PROTEIN BIOMARKERS PREDICTIVE OF MULTIVALENT RESPONSE TO A NOVEL DENGUE VACCINE

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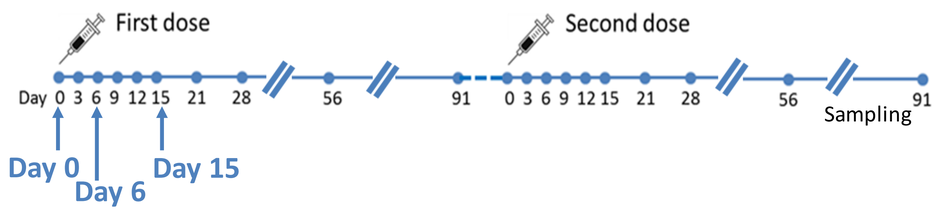
Introduction

- Dengue fever is the most prevalent mosquito-borne disease of humans
- 4 dengue virus (DENV) serotypes are known
- Protective antibodies against one DENV serotype offer limited protection against the others; immunity to one serotype may result in ab-dependent enhancement of infection
- Currently lacking assays & immune correlates that predict full protection in humans
- Deploy systems immunology approach to develop validated assays that predict DENV vaccine efficacy in clinical studies of naïve and exposed subjects

Study Design



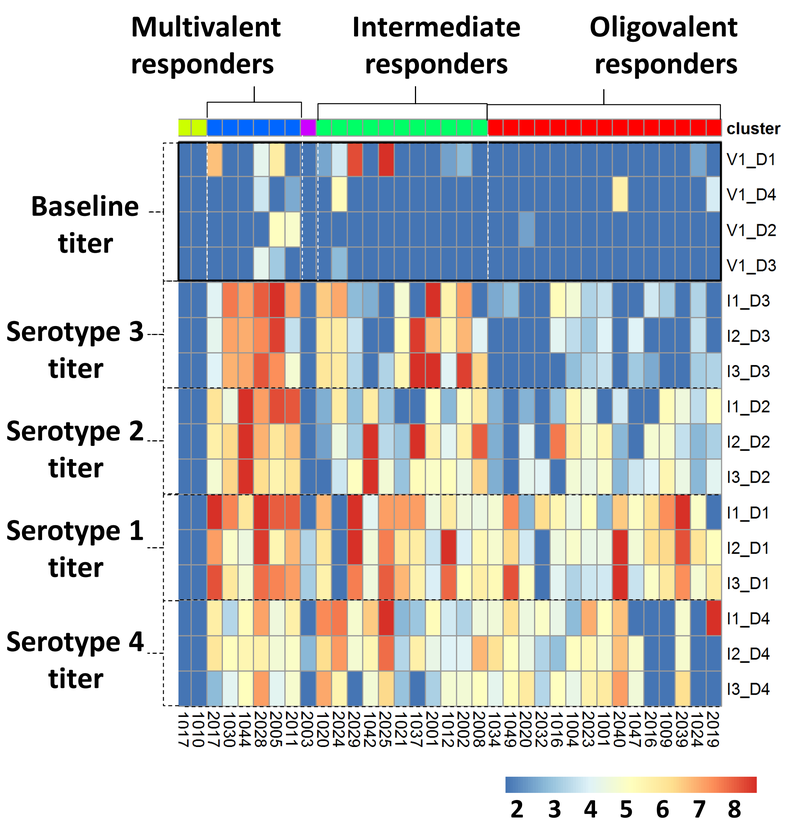
- Phase II trial in Brazil; healthy subjects 18-59 yrs (n=300)
- TV003 tetravalent attenuated live vaccine (NCT01696422)
- Selected DENV seronegative subjects (n=17) with a range of titer responses; focused proteomic analysis on samples from baseline and following the initial vaccination

- PBMC flow
- PBMC transcriptome
- Neutralization assays
- Plasma cytokines
- Plasma metabolome
- Plasma proteome

Multi-platform synthesis

Results

Unsupervised group clustering



- Groups were defined via machine learning using log titer values as input. Random Forest was used to calculate proximity values between subjects
- Dimensionality was reduced by Multidimensional Scaling (MDS) and hierarchical tree clustering was performed using Euclidean distances . Cluster membership per subject was determined as described (1)

1. Langfelder,P. et al. (2007) Defining clusters from a hierarchical cluster tree: The dynamic tree cut package for r. Bioinformatics, 24, 891–921.

Group differences Day 0

Gene	Oligovalent / Polyvalent	Intermediate / Polyvalent
FTL	**0.38**	**0.68**
MYH9	**0.64**	**0.41**
CORO1C	**0.65**	**0.47**
PPBP	**0.67**	**0.56**
ANXA5	**0.69**	**0.40**
COTL1	**0.70**	**0.60**
PDLIM1	0.71	0.50
PFN1	**0.73**	**0.55**
ARHGDIB	**0.74**	**0.59**
FLNA	**0.76**	**0.47**
ZYX	**0.76**	**0.55**
GSTP1	**0.76**	**0.56**
ENG	0.76	0.92
ENO1	**0.79**	**0.66**
CORO1A	**0.80**	**0.62**
SERPINB1	**0.81**	**0.68**
MBL2	0.81	0.68
PDIA3	0.83	0.73
TLN1	**0.83**	**0.61**
FERMT3	**0.85**	**0.58**
SRGN	**0.85**	**0.59**
VCL	**0.86**	**0.56**
COL18A1	0.88	1.07
APOE	**0.93**	**1.32**
SLC3A2	**0.94**	**1.11**
CLEC3B	1.00	1.23
APOA4	1.19	1.43
CSF1R	1.24	1.25
C3	**1.26**	**0.72**
CR2	1.30	1.67
F8	**1.31**	**1.67**
MMP9	**1.77**	**1.55**

Bold = p-value <0.05
Non-bold = p-value 0.05-0.09

Response pathway	Activation score: Oligovalent / Polyvalent	Activation score: Intermediate / Polyvalent	p-value
IL6	+ 1.924	+ 0.150	$1.51x10^{-8}$
IL1B	+ 1.820	+ 0.012	$4.67x10^{-10}$
TNF	+ 0.720	- 0.792	$1.10x10^{-14}$
IL4	- 0.267	- 0.831	$3.05x10^{-9}$
IFNγ	- 0.412	- 1.533	$2.80x10^{-7}$

+ = induced in numerator
- = induced in denominator

Group differences Day 6

Gene	Oligovalent / Polyvalent	Intermediate / Polyvalent
HMGB1	**0.42**	**0.22**
KRT1	**0.49**	**0.22**
CAT	**0.49**	**0.67**
MMP9	**0.51**	**0.67**
FABP5	0.65	0.45
RBP4	0.68	0.39
CD93	**0.86**	**0.97**
TKT	1.14	1.30
TNC	1.20	1.45
CTSD	**1.31**	**0.90**
IL1RAP	1.33	1.10
IGHM	1.72	2.27
ARHGDIB	**1.88**	**1.89**
VCL	2.02	2.67
CRP	**2.82**	**3.46**
SAA1	**2.87**	**2.76**

Response pathway	Activation score: Oligovalent / Polyvalent	Activation score: Intermediate / Polyvalent	p-value
IL6	+ 0.840	+ 0.840	$1.51x10^{-8}$
IL1B	+ 0.842	+ 0.842	$4.67x10^{-10}$
TNF	+ 1.553	+ 1.553	$1.10x10^{-14}$
MYC	- 2.000	- 1.000	$9.01x10^{-8}$

Group differences Day 15

Gene	Oligovalent / Polyvalent	Intermediate / Polyvalent
TF	**0.55**	**0.49**
KLKB1	**0.72**	**0.60**
LCP1	0.80	0.91
PIGR	**0.81**	**1.57**
IL1RAP	**0.92**	**0.66**
ENO1	1.08	1.46
CAT	1.11	1.78
PARK7	1.17	1.48
HSPA8	1.21	1.67
CFI	1.32	1.51
CA2	**1.38**	**1.89**
CST3	1.40	1.21
APCS	**1.43**	**1.26**
IGFBP3	**1.44**	**1.11**
PFN1	1.93	2.08
FTL	2.09	1.31
PPIA	2.10	1.13

Response pathway	Activation score: Oligovalent / Polyvalent	Activation score: Intermediate / Polyvalent	p-value
IL6	- 0.771	- 0.771	$1.51x10^{-8}$
IL1B	- 0.849	+ 0.000	$4.67x10^{-10}$
TNF	- 0.657	+ 0.184	$1.10x10^{-14}$
IFNγ	- 1.462	- 0.154	$2.80x10^{-7}$

- **Pre-vaccination:** Pro-inflammatory pathways (IL6, IL1B, TNF) were elevated in future oligovalent & intermediate responders compared to future polyvalent responders
- Elevated B cell & macrophage response pathways (IL4, IFNγ) in future polyvalent responders
- **Post vaccination:** Polyvalent responders had comparatively elevated activation (MYC, IFNγ) & pro-inflammatory pathways (IL6, IL1B, TNF); effect most pronounced by day 15

Methods

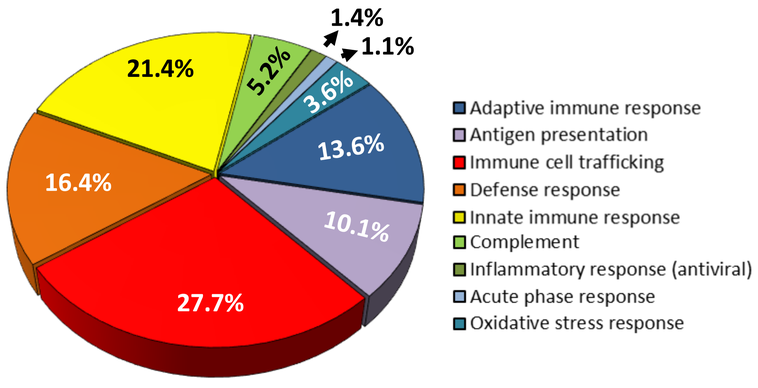
50 µl plasma

Sample processing
Abundant protein depletion
by IgY14-SuperMix
Trypsin digestion
MCX-mediated desalting
+ Stable isotope labeled (SIL) peptide mix
+ internal standard peptide (ISP) mix

Data acquisition
MRM-MS QTRAP 5500
Single injection
Immune response panel

Data analysis
Skyline, IPA,
Statistical analyses

Immune Response Panel composition



- 298 plasma proteins linked to immune response, described by 554 unique peptides
- Stable isotope labelled versions of each peptide included, to enable data normalization and subsequent assay validation

Platform performance

	Abundant protein depletion CV		MS injection Peak Area CV		Targeted protein detection rate	
	QC samples	Study samples	ISP	QC sample L/H	Total	>50% study samples
d0, d6	9.0%	19.9%	9.0%	19.9%	96.0%	71.5%
d0, d15	9.4%	16.4%	9.4%	16.4%	96.3%	69.5%

Conclusions

- Pre-existing inflammation may limit a robust response to the vaccination
- Identified candidate blood based biomarkers potentially usable for development of a test
- Synthesis with other platform data may offer additional insights
- Further testing planned with independent sets of samples

ClinicalTrials.gov Identifier: NCT01696422
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