54	59	100	139	116	137	<20	46	95	408	193	788	45	178	115	<20	177	25	402
JRCSF	4995	220	65	43	54	84	137	133	120	<20	69	120	590	293	1,045	65	201	114	<20	172	25	385
NL43	2131	3114	521	433	271	844	1394	464	385	97	232	1,852	7,649	486	1,182	1,750	1,361	1021	120	769	96	3598
NL43	1947	2126	511	392	203	888	1390	931	554	93	345	2,448	6,925	847	1,733	2,473	2,217	867	100	591	86	3172
aMLV	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	64	<20	<20	25	23	23	28	<20	<20	<20	<20	<100

M = MGRM in virus IDs

All human sera/plasmas were treated to minimize non-specific backgrounds

= extra blood collected from these subjects

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Characterize and evaluate antigenic variation of viruses that mediate new infection and identify epitopes on envelope proteins recognized by bNAbs (Berman Lab UCSC)

- Analyzed sequence data from VAX003 and VAX004 (GSID HIV Data Browser) for the purpose of new vaccine antigen development
- Collaborated with Keith Crandall (BYU) in a position by position analysis of amino acid sequence variation within gp120 from clade B and E viruses
- Analyzed the physical characteristics of the envelopes from each cohort to better understand the breadth of virus variation that must be addressed by a successful HIV vaccine
- Calculated new amino acid consensus sequences for clade B and E viruses using a hidden Markov model to account for insertions and deletions
- Identified viruses closest to the consensus sequence and most distant from the consensus sequence for antigen production and immunization studies

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Defining VAX004 Sequence Variation in New Infections: Virus Selection for Panel of New Immunogens

Characteristic	Specimens Analyzed
Randomly selected	28
Close to consensus sequence	10
Distant from consensus sequence	10
Early / acute infections	10
High virus loads	10
Unusual disulfide structures	5
Long or short V regions	5
Total:	78

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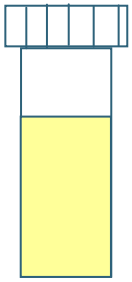
Characterize and evaluate antigenic variation of viruses that mediate new infection and identify epitopes on envelope proteins recognized by bNAbs (Berman Lab UCSC)

- Developed a new strategy (swarm analysis) that makes use of the swarm of naturally occurring virus variants within each individual to understand the molecular basis of susceptibility and resistance to bNAbs
- The new strategy depends on viruses from recent infections and is based on “clonal analysis” technology developed at Monogram Biosciences

Swarm Analysis Strategy

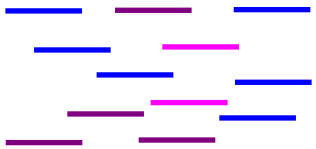


VAX004 plasma
Early transmission

plasma lysis

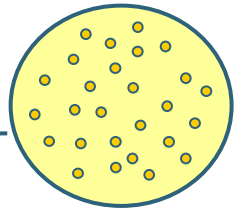
vRNA isolated

Population of gp160 genes
RT-PCR amplified and
ligated into vectors



- Dilute DNA
- Re-transform bacteria
- Grow on selection agar plate

Pick 24-48
gp160 clones



Infectivity
screen of
gp160 clones

Selection of 10
clones/subject and
neutralization
with HIV+ sera

Select and sequence
pairs of Neut-sensitive
and Neut-resistant
viruses for each subject

In vitro mutagenesis to
convert sensitive clones
to resistant & vice versa

Prepare pseudotype
viruses from mutants/
chimerae for
neutralization assays

Identify single nucleic
acids responsible for
neutralization
sensitivity & resistance

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Characterize and evaluate antigenic variation of viruses that mediate new infection and identify epitopes on envelope proteins recognized by bNAbs (Berman Lab UCSC)

- Mapped 3 novel mutations in clinical isolates that appear to induce conformational changes that result in improved exposure of epitopes recognized by bNAbs
  - Envelope genes with such mutations may represent an important source of new vaccine antigens.
  - Monomeric and oligomeric forms of these proteins have been produced and rabbit immunization studies have begun

O'Rourke SM, Schweighardt B, Scott WG, Wrin T, Fonseca DP, Sinangil F, Berman PW. Novel ring structure in the gp41 trimer of human immunodeficiency virus type 1 that modulates sensitivity and resistance to broadly neutralizing antibodies. **J Virol. 2009 Aug;83(15):7728-38**.

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