Sex bias in cancer:  
New findings from the X and Y chromosomes

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Why do males get more cancer?  
(a.k.a., What protects females from cancer?)

- 150,000 more cancers/yr in men than women in US
- Lifetime risk: 1 in 2 males, 1 in 3 females
- Crosses racial, demographic, and geographic differences
- Almost all tumor types affected

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Adult M:F ratio</th>
<th>Pediatric M:F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>1.3 : 1</td>
<td>Leukemia</td>
</tr>
<tr>
<td>Liver</td>
<td>2.7 : 1</td>
<td>Brain tumors</td>
</tr>
<tr>
<td>Bladder</td>
<td>3.9 : 1</td>
<td>Neuroblastoma</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>1.5 : 1</td>
<td>Germ cell tumors</td>
</tr>
<tr>
<td>Leukemia</td>
<td>1.6 : 1</td>
<td></td>
</tr>
</tbody>
</table>
Sex bias in cancer incidence and survival

- Incidence rate ratio
- Excess mortality ratio
- Higher male risk
- Worse male survival

Radkiewicz et al. Eur J Cancer 2017
Concordant epidemiology with environmental exposure

US SEER Data
Epidemiology independent of environmental exposures

Bladder
Year
Rate per 100,000
Male
All
Female

Kidney and Renal Pelvis
Year
Rate per 100,000
Male
All
Female

Brain
Year
Rate per 100,000
Male
All
Female

Oral Cavity and Pharynx
Year
Rate per 100,000
Male
All
Female

Leukemia
Year
Rate per 100,000
Male
All
Female

US SEER Data
Cancer genetics – somatic vs germline

**Somatic mutations**
- Occur in *nongermline* tissues
- Cannot be inherited if the mutation occurs after the germline differentiates

**Germline mutations**
- Present in egg or sperm
- Can be inherited
- Cause cancer family syndrome

**Mutation in tumor only (for example, breast)**

**Mutation in egg or sperm**

**All cells affected in offspring**

Adapted from NCI/ASCO
Blastic plasmacytoid dendritic cell neoplasm (BPDCN)

Skin

Lymph nodes

Bone marrow

> 3:1 Male:Female
Mutations in some chrX genes are markedly skewed toward men

BPDCN: >3:1 M:F and ~40% have ZRSR2 mutations – **all males**

Myelodysplastic syndrome: Loss-of-function mutations in ZRSR2 – **all males**

T-cell leukemia: *KDM6A* (UTX) mutations – **all males**

Taylor et al. *ASH* 2013
Van der Muelen et al. *Blood* 2015
Most genes on chrX are functionally hemizygous in both sexes.
X inactivation

Lee and Bartolomei. Cell 2013
X inactivation
Most genes on chrX are functionally hemizygous in both sexes.
Some genes ESCAPE X inactivation

- **e.g. STS**: 30–50 Myr ago
- **e.g. ZFX**: 80–130 Myr ago
- **UBE1X, SMCX**: 130–170 Myr ago
- **RPS4X, RBMX, SOX3**: 240–300 Myr ago

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ChrX and chrY have unique biology
Intra- and inter-individual variability in escape
EXITS (Escape from X-Inactivation Tumor Suppressor) hypothesis
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Dunford et al. *Nature Genetics* 2017
EXITS (Escape from X-Inactivation Tumor Suppressor) hypothesis
Central hypothesis

There are genes on the X chromosome that more frequently have Loss of Function (LOF) mutations in male cancers compared to female cancers.
Are there sex-biased genes in The Cancer Genome Atlas (TCGA)?

- SNV/InDel in 4126 cancer tumor/normal exome pairs
- SNV/InDel and copy number in 1994 cases

n=21 tumor types
Computational refinements and statistical approach

1. Improved copy number/ploidy calls for X and Y
2. Focused on loss-of-function
3. Tests:
   a. Permutation analysis
      - probability of observed vs expected male events
   b. Log-likelihood ratio test
      - likelihood of alternative vs null hypothesis for observed M:F ratio
   c. Normalized to background mutation rate, fraction of males in the set, # of X chr in the sample
4. “Pan-cancer” analysis, and in each individual tumor type
Central hypothesis

There are genes on the X chromosome that more frequently have Loss of Function (LOF) mutations in male cancers compared to female cancers.

At false discovery rate (FDR) <0.1:

- **No autosomal genes** had LOF mutations more frequently in male cancers
- **No genes on chrX** had a significantly increased frequency of silent coding mutations in male cancers
- **No genes on chrX** had non-silent LOF mutations more frequently in female cancers
- **No PAR genes** had LOF mutations more frequently in male cancers
“Pan-cancer” male-biased loss-of-function mutations

SNV/indel only

SNV/indel and CNV
Disease-specific male-biased loss-of-function mutations

Lower grade glioma (LGG)

Clear cell kidney cancer (KIRC)
Log likelihood ratio – a second test
Male-biased mutation enriched for X-inactivation escape genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Data set</th>
<th>LOF mutations</th>
<th>Total cancers</th>
<th>P value</th>
<th>Q (FDR) value</th>
<th>Escapes?</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRX</td>
<td>all</td>
<td>70 M : 47 F</td>
<td>2440 M : 1686 F</td>
<td>0.000001</td>
<td>0.00066</td>
<td>No/Some?</td>
</tr>
<tr>
<td>ATRX</td>
<td>LGG</td>
<td>45 M : 19 F</td>
<td>98 M : 72 F</td>
<td>0.000001</td>
<td>0.000071</td>
<td>No/Some?</td>
</tr>
<tr>
<td>CNKSR2</td>
<td>all</td>
<td>30 M : 10 F</td>
<td>2440 M : 1686 F</td>
<td>0.00037</td>
<td>0.049</td>
<td>Yes</td>
</tr>
<tr>
<td>DDX3X</td>
<td>all</td>
<td>34 M : 9 F</td>
<td>2440 M : 1686 F</td>
<td>0.000026</td>
<td>0.0075</td>
<td>Yes</td>
</tr>
<tr>
<td>KDM5C</td>
<td>all</td>
<td>31 M : 10 F</td>
<td>2440 M : 1686 F</td>
<td>0.000092</td>
<td>0.015</td>
<td>Yes</td>
</tr>
<tr>
<td>KDM5C</td>
<td>KIRC</td>
<td>14 M : 1 F</td>
<td>216 M : 118 F</td>
<td>0.0003</td>
<td>0.044</td>
<td>Yes</td>
</tr>
<tr>
<td>MAGEC3</td>
<td>all</td>
<td>15 M : 1 F</td>
<td>2440 M : 1686 F</td>
<td>0.000034</td>
<td>0.0075</td>
<td>Yes</td>
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<tr>
<td>KDM5C</td>
<td>all</td>
<td>24 M : 5 F</td>
<td>1225 M : 769 F</td>
<td>0.00022</td>
<td>0.079</td>
<td>Yes</td>
</tr>
<tr>
<td>KDM5C</td>
<td>KIRC</td>
<td>14 M : 1 F</td>
<td>216 M : 118 F</td>
<td>0.00047</td>
<td>0.08</td>
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<tr>
<td>KDM6A</td>
<td>all</td>
<td>50 M : 18 F</td>
<td>1225 M : 769 F</td>
<td>0.00025</td>
<td>0.079</td>
<td>Yes</td>
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All on chrX

(Nearly?) All escape
EXITS mutation in female tumors may be more likely biallelic

Loss of chrX (LOX) enriched in tumors with EXITS gene mutation

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<thead>
<tr>
<th></th>
<th>LOX</th>
<th>No LOX</th>
</tr>
</thead>
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<tr>
<td>EXITS mutation</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td>No EXITS mutation</td>
<td>45</td>
<td>681</td>
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$P=0.0005$
Individual EXITS genes are associated with a significant fraction of male cancer predominance.

These are almost definitely underestimates.
Only scratching the surface in the exome
Conclusion #1:

Loss-of-function mutations in EXITS genes contribute to a significant fraction of the difference in cancer incidence between men and women

(Escape from X-inactivation *may* have evolved to reduce cancer risk?)
Additional sex-biased/EXITS genes likely exist
EXITS mutations might disproportionately affect males who have lost Y

Loss of Y in tumors with Y homolog mutation

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<tr>
<td>Y homolog mut</td>
<td>9</td>
<td>79</td>
</tr>
<tr>
<td>No Y homolog mut</td>
<td>78</td>
<td>1277</td>
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P=0.019
Genetic predisposition to mosaic Y chromosome loss in blood

“epidemiological associations have been observed with various forms of cancer, autoimmune conditions, age-related macular degeneration, cardiovascular disease, Alzheimer’s disease, type 2 diabetes, obesity and all cause mortality.”
Fig. 1 | Prevalence of mosaic Y chromosome loss by age in male participants in the UK Biobank study. Bar chart shows the full age distribution of all male participants in the UK Biobank study (n = 205,011) at baseline.
Developed a score for risk of LOY based on genome wide association study

“genetically increased risk of LOY was associated with later age at menopause \((P = 0.003)\), with the CHEK2 locus individually reaching genome-wide significance for association with menopause \((P = 7.9 \times 10^{-22})\).”
“On the basis of our observations, we propose that LOY is determined by a ‘common soil’ of shared mechanisms that predispose cells to genomic instability and cancer across many cell types. Our identified CHEK2 association clearly illustrates this concept: loss of function of CHEK2 increases LOY in men, and in women delays age at menopause and increases the risk of breast cancer. These effects can all be attributed to inhibited apoptosis in the respective cell types.”

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*P=0.019*
Conclusion #2:

Loss-of-Y chromosome both directly contributes to some male cancers and is an *epiphenomenon/biomarker* of DNA damage and heightened risk for many types of disease.
### ATRX male bias is only in lower-grade glioma

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ATRX: escape restricted to brain/glial cells?

GTEx (Genotype-Tissue Expression) Project
Can we use sex bias to identify new cancer genes?

The long tail problem

CNKSR2
(Connector enhancer of kinase suppressor of RAS 2)
CNKSR2 has tumor suppressor function

**Knock-down**

- Relative expression

**MAPK activation**

- shControl
- shCnksr2 #1
- shCnksr2 #2

**Growth in semi-solid media**

- shControl
- shCnksr2 #1
- shCnksr2 #2

**Oncogenic signatures**

- Kniveson_Cancer_40th_June_2019
- Oncogenic signatures

John Cleary, Akinori Yoda
Role of EXITS in sex chromosome aneuploidies and cancer?

Table 2. Cancer risk among persons with Turner syndrome and Klinefelter syndrome

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Turner syndrome</th>
<th>Klinefelter syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>E</td>
</tr>
<tr>
<td>All malignancies</td>
<td>70</td>
<td>52.3</td>
</tr>
<tr>
<td>Solid tumors</td>
<td>64</td>
<td>48.4</td>
</tr>
<tr>
<td>Digestive system</td>
<td>4</td>
<td>7.88</td>
</tr>
<tr>
<td>Lung</td>
<td>3</td>
<td>2.60</td>
</tr>
<tr>
<td>Breast and female genital organ</td>
<td>17</td>
<td>24.3</td>
</tr>
<tr>
<td>Breast</td>
<td>2</td>
<td>16.3</td>
</tr>
<tr>
<td>Prostate</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>10</td>
<td>3.34</td>
</tr>
<tr>
<td>Nervous system</td>
<td>19</td>
<td>2.87</td>
</tr>
<tr>
<td>Hematological malignancy</td>
<td>6</td>
<td>3.98</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>2</td>
<td>1.68</td>
</tr>
<tr>
<td>Leukemia</td>
<td>4</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Bold type indicates 95% CI does not include 1.00.
What is the potential danger of ignoring sex as a variable?

Clayton et al, Phys & Behavior 2018
MuCullough et al, J Cereb Blood Flow Metab 2005
Need: design studies with power to detect sex differences

**Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation**

Median Overall Survival

- **Midostaurin**: 74.7 mo (95% CI, 31.5–NR)
- **Placebo**: 25.6 mo (95% CI, 18.6–42.9)

One-sided P=0.009 by stratified log-rank test

**FDA APPROVAL**

Unplanned subgroup analysis: *only males benefit?*

Stone RM et al. NEJM 2017
Conclusion #3:

Studying sex-specific differences can reveal new opportunities to understand sex-dependent and sex-independent biology.
Acknowledgements

- Andrew Dunford
- Mike Lawrence
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- John Cleary
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- Tim Sullivan
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- David Weinstock
- Gad Getz