Overview of XMRV

Conflict of Interest: Patents Licensed to Abbott Laboratories
2010 Estimated US Cancer Deaths

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>29%</td>
<td>26%</td>
</tr>
<tr>
<td>Prostate</td>
<td>11%</td>
<td>15%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>9%</td>
<td>9%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>6%</td>
<td>7%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Esophagus</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>All other sites</td>
<td>23%</td>
<td>All other sites 24%</td>
</tr>
</tbody>
</table>

Source: American Cancer Society, 2010.
The Hereditary Prostate Cancer 1 Gene Encodes the Antiviral Protein RNase L

Carpten, J.. et al., Nature Genetics, 30, 181-4, 2002
Prostate Cancer Tissue $\rightarrow$ RNA $\rightarrow$ cDNA $\rightarrow$ Virochip & RT-PCR

Red VP= homozygous for R462Q RNase L Variant

# Correlation of XMRV with RNase L Genotype in 86 Prostate Cancer Patients

<table>
<thead>
<tr>
<th>RNase L Genotype</th>
<th>XMRV Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous (RR)</td>
<td>1*/52 1.9%</td>
</tr>
<tr>
<td>Heterozygous (RQ)</td>
<td>0/14 0%</td>
</tr>
<tr>
<td>Homozygous (QQ)</td>
<td>8/20** 40%</td>
</tr>
</tbody>
</table>

*Highest tumor grade (8) of all XMRV positive cases

** p<0.00002 for association between QQ and virus
Xenotropic Murine Leukemia Virus-Related Virus

Related to Murine Leukemia Virus

Silverman et al., NATURE REVIEWS Urology, 2010
Discovery of XMRV in study of prostate cancer patients

Integration Sites

2002 - - - - 2006 2007 2008 2009 2010 2011

HPC1 Mapped To RNase L

Androgen effect, host restriction, antiretroviral drugs, MLV-related sequences in CFS blood, absence of XMRV in CFS blood, presence and absence in prostate cancer, assay development, in respiratory tract, contamination studies

Macaque study, mouse origin of 22Rv1 virus, no XMRV in CFS or prostate cancer, distinct XMRV variants in prostate cancer

36 so far 2011…

In 22Rv1 cell line, prostatic malignant epithelium, CFS blood, not in German prostate cancer cases

80 60 40 20 0

Year

2006 2007 2008 2009 2010

XMRV Publications

XPR1 receptor

80 60 40 20 0

2006 2007 2008 2009 2010

2011…
Evidence for XMRV Infections in Humans (Part I)

• PCR (PCa\textsuperscript{1-5}, CFS-ME\textsuperscript{1,6,7,9*}, RTI\textsuperscript{10}, donors\textsuperscript{6,7,9*})
• Full length viral genomes cloned using PCa RNA\textsuperscript{1,2}
• No mouse GAPDH DNA by PCR\textsuperscript{2}
• Clustering of PCa with RNase L variant QQ\textsuperscript{1,2,3}
• Distinct XMRV variants in prostate cancer patients\textsuperscript{11}

\textsuperscript{[1]}Cleveland Clinic, \textsuperscript{2}UCSF, \textsuperscript{3}Emory, \textsuperscript{4}Baylor, \textsuperscript{5}Columbia/Utah, \textsuperscript{6}WPI/UNevada, \textsuperscript{7}NCI, \textsuperscript{8}UCLA, \textsuperscript{9}FDA-NIH (*P-MLV), \textsuperscript{10}Hamburg, \textsuperscript{11}CDC
Evidence for XMRV Infections in Humans (Part II)

- IHC for viral antigen (Gag) in tissue (PCa\textsuperscript{1,5})
- FISH for viral nucleic acid in tissue (PCa\textsuperscript{1,3})
- Immune responses (PCa\textsuperscript{3}, CFS-ME\textsuperscript{6,7})
- Infectious virus in blood (CFS-ME\textsuperscript{6})
- MLV-related sequences in blood (CFS-ME\textsuperscript{9})
- Integration sites mapped in tissue (PCa\textsuperscript{8})

\[1\textsuperscript{Cleveland Clinic, 2UCSF, 3Emory, 4Baylor, 5Columbia/Utah, 6WPI/UNevada, 7NCI, 8UCLA, 9FDA-NIH (*P-MLV)}\]
Evidence for XMRV Infections in Humans (FISH)

Urisman A.


Green: XMRV
Blue: DAPI
Red: Cytokeratin

Scale Bar=10μ m

Stromal fibroblast
Dividing stromal cell
Stromal hematopoietic cell
Evidence for XMRV Infections in Humans (IHC)

<table>
<thead>
<tr>
<th>VP62 (QQ)</th>
<th>VP88 (QQ)</th>
<th>VP51 (RR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>C</td>
<td>I</td>
</tr>
<tr>
<td>B</td>
<td>D</td>
<td></td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>

Urisman A.
Evidence for XMRV Infections in Humans (Serology)

CFS-ME
Lombardi et al., Science 2009

Prostate cancer
Arnold et al., Urology 2010

RNase L Variant

Graph 1: Barf3ER-SFFV Env (MFI) vs Human plasma 1:10 dilution

Graph 2: % Neutralization (RLU) vs Group of human sera (QQ, RQ, RR)
## Evidence for XMRV Infections in Humans (Integration Sites)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Site of integration</th>
<th>Gene(s) within indicated distance of integration site (distance [kbp]) $^c,d$</th>
<th>miRNA within ±2 Mbp of integration site</th>
<th>Common fragile site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytoband$^b$</td>
<td>Nucleotide position</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP29</td>
<td>3p13$^c$</td>
<td>73283567</td>
<td>FLJ10213 (90.1), PPP4R2</td>
<td></td>
</tr>
<tr>
<td>VP229</td>
<td>16q22.1$^f$</td>
<td>66531394</td>
<td>PSMIB10$^b$ (−3.1), LCAT$^b$, CTRL$^b$, SLC12A4$^b$, PSKH1, DPEP3, DPEP2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NRR1L, EDC4$^c$, DDX28, DUS2L, NUTF2$^c$, THAP11, CENPT</td>
<td>mir-328</td>
</tr>
<tr>
<td>VP234</td>
<td>17q23.2$^c$</td>
<td>55946474</td>
<td>APPBP2 (11.9)</td>
<td></td>
</tr>
<tr>
<td>VP268</td>
<td>11q13.4$^f$</td>
<td>72182279</td>
<td>PPMID$^b$, C17orf64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16q22.1$^c$</td>
<td>66678692</td>
<td>STARD10$^b$ (0.2), ATG16L2, FCHSD2$^c$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7p15.1$^c$</td>
<td>28689605</td>
<td>CENTD2$^b$,</td>
<td></td>
</tr>
<tr>
<td>VP283</td>
<td>19p13.2$^f$</td>
<td>11115762</td>
<td>SPC24 (11.7), ANKRD25, LDLR$^c$</td>
<td></td>
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<tr>
<td></td>
<td>3q29</td>
<td>198606680</td>
<td>LOC55908, DOCK6, SMARCA4, DLG1 (−96.8)</td>
<td></td>
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<tr>
<td>VP338</td>
<td>12q13.11$^c$</td>
<td>45110969</td>
<td>SLC38A2$^c$ (−58.2)</td>
<td></td>
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<tr>
<td>VP363</td>
<td>16q22.1$^f$</td>
<td>67648746</td>
<td>HAS3 (−48.9), TMCO7</td>
<td></td>
</tr>
<tr>
<td>VP432</td>
<td>1q32.1$^c$</td>
<td>202666825</td>
<td>PPP1R15B (−19.3), PIK3C2B$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6q21$^f$</td>
<td>111385428</td>
<td>PLEKHA6, MDM4$^b$</td>
<td></td>
</tr>
<tr>
<td>VP433</td>
<td>14q12</td>
<td>30803190</td>
<td>GTF3C6 (−1.0), BXDC1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15q22.31$^f$</td>
<td>63070335</td>
<td>HECTD1$^c$ (−56.8), HEATR5A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OSTbeta, RASL12, LOC390594</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Includes only integrations with evidence of proviral DNA in paired normal tissues. $^b$ Chromosome arm and band. $^c$ Distance from integration site to gene start or stop. $^d$ Gene is known to be expressed in prostate tissue. $^f$ Integrations with evidence of proviral DNA in paired normal tissues are marked with a superscript $f$. $^c$ Integrations with evidence of proviral DNA in paired normal tissues are marked with a superscript $c$.
XMRV Integration Site in APPBP2 (PAT1/ARA67) in Prostate Tumor DNA (Patient VP234)

XMRV U5 End

Human Genomic DNA

5’-actaccagctggggtctttcaGCCTAGCCTCCATTGTGGGTTTTGTTTTGAGACAGGGTC - 3’

Dong et al., 2007
Distinct XMRV Variants Identified in Prostate Cancer Patients

“a viral strain in three prostate cancer patients that is distinct from the XMRV seen in previous studies is significant and demonstrates a broader viral diversity…..an expected result consistent with virus evolution during spread and persistence.”

Switzer et al., PLoS ONE, 2011
Evidence Against XMRV Infections in Humans (Part I)

- PCR negative (PCa\textsuperscript{1-5}, CFS-ME\textsuperscript{1,4-11})
- Serology negative (PCa\textsuperscript{1,11}, CFS-ME\textsuperscript{5,11})
- IHC negative (PCa)\textsuperscript{2,3}
- Evidence of lab contamination\textsuperscript{3,4,11}
- Evidence of reagent contamination\textsuperscript{5,11}
- Lack of live virus in plasma (CFS-ME)\textsuperscript{11}

\textsuperscript{1}Robert Koch-Institute, \textsuperscript{2}NCI, \textsuperscript{3}Mayo Clinic, \textsuperscript{4}Imperial College London, \textsuperscript{5}Japanese Red Cross, \textsuperscript{6}MRC, \textsuperscript{7}Radboud University, \textsuperscript{8}CDC, \textsuperscript{9}Chinese Academy of Medical Sciences, \textsuperscript{10}Brigham and Women’s Hospital, \textsuperscript{11}University of Utah
Evidence Against XMRV Infections in Humans (Part II)

• XMRV in 22Rv1 identical (almost) to strain VP62\textsuperscript{1-4}
• Lack of sequence diversity typically expected for independently acquired infections\textsuperscript{4}
• Evidence that XMRV in 22Rv1 cell line derived from mice and not the patient\textsuperscript{1,2}

\textsuperscript{1}NCI, \textsuperscript{2}Tufts, \textsuperscript{3}Hutchinson, \textsuperscript{4}University College London
Evidence Against XMRV Infections in Humans (IHC)

Prostate Cancer

Aloia et al., Cancer Research, 2010
### TABLE 4. Characteristics of XMRV integration sites identified in human prostate cancer tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Site of integration</th>
<th>Gene(s) within indicated distance of integration site (distance [kbp])</th>
<th>miRNA within ±2 Mbp of integration site</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytoband&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Nucleotide position</td>
<td>±50 kbp</td>
<td>±100 kbp</td>
</tr>
<tr>
<td>VP29</td>
<td>3p13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73283567</td>
<td>FLJ10213 (90.1), PPP4R2</td>
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<td>CENTD2&lt;sup&gt;ψ&lt;/sup&gt;,</td>
</tr>
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<td>16q22.1&lt;sup&gt;c&lt;/sup&gt;, 7p15.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66678692, 28689605</td>
<td>NFATC3&lt;sup&gt;c&lt;/sup&gt; (1.8), DUS2L, CREB5&lt;sup&gt;c&lt;/sup&gt; (−2.6)</td>
<td>DOX28, DPEP2</td>
</tr>
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<td>19p13.2&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>SPC24 (11.7), ANKRD25, LDLR&lt;sup&gt;*&lt;/sup&gt;</td>
<td>LOC55908, DOCK6, SMARCA4, DLG1 (−96.8)</td>
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</tr>
</tbody>
</table>
Large Differences in Detection Rates
(possible reasons)

1. Positives are lab contamination and XMRV is not an actual human infection or:

2. XMRV is a human infection but results vary because of:
   - Geographical distribution
   - Sequence variants
   - Clinical criteria for patient selection
   - Lack of standardized methods
   - Different types of human specimens assayed
   - Lack of widely available positive control human specimens

3. Different gammaretrovirus(es) are present in humans
XMRV in 22Rv1 Cells and Patient Specimens
(Possible Explanations)

1. “It’s all contamination”—John Coffin, or:

2. The hypothetical recombination events that generated the 22Rv1 virus in the lab also occurred in nature

3. The relative lack of sequence diversity among XMRV isolates might reflect:
   - Infections but minimal or no replication
   - Replication in cells lacking APOBEC3 (e.g. prostate cancer cells)
   - Direct infection from mice or from biologic products produced in mice or in mouse cells

4. There is more sequence diversity than is apparent (Switzer et al., 2011)
IV Inoculation of Rhesus Macaques with XMRV

Animal Protocol:

Baseline bleeds

Blood collections

XMRV i.v.

XMRV vaccine

XMRV reinfection i.v. n=2

LN coll.

Necropsies:
- x
- RLm-1
- ROu-4
- x
- RLq-10
- xx
- RII-10
- RYh-10
Rhesus Macaque: XMRV Infection of Blood Cells

Viremia:

Days Post-Infection:

PBMC Infection:

Animal:

- RII-10
- RLq-10
- RYh-10
- Limit of detection

Days Post-Infection:

-12  4   7   9   11  14  21  28  35  42  C

Animal:

- RYh
- RIL
- RLq

Env
GAPDH
Env
GAPDH
Env
GAPDH
Biology: XMRV Traffics to Monkey Prostate

GAG STAINING

Monkey RLm-1

Prostate Epithelium Is An Early Target for XMRV

Monkey Rou-4
## XMRV Infection in Primates

<table>
<thead>
<tr>
<th>Early Targets</th>
<th>Late Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid Organs</td>
<td></td>
</tr>
<tr>
<td>Peripheral Blood (CD4+)</td>
<td></td>
</tr>
<tr>
<td>Spleen &amp; Lymph Nodes</td>
<td></td>
</tr>
<tr>
<td>Bone Marrow</td>
<td></td>
</tr>
<tr>
<td>Large &amp; Small Intestine</td>
<td></td>
</tr>
<tr>
<td>Lung (macrophages)</td>
<td></td>
</tr>
<tr>
<td>Pancreas (epithelium)</td>
<td></td>
</tr>
<tr>
<td>Genital Tract</td>
<td></td>
</tr>
<tr>
<td>Prostate (stroma only)</td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td></td>
</tr>
<tr>
<td>Seminal vesicle &amp; Epididymis</td>
<td></td>
</tr>
<tr>
<td>Cervix &amp; Vagina (epithelium)</td>
<td></td>
</tr>
</tbody>
</table>
IV Inoculation of Rhesus Macaques with XMRV

Animal Protocol:

Baseline Bleeds

Blood collections

XMRV i.v. n=2

XMRV reinfection i.v.

LN coll. x x x x

Necropsies: xx
RLm-1
ROu-4
RLq-10
RYh-10

XMRV vaccine

-2 M -1 days 0 7 14 21 28 35 42 56 70 ....144 160 174 188 202 216 275 291
Biology: Immune Stimulation Reactivates XMRV

(Rhesus macaque 291 Days Post-Infection; 16 d After Injection of XMRV Proteins)
Questions

• Is XMRV a real virus?
• Does XMRV have interesting properties?
• Is XMRV capable of infecting humans?
• Has XMRV infected humans?
• Is XMRV associated with human disease?
• Does XMRV cause human disease? If so, how, and what can be done about it?
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Cristina Magi-Galluzzi
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Yerkes/Emory
Francois Villinger
Prachi Sharma
Nattawat Onlamoon

UCLA
Sam Chow
Sanggu Kim
Alice Rusmevichientong

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-Gerald Schochetman
-Xiaoxing Qiu
-Sushil Devare

TGEN -John Carpten, Jeff Trent

JHMI -William Isaacs

USC - Graham Casey

WPI-Judy Mikovits,

Vincent Lombardi

NCI-Frank Ruscetti

UCSF
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-Don Ganem
-Nicole Fischer (UMC Hamburg)
-Anatoly Urisman

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