

Adapting LIBRA-seq/BEAM to whole BK polyomavirus particles to identify broadly-specific human monoclonal antibodies.

Sarah MARCHAND

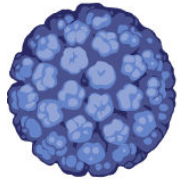
Supervisor : Dorian McILROY

ANR project : REP2EPS

Thesis : Analysis of specific B-repertoires for human polyomaviruses and papillomaviruses

CONTEXT

BK POLYOMAVIRUS (BKPyV) :



- Non-enveloped, double stranded DNA virus
- Prevalence $\geq 90\%$ (Laine et al, Scientific Reports, 2023)
 - Four genotypes (I, II, III and IV)



- Reactivates and replicates in immunocompromised individuals, particularly kidney transplant patients
 - Polyomavirus-associated nephropathy (PyVAN) → deterioration of graft function, graft loss



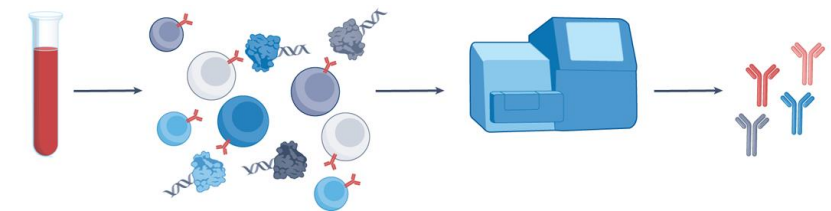
- Treatment :
 - No specific antiviral treatment
 - Management of immunosuppressive therapies



Can lead to immune response against the graft (Rampersad, Clin Trans, 2024)

→ Need for new therapies : Monoclonal antibodies ?

↗ Strategies to generate human therapeutic antibodies for viral infections : **Single cell immune profiling**



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CONTEXT

Single cell immune profiling approach

LIBRA-seq (Setliff et al. Cell. 2019)

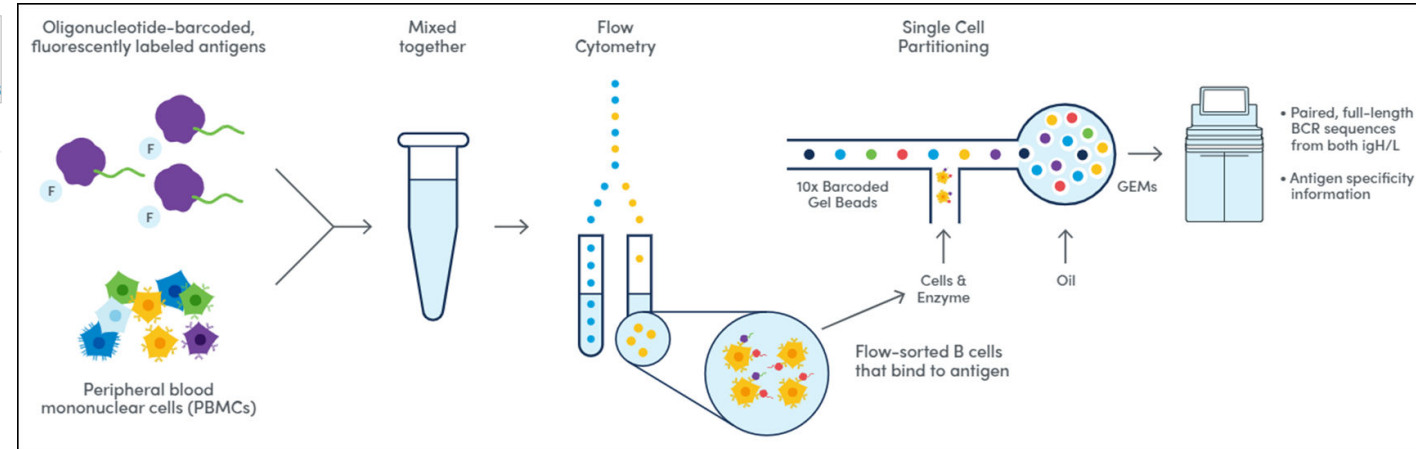
Cell

Volume 179, Issue 7, 12 December 2019, Pages 1636-1646.e15

Resource

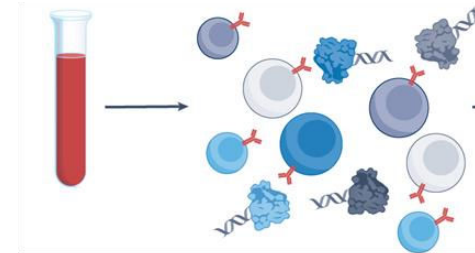
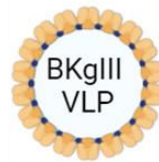
High-Throughput Mapping of B Cell Receptor Sequences to Antigen Specificity

Ian Setliff^{1,2,16}, Andrea R. Shiakolas^{1,3,16}, Kelsey A. Pilewski^{1,3}, Aryn A. Murji^{1,3}, Rutendo E. Mapengo⁴, Katarzyna Janowska⁵, Simone Richardson^{4,11}, Charissa Oosthuisen^{4,11}, Nagarajan Raju^{1,3}, Larance Ronsard⁷, Masaru Kanekiyo⁸, Juliana S. Qin¹, Kevin J. Kramer^{1,3}, Allison R. Greenplate¹, Wyatt J. McDonnell^{3,9,17}, Barney S. Graham⁸, Mark Connors¹⁰, Daniel Lingwood⁷, Priyamvada Acharya^{5,6}, Lynn Morris^{4,11,12}, ...Ivelin S. Georgiev^{1,3,13,14,15,18} ✉



CLINICAL
RESEARCH

→ Identify broadly neutralizing antibodies against 4 genotypes of BKPyV :

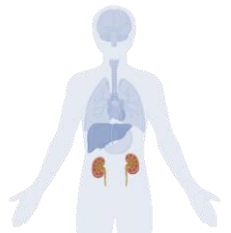


LIBRA-seq

FUNDAMENTAL
RESEARCH

→ Better understanding host responses by analyzing the BCR repertoire :

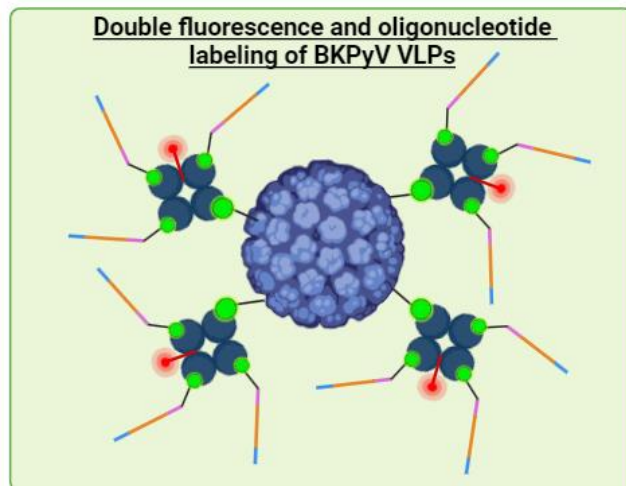
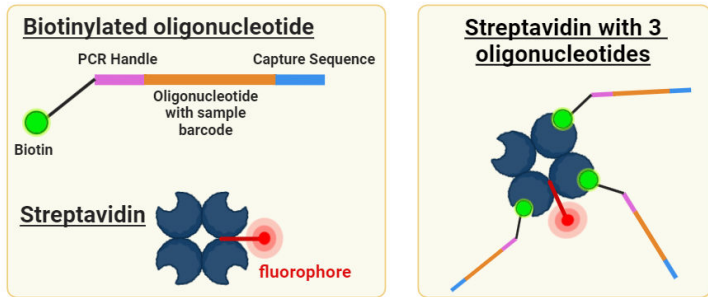
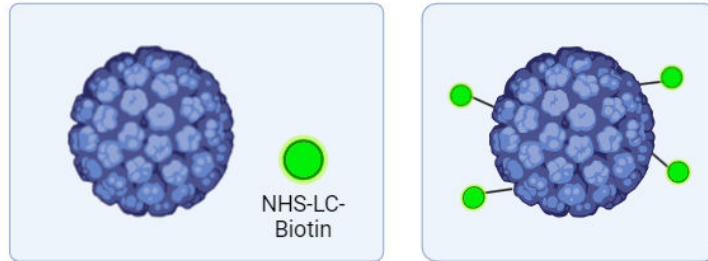
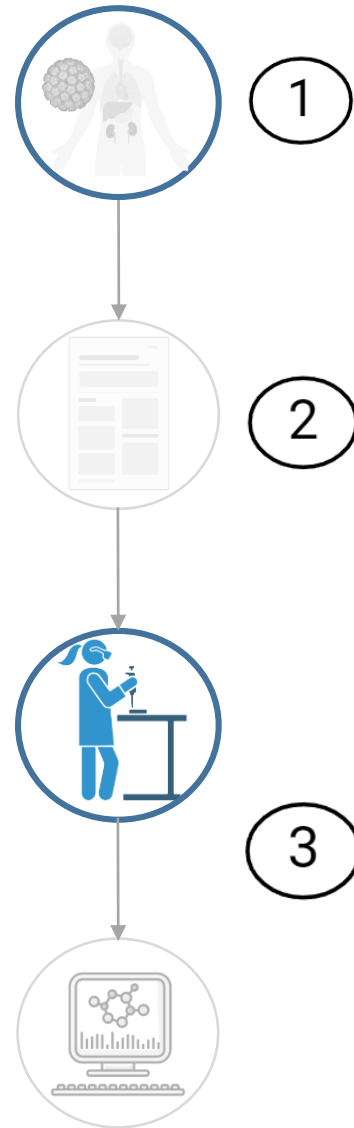
- Are virus-specific antibody repertoires distinct between clinically relevant patient groups ?
- Why do some viruses induce a memory IgM response ?
- ...



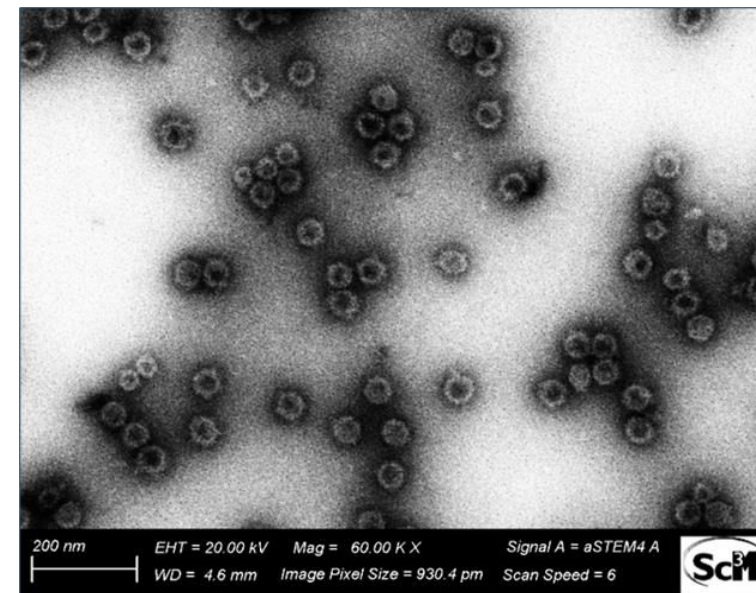
Adapting LIBRA-seq/BEAM to whole BK polyomavirus particles

METHOD

→ Adapting LIBRA-seq/BEAM to non enveloped viruses like BK polyomavirus



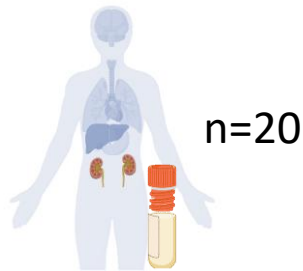
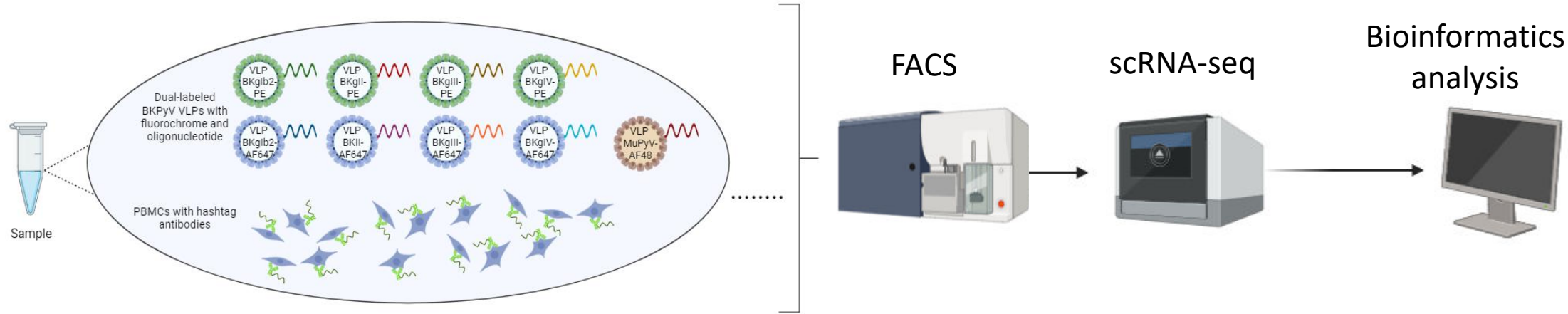
- ✓ 1) Production of biotinylated BKPyV VLPs
- ✓ 2) Couple with streptavidin-fluorophore-oligonucleotides
- ✓ 3) Check how the complexes look (electron microscopy)
- ✓ 4) Validate binding properties + different binding conditions optimised
 - to mAb (ELISA)
 - to 293TT cells (flow cytometry)
 - to PBMCs (flow cytometry)



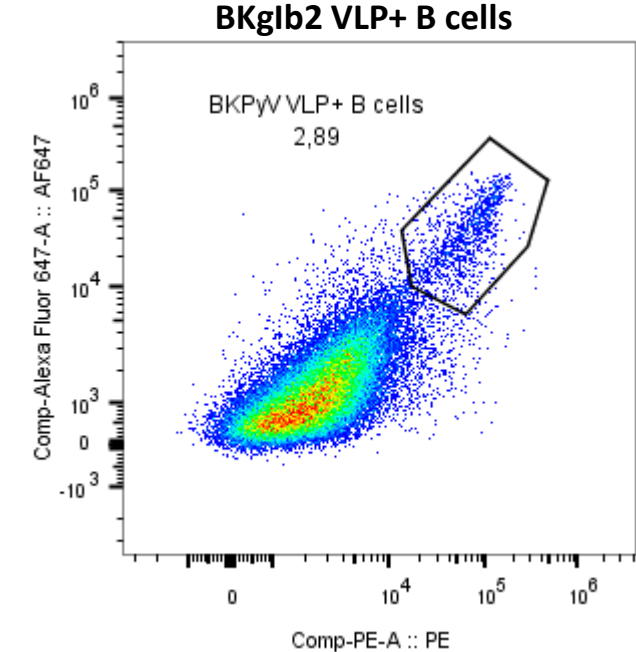
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METHOD

→ Single-cell experiments

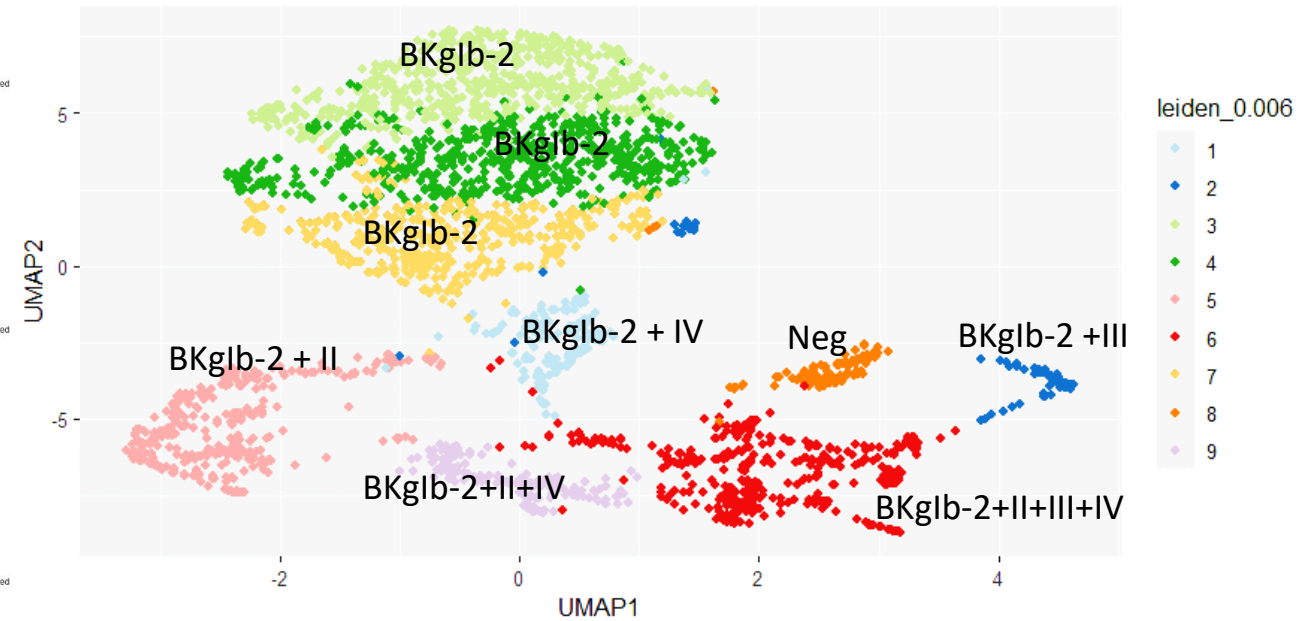
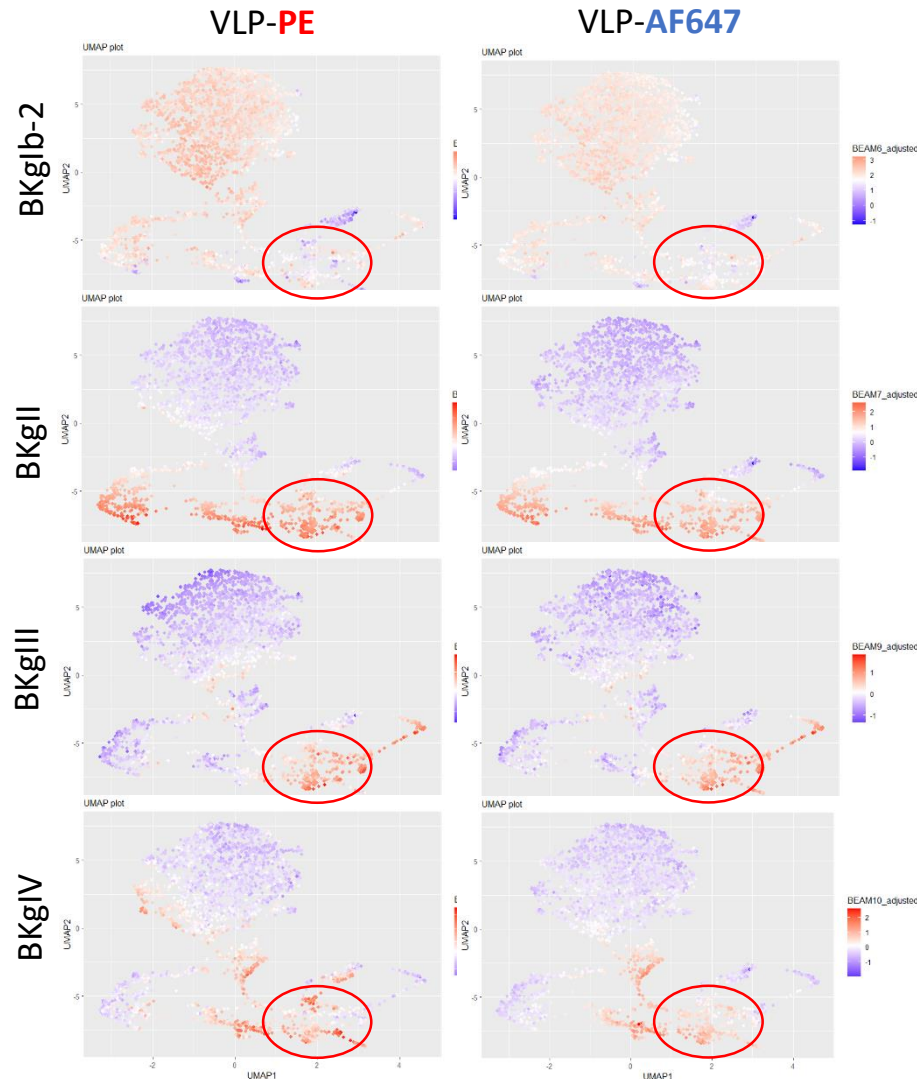


- Patients with persistent high-level viremia (**non-controllers = NC**)
- Patients who suppressed viremia (**controllers = C**)



RESULTS

→ Bioinformatics analysis

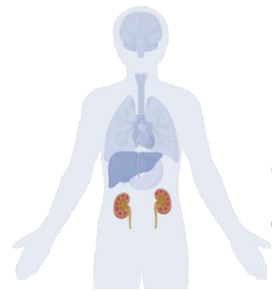
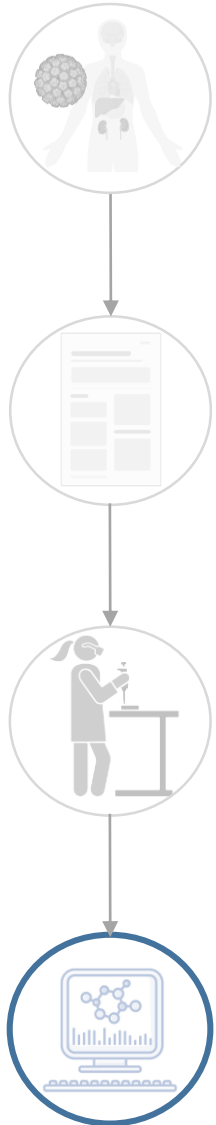


Cluster 6 : BKgIb-2 + BKgII + BKgIII + BKgIV

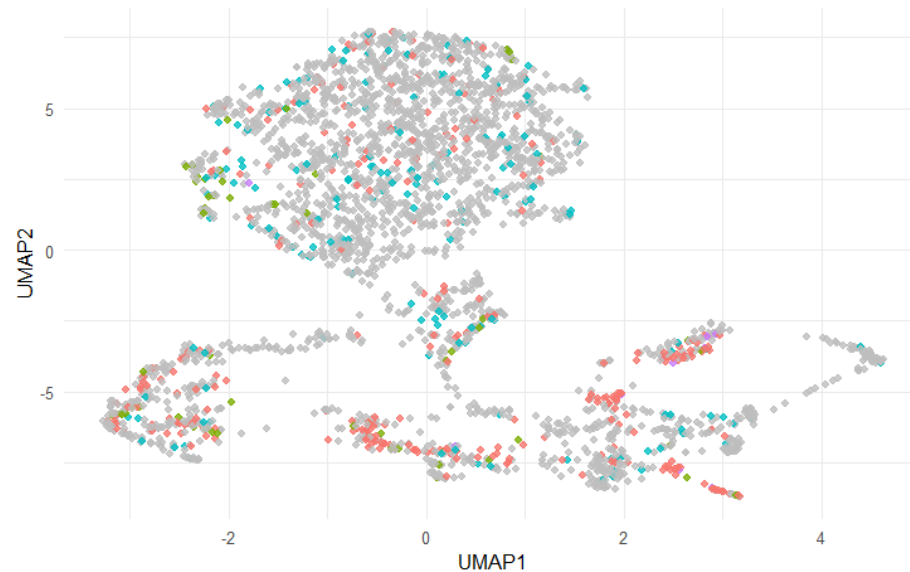
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RESULTS

→ Bioinformatics analysis

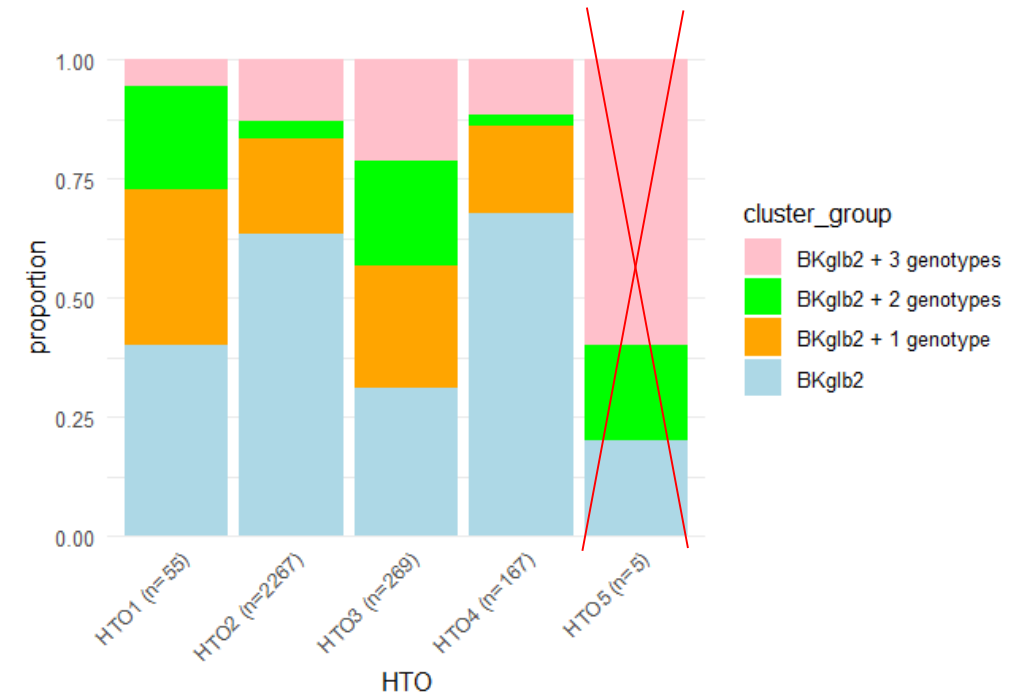
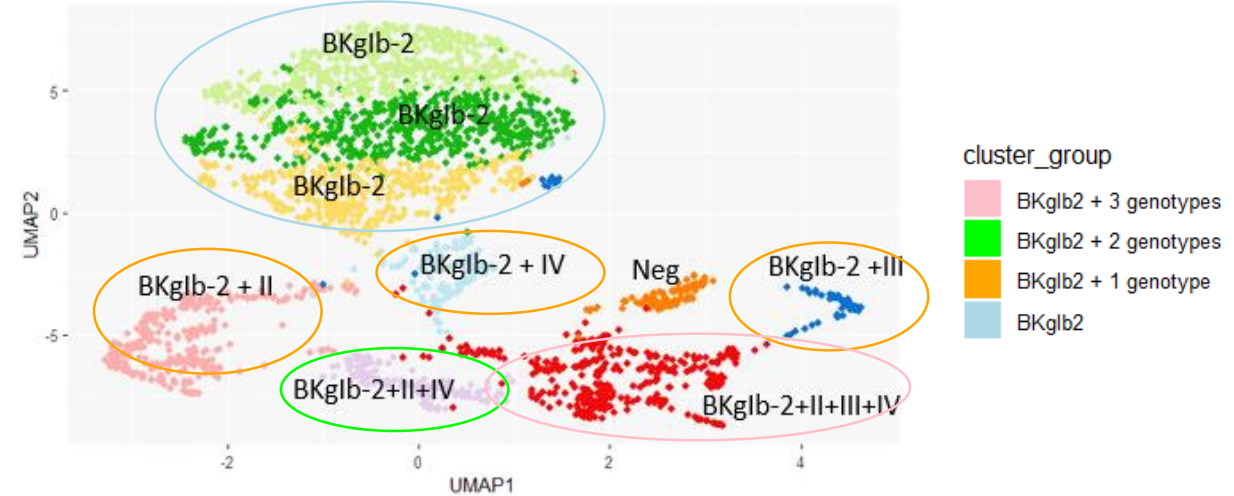


One patient = one hashtag oligonucleotide = HTO



HTO Categories

- HT01
- HT02
- HT03
- HT04
- HT05



CONCLUSION

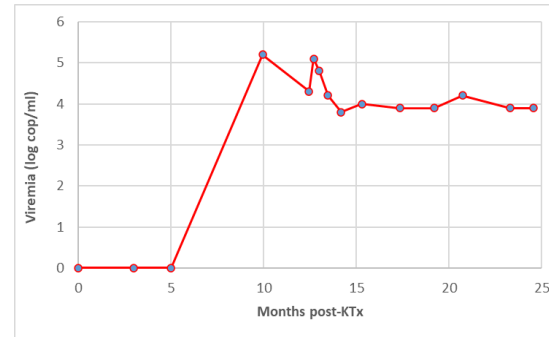
CLINICAL RESEARCH

→ Identify broadly neutralizing antibodies against 4 genotypes of BKPyV

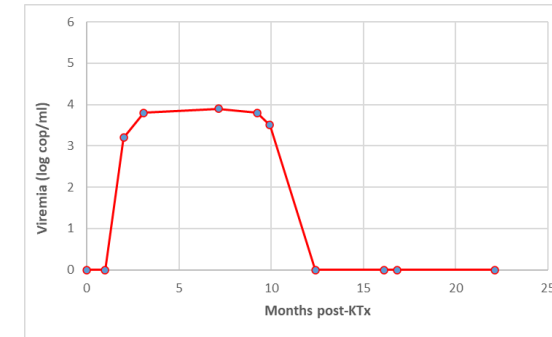
What was one of our initial hypotheses ?

→ Patients with persistent high-level viremia (**non-controllers** = NC) may have a restricted antibody repertoire with limited cross-reactivity, compared to patients who suppressed viremia (**controllers** = C), who may possess antibodies with a broader spectrum of reactivity.

NC



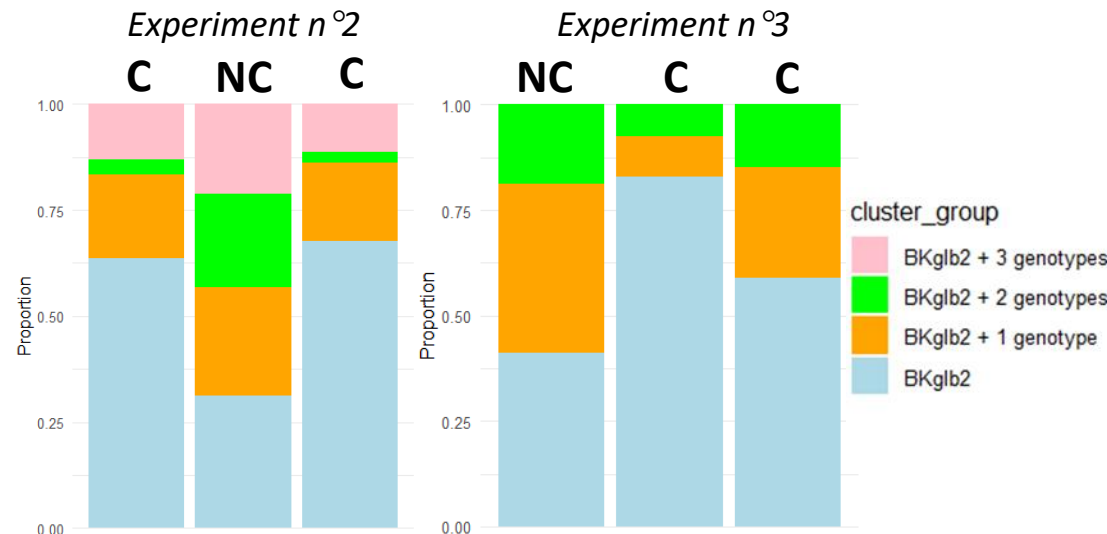
C



FUNDAMENTAL RESEARCH

→ Better understanding host responses by analyzing the BCR repertoire :

Are virus-specific antibody repertoires distinct between clinically relevant patient groups ?



It's quite the opposite!

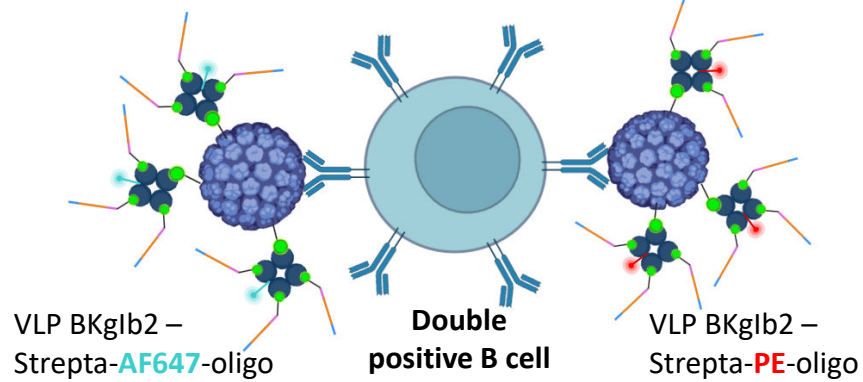
- Non-controller patient seems to have a broader spectrum of antibody repertoire.
- Despite this, they were unable to control their viremia.

ACKNOWLEDGMENT

Thank you for your attention

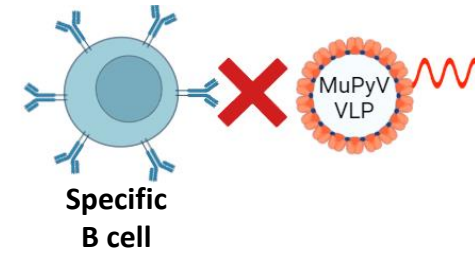


Two labeled VLP for each genotype

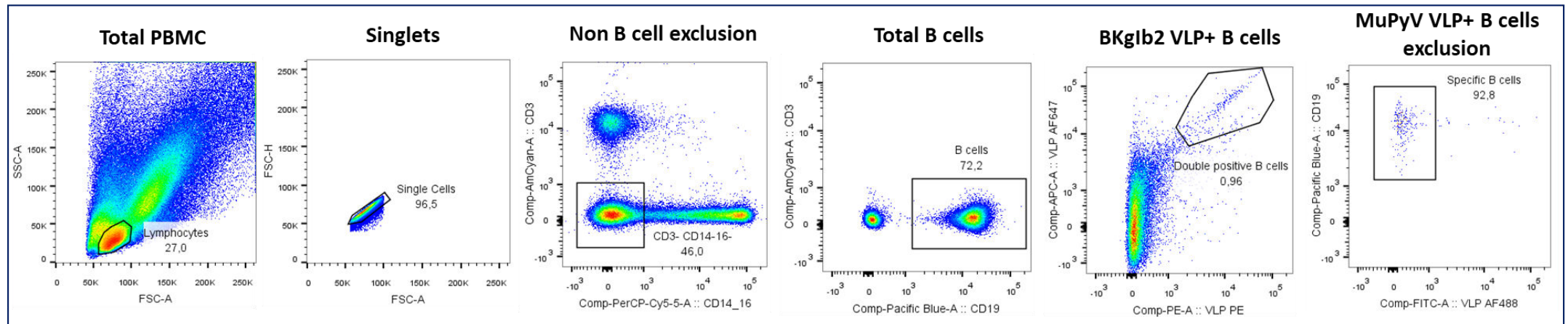


Dual labeled MuPyV VLP :

to exclude B-cells that bind VLPs non-specifically



GATING STRATEGY



→ BKPyV-specific B cells were detected.