A conversation between cancer registries and scientists
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The chapters of the story
- Registry data shows a very clear finding
- Patient-based studies try to make sense of the finding
- Scientists try to model the finding in the lab
- Scientists come to a new conclusion
- The new idea can best be tested through registry data
- More investigations
- Conclusions

To begin...
- Let's take a simple look at gender differences in cancer rates, through the public-facing portal for the NCDB.
- It only includes a limited set of variables, but gender is a pretty reliable data field.
Age Group by Gender of Urinary Bladder Cancer Diagnosed in 2006 to 2015

table

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male</th>
<th>Female</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Under 20</td>
<td>158</td>
<td>118</td>
<td>276</td>
</tr>
<tr>
<td>2.</td>
<td>20 - 29</td>
<td>619</td>
<td>276</td>
<td>895</td>
</tr>
<tr>
<td>3.</td>
<td>30 - 39</td>
<td>2509</td>
<td>1048</td>
<td>3557</td>
</tr>
<tr>
<td>4.</td>
<td>40 - 49</td>
<td>12197</td>
<td>4427</td>
<td>16624</td>
</tr>
<tr>
<td>5.</td>
<td>50 - 59</td>
<td>48632</td>
<td>15869</td>
<td>64501</td>
</tr>
<tr>
<td>6.</td>
<td>60 - 69</td>
<td>102668</td>
<td>30340</td>
<td>133008</td>
</tr>
<tr>
<td>7.</td>
<td>70 - 79</td>
<td>126338</td>
<td>37321</td>
<td>163659</td>
</tr>
<tr>
<td>8.</td>
<td>80 - 89</td>
<td>90316</td>
<td>32127</td>
<td>122443</td>
</tr>
<tr>
<td>9.</td>
<td>90 and over</td>
<td>14489</td>
<td>7338</td>
<td>21827</td>
</tr>
<tr>
<td>TOTAL</td>
<td>397926</td>
<td>128864</td>
<td>526790</td>
<td>100%</td>
</tr>
</tbody>
</table>

http://oliver.facs.org/BMPub/

What could account for the gender difference?

- Smoking rates? The NCDB does not include smoking information as a searchable data field, either for the public-facing dataset or the facility Participant User File. Local registries can be consulted, or patient interviews used.

Could women have a protective factor?

A reduced risk was observed among parous women (HR=0.76; 95% CI 0.62-0.93)

These findings were generally in agreement with the meta-analytic results for which the combined relative risk (RR) estimate was reduced among ever parous women (combined RR estimate for ever parous versus nulliparous=0.66, 95% confidence intervals [95% CI] 0.55-0.79)

The incidence of BCa decreased with increasing parity and older age at first birth. Although smoking habits may partly explain some of the associations, our findings provide support for yet-to-be-identified protective mechanisms associated with childbearing, possibly mediated by hormonal or structural changes following pregnancy.
Developing a mouse model: MB49 cells

Tumor growth looks restricted in females, matching with the idea that there could be a protective aspect to female biology.

Can we reproduce any protective effects of pregnancy?

No clear difference in tumor take rates or sizes based on pregnancy. It is a pretty complex phenomenon; could we test something a little simpler? What about the pregnancy hormone hCG (human chorionic gonadotropin)?

Not only no protective effect, but the opposite!
Why did the opposite of what we expected happen? Let's look at the cells alone in a dish and see if we can understand…

Just putting hCG in the culture media did NOT stimulate additional growth. No real difference was seen at all, completely opposite of what we saw in the mice. That doesn’t help us to study the growth-enhancing mechanism of hCG, but it is pretty interesting…

Key hCG information was revealed after reading a lot of papers on the subject

- The receptor of hCG is lost in a lot of bladder cancers, nobody really knows why.
- If our bladder cancer cells lost their hCG receptor, then it must be responding in vivo to some metabolite of hCG, not hCG itself.
- That would explain why an hCG injection into an animal could result in an effect on the tumor cells, but hCG in the culture media had no effect.

Key hCG information was revealed after reading a lot of papers on the subject

- The reason we used testes tissue as a positive control? Testes have a TON of hCG receptors.
- When hCG binds to testes tissue, it stimulates the production of testosterone.
- Given the way tumor in male animals grew when hCG was given, maybe those cells are responding to testosterone.
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- The reason we used testis tissue as a positive control? Testes have a TON of hCG receptors.
- When hCG binds to testis tissue, it stimulates the production of testosterone.
- Given the way tumor in male animals grew when hCG was given, maybe those cells are responding to testosterone.

We started thinking about bladder cancer in a new way:

- Could androgen ablation help with bladder cancer? There is a population of men undergoing chemical castration, and that population is prostate cancer patients. Men taking Lupron for prostate cancer might hold answers about bladder cancer. How would we find out?
- If only there were a searchable database with information about cancer patients, their treatments, and their outcomes 😊

So, this got us thinking...
Registries to the rescue

- Registries could help answer some questions by looking at a group of patients who are sometimes on Lupron and sometimes not, and seeing if that single element made a difference in bladder cancer outcomes.
- Query for prostate cancer patients with seq01 prostate cancer
- Sort according to Lupron +/-
- Assess the seq02+ cancers in each group
- Is bladder cancer less common in the Lupron group?

Someone did a version of this study

"Although accumulating preclinical evidence indicates the involvement of androgen receptor signals in bladder cancer (BC) development, its clinical relevance remains unclear. We aimed to evaluate the predictive role of androgen deprivation therapy (ADT) in BC recurrence in prostate cancer (PC) patients. We retrospectively reviewed 20,328 patients with PC, diagnosed during 1991-2013, and 239 (1.2%) men with primary BC were identified. After excluding ineligible patients, 162 patients made up a final cohort. With a median follow-up of 62 months, 38 (50%) of 76 control patients without ADT experienced BC recurrence, while 19 (22%) of 86 did in ADT group. Thus, patients having received ADT for their PC showed a significantly lower risk of BC recurrence (5-year actuarial recurrence-free survival: 76% vs 45%; P = 0.001) and also had a significantly smaller number of recurrence episodes (5-year cumulative recurrence: 0.44 vs 1.54; P < 0.001), compared to the control patients. A multivariable analysis revealed ADT as an independent prognosticator (hazard ratio, 0.29; 95% confidence interval, 0.17-0.49) for BC recurrence. This is the first clinical study showing that ADT significantly reduces the risk of BC recurrence."
Results: survival

Results: bladder cancer recurrence

Of course, in biology nothing is actually as simple as one thing!

One group wanted to look at the difference between males and females at two levels: both the hormone level and the genetic level. They manipulated the gene Sry, which is located on the Y chromosome and holds the instructions for male development.

<table>
<thead>
<tr>
<th>Normal options</th>
<th>XY</th>
<th>YY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ovaries</td>
<td>X</td>
<td>Y</td>
</tr>
<tr>
<td>Testes</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Normal testes</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Ovaries</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

XX | XY | XX | XY

XX | XY | XX | XY

Sry protects against bladder cancer in males via an androgen-dependent mechanism.
Of course, in biology nothing is actually as simple as one thing!

Evidence that sex hormones play a key role, and so do sex chromosomes.

- **XX is protective compared to XY**, even if testosterone is held to the same level.
- So there is some female specific protection after all.

The group found that the X chromosome has a copy of a protective gene. Those with XX have two copies of the gene KDM6A, those with XY only have one. The XX genotype becomes less protective if KDM6A is mutated.

Another group found another factor.

**BACKGROUND:**
Males have a higher incidence of bladder cancer than females, but the reasons remain unknown. Unlike prostate cancer, human bladder cancer is not generally considered to be dependent on hormone activity. We investigated the possible involvement of androgens and the androgen receptor (AR) in bladder cancer.

**METHODS:**
We used N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) to induce bladder cancer in wild-type male and female mice, with and without castration in males, and in AR knockout (ARKO) male and female mice, with and without dihydrotestosterone (DHT) supplementation in males. We also treated human bladder cancer cell lines, including TCC-SUP and UMUC3, and mouse xenograft models established from these lines with androgen deprivation therapy (androgens inhibited or castrated), or the anti-AR molecule ASC-J9, which causes selective degradation of the AR.

**RESULTS:**
More than 92% of wild-type male and 42% of wild-type female mice treated with BBN eventually developed bladder cancer, whereas none of the male or female ARKO mice did. Treatment with BBN induced bladder cancer in 25% of ARKO mice supplemented with DHT and in 50% of castrated wild-type male mice.

Androgen deprivation of AR-positive human bladder cancer cells by androgen deprivation in vitro or castration in mice and/or by treatment with the antiandrogen flutamide in vitro or in vivo, as well as AR knockdown by AR-siRNA or by ASC-J9, suppressed cell proliferation in vitro and xenograft tumor growth in vivo.

**CONCLUSIONS:**
Our findings implicate the involvement of both androgens and the AR in bladder cancer. Targeting AR and androgens may provide novel chemopreventive and therapeutic approaches for bladder cancer.

Results: tumor growth

<table>
<thead>
<tr>
<th>Group</th>
<th>12 mice per group</th>
<th>Cancers, mice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT male</td>
<td>11 (92)</td>
<td></td>
</tr>
<tr>
<td>AAV100 male</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>AAV100 male = DHT</td>
<td>3 (23)</td>
<td></td>
</tr>
<tr>
<td>WT female</td>
<td>5 (42)</td>
<td></td>
</tr>
<tr>
<td>AAV100 female</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Results: tumor growth

So, if the androgen receptor is so important in bladder cancer...

- ...we are right back to thinking about prostate cancer. Anti-AR therapy is a focus of the lab I'm in right now, with a focus on prostate cancer. Could such a therapy be useful in bladder cancer, too?
- ABC294640 is a drug that downregulates AR, even the hyperactive mutant versions (the most dangerous)
Phase I trial (focus on safety)

- Across all dose levels, the most common treatment-related toxicities were nausea, fatigue, and vomiting.
- Six patients (40%) had a best response of stable disease: one metastatic urothelial carcinoma patient (at 250 mg qd) ultimately developed progressive disease after 12 cycles; one cholangiocarcinoma patient (at 500 mg bid) came off study in cycle 3 for toxicity; and four patients (1 at 250 mg qd, 2 at 250 mg bid, and 1 at 500 mg bid) came off study for progressive disease at the second disease evaluation (8 weeks).

Primary Tumor Type [n(%)]

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon/Rectum</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Urothelial</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Other*</td>
<td>5</td>
<td>24</td>
</tr>
</tbody>
</table>

Conclusions: research recap

- Bladder cancer is more prevalent in males 3:1
- Bladder cancer growth is enhanced by testosterone
- Androgen ablation therapy may be helpful for bladder cancer
- Downregulation of the androgen receptor may be helpful for bladder cancer
- The new drug ABC294640 downregulates the androgen receptor
- Females may have some protection against bladder cancer due to their XX genotype

ABC mechanism of action
Conclusions: research process

- Registry data is rich in potentially important observations
- Observations made with registry data can be explored with
  - Interview tools
  - Tissue bank samples linked to registry data
  - Molecular biology
- A new insight made at the bench can be fed back into registry-based research
- The more scientists, registrars, physicians, and patients all connect, the better the research gets!!

Acknowledgements

- Laura Kasman, PhD, lead on the bladder project
- Christina Johnson, PhD, lead on the ABC project
- Laurie Josiger, CTR and Roper St. Francis registry team for keeping me involved in the registry world
- Conference organizers

Let’s keep those collaborations going!