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PROSPECTS FOR DIGITAL PCR IN ABSOLUTE QUANTIFICATION OF DNA

LESSONS LEARNED FROM HIV CURE AND FOLLOW-UP

Ward De Spiegelaere,
5th qPCR & Digital PCR congress

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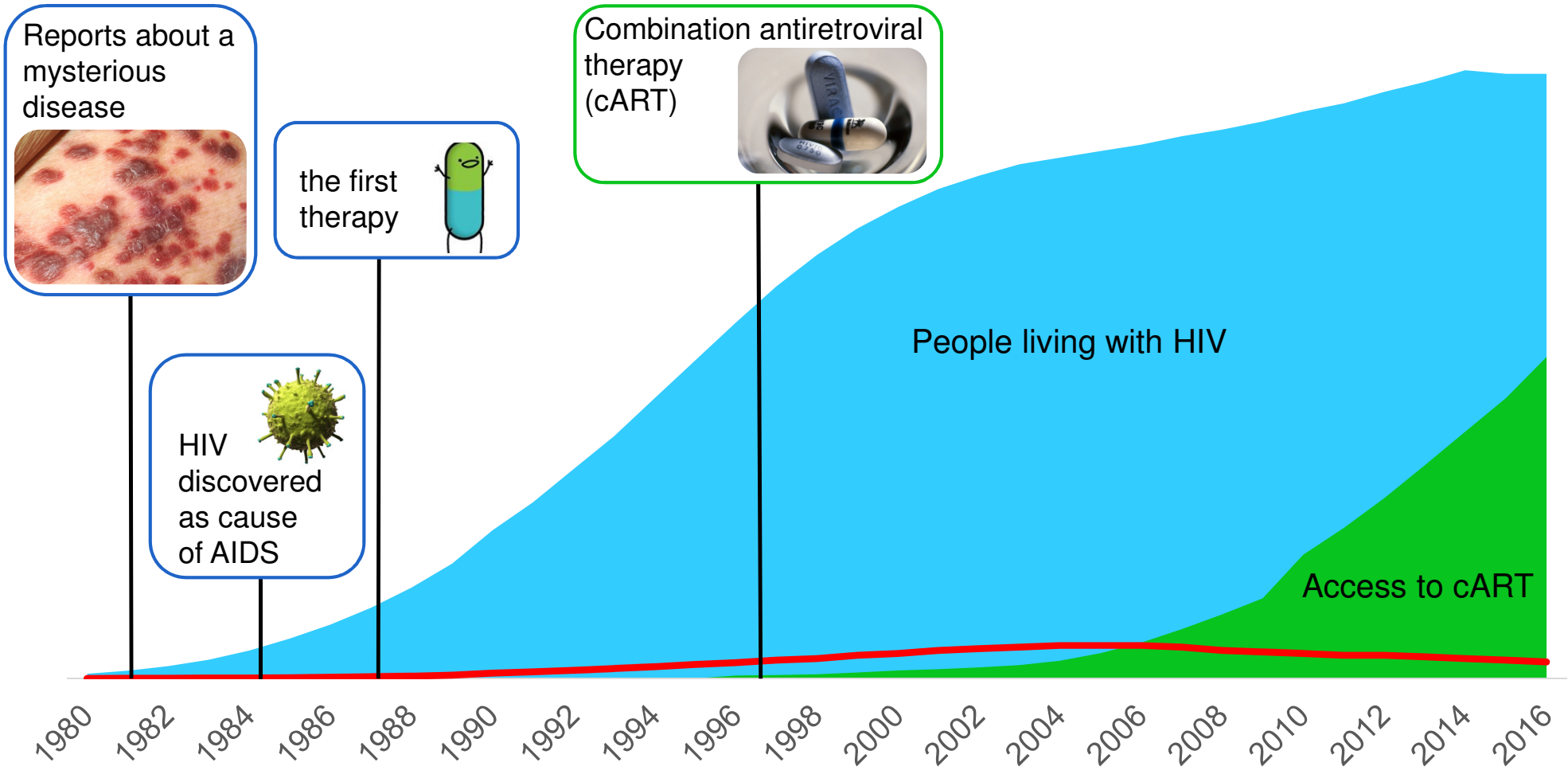
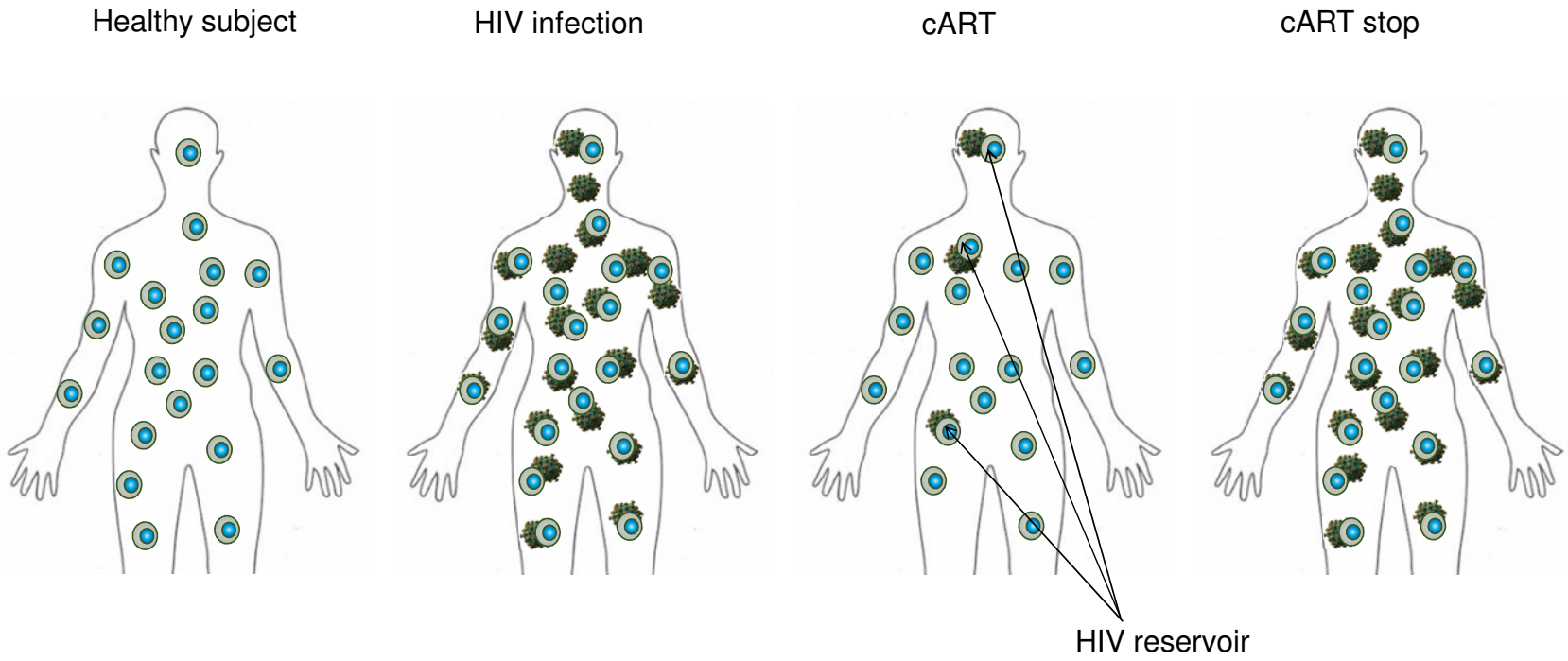
UZ

HIV Cure Research Center

THE HIV/AIDS PANDEMIC



HIV TREATABLE, BUT INCURABLE



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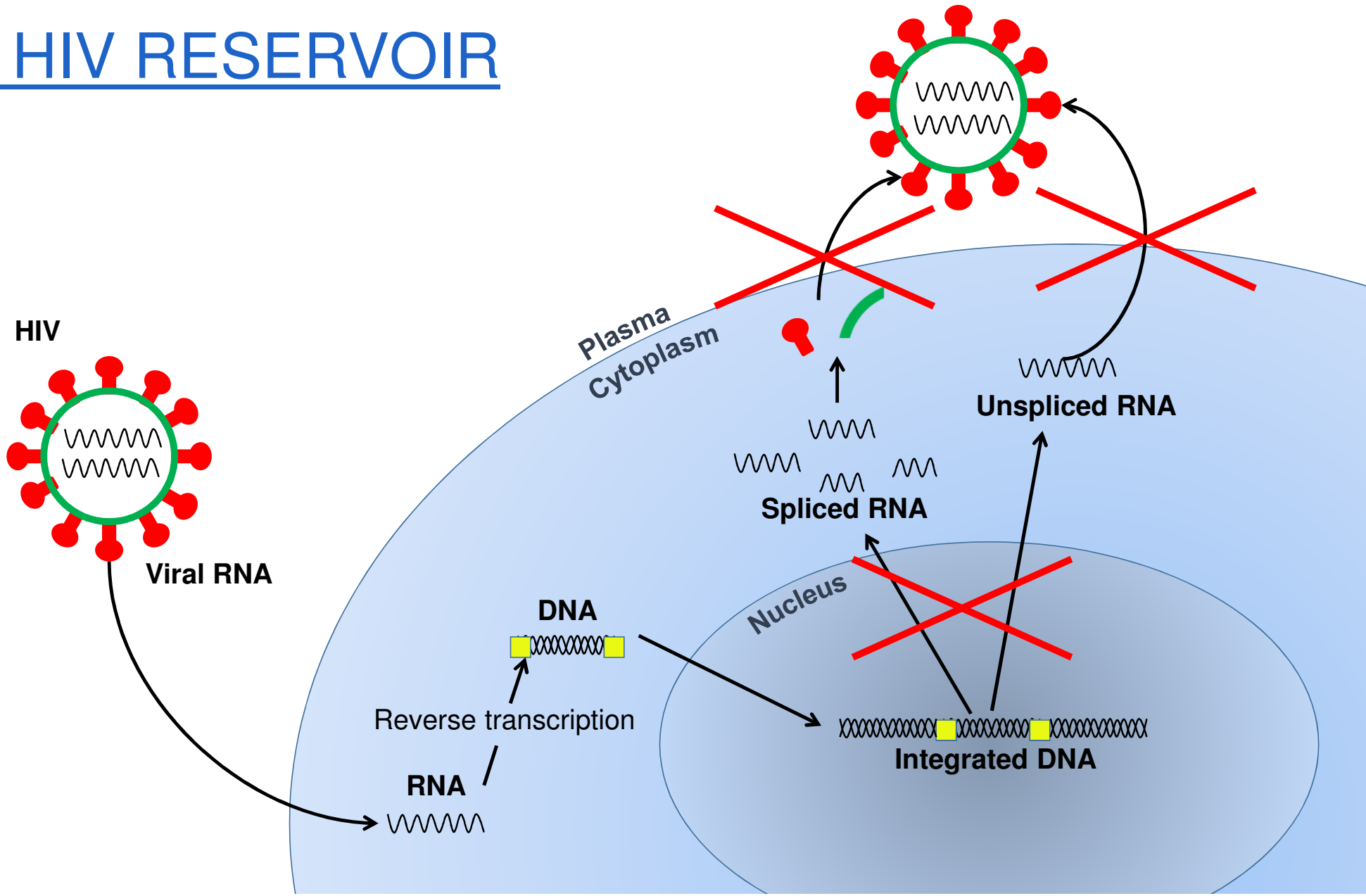
THE HIV RESERVOIR



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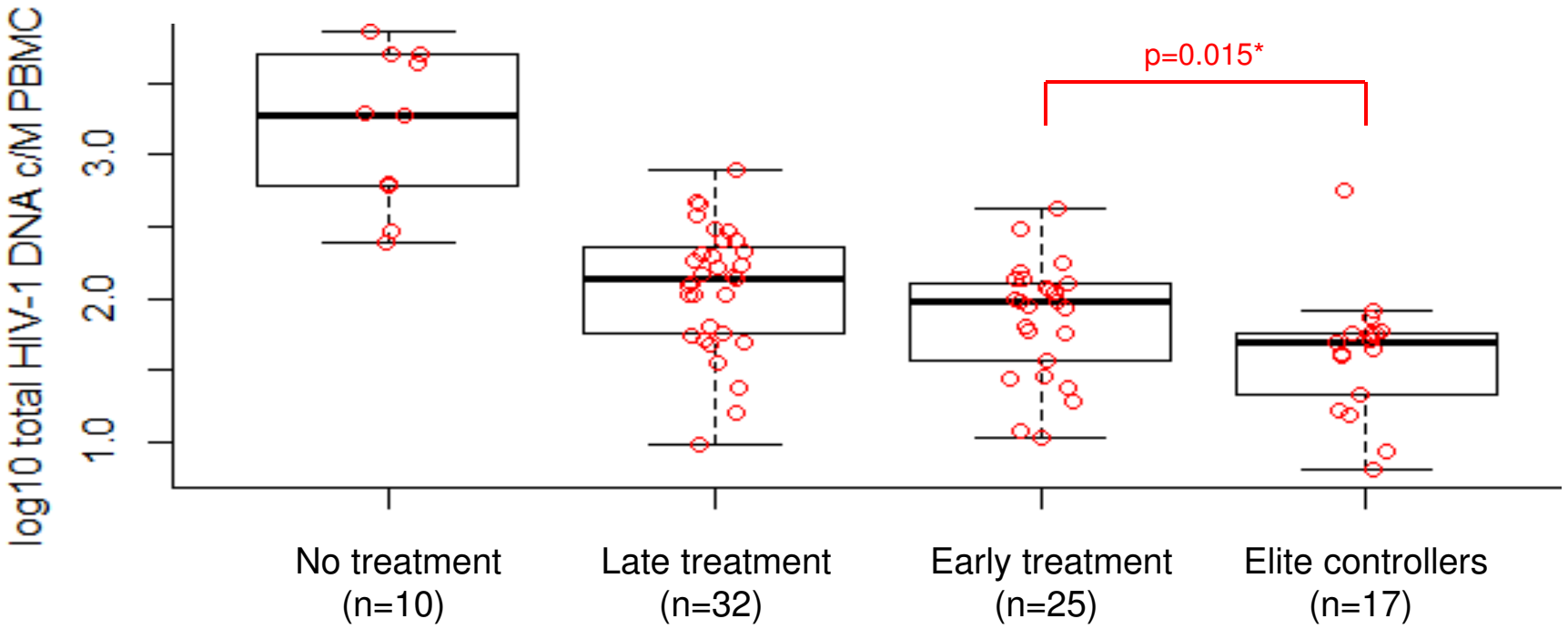
HOW BIG IS THE HIV RESERVOIR



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Malatinkova, E., Spiegelaere, W.D., Bonczkowski, P., Kiselinova, M., Vervisch, K., Trypsteen, W., Johnson, M., Verhofstede, C., Looze, D., Murray, C., Loes, S.K., Vandekerckhove, L., 2015. Impact of a decade of successful antiretroviral therapy initiated at HIV-1 seroconversion on blood and rectal reservoirs. eLife 4, e09115.

eLIFE

DPCR IS MORE PRECISE THAN QPCR

Comparison of ddPCR versus seminested qPCR for HIV DNA quantification

Coeficient of Variation (CV) between triplicates

	n	replicates	CV ddPCR	CV qPCR	Fold difference
Standard curve	7	3	14%	43%	3
Patient samples	17	3	8%	29%	3.5

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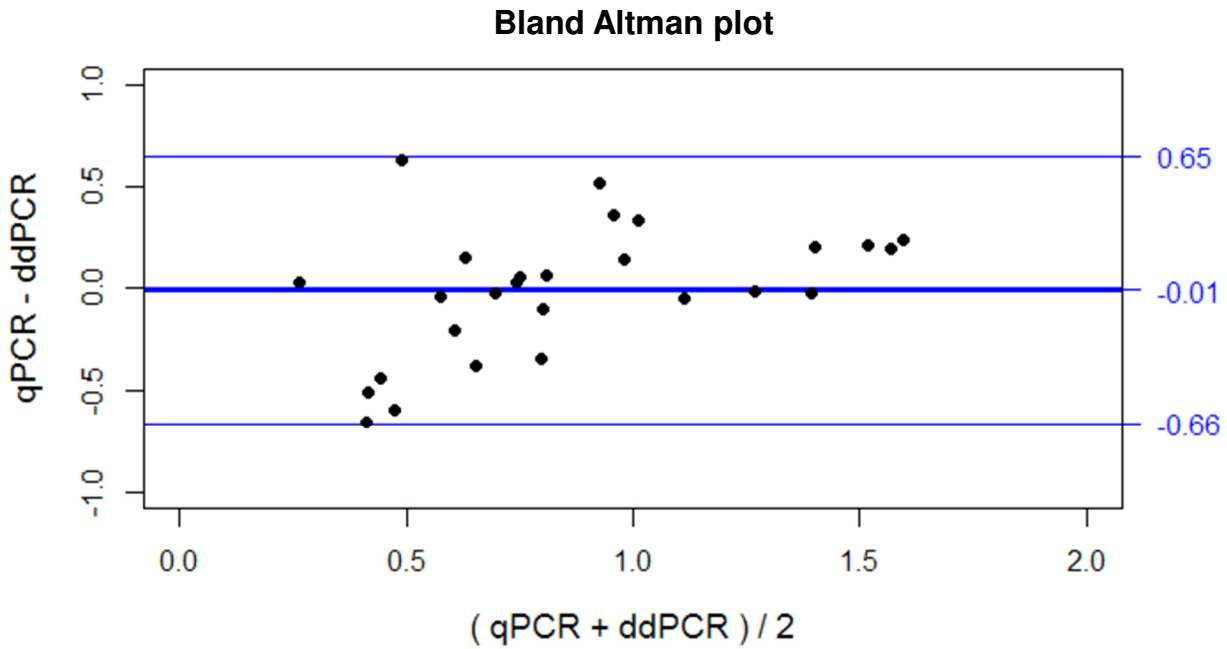
Bosman et al., Scientific Reports 2015 5:13811

ACCURACY

No significant bias compared to qPCR on HIV DNA measurement



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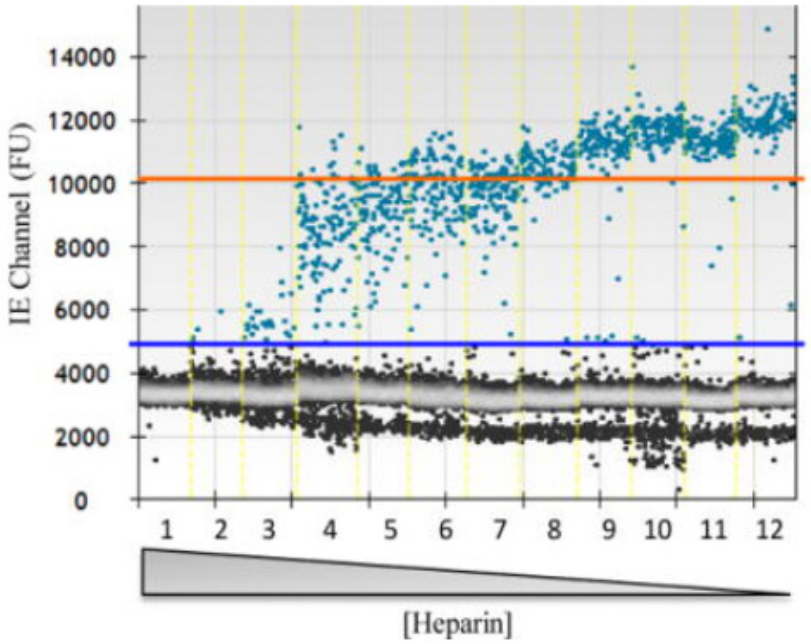
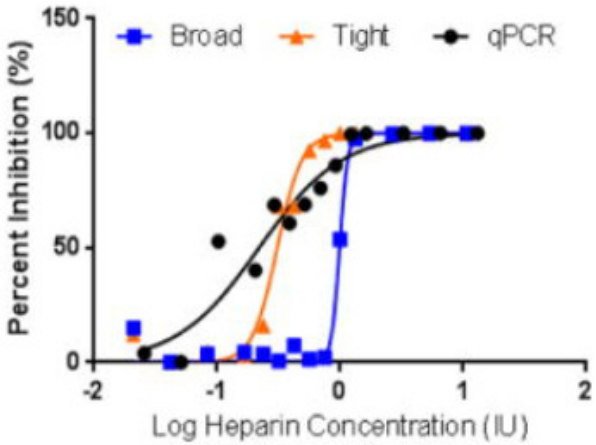
Bosman et al., Scientific Reports 2015 5:13811

DPCR AND INHIBITION

dPCR outperforms qPCR comparing inhibitory substances
(SDS, EDTA and Heparin)





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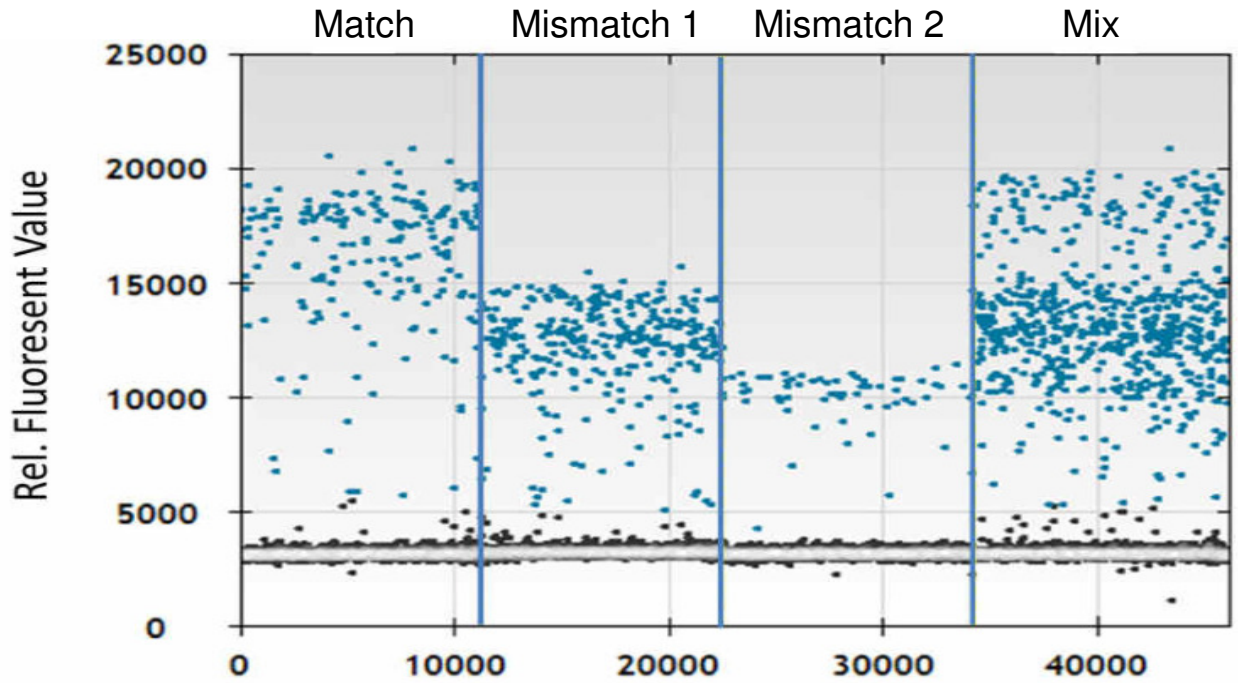
Dingle et al., Clin Chem (2013)59:1670-2

DPCR AND INHIBITION: MISMATCHES

Probe mismatches



Lower fluorescence would harm qPCR but less harm to ddPCR

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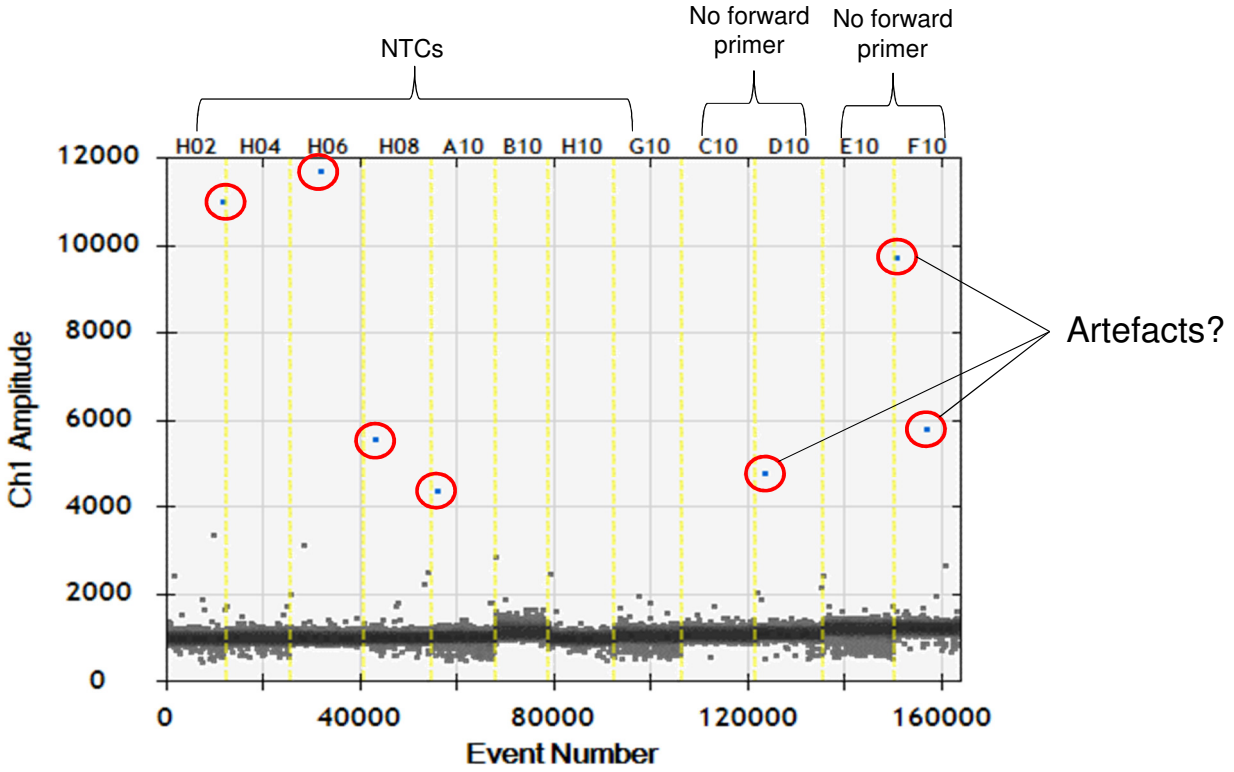
Trypsteen et al., Anal Bioanal Chem (2015) 407:5827-34 (suppl data)

SENSITIVITY: FALSE POSITIVES IN DDPCR



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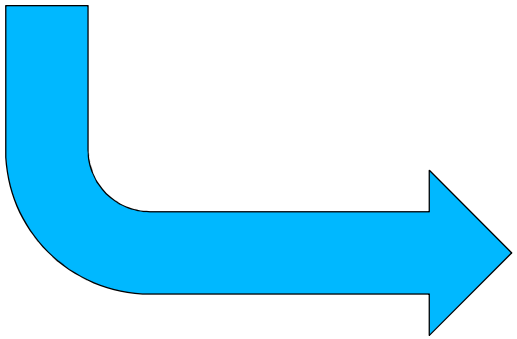
Kiselinova et al., Plos One (2014) 9: e85999

DPCR PERFORMANCE

-Better accuracy and precision ✓

-Less refractory to inhibition ✓

-More sensitive? X



When to use dPCR or qPCR depends on the question:

Template present? → go for standar PCR or nested

How much? → go for digital PCR

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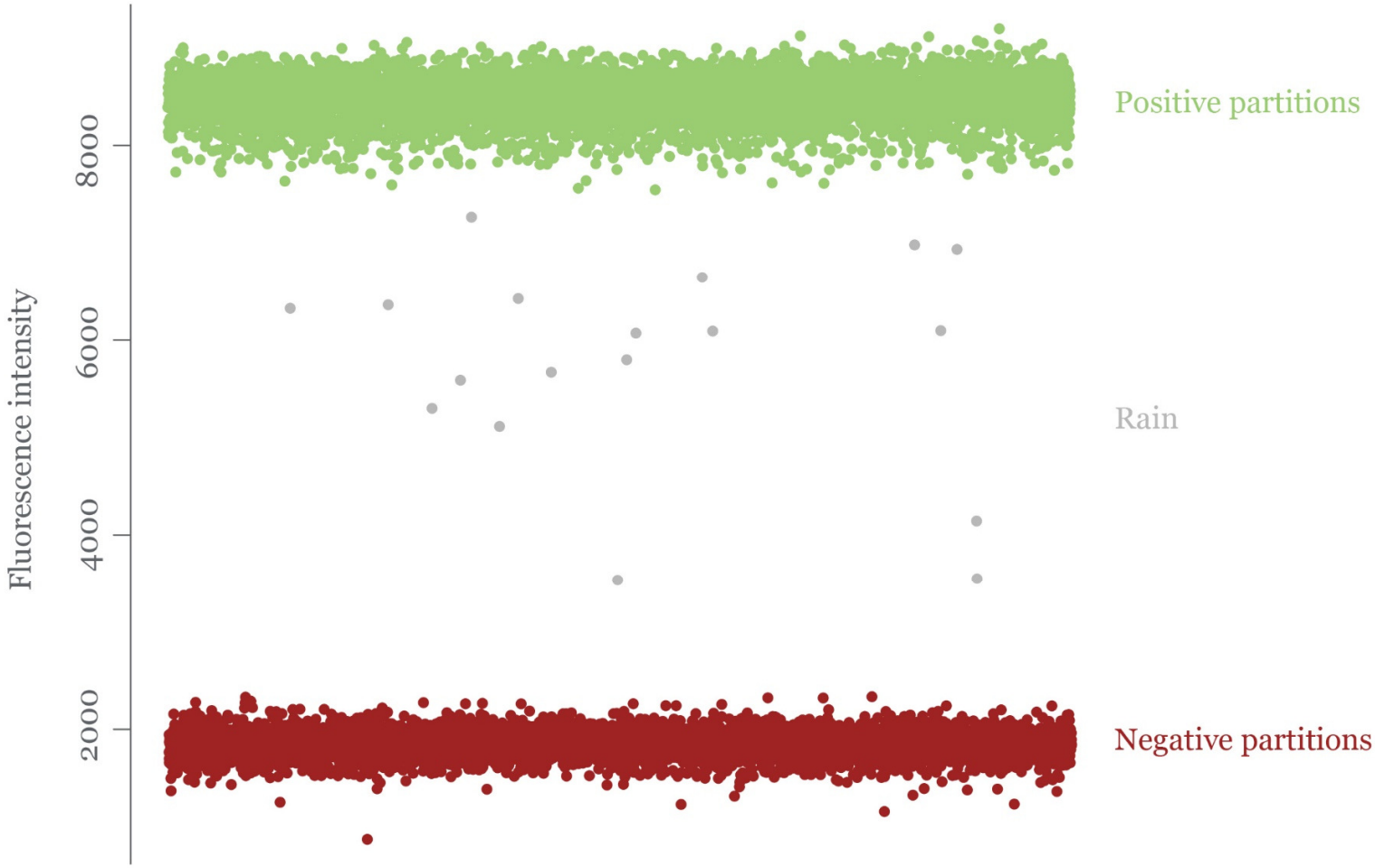
SETTING A TRESHOLD FOR DIGITAL PCR



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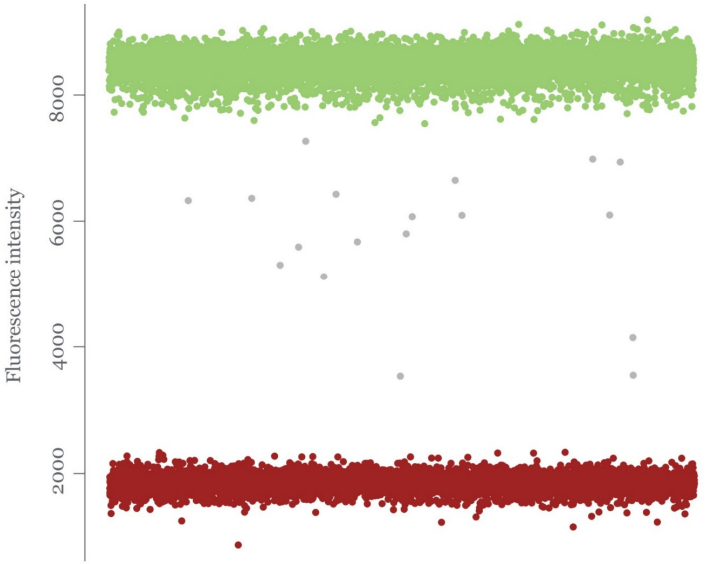
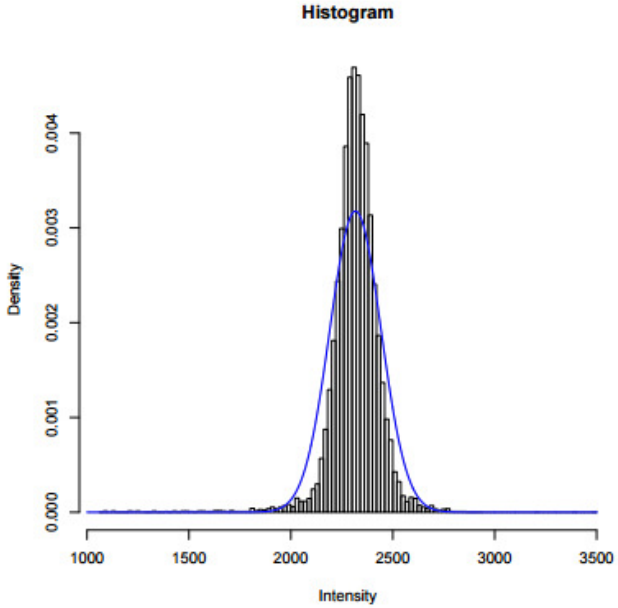
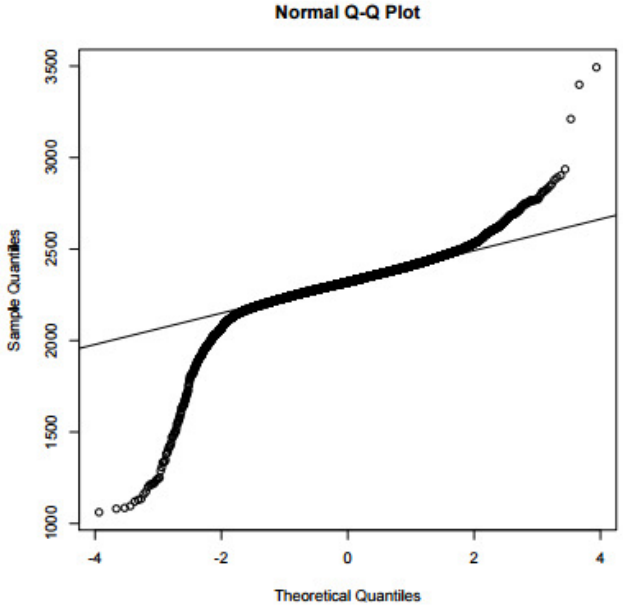
DATA DRIVEN METHODS:HARD TRESHOLDING

Do not use parametric distributions!







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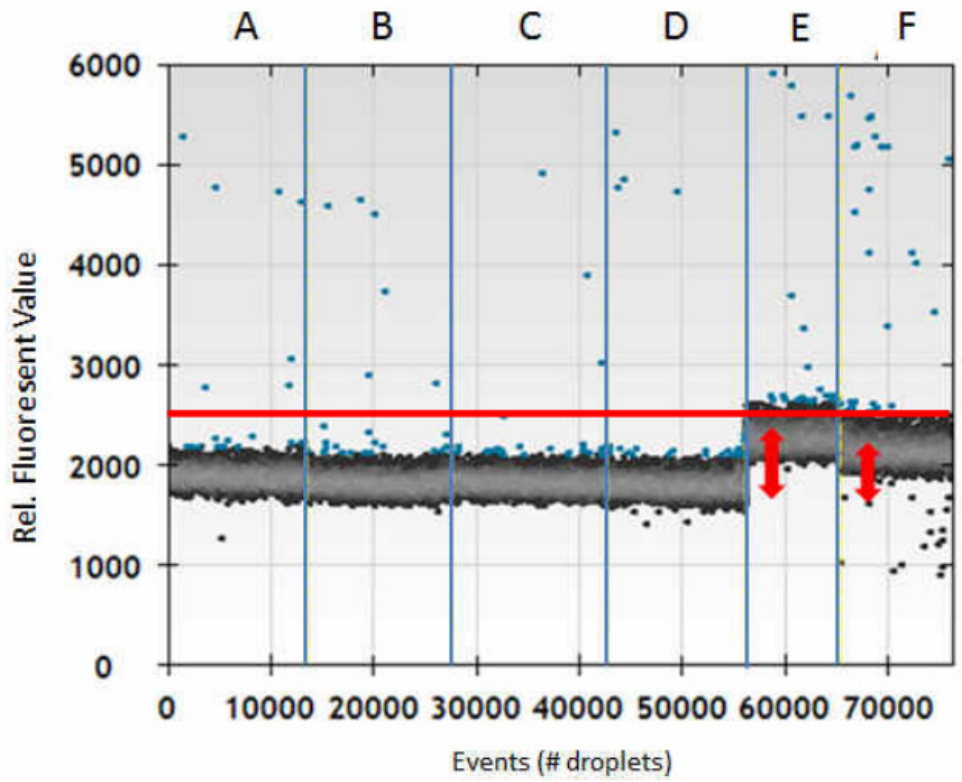
www.ddpcrquant.ugent.be

www.dPCR.ugent.be

Trypsteen et al., Anal Bioanal Chem (2015) 407:5827-34

BACKGROUND SHIFT



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DDPCRQUANT

ddpcRquant

Input Data

Choose Input Data

Upload ddpcrfiles

Upload CSV Files

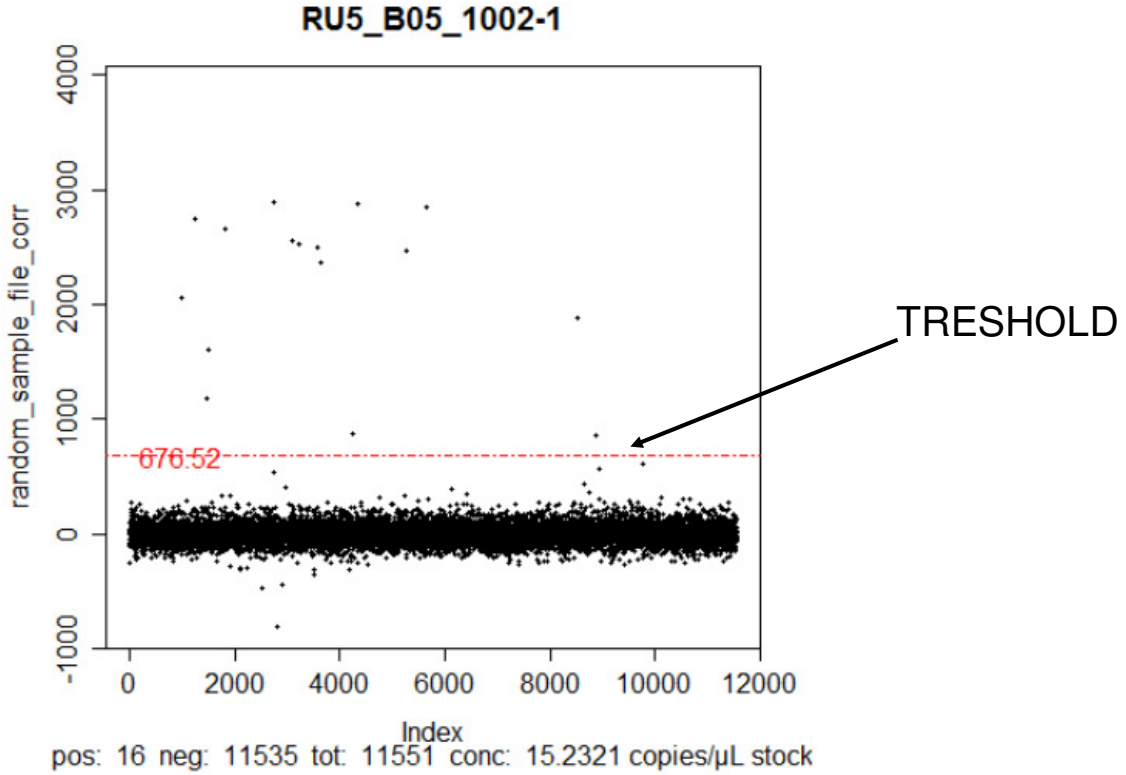
Browse... No file selected

Choose BioRad QX system (demodata = qx100)

qx200

Start Analysis Reset Analysis

Data Input | Overview Assays | NTC Threshold Analysis | Sample Analysis | Summary | Replicate Analysis | How To Use



TRESHOLD

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www.ddpcrquant.ugent.be

Trypsteen et al., Anal Bioanal Chem (2015) 407:5827-34

SOFT TRESHOLDING (MODELLING THE RAIN)

analytical chemistry

Article

pubs.acs.org/ac

Model-Based Classification for Digital PCR: Your Umbrella for Rain

Bart K. M. Jacobs,[*,†] Els Goetghebeur,[†] Jo Vandesompele,[‡,¶,§] Ariane De Ganck,[¶] Nele Nijs,[¶] Anneleen Beckers,[¶] Nina Papazova,[∥] Nancy H. Roosens,[∥] and Lieven Clement[*,†,§]

[†]Department of Applied Mathematics, Computer Science and Statistics, Ghent University, Ghent, Belgium

[‡]Center for Medical Genetics Ghent (CMGG), Ghent University, Ghent, Belgium

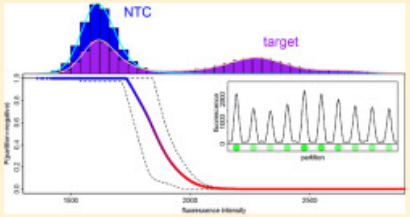
[¶]Biogazelle, Zwijnaarde, Belgium

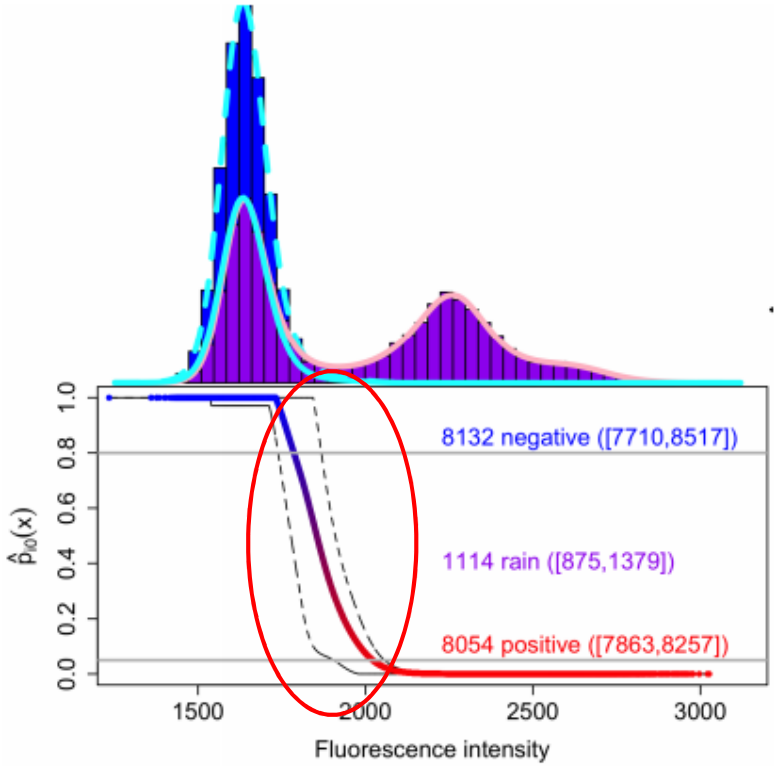
[§]Bioinformatics Institute Ghent From Nucleotides to Networks (Big N2N), Ghent University, Ghent, Belgium

[∥]Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium

Supporting Information

ABSTRACT: Standard data analysis pipelines for digital PCR estimate the concentration of a target nucleic acid by digitizing the end-point fluorescence of the parallel micro-PCR reactions, using an automated hard threshold. While it is known that misclassification has a major impact on the concentration estimate and substantially reduces accuracy, the uncertainty of this classification is typically ignored. We introduce a model-based clustering method to estimate the probability that the target is present (absent) in a partition conditional on its observed fluorescence and the distributional shape in no-template control samples. This methodology acknowledges the





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MEASURING THE HIV RESERVOIR

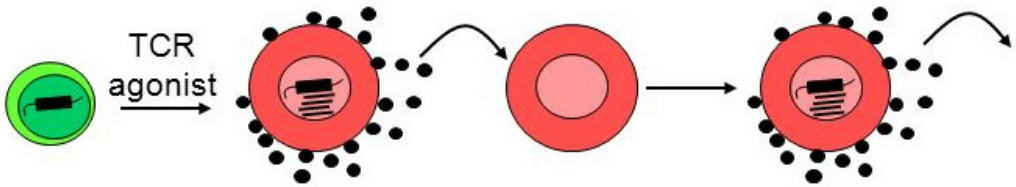
1) HIV cure research
   - Monitoring efficacy of experimental treatment

   Question: does the DNA reservoir correlate with the replication competent reservoir?

2) Current clinical follow-up
   - Stratisfying high risk vs low risk
   - Regimen change to monotherapy

DDPCR IN HIV CURE STUDIES?

1) Validation of HIV DNA as a relevant marker for the HIV reservoir
   Comparison with an *ex vivo* viral outgrowth assay



2) Analysis of HIV DNA levels in cure studies

Clinical Infectious Diseases
MAJOR ARTICLE

Short-Course Toll-Like Receptor 9 Agonist Treatment Impacts Innate Immunity and Plasma Viremia in Individuals With Human Immunodeficiency Virus Infection

Line Vibholm,[1,2,a] Mariane H. Schleimann,[1,2,a] Jesper F. Højen,[1,2] Thomas Benfield,[3] Rasmus Offersen,[1,2] Katrine Rasmussen,[1] Rikke Olesen,[1] Anders Dige,[2,4] Jørgen Agnholt,[2,4] Judith Grau,[5] Maria Buzon,[5] Burghardt Wittig,[6] Mathias Lichterfeld,[7] Andreas Munk Petersen,[8,9] Xutao Deng,[10,11] Mohamed Abdel-Mohsen,[10,11,12] Satish K. Pillai,[10,11] Sofie Rutsaert,[13] Wim Trypsteen,[13] Ward De Spiegelaere,[13,14] Linos Vandekerckhove[13]

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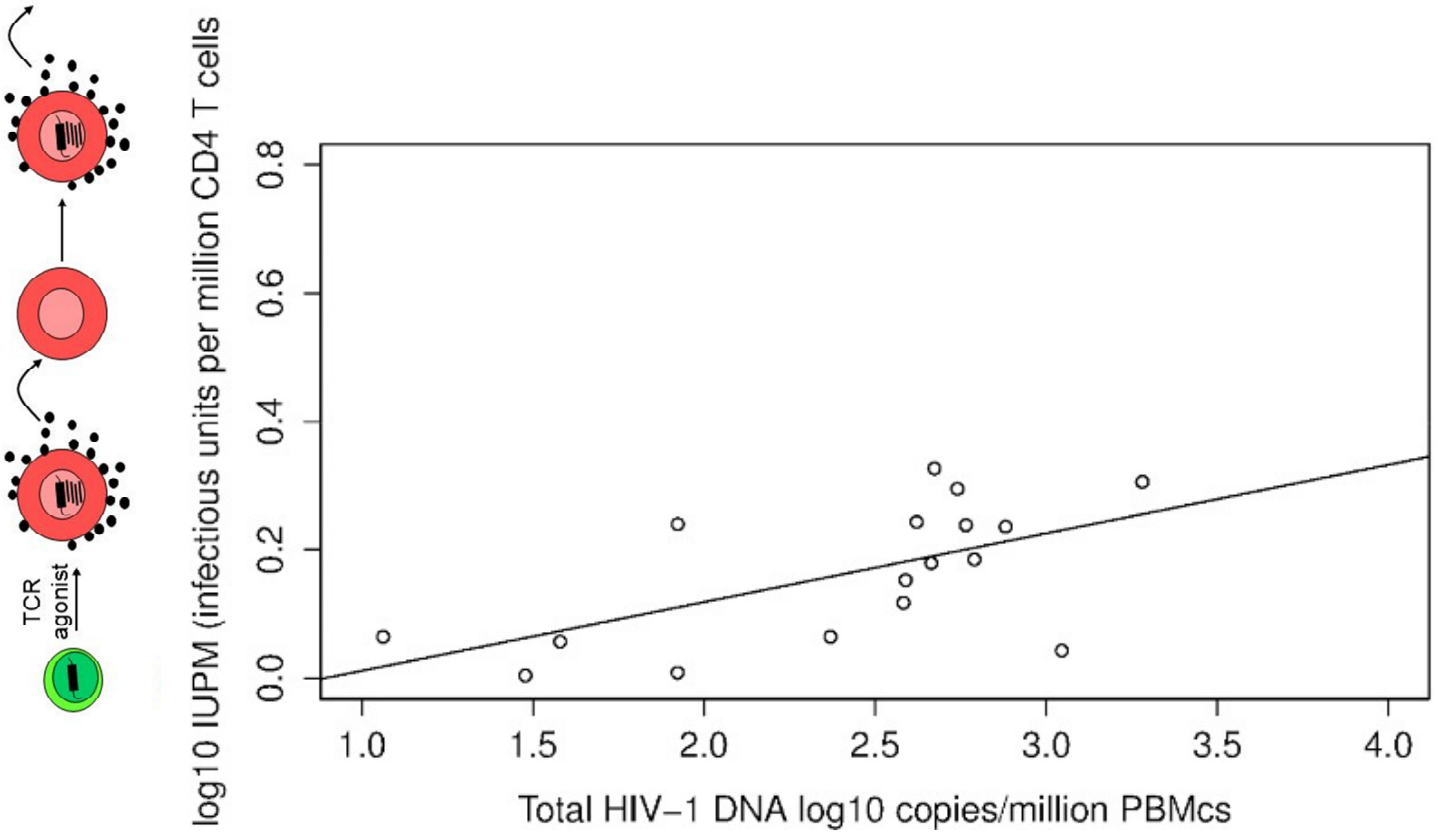
TOTAL HIV DNA CORRELATES WITH VIRAL OUTGROWTH



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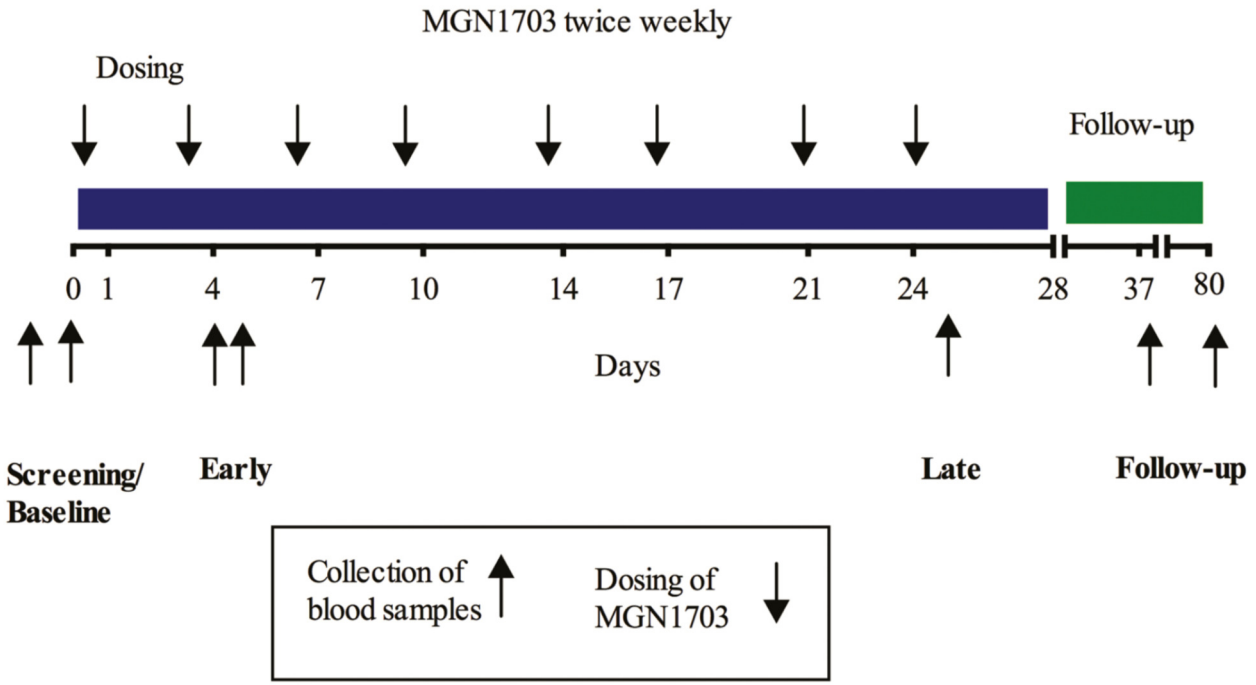
Kiselinova M, De Spiegelaere W, Buzon MJ, Malatinkova E, Lichterfeld M, et al. (2016) Integrated and Total HIV-1 DNA Predict Ex Vivo Viral Outgrowth. PLOS Pathogens 12(3): e1005472.

PLOS | PATHOGENS

SHORT-COURSE TOLL-LIKE RECEPTOR 9 AGONIST TREATMENT



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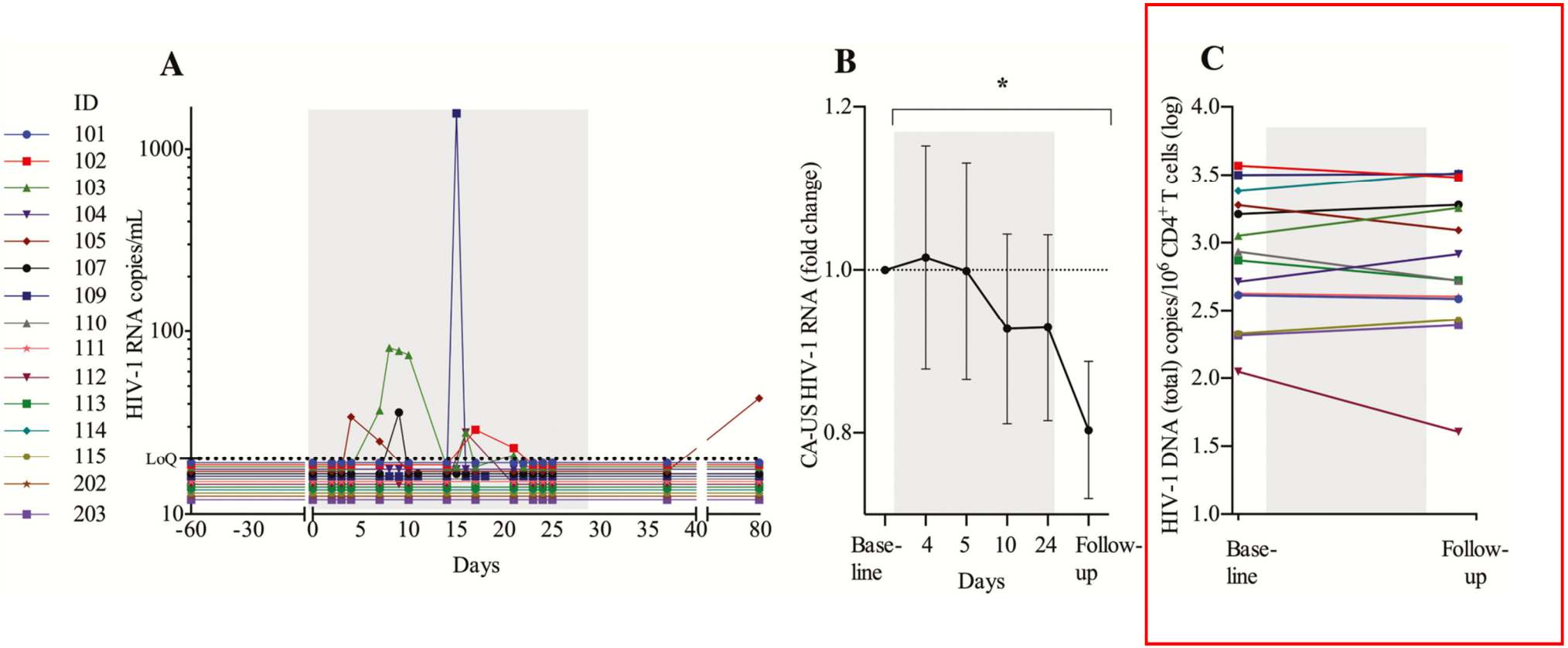
From: Short-Course Toll-Like Receptor 9 Agonist Treatment Impacts Innate Immunity and Plasma Viremia in Individuals With Human Immunodeficiency Virus Infection

Clin Infect Dis. 2017;64(12):1686-1695. doi:10.1093/cid/cix201

SHORT-COURSE TOLL-LIKE RECEPTOR 9 AGONIST TREATMENT



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Clin Infect Dis. 2017;64(12):1686-1695. doi:10.1093/cid/cix201

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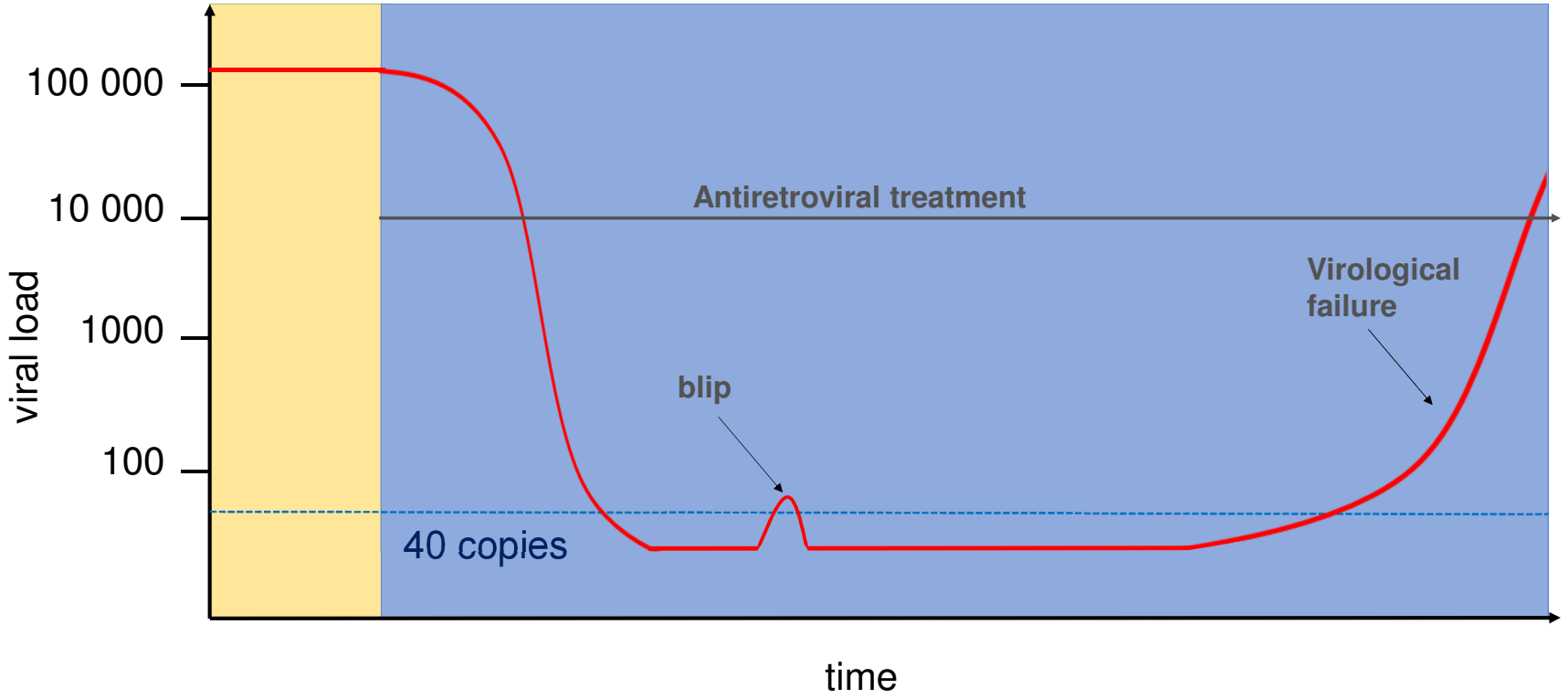
IN FOLLOW-UP

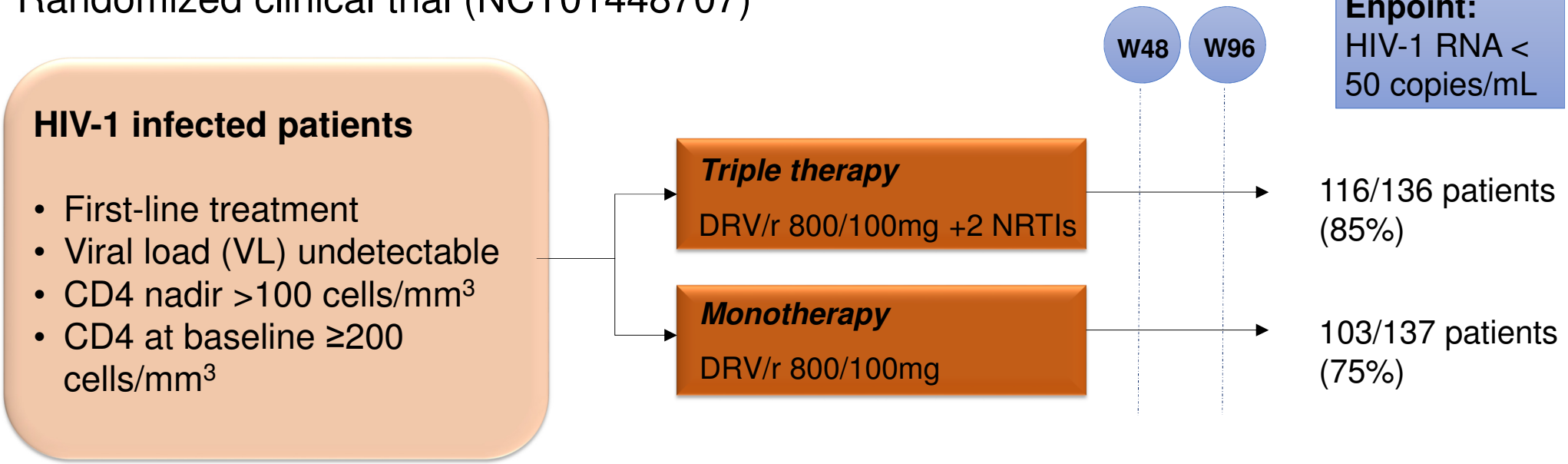
- Patient stratification



triple therapy → monotherapy?

MONOTHERAPY: PROTEA STUDY

- Randomized clinical trial (NCT01448707)



→ Switch to monotherapy shows lower efficacy but is safe

→ **Can we predict this outcome upfront?**

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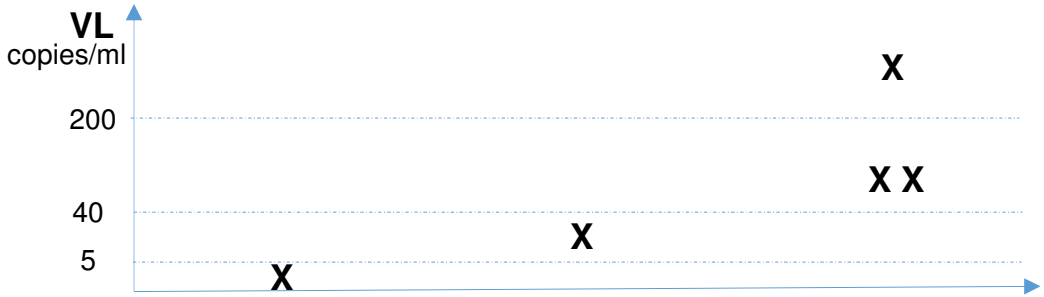
PROTEA SUBSTUDY

Patient characteristics



	Undectable VL	Detected VL	Failers
N	27	25	25
Age, years, mean (SD)	45 (10.8)	42 (9.3)	48 (8.8)
Male sex, N (%)	21 (77.8)	20 (80)	20 (80)
CD4-nadir, cells/mm^3	245 (204-309)	250 (190-357)	217 (169-311)
CD4 at baseline, cells/mm^3	585 (399-644)	633 (522 - 796)	570 (447 - 765)
Time on cART, years	5.7 (3.3-9.45)	4.5 (2.8-7.4)	4.6 (2.4-8.3)

Data documented as median (Q1,Q3), unless stated otherwise.

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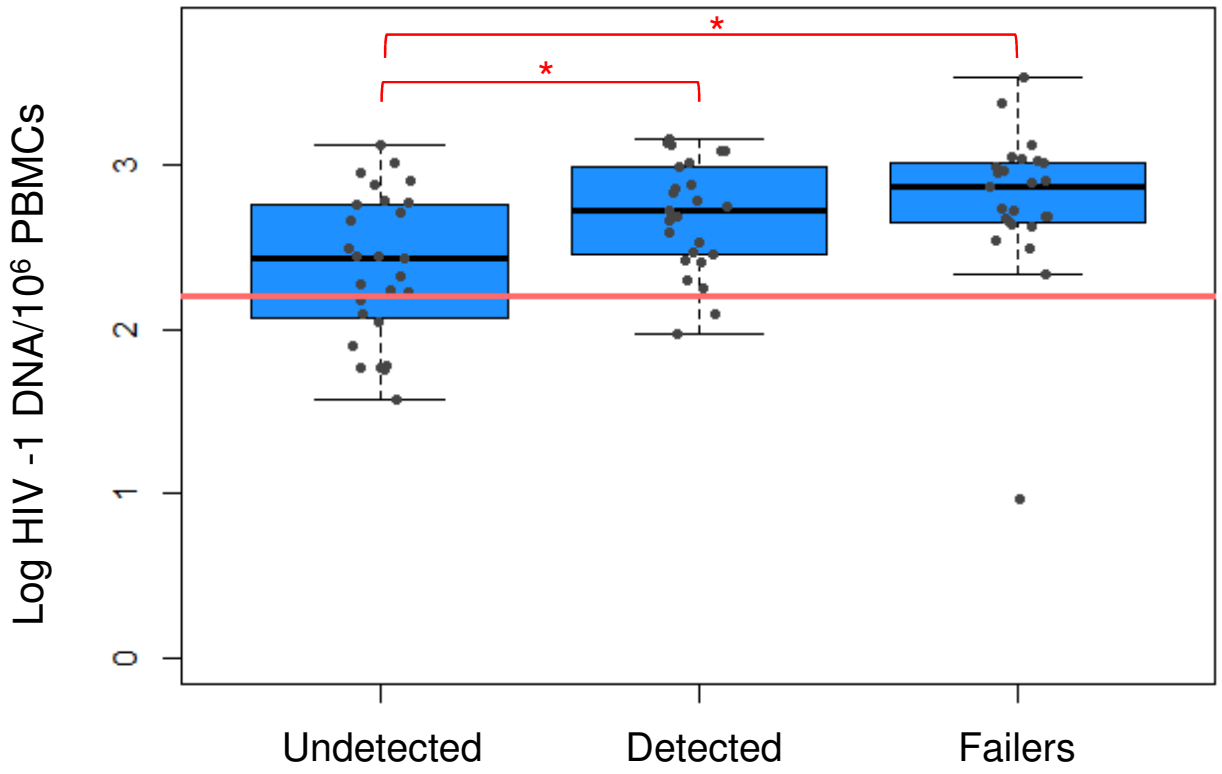
TOTAL HIV-1 DNA

Level of total HIV-1 DNA in patients on DRV/r monotherapy



* : p<0,05

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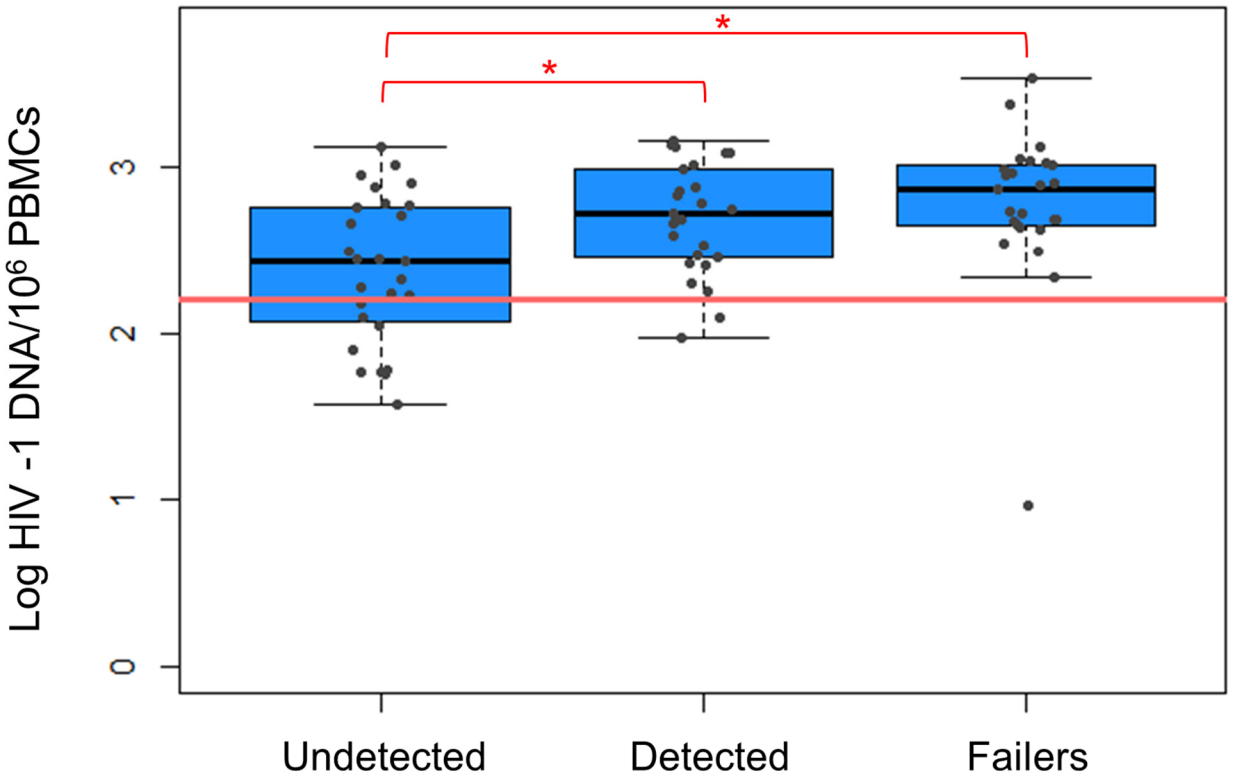
TOTAL HIV-1 DNA

Cut-off of **2.2 log** total HIV DNA/10^6PBMCs.

DRV/r monotherapy:

66% of patients

success rate: 99%.



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CONCLUSION

dPCR in HIV research

- Good performance for HIV DNA quantification
- Promising tool in current HIV follow-up
- Validation in HIV cure setting still requiered

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HIV Cure Research Center

www.dPCR.ugent.be

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Prof. Chris Verhofstede

The AIDS Reference Center
Prof Dirk Vogelaers
Prof Chris Verhostede

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amfAR AIDS RESEARCH

HIVera

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janssen PHARMACEUTICAL COMPANIES OF Johnson&Johnson