THE LANCET Infectious Diseases

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: McMullan LK, Flint M, Chakrabarti A, et al. Characterisation of infectious Ebola virus from the ongoing outbreak to guide response activities in the Democratic Republic of the Congo: a phylogenetic and in vitro analysis. *Lancet Infect Dis* 2019; published online July 9. http://dx.doi.org/10.1016/S1473-3099(19)30291-9.

Appendix

Characterisation of infectious Ebola virus from the ongoing outbreak to guide response activities in the Democratic Republic of the Congo: a phylogenetic and in vitro analysis

Laura K. McMullan[1#], Mike Flint[1], Ayan Chakrabarti[1], Lisa Guerrero[1], Michael K. Lo[1], Danielle Porter[2], Stuart T. Nichol[1], Christina F. Spiropoulou[1], Cesar Albarino[1]

[1]Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, MS G-14, Atlanta, GA 30329, USA.
[2]Gilead Sciences, Inc, 333 Lakeside Drive, Foster City, CA 94404, USA

[#]Corresponding author. Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, MS G-14, Atlanta, GA 30329, USA.
LKM: +1-404-639-4570, lim8@cdc.gov

Table S1. Ebola viruses used in the phylogenetic analysis and displayed in figure 1. Outbreak location, year of outbreak, taxa, and GenBank accession number are listed.

Outbreak	Year	Taxa	Accession No
Sierra Leone	2014	a	KM034550
Liberia	2014	a	KP178538
Sierra Leone	2014	b	KM034559
Sierra Leone	2014	c	KM034561
Guinea	2014	a	KJ660347
Guinea	2014	b	KJ660348
Gabon	2002	a	KC242800
Republic of the Congo	2003	a	KF113528
Republic of the Congo	2003	b	KF113529
Luebo	2008	a	HQ613402
Luebo	2007	f	KC242788
Luebo	2007	b	HQ613403
Luebo	2007	e	KC242787
Luebo	2007	d	KC242789
Luebo	2007	c	KC242786
Ituri	2018	d	MK007331
Ituri	2018	a	MK007329
Ituri	2018	b	MK007330
Ituri	2018	c	MK007332
Likati	2017	a	MH481611
Yambuku	1976	b	KM655246
Yambuku	1976	a	AF086833
Bonduni	1977	a	KC242791
Yambuku	1976	c	KC242801
Gabon	1996	c	KC242794
Gabon	1994	a	KC242792
Gabon	1996	b	KC242797
Kikwit	1995	d	KC242796
Kikwit	1995	c	KU978803
Kikwit	1995	a	AY354458
Kikwit	1995	b	JQ352763
Bikoro	2018	c	MH733478
Bikoro	2018	d	MH733480
Bikoro	2018	a	MH733477
Bikoro	2018	b	MH733479
Boende	2014	b	KP271018
Boende	2014	a	KM519951
Boende	2014	c	KP271020
Boende	2014	d	KR819004

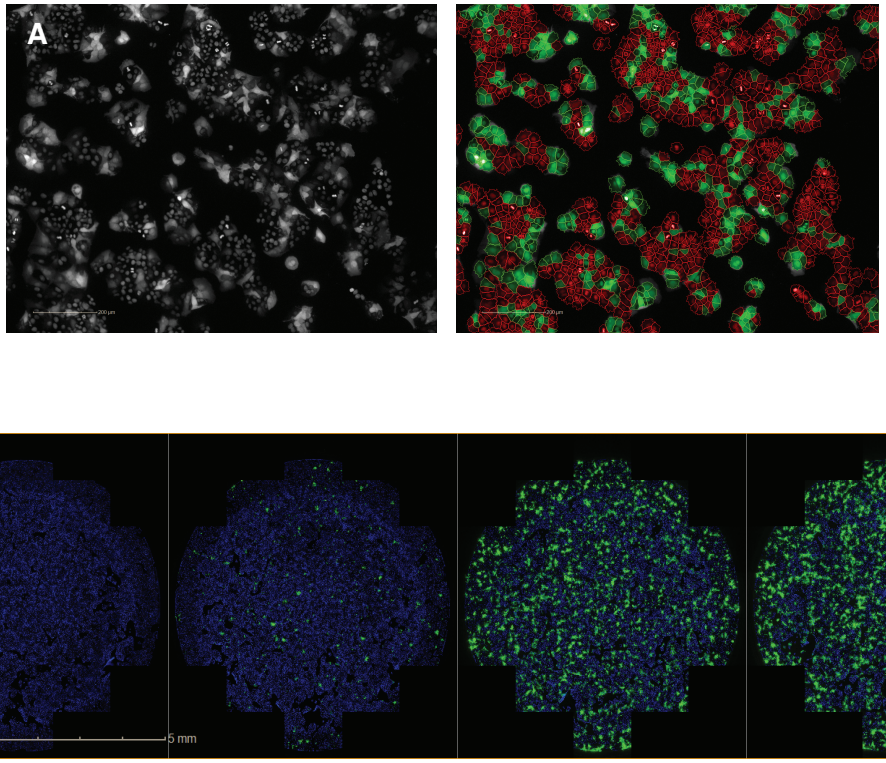

Figure S1. The EBOV-Ituri virus is inhibited by remdesivir. (a) Segmentation and detection of ZsG in cells infected with the recombinant reporter EBOV-Ituri virus. (b) Concentration-dependent inhibition of ZsG expression from the reporter EBOV-Ituri virus by remdesivir. Green, ZsG; blue, nuclei stained with DAPI. (c) Concentration-response graph for remdesivir against EBOV-Makona (blue squares) and -Ituri (red circles) viruses (left y-axis), and for cell viability (black triangles, right y-axis), relative to vehicle treated controls. Data represent the means of 4 biological replicates ± standard deviation.

A

Ituri	**A**	A	T	G	A	G	T	D	E	P	L	P	E	T	A	P	T	**T**	P	**T**	**A**	S	T	L	P	H	**K**	A	**R**	A	T
Bikoro	V	A	T	R	A	G	T	G	E	P	P	P	E	T	T	P	M	P	L	A	T	G	T	L	P	H	E	T	K	A	T
Makona	V	V	A	G	P	E	N	D	K	P	P	T	G	A	A	P	T	P	L	A	T	S	A	S	L	Y	E	A	K	V	T
Mayinga	V	A	T	R	A	G	T	G	E	L	L	P	E	T	A	S	T	P	P	A	T	S	T	F	P	H	E	A	K	A	I
AA for GP	**3**	**82**	**262**	**314**	**315**	**331**	**336**	**338**	**360**	**368**	**378**	**382**	**405**	**411**	**417**	**422**	**424**	**429**	**430**	**432**	**435**	**440**	**441**	**443**	**446**	**455**	**457**	**474**	**478**	**503**	**544**

B

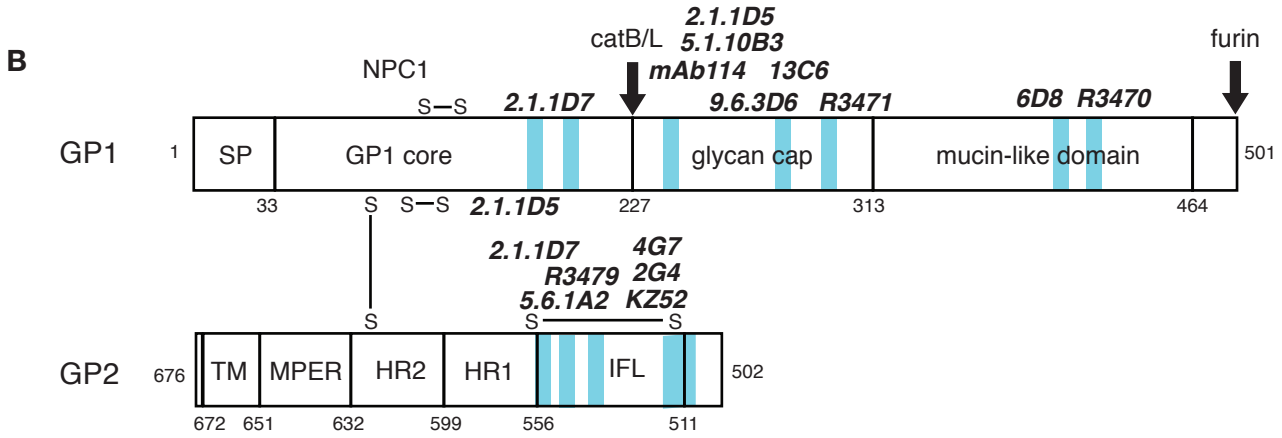

Figure S2. (a) Glycoprotein amino acid differences between EBOV-Ituri and other EBOV strains. (b) Schematic of EBOV glycoprotein subunits 1 and 2 (GP1 and GP2). The epitopes recognized by the mAbs 5.6.1A2, 2.1.1D5, 2.1.1D7, 9.6.3D6, mAb114, 13C6, 6D8, R3470, R3471, R3479, 4G7, 2G4, and KZ52 are indicated by blue shading. Amino acid residue numbering is indicated below each schematic. SP, signal peptide; NPC1, Neimann-Pick C1 receptor binding site; CatB/L, cleavage sites for cathepsin B and L; furin, cleavage site for cellular furin; IFL, internal fusion loop domain; HR1, heptad repeat 1; HR2, heptad repeat 2; MPER, membrane-proximal envelope region; TM, transmembrane domain.

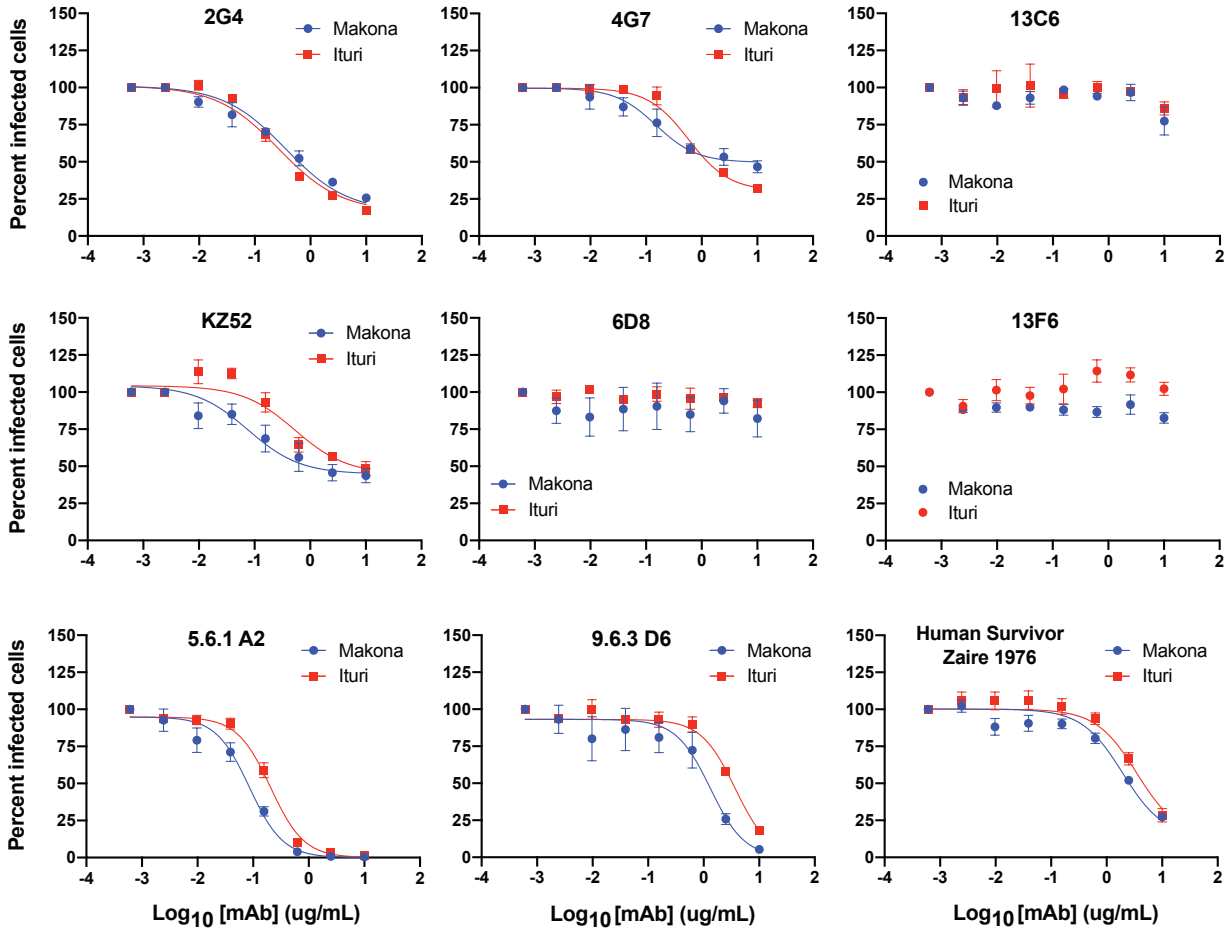

Figure S3: Neutralisation of EBOV-Ituri and -Makona virus. EBOV-Ituri (red squares) and -Makona (blue circles) viruses with antibodies: mAb 2G4, mAb 4G7, mAb 13C6, mAb KZ52, mAb 6D8, mAb 13F6, mAb 5.6.1A2, mAb 9.6.3D6, and convalescent patient serum from Zaire 1976. Each point represents the mean from at least 3 biological replicates ± SD. Each graph is a representative of at least two independent experiments. EC50 values are listed in Table 1.

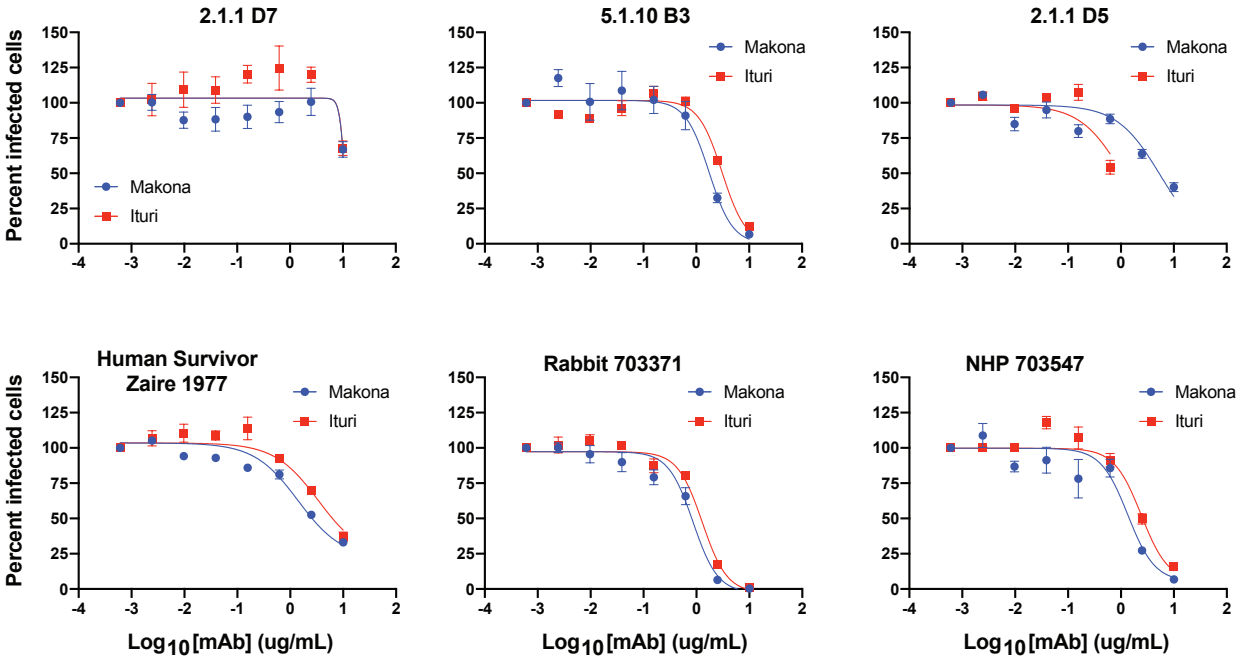

Figure S4. Neutralisation of EBOV-Ituri and -Makona virus. EBOV-Ituri (red squares) and –Makona (blue circles) viruses with monoclonal and polyclonal antibodies: mAb 2.1.1 D7, mAb 5.1.10 B3, mAb 2.1.1 D5. Ebola convalescent antibodies from Zaire Jan 1977 (703200) is a subsequent sample taken in Jan 1977 from the same Ebola survivor shown in Fig S3 (Nov 1976, 703201). Polyclonal antibodies from EBOV-inoculated rabbit (703371) and polyclonal antibodies from EBOV-inoculated non-human primate (NHP) (703547) are shown. Each point represents the mean from at least 3 biological replicates ± SD. Each graph is a representative of at least two independent experiments. IC50 values are shown in Table 2.

A Ebola Xpert assay NP

	For Primer (880-902)	Probe (924-946)	Rev Primer (rev comp) (956-976)
	GCTCCTTTCGCCCGACTTTTGAA	**TGAGCATGGTCTTTTCCCTCAAC**	**TTGCACTTGGAGTCGCCACAG**
Ituri	**GCTCCTTCCGCCCGACTTTTGAA**	**TGAGCATGGTCTTTTCCCTCAAC**	**TTGCACTTGGAGTCGCCACAG**
Bikoro	**GCTCCTTTCGCCCGACTTTTGAA**	**TGAGCATGGTCTTTTCCCTCAAC**	**TTGCACTTGGAGTCGCCACAG**
Makona	**GCTCCTTTCGCCCGACTTTTGAA**	**TGAGCATGGTCTTTTCCCTCAAC**	**TTGCACTTGGAGTCGCCACAG**
Mayinga	**GCTCCTTTCGCCCGACTTTTGAA**	**TGAGCATGGTCTTTTCCCTCAAC**	**TTGCACTTGGAGTCGCCACAG**

B Ebola Xpert assay GP

	For Primer (311-339)	Probe (346-368)	Rev Primer (rev comp) (380-397)
	GGGCTGAAAACTGCTACAATCTTGAAATC	**CCTGACGGGAGTGAGTGTCTACC**	**ACGGGATTCGGGGCTTCC**
Ituri	**GGGCTGAAAACTGCTACAATCTTGAAATC**	**CCTGACGGGAGTGAGTGTCTACC**	**ACGGGATTCGGGGCTTCC**
Bikoro	**GGGCTGAAAACTGCTACAATCTTGAAATC**	**CCTGACGGGAGTGAGTGTCTACC**	**ACGGGATTCGGGGCTTCC**
Makona	**GGGCTGAAAACTGCTACAATCTTGAAATC**	**CCTGACGGGAGTGAGTGTCTACC**	**ACGGGATTCGGGGCTTCC**
Mayinga	**GGGCTGAAAACTGCTACAATCTTGAAATC**	**CCTGACGGGAGTGAGTGTCTACC**	**ACGGGATTCGGGGCTTCC**

C CDC assay NP2

	For Primer (356-380)	Probe (Rev comp) (393-424)	Rev Primer (Rev comp) (451-475)
	AATTGCTGCCAGCAGTATCTAGTGG	**GAGAACACTTGCTGCCATGCCGGAAGAGGAGA**	**CTCTCYTTTGCAAGTCTATTCCTTC**
Ituri	**AATTGCTGCCAGCAGTATCTAGTGG**	**GAGAACACTTGCTGCCATGCCGGAAGAGGAGA**	**CTCTCCTTTGCCAGTCTATTCCTTC**
Bikoru	**AATTGCTGCCAGCAGTATCTAGTGG**	**GAGAACACTTGCTGCCATGCCGGAAGAGGAGA**	**CTCTCCTTTGCAAGTCTATTCCTTC**
Makona	**AATTGCTGCCAGCAGTATCTAGTGG**	**GAGAACACTTGCTGCCATGCCGGAAGAGGAGA**	**CTCTCCTTTGCAAGTCTATTCCTTC**
Mayinga	**AATTGCTGCCAGCAGTATCTAGTGG**	**GAGAACACTTGCTGCCATGCCGGAAGAGGAGA**	**CTCTCCTTTGCAAGTCTATTCCTTC**

D CDC assay VP40

	For Primer (608-624)	Probe (627-650)	Rev Primer (Rev comp) (652-674)
	TGCGYCCAGGAATTTCA	**TCATCCAAAACTKCGCCCCATTCT**	**TTACCCAACAARAGTGGGAAGAA**
Ituri	**TGCGTCCAGGAATTTCA**	**TCATCCAAAACTTCGCCCCATTCT**	**TTGCCCAACAAAAGTGGGAAGAA**
Bikoro	**TGCGCCCAGGAATTTCA**	**TCATCCAAAACTTCGCCCCATTCT**	**TTACCCAACAAGAGTGGGAAGAA**
Makona	**TGCGTCCAGGAATTTCA**	**TCATCCAAAACTTCGCCCCATTCT**	**TTACCCAACAAAAGTGGGAAGAA**
Mayinga	**TGCGTCCAGGAATTTCA**	**TCATCCAAAACTTCGCCCCATTCT**	**TTACCCAACAAAAGTGGGAAGAA**

Figure S5. The EBOV-Ituri sequence contains nucleotide mismatches in the primer-binding sites of two diagnostic assays. (a) Comparison of primer binding sites used in the Xpert Ebola NP and (b) GP assays, and the (c) CDC NP2 RT-qPCR assay and the (d) CDC VP40 RT-qPCR assay. Red nucleotides indicate mismatches present in the Ituri sequence.