



RESEARCH ARTICLE

REVISED Discovery and description of the first human Retro-Giant virus [version 2; referees: 1 approved]

Elena Angela Lusi ¹, Federico Caicci ²

¹St Vincent Health Care Group, University College Dublin, Dublin, Ireland

²Department of Biology-Electron Microscopy Unit, University of Padua, Padova, Italy

v2 First published: 04 Jul 2018, 7:1005 (<https://doi.org/10.12688/f1000research.15118.1>)
 Latest published: 20 Sep 2018, 7:1005 (<https://doi.org/10.12688/f1000research.15118.2>)

Abstract

Background: Robert Gallo reported the first human retrovirus HLTV in 1980. What we report here is the first human giant virus, Mimivirus-like, with a retroviral core.

Methods: The isolation of human giant viruses from human T cells Leukaemia was performed on 25% sucrose gradient. The purified viral pellet was examined using electron microscopy (EM), after immunolabelling with anti-FeLV gag p27 moAb, used for its ability to bind conserved epitopes among different mammalian retroviruses. These human giant viruses were tested for reverse transcriptase activity. RNA extracted from the viral particles was initially amplified with the Pan Retrovirus PCR technique. In addition, a shotgun whole genome sequence was performed.

Results: EM showed the presence of ~400 nm giant viruses, mimivirus-like, specifically labelled by anti-FeLV gag p27 Ab. The giant viruses had the reverse transcribing property. Whole genome sequence showed the presence of transforming retroviral genes in the large viral genome confirming that the Retro-Giant viruses are a distinct branch, missing from the current classification of retroviruses.

Conclusions: Although sharing some of the morphological features with Mimiviruses, this human giant virus differs substantially from environmental DNA-giant viruses isolated so far, in that it manifests a unique mammalian transforming retroviral core and T cell tropism. The virus should not be confused with a classic human retrovirus nor even a large human retrovirus, but an ancestral human giant virus, mimivirus-like, with a mammalian retroviral core. Certainly, the oncogenic potential of the viral particle and its T cell tropism is of concern and further studies are needed to clarify the role of this giant virus in human diseases and evolution of archetypal retroviruses.

Keywords

Retroviruses, Mimiviruses, Giant Viruses, anti-FeLV gag, human T cell Leukaemia, Retro-Giant Virus

Open Peer Review

Referee Status:

Invited Referees

1

REVISED

version 2

published
20 Sep 2018

report



version 1

published
04 Jul 2018

report

¹ **Didier Raoult**, Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, France

Discuss this article

Comments (0)

Corresponding author: Elena Angela Lusi (elenaangelalusi@yahoo.it)

Author roles: **Lusi EA:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Caicci F:** Methodology

Competing interests: No competing interests were disclosed.

Grant information: This work was supported in part by St Vincent Health Care Group of Dublin, Ireland.

Copyright: © 2018 Lusi EA and Caicci F. This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

How to cite this article: Lusi EA and Caicci F. **Discovery and description of the first human Retro-Giant virus [version 2; referees: 1 approved]** *F1000Research* 2018, **7**:1005 (<https://doi.org/10.12688/f1000research.15118.2>)

First published: 04 Jul 2018, **7**:1005 (<https://doi.org/10.12688/f1000research.15118.1>)

REVISED Amendments from Version 1

This new version of the manuscript contains the Whole Genome Shotgun sequences of a human oncogenic Retro-Giant Virus isolated from human T cell leukaemia ([Dataset 4](#) and [Dataset 5](#)).

This complete sequence confirms my preliminary findings.

See referee reports

Introduction

Our previous paper described the presence of unusual Mimiviruses-like structures in human tissues¹. Like Mimiviruses (~450 nm giant viruses found in the amoebas), these human structures had the ability to retain Gram staining, and mass spectrometry revealed the presence of histone peptides that had the same footprints as giant viruses²⁻⁹. However, the human giant virus-like structures displayed a distinct and unique mammalian retroviral antigenicity.

Our initial discovery in human tissues presented the conundrum of whether the structures were giant viruses with a retroviral nature or cellular components having a viral footprint. The distinction between the virus and the cells was blurred. The most difficult part to explain arose from the unique mammalian retroviral antigenicity associated with the human Mimivirus-like structures.

There was only one possibility to solve the dilemma: isolate the viruses (if really present) and verify if they contain genetic material. Consequently, in the present study we chose the traditional way of isolating virus using a sucrose gradient, following the same protocols and steps described by Prof Robert Gallo in his discovery of the first human retrovirus, human T-lymphotropic virus (HTLV)¹⁰.

The gigantic dimension of our viral particle excluded it from the orthodox understanding of retroviruses but simultaneously presented the antipodean challenge to establish if this giant virus's retroviral properties signified the discovery of the first human Retro-Giant virus.

In this manuscript, we report the isolation of a human giant virus, mimivirus-like, with a retroviral core from human T cell acute lymphoblastic leukaemia, and the various experiments on the purified giant virus, including immunogold electron microscopy, nucleic acid extraction, reverse transcriptase assay, whole genome sequencing and phylogenetic analyses.

The experiments, validated by independent operators kept blind, determined the retroviral nature of a human giant virus with associated viral factory that is ancestral to archetypal retroviruses.

Methods

Purification of human giant viruses on sucrose gradient

10⁸ human T cell leukaemia (HPB-ALL, DSMZ, Germany), grown at 37°C in RPMI-1640, 10% fetal bovine serum and 2 mM L-glutamine, were centrifuged at 1,500 rpm g for 5 minutes at 4°C. The cell pellet was washed with 1x PBS. The cells were lysed (vortexed) with 2.5 ml PBS in the presence of 25µl of protease inhibitor cocktail (Abmgood, Richmond BC, Canada). Cell suspension was vortexed and incubated at 4°C for 30 minutes. Cell lysis was monitored using a phase contrast light microscope. The resulting crude extract was centrifuged at 3,000 rpm for 5 minutes. The pellet containing the cellular nuclei was discharged.

The resulting supernatant was collected and slowly dripped over 9 ml of a 35-30-25% sucrose gradient (Sigma, Milan, Italy) and centrifuged at 10,000 rpm for 5 h in a 15 ml Corex glass centrifuge tubes (Fisher Scientific, Dublin, Ireland). Once a visible white disk, corresponding to 25% sucrose fraction, was observed, the viral pellet was collected after centrifugation at 14,000 rpm for 30 min, at 4°C.

RNA extraction from the giant viral particles

The viral pellet was lysed with 1ml of RNA-XPress Reagent (Himedia, Mumbai, India), a monophasic solution of phenol-guanidine thiocyanate, and incubated at room temperature (RT) for 5 minutes. This was followed by the addition of 200 µL chloroform, vortexing for 15 sec and incubation at RT for 10 min. The organic and aqueous phases were separated by centrifuging the sample at 11,000 rpm for 15 minutes at 4°C. The aqueous phase, containing RNA,

was harvested and precipitated with 600 µl of isopropyl alcohol and glycogen. After incubation for 1h at 20°C, RNA was pelleted by centrifugation at 11,000 rpm for 10 minutes. The RNA pellet was washed with 75% of ethanol, air dried and resuspended in RNase free H₂O (Himedia). One aliquot was utilized for concentration determination in a MaestroNano Spectrophotometer (Maestrogen Inc, Hsinchu City, Taiwan).

cDNA synthesis

1 µg of total RNA was utilized for cDNA synthesis using EasyScript cDNA Synthesis Kit (Abmgood) according to the manufacturer's instructions. Briefly, 20 µl of reaction contained 200 units of reverse transcriptase, 0.5 µM of random primers, 20 units of ribonuclease inhibitors, 500 µM dNTP. The reaction was carried out at 25°C for 10 min, then at 42°C for 50 min.

Pan-retrovirus PCR from RNA of the human giant virus

We performed a Pan-retrovirus PCR from the RNA extracted from the giant viruses. To amplify a segment of the Pol gene, we used degenerate primers targeting a conserved region, of approximately 140bp, between the most conserved domain VLPQG and YMDD in the Pol gene of retroviruses. The oligonucleotide primers and conditions were derived from those described by Tuke *et al.*¹¹. The first PCR mixture was performed by amplifying 1 µl of the double-stranded cDNA reaction with the following reagents: 1 µM primer PAN-UO (5'-CTT GGATCCTGGA AAGTGCTAAGCCAC-3') and 1µM primer PAN-D1 (5'-CTCAAGCTTCAG CGATGGTCATCCATCGTA-3') with 1.25 unit of thermostable DNA polymerase (Precision DNA Polymerase, Abmgood). The above mixture was brought to a final volume of 25µl with a PCR mix (Abmgood, Richmond, BC, Canada) containing 0.2 mM dNTPs/2.0mM MgCl₂ in 1X PCR reaction buffer. The PCR was performed in a Thermal Cycler (GET3X Triple Block Thermal Cycler, Bio-Gener, China) using the following conditions: 1 cycle of 95°C for 10 minutes; 35 cycles of 95°C for 1 minute, 34°C for 1 minute, 72°C for 1 minute; 1 cycle of 72°C for 10 minutes.

In total, 1 µl of this reaction was re-amplified in a semi-nested reaction using the PAN-UI (5' CTTGGATCCAGT-GTCTAGCCCACAAGGG-3') primer in combination with PAN-D1. Conditions for the semi-nested PCR were: 1 cycle of 10 minutes at 95°C; 40 cycles of 95°C for 1 minute, 45°C for 30 seconds, 72°C for 1 minute; 1 cycle of 72°C for 10 minutes.

A 10-µl aliquot of the resulting PCR product was analyzed after electrophoresis on a 2.5% MS8 agarose gel (Laboratorios Conda, Madrid, Spain). The amplified bands were recovered from the gel with UltraPrep Agarose Gel Extraction Kit (AHN Biotechnologie GmbH, Nordhausen, Germany) according to the manufacturer's instructions. Briefly, the DNA was excised from the agarose gel and weighted. Three volumes of buffer (volume: weight of the excised gel band size) was added and the mixture was incubated at 50°C for 10 minutes. The DNA was bound to a column and centrifuged at 13,000 rpm for 1 minute. After wash with 700 µl of washing buffer, the DNA was recovered from the column with 50 µl of elution buffer.

Sanger sequencing and molecular phylogenetic analyses

DNA sequencing was performed on an ABI 3500 Automatic Sequencer (Applied Biosystems, Foster City, CA, USA) using Big Dye Terminator v3.1 (Applied Biosystems).

Molecular phylogenetic analyses were made at BMR Genomics Institute (Padua, Italy). Our sequences were aligned against other retroviral viral sequences. Sequence accession numbers used in the alignment between 150 bp segment from retro- giant viruses with equivalent VLP—YMDD Polregion (RT) of different retroviruses, amplified with the same Pan Retrovirus-PCR, are reported in [Dataset 1](#)¹². For the phylogenetic analysis of the 400 bp amplicon, retroviral sequences and accession numbers are displayed in [Dataset 2](#)¹³.

Phylogenetic tree for the 150bp VLPQ-YMDD interval was made using [Phylogeny.fr](#) (A La Carte Mode). [T-Coffee](#) was used for multiple alignment, [Gblocks](#) v 0.91b for alignment curation, [PhyML](#) 3.1 for phylogeny and [TreeDyn](#) 198.3 for tree drawing. A non-parametric, Shimodaira-Hasegawa-like approximate Likelihood-Ratio branch test (SH-like aLTR) was used as a statistical test.

For the 400 bp amplicon, phylogenetic tree was made using [Phylogeny.fr](#). [Muscle](#) v3.8.31 was used for multiple alignment, [Gblocks](#) v 0.91b for alignment curation, [PhyML](#) 3.1 for phylogeny and [TreeDyn](#) 198.3 for tree drawing. A non-parametric, Shimodaira-Hasegawa-like approximate Likelihood-Ratio branch test (SH-like aLTR), default HKY85, was used as a statistical test.

Dataset 1. 150 bp amplicon alignment against other VLPQG-YMDD Pol sequences of different retroviruses<http://dx.doi.org/10.5256/f1000research.15118.d208073>**Dataset 2. 400bp amplicon sequence and its alignment against other retroviral families**<http://dx.doi.org/10.5256/f1000research.15118.d208074>**Electron microscopy (EM) immunogold of viral particles**

25 µl of the 25% sucrose isolated viral pellet was placed on Holey Carbon film on Nickel 400 mesh. The grids were treated for 30 minutes at room temperature with the primary monoclonal antibody (moAb) anti-Feline Laekemia Virus p27gag (catalog number, PF12J-10A; Custom Monoclonals International, West Sacramento, CA, USA) and subsequently with a secondary anti-mouse gold conjugated antibody (BB international anti-mouse IgG 15 nm gold conjugate; catalog number, EM.GMHL15, Batch 4838). After staining with 1% uranyl acetate, the sample was observed with a Tecnai G2 (FEI) (Thermo Fisher) transmission electron microscope, operating at 100 kV. Images were captured with a Veleta (Olympus Soft Imaging System) digital camera.

Gram positive stain of the giant viral particles

Gram positive staining of purified human giant viruses was performed with Colour Gram 2 Biomerieux kit, following the manufactures instructions. Before staining, slides were heated fixed 3–4 times through the Bunsen burner flame.

Reverse transcriptase (RT) assay of the human giant viruses

After sucrose gradient isolation, the viral pellet was lysed in 20 µl of 20 mM Tris-HCL pH7.5, 100 mM NaCl, 0.1 mM EDTA, 1mM DTT, 50% (v/v) glicerol, 0.25% Triton X-100 (Sigma). To test the ability of the human giant viruses to retro-transcribe, 10 µl of the viral lysate, instead of a reverse transcriptase enzyme, were used to retro-transcribe 1 µg of total RNA from Human Liver Total RNA (ThermoFisher Scientific, Waltham, MA, USA). The reverse transcriptase reaction for the viral pellet was carried out with random primers using a commercial kit (EasyScript cDNA Synthesis kit; Abmgood), deprived of the supplied reverse transcriptase enzyme. The reverse transcriptase reaction was carried at 25°C for 10 minutes, then at 42°C for 50 minutes. The reaction was stopped by heating at 85°C for 5 minutes. The viral reverse transcriptase activity was compared to positive controls where a commercial RT enzyme was included (EasyScript RTase; Amgood).

After the reverse transcription, 2 µl of the obtained single stranded cDNA was further amplified in presence of 10 pmol of primers for GAPDH, 1.25 units of thermostable DNA polymerase (Precision DNA Polymerase; Abmgood), 0.2 mM dNTPs/2.0 mM MgCl₂ in 1X PCR buffer in a final volume of 25µl. PCR conditions were: 1 cycle of 95°C for 5 minutes; 40 cycles of 94°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute; 1 cycle of 72°C for 5 minutes. 20 µl of the PCR reaction was loaded on a 1% agarose gel for electrophoresis.

Dataset 3. Uncropped and unedited blots<http://dx.doi.org/10.5256/f1000research.15118.d208075>**Whole genome shotgun sequencing**

Sequencing and bioinformatics analysis were performed by Genomix4Life (University of Salerno). In detail, the libraries were prepared using DNA as starting material, with Nextera flex Kit (Illumina Inc). Libraries were sequenced (paired-end, 2 × 75 cycles) on NextSeq platform (Illumina Inc.) Fastq underwent Quality Control using **FastQC tool**. *De novo* assembly was performed using Geneious (version 11.1) software. The assembler was used with standard parameters. **Blast2Go** was used to perform the blast alignment sequences. Annotation with respect to the NCBI Viruses database (taxa 10239) was made through **Gene Ontology**. The algorithm used was blastx-fast and the statistical significance threshold for reporting matches against a sequence viruses database was set 1.0E-3. The number of sequence alignments to retrieve was set to 20. The other parameters was set as default. All statistical values regarding assembled contigs were computed using QUAST (QUALity Assessment Tool).

Dataset 4. Illumina FastQ and FastQC raw data<https://dx.doi.org/10.5256/f1000research.15118.d218559>

Results

Virus purification from acute lymphoblastic T cell leukaemia cells and EM immunogold of the viral particles

Giant viral particles, isolated from human T cell leukemia (HPB-ALL) cells, formed a white ring on 25% of sucrose gradient. Only the 25% fraction was collected. This fraction was pure and did not contain any contamination such as cellular nuclei; the nuclear fraction was discharged in the first step of differential centrifugation, before layering onto the sucrose gradient.

EM immunogold of the viral pellet depicted giant viral particles (~400nm) that were specifically marked by an anti-Feline Leukaemia virus core p27 gag moAb (Figure 1A). The purified human giant viruses retained the Gram stain, like Mimiviruses in amoebas (Figure 1B). In some micrographs, the predominant 400 nm viral particles are ‘budding’ around a much larger giant particle that also displays the retroviral antigens. This suggests a possible reproduction of the Retro-Giant viruses that strongly recalls the archaea features.

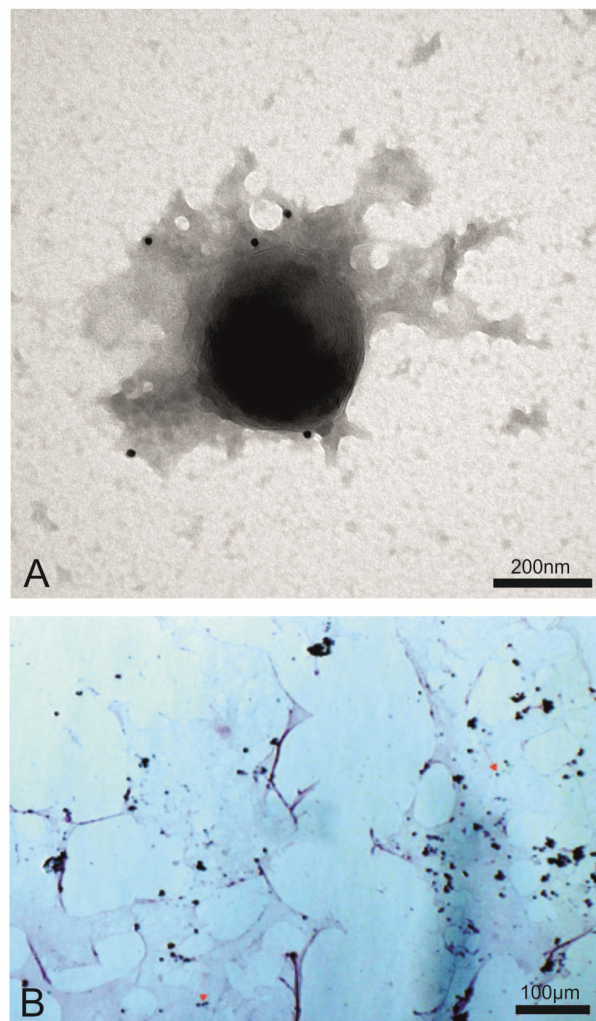
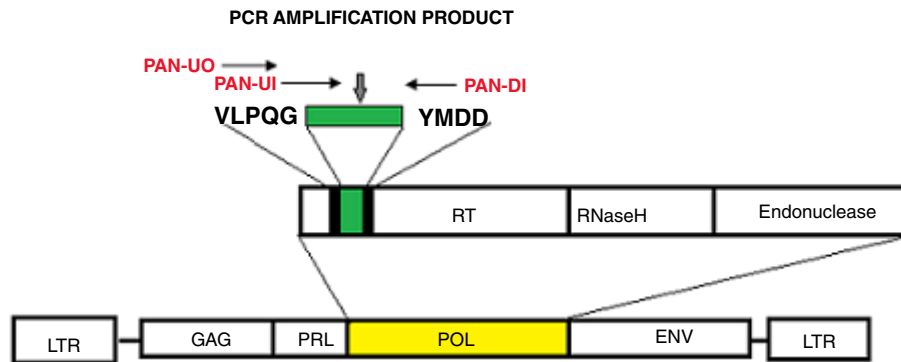


Figure 1. Purified human 400 nm giant virus labeled with anti FeLV p27 gag moAb. (A) Electron microscopy immunogold shows a ~400 nm giant virus isolated from human T cell leukemia marked with anti-FeLV p27gag moAb (picture representative of 100 repeats). (B) The same viral pellet during Gram staining shows blue granules that diagnose giant viruses (red arrows indicate some of these, but blue granules can be seen all over the slide). *Mimiviruses* (giant viruses) were first discovered in the amoebas. The amoebas had Gram positive granules that proved to not be bacteria but giant viruses, mimicking microbes. In the previous manuscript¹, we showed the presence in human cells of Gram positive giant viral particles associated with viral factories, both sharing the retroviral antigenicity. The viral factories are located inside the cells. What we are presenting here are giant viral particles isolated from human T cell acute lymphoblastic leukaemia by sucrose gradient. This human giant virus differs from amoebas's giant viruses in that it displays the properties of classical retroviruses.

RNA extraction from the giant viral particles, Pan-retrovirus PCR, sequencing and molecular phylogenetic analyses

The human giant particles contained retroviral RNA. Identification of the retroviral sequences, extracted from the isolated giant viral particles, was accomplished by PCR with degenerate primers targeting a mostly highly conserved sequence in the reverse transcriptase gene of retroviruses, between two conserved domains VLPQG and YMDD. This amplification approach with degenerate primers was initially described by Tuke *et al.* and it is called Pan-retrovirus PCR¹¹. This PCR system has the ability to detect a ~140 bp amplicon of the Pol gene across many different retroviruses. HIV-1, HTLV-1, Simian D type virus Mason Pfizer monkey virus, Moloney murine leukaemia virus, HERV-W, ERV9 and unknown lymphoma associated retroviruses have been successfully detected with this approach¹⁴⁻¹⁶. The principles of the technique and the primers are illustrated in Figure 2. We performed the Pan-retrovirus -PCR experiments exclusively on sucrose gradient purified giant viruses that were first examined using EM immunogold.



>RGV

GCTGTGCAGATGTAACAGAATCTTCTGCTAATGCCCAAAGAAGTGTAGCAAGACTGGAAAGTAGCATCT
TCTTCATGCACCCATTATGGATGACCATCGCTGAAGCTTGAGACT

>HIV-1

ATGGAAAGGATCACCAGCAATATTCAAAGTAGCATGACAAAATCTTAGAGCCTTTAAAAACAAAATCC
AGACATAGTTATCTATCAA

>HTLV-1

GTTTAAAAATAGTCCACCCCTGTTTCAAATGCAGCTGGCCCATATCTGCAGCCATTCCGGCAAGCTTTCCC
CCAATGCACTATTCTCAG

>ERV-9

GTTTAGGGATAGCCCTCATCTGTTTGGTCAGGCCCTAGCCAAAGATCTAAGGCCACTTCTCAAGTCCAGGC
ACTCTCCTGGTTCAA

>MSRV

GTTTAGGGATAGCCCTCATCTGTTTGGTCAGGCCCTAGCCAAAGACTTGAGCCAATTCTCATACCTGGACAC
TCTTGTCCTCAG

>MoML V

TTTCAAAAACAGTCCACCCCTGTTTGGATGACCCACTGCAACAGAGACCTAGCAGACTTCCGGATCCAGCACC
CAGACTTGATCCTGCTACAG

>MPMV

TATGGCCAACAGTCCCTACCTTATGTCAAAAATATGTGGCCACAGCCATACATAAGGTTAGACATGCCTGGAA
ACAAATGTATATTATACAT

>HERV-W

GTTTAGGGATAGCCCTCATCTGTTTGGTCAGGCCCTAGCCAAAGACTTGAGCCAGTCTCATACCTGGACAC
TCTTGTCCTTCGG

>FRD

TTTCAGAGATAGTCCCATTTGTTTGGGCAAGCCTTGGCTAGATATTTGCAGGACCTAAGTCTTTATATGGGA
GGGCATCTCCTACAG

Figure 2. The Pan-retrovirus PCR approach as described by Tuke *et al.*¹¹. The technique uses degenerate primers capable of amplifying a region in Reverse Transcriptase, between two conserved motifs VLPQ and YMDD in the Pol gene across different retroviruses. The Pol sequence amplified from the human giant viruses is indicated as RGV (bold red). Corresponding same size region of different retroviruses, amplified with the same technique^{11,14}, are reported.

A predominant band of the expected size of >150 bp was amplified from RNA extracted from the human giant viruses (Figure 3, lane 1). Multiple alignments with equivalent and already established Pol region of retroviruses, amplified by the same technique, confirm that our 150 bp amplicon is a Pol-like gene. A molecular phylogenetic analysis based on this region suggests that this amplicon (indicated as RGV) belongs to a distinct evolutionary branch among the whole retroviral families (Figure 4).

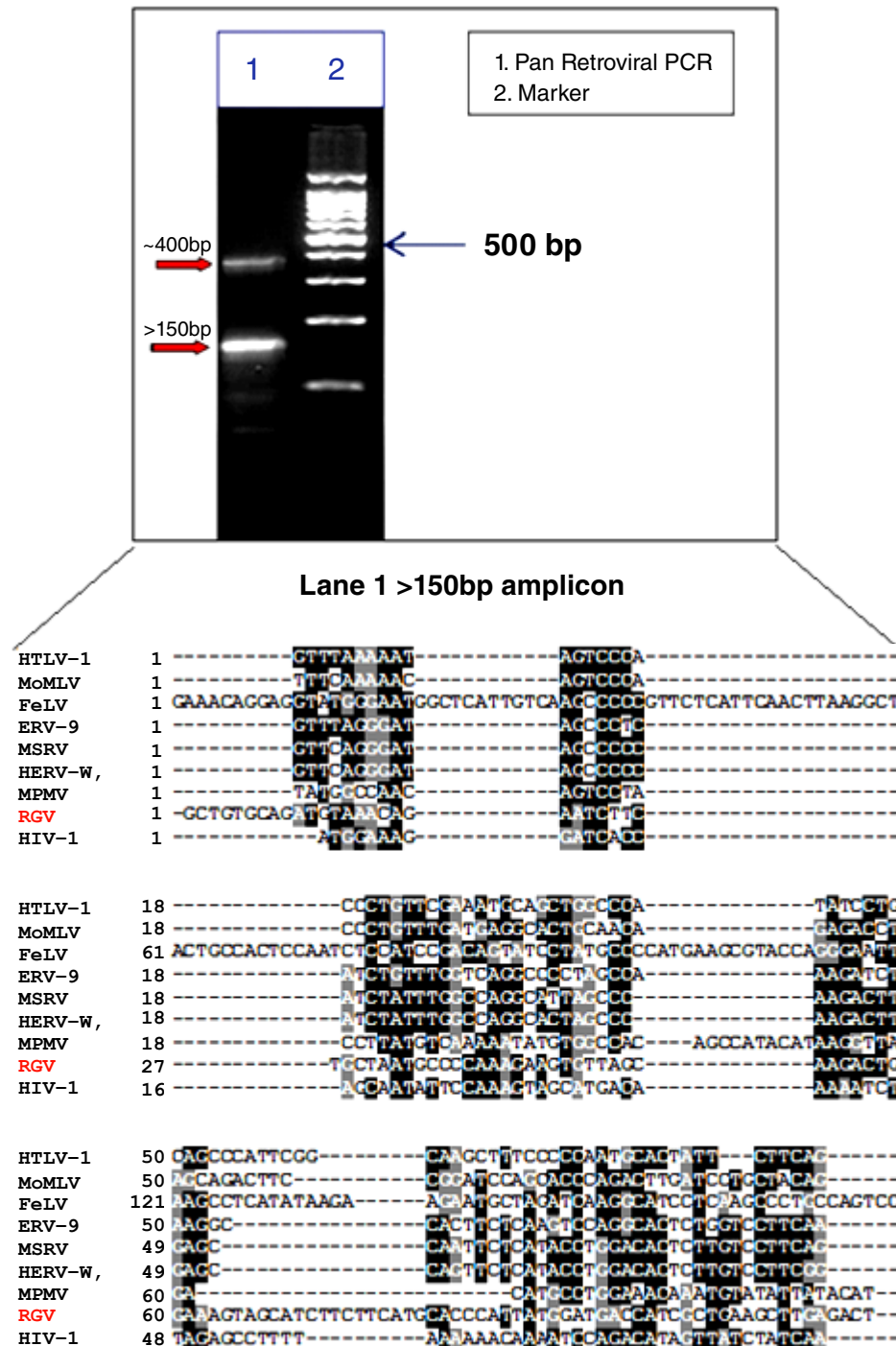


Figure 3. RNA extracted from giant viruses was amplified with a Pan-retrovirus PCR, as described by Tuke *et al.*¹¹. A ~400 bp band and a >150 bp band were amplified, lane 1 on the agarose gel. Lane 2 is the marker (100-200-300-400-500-600-700-800-900-1000-1500bp). Multiple alignments of our 150 bp band (RGV bold red) with equivalent and already established Pol region of retroviruses, amplified by the same technique, confirm that our 150 bp amplicon is a Pol-like gene.

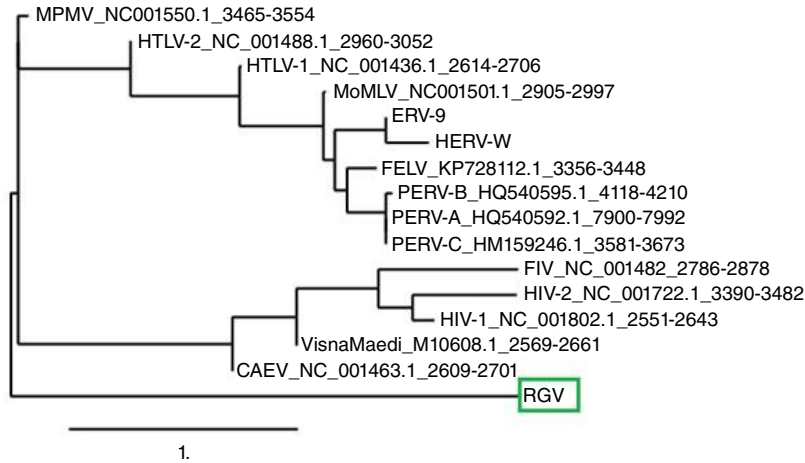


Figure 4. Phylogenetic tree of Pol region between the two conserved motifs VLPQG and YMDD of the reverse transcriptase. The Retro-Giant Virus (RGV) >150 amplicon (red circle) was analyzed and compared with the same conserved region of other retroviruses. See the *Methods* section for information on phylogenetic analysis. The RGV amplicon (green box) appears as a new, distinct, ancestral branch.

Along with the 150 bp band, a ~400bp amplicon was also detected (Figure 3, lane 1). Multiple alignment and phylogenetic analysis showed that the 400 bp band aligns entirely on the human chromosome 7 and clusters with human endogenous retroviruses (HERVs) genes (Figure 5). This finding replicates consolidated reports of almost intact human endogenous retrovirus genomes in chromosome 7¹⁷⁻²⁴. Additional information is in [Dataset 1–Dataset 3](#).

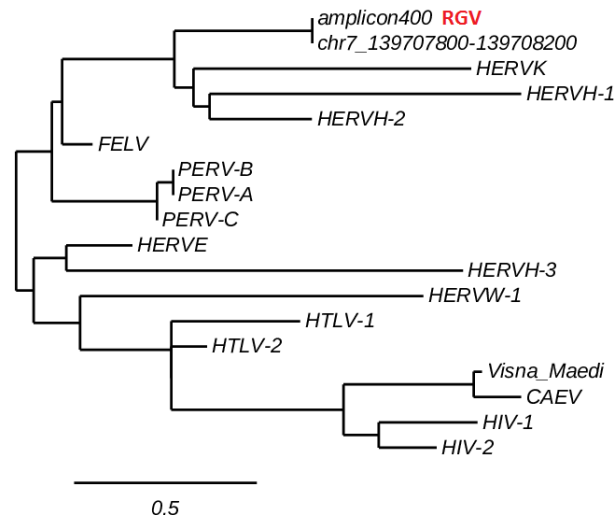


Figure 5. Phylogenetic relationship of the ~ 400 bp amplicon amplified with the Pan-retrovirus PCR from the RNA of the giant viruses. The ~400bp amplicon aligns entirely on a fragment of the human chromosome 7 and clusters with HERVs. This finding replicates established data of HERVs mapping in the human chromosome 7. See the *Methods* section and [Dataset 2](#) for information on phylogenetic analysis.

Phylogenetic tree was made with webserver <http://www.phylogeny.fr>. Musclev3.8.31 was used for multiple alignment, Gblocks for alignment curation, PhyML for phylogeny and TreeDyn for treedrawing. A non-parametric, Shimodaira-Hasegawa-like approximate Likelihood-Ratio branch test (SH-like aLTR) was used as statistic test.

Reverse transcriptase activity

The retro-giant viruses has reverse transcriptase activity. 10 µl the lysated viral pellet produced cDNA from an RNA template (Figure 6).

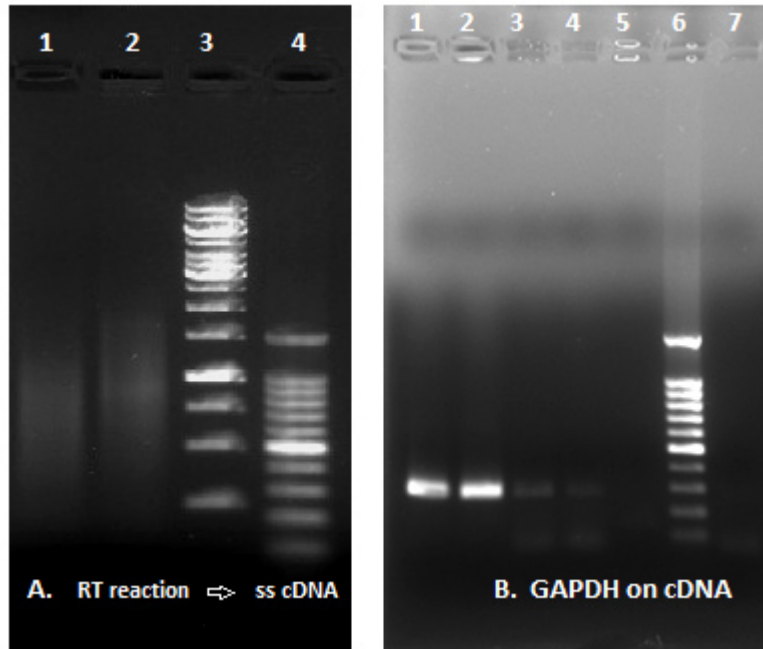


Figure 6. Reverse Transcriptase (RT) activity of the human Retro-Giant viruses. (A) RT reaction and synthesis of ss c-DNA: Lane 1, reaction with a commercial RT enzyme; Lane 2, reaction with viral pellet (reaction without RT enzyme); Lane 3, GeneRuler 1Kb DNA ladder; Lane 4, 100bp DNA ladder. (B) GAPDH amplification from ss-cDNA template: Lanes 1 and 2, reaction with commercial RT enzyme; Lanes 3 and 4, reaction with the lysated viral pellet; Lane 5, negative control; Lane 6, DNA ladder; Lane 7, additional negative control.

Summary of results (Figure 7 and Figure 8)

1. The fraction extracted from human T cell leukaemia cells and purified through 25% sucrose gradient are human giant viruses with a retroviral core.
2. The anti FeLV p27 gag antibody labelled the giant viral particles at EM immunogold
3. The Retro-Giant viruses have reverse transcriptase activity.
4. Pan-retrovirus PCR (Tuke protocol) suggested the presence inside the giant particles of retroviral amplicons.
5. The whole genome shotgun sequencing confirmed the presence of oncogenic retroviral core genes.
6. Like giant mimiviruses in the amoebas, these human giant viruses retain the Gram stain and they are associated with viral factories, but the substantial difference is their T tropism and the retroviral core: they are human Retro-Giant viruses (RGV), missing from the current retroviral classification.

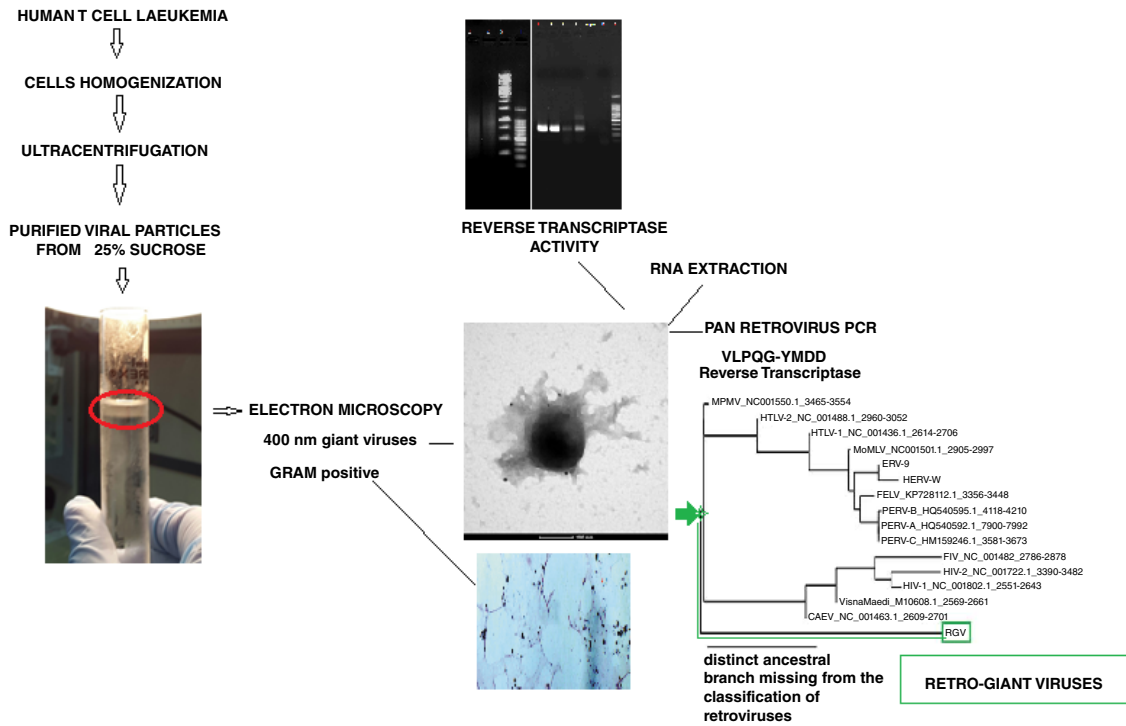


Figure 7. Steps for purification and characterization of human Retro-Giant viruses. Giant viruses were isolated from human leukaemia T cells on 25% sucrose gradient (sedimentation fraction of giant viruses in general). Cell nuclei was discharged before layering onto the sucrose. The isolated viral pellet was examined using EM immunogold, which confirmed the presence of ~400 nm giant viruses with retroviral antigens (anti-FelV gag). The viral pellet was also stained with the Gram stain. The viral lysate had reverse transcriptase activity. A Pan retroviral PCR of the RNA extracted from the giant viral particles and whole genome sequencing showed the presence in the giant viruses of retroviral core genes.

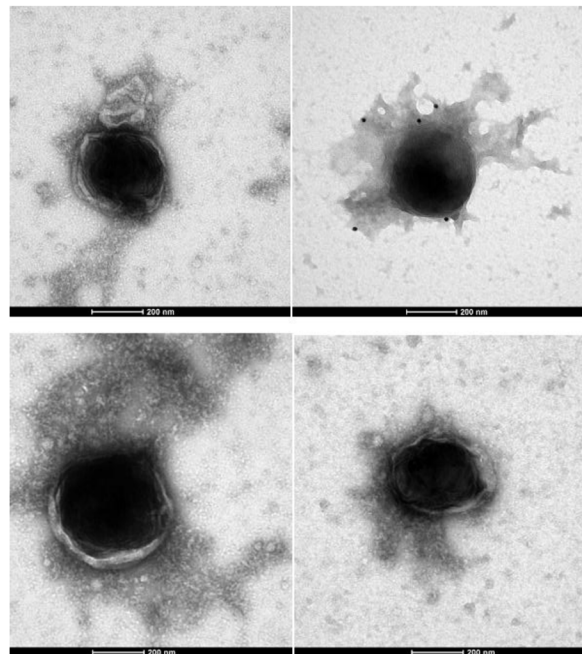


Figure 8. EM of isolated Retro-Giant viruses from human T cell acute lymphoblastic leukaemia by sucrose gradient. These pictures are representative of 100 micrographs. Contact the corresponding author to inspect the entire collection. These human giant viruses have a retroviral antigenicity (positive immunogold with moAbs anti-FelV retroviral antigens, black dots in the picture), reverse transcriptase activity and retroviral core genes. The human Retro-Giant viruses retain the Gram stain and inside the cells they are associated to their viral factories, also displaying the retroviral antigenicity¹.

Whole genome shotgun sequences

The sequences resulting from the whole genome shotgun sequences are reported in [Dataset 5](#). The very large genome (~1861 ORFs) appears more complex than Mimiviruses and contains retroviral core genes. Sequences indicated in yellow are the retroviral core genes (see [Dataset 5: Shotgun annotations](#)).

In particular, the oncogenic Gag-AKT fusion gene, related to FeLV ([Dataset 5: Gag-AKT fusion protein analogy](#); E-value 2.00E-33; >95.04% identity), confirms our proteomics results and the documented retroviral antigenicity at the EM immunogold.

Mimiviruses and Archaea genes are also present. The Archaea features are very dominant (thermosome, operons, histone-like proteins, plasmids and anaerobic metabolism). The viral transcription apparatus is also well represented. Viral Notch and other viral oncogenes involved in retroviral induced leukaemia are also present. Some sequences also suggest that this giant microbial entity has its own metabolic apparatus and its own immune system.

In conclusion, this complex human giant is the summary of giant viruses, mimiviruses and archaea. The presence of reverse transcriptase and mammalian core retroviral gene establish the presence in acute human T cells Leukaemia of oncogenic Retro-Giant Viruses with archaea features.

Dataset 5. Gag-AKT fusion protein analogy, Shotgun annotations, and Seq-ORF.fasta files

<https://dx.doi.org/10.5256/f1000research.15118.d218560>

Discussion

Robert Gallo reported the first human retrovirus HLTV in 1980. What we report here is the discovery of the first Mimivirus-sized human giant virus with a retroviral core.

In our previous work, conducted initially on human tissues with anti-FeLV gag p27 moAb, EM depicted previously unreported ~400 nm gigantic particles associated with large aggregates, resembling viroplasms, recognized by anti-FeLVp27 gag Ab¹. The particle diameters were more than four times the 100 nm size expected in retroviruses. These large particles and associated structures discovered in human cells appeared to morphologically parallel previously reported amoebas Mimiviruses (giant viruses and their viral factories)⁷.

Gram positive blue granules that disclosed the existence of giant viruses in the amoeba, similarly detected this newly giant virus in human cells both in our previous study¹ and current study.

Proteomic analyses suggested the presence of histone H4 variants common to environmental giant DNA viruses, but the striking difference was the unique mammalian retroviral nature of the human giant particles.

However, working on human tissues was confusing and the distinction between the virus and the cells was blurred. How to prove if we were really facing ancestral giant viruses with a retroviral core?

In order to distinguish the giant agent from the human cells, in the present study we isolated the viruses, examined their morphology using EM, extracted their nucleic acid performed a Pan-retrovirus PCR and a whole genome sequencing.

The presence of human Retro-Giant viruses was confirmed step by step. A white ring sedimented on a 25% of sucrose gradient - the same sedimentation fraction of the giant DNA viruses isolated from the amoebas⁷. EM depicted ~400nm giant viral particles that showed the ability to retain the Gram stain, but the striking difference was their unique mammalian retroviral nature. Distinct from the amoebas' Mimiviruses, the viral particles were immunolabelled with anti FeLV p27gag moAb, they contained retroviral RNA and showed reverse transcriptase activity.

RNA extracted exclusively from the viral particles, isolated on a sucrose gradient, was initially amplified with a Pan-retrovirus PCR, able to detect a conserved fragment in the Reverse transcriptase across different retroviral genera, and subsequently a shotgun whole genome sequencing was performed. To avoid any other source of contamination, we made sure that the cells' nuclei were removed before layering on the sucrose gradient. The shotgun whole genome sequencing confirmed the presence in the giant viruses of retroviral core genes and acute transforming gag-akt fusion proteins related to feline leukaemia retrovirus lineage.

The T tropism of the Retro-Giant viruses relies on their retroviral nature, however, it is very improbable, as recently described²⁵, to find DNA mimiviruses in human T lymphocytes. Nevertheless, the discovery of the Retro-Giant viruses was made, not only because of their ability to bind the anti-FeLV antibodies, but also for fundamental elements that we took from the discovery of the amoebas giant mimiviruses in 2003². How could we conceive the possibility of colouring the Retro-Giant viruses with the Gram staining without the previous discovery of mimiviruses in the amoeba? Our 400 nm particles would have been erroneously perceived as giant vesicles and not as gram positive giant viruses with prokaryotes-archaea features. The Retro-Giant viruses represent a unique viral entity that suggests that defective retroviruses were possibly not sufficient for replication and required the interchange of genetic information with giant viruses' large biosynthetic assortment.

It follows by viewing our human Retro-Giant virus as a system that evolved from ancestral viruses to surround and shuttle retroviruses, providing a wider pathway for their dissemination. The `viral factories` and viral histone H4, described in our previous study¹, suggests a protected system that hijacks host immunity and epigenetics to enhance viral replication.

The fact that the Retro-Giants can be detected with an anti-FeLV gag is simply an amusing co-incidence. It might be that prototypical leukaemia viruses were the first organisms to put fragments of an evolving protein machinery together to make something useful shared among ancient retroviruses, prokaryotes and giant viruses. Feline retroviruses share conserved ancestral epitopes among different mammalian retroviruses²⁶⁻³¹. In addition, the presence of a shared 5'-leader sequence in ancestral human and mammalian retroviruses and its transduction into Feline Leukemia virus has been recently documented³². The constant presence in our giants of gag-pol leukaemogenic sequences was demonstrated by EM immunogold, genomics and proteomics.

Conclusion

In conclusion, we report not an archetypal human retrovirus nor even a large human retrovirus, but a human giant virus with a mammalian retroviral core. Although sharing some morphological features with mimiviruses and archaea, this human giant virus differs substantially from DNA amoebal giant viruses for its unique presence of mammalian retroviral core genes.

The whole genome shotgun sequencing confirmed the discovery of a human Retro-Giant in human acute T cells leukemia. This human giant microbial entity is the expression of a fascinating synthesis between archaea, giant viruses and retroviruses. The Retro-Giant Viruses possibly reflects the transition from the RNA to DNA world. This discovery may change our perspective in retrovirology and the way we conceive retroviruses.

With the Retro-Giants, the concept of giant virus is applied for the first time to the dogmas of retrovirology. Not the basic gag-polenv backbone and the small dimension anymore, but Gram positive giant particles with the archaeal-mimiviruses features and mammalian retroviral genes.

Temin in his 1975 Nobel address showed that a normal non carcinogenic avian virus interacts with something in human cell to create Rous Sarcoma Virus. He also suspected an ancestor for the reverse transcriptase. However, at that time the concept of Giant Viruses was missing, we had to wait till 2003, but the Retro-Giants seem to confirm Temin's prediction about the ancestors and the mechanism of cross-speciation transition of archetypal retroviruses.

The unusual features of the Retro-Giant viruses challenge our current concepts of retrovirology and the Retro-Giants will not have an easy life. It is difficult to accept the concept of viruses being giant, but it becomes almost unbearable when the giants are *Retro-Giants*.

“What? A giant virus with prokaryotic features and a retroviral core? If they are so gigantic, why has nobody seen them before?” How to accept the provocative idea that the Retro-Giant viruses, could be ancestral creatures evolved earlier than archetypal retroviruses, playing a role in human leukaemia ? These kind of questions reveal how complex scientific processes shape contemporary medical discoveries and their reception.

The giant mimiviruses in the amoeba are prehistorical creatures, evolved millions of years ago, since the dawn of evolution of eukaryotic cells^{33,34}. They are gigantic, yet nobody saw them until 2003².

For the discovery of the *Retro-Giant* viruses, their ability to bind a screen of antibodies anti-Feline retroviruses and some of the biochemical properties of giant viruses proved to be lucky.

The Retro-Giant viruses despite their archaea- giant viruses features should be included in the current classification of retroviruses because their oncogenic retroviral genes may be the ones responsible for some types of human leukaemia.

We could isolate constantly the Retro-Giants form acute human T cells leukaemia just with a routine sucrose gradient. Every time, in every replicate fulfilling some of Koch's postulates.

Actions to eradicate leukaemogenic retroviruses is currently not possible, but targeting the constant presence of the ancestors may provide a new methodology.

Not archetypal retroviruses, but Gram positive ancestral creatures with leukaemogenic retroviral core genes: this is the essence of the human *Retro-Giants* that were missing.

Data availability

All slides and EM grids are available to be examined; please contact the corresponding author.

F1000Research: Dataset 1. 150 bp amplicon alignment against other VLPQG-YMDD Pol sequences of different retroviruses. DOI, [10.5256/f1000research.15118.d208073](https://doi.org/10.5256/f1000research.15118.d208073)¹²

F1000Research: Dataset 2. 400bp amplicon sequence and its alignment against other retroviral families. DOI, [10.5256/f1000research.15118.d208074](https://doi.org/10.5256/f1000research.15118.d208074)¹³

F1000Research: Dataset 3. Uncropped and unedited blots. DOI [10.5256/f1000research.15118.d208075](https://doi.org/10.5256/f1000research.15118.d208075)³⁵

F1000Research: Dataset 4. Illumina FastQ and FastQC raw data. [10.5256/f1000research.15118.d218559](https://doi.org/10.5256/f1000research.15118.d218559)³⁶

F1000Research: Dataset 5. Gag-AKT fusion protein analogy, Shotgun annotations, and Seq-ORF.fasta files. [10.5256/f1000research.15118.d218560](https://doi.org/10.5256/f1000research.15118.d218560)³⁷

Grant information

This work was supported in part by St Vincent Health Care Group of Dublin, Ireland.

Acknowledgements

The anti-FeLV-related moAbs were kindly provided as a gift by Dr Chris Grant of Custom Monoclonals International (West Sacramento, CA 95691, USA).

We thank Microgem Laboratory Research (Napoli, Italy) for their technical assistance.

References

- Lusi EA, Maloney D, Caicci F, *et al.*: **Questions on unusual Mimivirus-like structures observed in human cells [version 1; referees: 2 approved]**. *F1000Res*. 2017; 6: 262. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- La Scola B, Audic S, Robert C, *et al.*: **A giant virus in amoebae**. *Science*. 2003; **299**(5615): 2033. [PubMed Abstract](#) | [Publisher Full Text](#)
- Yamada T: **Giant viruses in the environment: their origins and evolution**. *Curr Opin Virol*. 2011; **1**(1): 58–62. [PubMed Abstract](#) | [Publisher Full Text](#)
- Van Etten JL, Lane LC, Dunigan DD: **DNA viruses: the really big ones (giruses)**. *Annu Rev Microbiol*. 2010; **64**: 83–99. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Claverie JM, Ogata H, Audic S, *et al.*: **Mimivirus and the emerging concept of “giant” virus**. *Virus Res*. 2006; **117**(1): 133–44. [PubMed Abstract](#) | [Publisher Full Text](#)
- Raoult D, Audic S, Robert C, *et al.*: **The 1.2-megabase genome sequence of Mimivirus**. *Science*. 2004; **306**(5700): 1344–50. [PubMed Abstract](#) | [Publisher Full Text](#)
- Campos RK, Boratto PV, Assis FL, *et al.*: **Samba virus: a novel mimivirus from a giant rain forest, the Brazilian Amazon**. *Virology*. 2014; **11**: 95. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Thomas V, Bertelli C, Collyn F, *et al.*: **Lausannevirus, a giant amoebal virus encoding histone doublets**. *Environ Microbiol*. 2011; **13**(6): 1454–66. [PubMed Abstract](#) | [Publisher Full Text](#)
- Hepat R, Song JJ, Lee D, *et al.*: **A viral histone h4 joins to eukaryotic nucleosomes and alters host gene expression**. *J Virol*. 2013; **87**(20): 11223–30. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Poiesz BJ, Ruscetti FW, Gazdar AF, *et al.*: **Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma**. *Proc Natl Acad Sci U S A*. 1980; **77**(12): 7415–9. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tuke PW, Perron H, Bedin F, *et al.*: **Development of a pan-retrovirus detection system for multiple sclerosis studies**. *Acta Neurol Scand Suppl*. 1997; **169**: 16–21. [PubMed Abstract](#) | [Publisher Full Text](#)
- Lusi EA, Caicci F: **Dataset 1 in: Discovery and description of the first human Retro-Giant virus**. *F1000Research*. 2018. <http://www.doi.org/10.5256/f1000research.15118.d208073>
- Lusi EA, Caicci F: **Dataset 2 in: Discovery and description of the first human Retro-Giant virus**. *F1000Research*. 2018. <http://www.doi.org/10.5256/f1000research.15118.d208074>

14. Perron H, Garson JA, Bedin F, *et al.*: **Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis.** The Collaborative Research Group on Multiple Sclerosis. *Proc Natl Acad Sci U S A.* 1997; **94**(14): 7583. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
15. Donehower LA, Bohannon RC, Ford RJ, *et al.*: **The use of primers from highly conserved pol regions to identify uncharacterized retroviruses by the polymerase chain reaction.** *J Virol Methods.* 1990; **28**(1): 33–46. [PubMed Abstract](#) | [Publisher Full Text](#)
16. Shih A, Misra R, Rush MG: **Detection of multiple, novel reverse transcriptase coding sequences in human nucleic acids: relation to primate retroviruses.** *J Virol.* 1989; **63**(1): 64–75. [PubMed Abstract](#) | [Free Full Text](#)
17. Reus K, Mayer J, Sauter M, *et al.*: **Genomic organization of the human endogenous retrovirus HERV-K(HML-2.HOM) (ERV6) on chromosome 7.** *Genomics.* 2001; **72**(3): 314–20. [PubMed Abstract](#) | [Publisher Full Text](#)
18. Mayer J, Sauter M, Rácz A, *et al.*: **An almost-intact human endogenous retrovirus K on human chromosome 7.** *Nat Genet.* 1999; **21**(3): 257–8. [PubMed Abstract](#) | [Publisher Full Text](#)
19. Tönjes RR, Czaderma F, Kurth R: **Genome-wide screening, cloning, chromosomal assignment, and expression of full-length human endogenous retrovirus type K.** *J Virol.* 1999; **73**(11): 9187–95. [PubMed Abstract](#) | [Free Full Text](#)
20. Alliel PM, Périn JP, Goudou D, *et al.*: **The HERV-W7q family in the human genome. Potential for protein expression and gene regulation.** *Cell Mol Biol(Noisy-le-grand).* 2002; **48**(2): 213–7. [PubMed Abstract](#)
21. Yu H, Liu T, Zhao Z, *et al.*: **Mutations in 3'-long terminal repeat of HERV-W family in chromosome 7 upregulate syncytin-1 expression in urothelial cell carcinoma of the bladder through interacting with c-Myb.** *Oncogene.* 2014; **33**(30): 3947–58. [PubMed Abstract](#) | [Publisher Full Text](#)
22. Mayer J, Stühr T, Reus K, *et al.*: **Haplotype analysis of the human endogenous retrovirus locus HERV-K(HML-2.HOM) and its evolutionary implications.** *J Mol Evol.* 2005; **61**(5): 706–15. [PubMed Abstract](#) | [Publisher Full Text](#)
23. Weiss RA, Stove JP: **Virology. Our viral inheritance.** *Science.* 2013; **340**(6134): 820–1. [PubMed Abstract](#) | [Publisher Full Text](#)
24. Bock M, Stoye JP: **Endogenous retroviruses and the human germline.** *Curr Opin Genet Dev.* 2000; **10**(6): 651–5. [PubMed Abstract](#) | [Publisher Full Text](#)
25. Abrahão J, Silva L, Oliveira D, *et al.*: **Lack of evidence of mimivirus replication in human PBMCs.** *Microbes Infect.* 2018; **20**(5): 281–283. [PubMed Abstract](#) | [Publisher Full Text](#)
26. Donner L, Fedele LA, Garon CF, *et al.*: **McDonough Feline sarcoma virus: characterization of the molecularly cloned provirus and its feline oncogene (v-fms).** *J Virol.* 1982; **41**(2): 489–500. [PubMed Abstract](#) | [Free Full Text](#)
27. Sherr CJ, Fedele LA, Benveniste RE, *et al.*: **Interspecies antigenic determinants of the reverse transcriptases and p30 proteins of mammalian type C viruses.** *J Virol.* 1975; **15**(6): 1440–8. [PubMed Abstract](#) | [Free Full Text](#)
28. Geering G, Aoki T, Old LJ: **Shared viral antigen of mammalian leukaemia viruses.** *Nature.* 1970; **226**(5242): 265–266. [PubMed Abstract](#) | [Publisher Full Text](#)
29. Ishida T, Pedersen NC, Theilen GH: **Monoclonal antibodies to the v-fes product and to feline leukemia: virus P27 interspecies-specific determinants encoded by feline sarcoma viruses.** *Virology.* 1986; **155**(2): 678–87. [PubMed Abstract](#) | [Publisher Full Text](#)
30. Davis J, Gildea RV, Oroszlan S: **Multiple species-specific and interspecific antigenic determinants of a mammalian type C RNA virus internal protein.** *Immunochemistry.* 1975; **12**(1): 67–72. [PubMed Abstract](#) | [Publisher Full Text](#)
31. Wünsch M, Schulz AS, Kock W, *et al.*: **Sequence analysis of Gardner-Arnstein feline leukaemia virus envelope gene reveals common structural properties of mammalian retroviral envelope genes.** *EMBO J.* 1983; **2**(12): 2239–2246. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
32. Kawasaki J, Kawamura M, Ohsato Y, *et al.*: **Presence of a Shared 5'-Leader Sequence in Ancestral Human and Mammalian Retroviruses and Its Transduction into Feline Leukemia Virus.** *J Virol.* 2017; **91**(20): pii: e00829-17. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Moreira D, López-García P: **Evolution of viruses and cells: do we need a fourth domain of life to explain the origin of eukaryotes?** *Philos Trans R Soc Lond B Biol Sci.* 2015; **370**(1678): 20140327. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
34. Forterre P, Gaïa M: **Giant viruses and the origin of modern eukaryotes.** *Curr Opin Microbiol.* 2016; **31**: 44–9. [PubMed Abstract](#) | [Publisher Full Text](#)
35. Lusi EA, Caicci F: **Dataset 3 in: Discovery and description of the first human Retro-Giant virus.** *F1000Research.* 2018. <http://www.doi.org/10.5256/f1000research.15118.d208075>
36. Lusi EA, Caicci F: **Dataset 4 in: Discovery and description of the first human Retro-Giant virus.** *F1000Research.* 2018. <http://www.doi.org/10.5256/f1000research.15118.d218559>
37. Lusi EA, Caicci F: **Dataset 5 in: Discovery and description of the first human Retro-Giant virus.** *F1000Research.* 2018. <http://www.doi.org/10.5256/f1000research.15118.d218560>

Open Peer Review

Current Referee Status: 

Version 2

Referee Report 12 November 2018

<https://doi.org/10.5256/f1000research.17785.r38527>



Didier Raoult

Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, Marseille, France

Accept as it is.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 13 July 2018

<https://doi.org/10.5256/f1000research.16470.r35746>



Didier Raoult

Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, Marseille, France

To authors

This work reports the description of a giant virus with cross-reactivity and genes in common with retroviruses. The authors propose to describe the first giant retrovirus obtained from human cells. This work is very preliminary, and it is unfortunate not to have the complete genome of this virus. The reviewer is very supportive of this preliminary work. However, he thinks that, at least, the RNA polymerase sequence of the giant viruses, for which there are primers that have been described for mimiviridae, would be added, which would give a much higher value to this paper.

All in all, this is a work that can attract the attention of the scientific community to an interesting emerging hypothesis.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 17 Jul 2018

Elena Angela Lusi, 1. St Vincent Health Care Group, University College of Dublin, Ireland., Ireland

Dear Prof Raoult,

Thank you for taking the time to review my manuscript. Your suggestions are well received and greatly appreciated. I expect to have the genome sequenced in a few weeks.

What I have accomplished so far in the characterization of this human Retro-Giant virus is :

1. Isolation of the giant viral particles from human T cell leukaemia;
2. Electron Microscopy immunogold of the giant viruses with a screen of anti-FeLV retroviruses Abs;
3. Gram stain of the viral pellet;
4. Amplification of the VLPQ-YMDD region of RT transcriptase gene from the RNA of the giant viruses;
5. Reverse Transcriptase activity of the giant viral particles;

all of which I believe to be of a great significance.

I have also completed the proteomics analyses of the isolated viral particles. Retroviral as well as giant viruses peptides were confirmed. One of the identified retroviral peptides has oncogenic properties.

I will provide you with additional data once this last round of experiments has been completed.

Might these Retro-Giants be the ancestors of archetypal retroviruses? That is the question and an interesting scenario in retrovirology.

Your discovery of the Giant Viruses and my finding of human Retro-Giant viruses seem to be consistent with [Howard Temin's views](#).

Best Regards
Elena Angela Lusi

Competing Interests: No competing interests.

Author Response 10 Sep 2018

Elena Angela Lusi, 1. St Vincent Health Care Group, University College of Dublin, Ireland., Ireland

Dear Prof Raout,

As per your request, please find the results of the whole genome sequence attached in the revised manuscript.

With this shotgun sequencing I can confirm the discovery of a human Retro-Giant in human T cells leukaemia.

This human virus is not a classic retrovirus, but a Gram positive Giant Virus, Mimivirus-like, with a unique mammalian transforming retroviral core and a T cells tropism.

The ORFs indicated in yellow are the retroviral core genes.

The presence of leukaemogenic retroviral sequences confirms the numerous repeats of the EM immunogold, the documented retroviral antigenicity, the preliminary PCR results and reverse transcriptase activity of the giant viral particles.

The viral genome and the morphological analyses show the peculiar features of this giant microbial entity that is expression of a fascinating synthesis between archaea, prokaryotes and giant viruses. In some aspects, this human giant virus is more similar to an archaea than to a virus, even though the viral apparatus is well present.

We are facing ancestral creatures with a retroviral nature. The Retro-Giant Viruses are possibly the expression of the transition from the RNA to DNA world.

This discovery may even change our perspective in retrovirology and the way we conceive retroviruses. Not the basic gag-pol-env backbone and the small dimension anymore, but Gram positive giant particles with prokaryotes features and retroviral genes related to mammalian oncogenic retroviruses.

Just observe and consider Temin's last diagram in his Nobel address. He shows that a normal non carcinogenic avian virus interacts with something in human cell to create Rous Sarcoma Virus. He also suspected an ancestor for the Reverse Transcriptase.

I have assumed that most phylogenies are hypotheses and are based on indirect evidence. However, your discovery of Giant Viruses and my Retro-Giants could be that something I suspect

Temin would think. Phylogeny is sometimes shield against the darkness, but our findings may have melted these shields.

A part from the fact that the Retro Giant viruses are ancestral to retroviruses, as a medical doctor working with people, I point to their leukaemogenic role. This really matters. We could isolate constantly the Retro-Giants from acute human T cells leukaemia just with a routine sucrose gradient. Every time, in every replicate fulfilling some of the Koch's postulates.

The Retro-Giant viruses have oncogenic retroviral genes. Actions to eradicate leukaemogenic retroviruses are currently not possible, but targeting the constant presence of the ancestors may provide a new methodology.

We can not ignore the presence of the Retro-Giants, their oncogenes and their presence in human leukaemia. Science seek truths and patients need a diagnosis and treatment.

The concept of human giant viruses with prokaryotes features and an oncogenic retroviral core is not an easy one.

Fundamental elements and concepts of your discovery of the Giant Viruses helped me in understanding that this time we were facing exceptional ancestral creatures where prototypical leukaemia viruses were the first organisms to put together fragments of an evolving protein machinery shared among ancient retroviruses, archaea and prokaryotes.

The Retro-Giant viruses confirm Temin's prediction and, despite their prokaryotes and giant viruses features, they should be included in the current classification of retroviruses because their oncogenic retroviral genes may be the ones responsible for some types of human leukaemia.

Best Regards
Elena Angela Lusi

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research