Isolation and longitudinal characterization of the development of HIV broadly neutralizing antibodies.

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1. Introduction

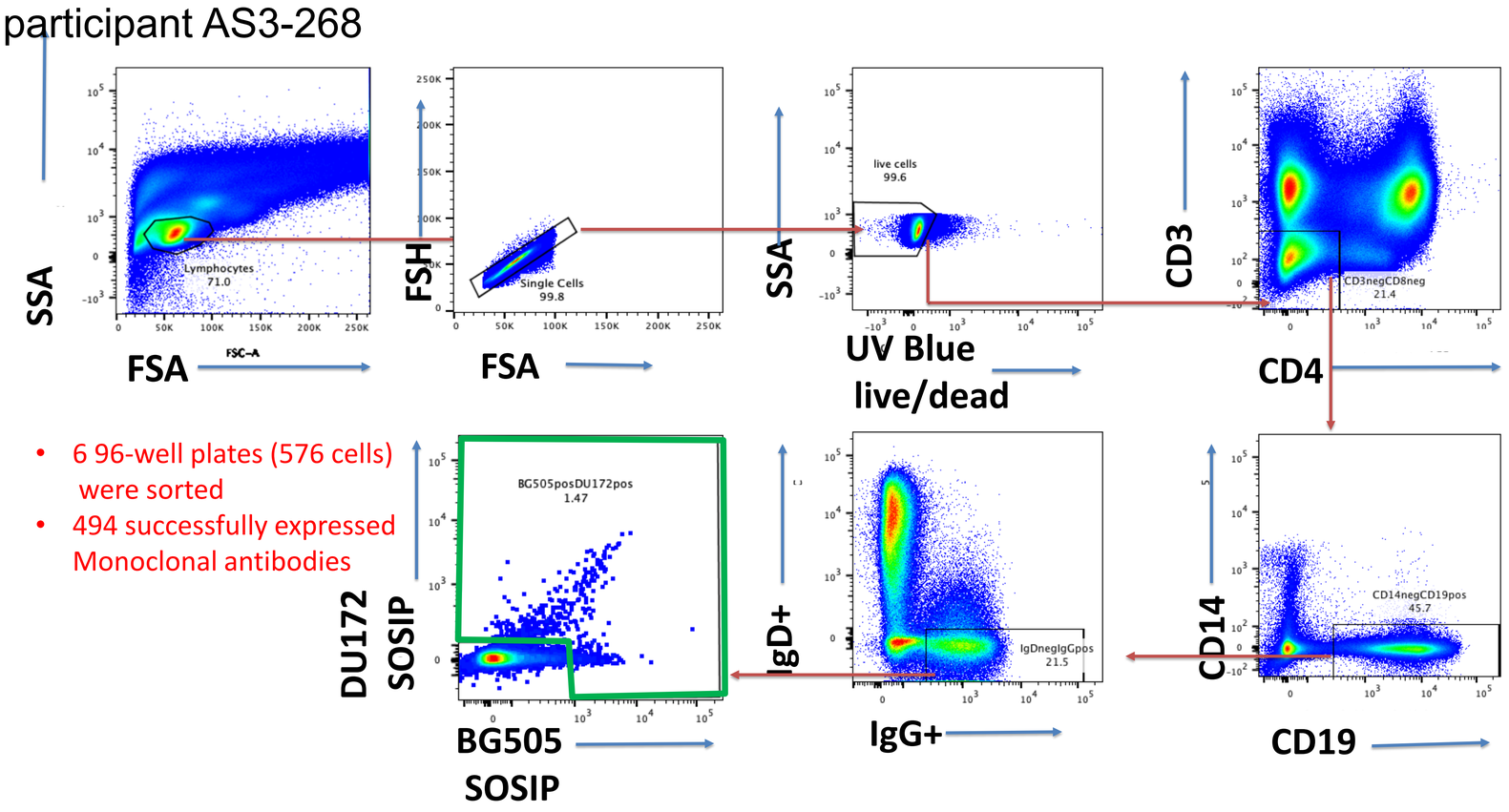
- The main aim of HIV vaccine development is to induce broadly neutralizing antibodies (bNAbs) in vaccinated individuals.
- However, no vaccine has been successful in inducing bNAbs thus far
- Major challenges in inducing bNAbs include:
  a. Viral factors
     - High viral diversity
     - Viral envelope glycan shield
  b. Unusual bNAbs features
     - Takes long to develop >2 years
     - High somatic hypermutations
     - Long CDHR3 region
     - Poly/autoreactive
  c. HIV induced B cell defects
     - Hypergammaglobulinemia
     - B cell subsets variations
- Our aim is to characterize bNAbs and study their longitudinal development to guide immunogen design

2. Methods

- Participant (AS3-268) was one of the six participants with plasma neutralization breadth (89%) from the HPP HIV Acute cohort.
- HIV specific memory B cell index sorting was done by FACS using DU172 and BG505 sosip trimers
- Rapid assembly, transfection, and production of immunoglobulins (RATP-Ig) method was used to isolate monoclonal antibodies from sorted B cells
- Single point neutralization assay was used to screen for potential bNAbs from RATP-Ig isolated monoclonal antibodies.

3. Results

Fig 1: Gating strategy for sorting HIV specific memory B cells from participant AS3-268

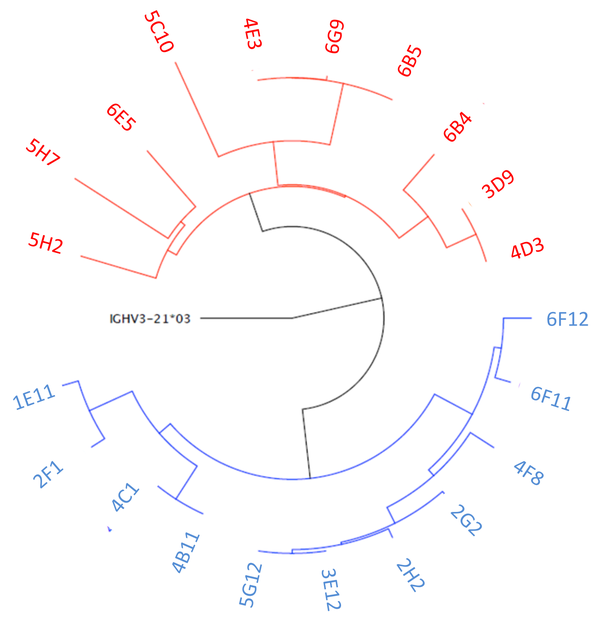


- 6 96-well plates (576 cells) were sorted
- 494 successfully expressed Monoclonal antibodies

Table 1: Identified potential bNAbs from participant AS3-268

PLATE&WELL NO:	VIRUSES					
	Q842	TRO.11	DU172	CAP45	BG505	SIV
1E11	101%	96%	-3%	35%	100%	23%
3D9	101%	100%	89%	100%	100%	22%
3E12	101%	101%	93%	100%	100%	5%
4B11	88%	98%	32%	5%	99%	-3%
4D3	101%	101%	88%	100%	100%	1%
4E3	101%	101%	81%	100%	100%	11%
4F8	96%	98%	28%	95%	98%	2%
5C10	99%	-4%	19%	-2%	97%	4%
6B4	101%	100%	94%	100%	100%	-3%
6B5	102%	100%	81%	100%	100%	13%
6E5	94%	91%	57%	98%	89%	50%
6F11	98%	84%	28%	100%	100%	19%
6F12	100%	87%	28%	91%	99%	25%
6G9	101%	100%	72%	26%	100%	20%
6G10	75%	73%	34%	49%	73%	41%
2G2	100%	101%	91%	-10%	100%	25%
2H2	100%	100%	94%	11%	99%	-7%
4C1	65%	90%	15%	8%	95%	26%
5H2	97%	40%	18%	28%	94%	51%
VRC01	100%	3%	15%	66%	100%	-34%

Figure 3: Phylogenic tree of the identified potential bNAbs from participant AS3-268



4. Summary and future work

- Using RATP-Ig and Single point neutralization methods we identified 19 potential bNAbs from participant AS3-268
- All these antibodies use the VH3-21*03 gene and belong to the same lineage
- These data will directly inform ongoing efforts to develop effective vaccines against HIV by informing the selection of appropriate vaccine formulations to favor the induction of bNAbs
- Future work
  a. Clone and test neutralization breadth of the identified potential bNAbs using 18 pseudotyped virus panel
  b. VDJ next generation sequencing of longitudinal samples to track maturation pathway of AS3-268 bNAbs
  c. Deep sequencing of HIV envelope from longitudinal plasma sample to determine antibody-virus co-evolution
  d. Compare transcriptional profile of AS3-268 bNAb and non-bNAb producing B cells

5. References

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6. Acknowledgements

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