Year in Review: Top Basic/Translational Research Studies in Cardio-Oncology

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Relevant Disclosures

• None
• Question: Is clonal hematopoiesis (CHIP) causally linked to atherosclerotic cardiovascular disease?

• Methods: Nested case-control analyses of whole exome sequencing data from 4 cohorts (BioImage/MDC & ATVB/Promis) in 4726 coronary disease cases & 3529 controls

• Gene-level analyses in BioImage/MDC & JHS, FUSION, FHS

• CHIP carriers based on prespecified variants in 74 genes

• \textit{Lldr} KO mice underwent BMT from control or \textit{Tet2} heterozygous or homozygous knockout
Associations between CHIP and Coronary Disease and Early Onset MI

- **Results:** Risk on the order of 2-fold for coronary disease; 4-fold for early onset disease
Associations between CHIP and Coronary Disease and Early Onset MI

**Results:** Specific mutations strongly associated with CV risk – 4 critical genes; CHIP status also associated with CAC

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of Participants with Myocardial Infarction/No. at Risk</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biolmage</td>
<td>94/326</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDC</td>
<td>299/607</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JHS/FUSION/FHS</td>
<td>169/3505</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| DNMT3A                    |                                                            |                       |         |
| Biolmage                  | 5/14                                                       | 1.7 (0.7–4.1)         | 0.27    |
| MDC                       | 11/13                                                      | 2.5 (1.4–4.7)         | 0.003   |
| JHS/FUSION/FHS            | 8/99                                                       | 1.1 (0.5–2.2)         | 0.90    |
| Fixed-effects meta-analysis|                                                           | 1.7 (1.1–2.6)         | 0.01    |

| TET2                      |                                                            |                       |         |
| Biolmage                  | 3/7                                                        | 1.6 (0.5–5.0)         | 0.46    |
| MDC                       | 2/6                                                        | 0.8 (0.2–3.3)         | 0.76    |
| JHS/FUSION/FHS            | 4/36                                                       | 3.5 (1.3–9.6)         | 0.01    |
| Fixed-effects meta-analysis|                                                           | 1.9 (1.0–3.7)         | 0.06    |

| ASXL1                     |                                                            |                       |         |
| Biolmage                  | 4/6                                                        | 2.1 (0.7–5.4)         | 0.16    |
| MDC                       | 3/6                                                        | 1.4 (0.5–4.6)         | 0.53    |
| JHS/FUSION/FHS            | 2/10                                                       | 2.8 (0.7–11.4)        | 0.15    |
| Fixed-effects meta-analysis|                                                           | 1.9 (1.0–3.9)         | 0.05    |

| JAK2                      |                                                            |                       |         |
| Biolmage                  | 0/0                                                        | 10.0 (2.4–41.5)       | 0.001   |
| MDC                       | 2/2                                                        | 17.4 (2.4–127.6)      | 0.005   |
| JHS/FUSION/FHS            | 1/3                                                        | 12.0 (3.8–38.4)       | 0.001   |
| Fixed-effects meta-analysis|                                                           |                       |         |

| Other                     |                                                            |                       |         |
| Biolmage                  | 7/17                                                       | 1.8 (0.8–3.9)         | 0.16    |
| MDC                       | 3/4                                                        | 1.9 (0.6–6.0)         | 0.28    |
| JHS/FUSION/FHS            | 6/35                                                       | 3.0 (1.3–6.9)         | 0.009   |
| Fixed-effects meta-analysis|                                                           | 2.2 (1.3–3.7)         | 0.002   |
Murine Studies Corroborate Human Level Observations

- Results: \textit{Ldlr} KO mice fed a high cholesterol diet who had received bone marrow from \textit{Tet2} KO mice had larger aortic lesion sizes; also true for heterozygotes

- \textit{Tet2} KO mice also had evidence for increased systemic inflammation
Conclusions and Perspective

- **Conclusions**: Robust evidence for an association between somatic mutations in hematopoietic cells and human atherosclerosis

- Association appears to be causal, as demonstrated by murine studies

- **Perspective**: What is the role of the inflammasome? What are the precise mediators?

- Are there targeted therapies to reduce both CV disease and cancer risk?
Heart Failure Stimulates Tumor Growth by Circulating Factors

• **Question:** Is there a causal relationship between cardiovascular disease and cancer?

• **Methods:** HF induced with anterior MI in APC\textsuperscript{min} mice (prone to intestinal tumors), compared to sham

• Transplant of failing heart also used to isolate effect of cardiac secretome (and exclude hemodynamic effect)

• Evaluated tumor burden after 5 weeks

• Candidate proteins selected via proteomic studies and validated in humans with heart failure (VitD-CHF)
Increased Tumor Burden in HF APC\textsubscript{min} Mice

- **Results:** LVEF markedly decreased in MI mice, increased inflammatory/fibrosis gene expression.

- After 6 weeks, more polyps and increased tumor load in HF mice; fibrosis and LVEF correlated with load.
**Transplanted Hearts Demonstrate Similar Effect; Inflammatory Proteins Identified**

- **Results:** Heart transplant also performed of infarcted heart into APC\textsuperscript{min} mice to exclude hemodynamic effect

- Increased tumor burden also observed; correlations between tumor load and fibrosis and LVEF

- Increased expression of LV gene expression in HF: \(\alpha-1\) antitrypsin (SerpinA1), \(\alpha-1\) antichymotrypsin (SerpinA3), fibronectin, ceruloplasmin, paraoxonase

- In vitro experiments demonstrate increased cell growth with SerpinA3 stimulation
Human Studies Corroborate Murine Level Observations

• **Results:** Human studies corroborate increased inflammation in chronic heart failure.

• Patients with worse heart failure at increased risk of developing all-cancer.

• Smaller study of 180 healthy subjects and 101 with chronic heart failure demonstrate differences in candidate proteins.
Conclusions and Perspective

• **Conclusions:** Causal relationship between HF and tumor growth as evidenced by MI-induced model in an animal susceptible to adenoma formation

• A number of candidate proteins identified, focused on inflammatory pathway

• **Perspective:** More evidence to suggest there is a mechanistic link

• Can we develop better prognostic/predictive biomarkers and therapies to target both pathways? Role of inflammation?
**Question:** Can patient-specific iPSC be used as a preclinical platform to screen for cardiotoxicity?

**Methods:** Skin biopsies or blood draws obtained from 11 healthy individuals and 2 cancer patients receiving TKIs to generate hiPSC

- Skin fibroblasts or PBMC $\rightarrow$ hiPSC $\rightarrow$ hiPSC-CM (cardiomyocytes); or hiPSC-CFs (cardiac fibroblasts); or hiPSC-ECs (endothelial cells)

**Assays:** viability, contractility, calcium imaging, electrophysiology, kinase phosphorylation, and gene expression
Methods: Human iPSC and Toxicity Assessment Workflow

1. Somatic tissue isolation
2. Cellular reprogramming (OKSM)
3. hiPSC colonies differentiation
4. hiPSC–cardiomyocytes
5. hiPSC–endothelial cells
6. hiPSC–cardiac fibroblasts
7. Gene expression analysis
8. Tyrosine kinase inhibitor panel treatment
9. Contractility, calcium imaging, electrophysiology
10. Qualitative imaging
11. Quantitative toxicology
12. Cardioprotective drug screening
13. High-throughput phosphorylation arrays

Patient cohort
Results: TKI Toxicity Screening

- **Results:** Screened 21 small molecule TKIs using hiPSC-CMs; doxorubicin served as positive control
- Developed a cardiac safety index; normalized toxicity to Cmax; <0.10 more severely toxic

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cessation of beating (µM)</th>
<th>Effective concentration (µM)</th>
<th>Amplitude of effect</th>
<th>LD₅₀ (µM)</th>
<th>Cmax (µM)</th>
<th>Cardiac safety index</th>
<th>Clinically reported cardiotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vemurafenib</td>
<td>33</td>
<td>11.00</td>
<td>0.34</td>
<td>32.10</td>
<td>126.04</td>
<td>0.003</td>
<td>QT, LV, HF, MI, Hy</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>3.7</td>
<td>2.51</td>
<td>1.03</td>
<td>3.40</td>
<td>8.43</td>
<td>0.004</td>
<td><strong>HF, LV</strong></td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>3.7</td>
<td>1.20</td>
<td>0.60</td>
<td>0.78</td>
<td>2.93</td>
<td>0.010</td>
<td><strong>MI, Hy</strong></td>
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<tr>
<td>Regorafenib</td>
<td>11</td>
<td>3.70</td>
<td>0.84</td>
<td>7.10</td>
<td>8.08</td>
<td>0.010</td>
<td><strong>QT, Tdp, Scd, HF, Hy</strong></td>
</tr>
<tr>
<td>Vandetanib</td>
<td>33</td>
<td>5.68</td>
<td>2.47</td>
<td>20.60</td>
<td>4.26</td>
<td>0.041</td>
<td>QT, Brady</td>
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<tr>
<td>Crizotinib</td>
<td>11</td>
<td>1.91</td>
<td>0.59</td>
<td>8.60</td>
<td>1.24</td>
<td>0.063</td>
<td><strong>QT, LV, Vas</strong></td>
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<tr>
<td>Nilotinib</td>
<td>100</td>
<td>8.31</td>
<td>2.65</td>
<td>29.00</td>
<td>4.27</td>
<td>0.104</td>
<td></td>
</tr>
<tr>
<td>Imatinib</td>
<td>100</td>
<td>33.00</td>
<td>1.59</td>
<td>78.20</td>
<td>5.11</td>
<td>0.126</td>
<td>LV (rare)</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>33</td>
<td>11.00</td>
<td>0.40</td>
<td>100.76</td>
<td>2.30</td>
<td>0.209</td>
<td>#LV, QT</td>
</tr>
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<td>Sunitinib</td>
<td>3.7</td>
<td>0.81</td>
<td>1.33</td>
<td>12.70</td>
<td>0.18</td>
<td>0.218</td>
<td>#HF, LV, MI, QT, Hy</td>
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<td>Bosutinib</td>
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<td>4.73</td>
<td>1.92</td>
<td>12.39</td>
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<td>0.315</td>
<td>PE</td>
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<tr>
<td>Gefitinib</td>
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<td>3.11</td>
<td>1.24</td>
<td>26.30</td>
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<td>0.409</td>
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<tr>
<td>Afatinib</td>
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<td>1.65</td>
<td>1.11</td>
<td>12.30</td>
<td>0.10</td>
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<tr>
<td>Dabrafenib</td>
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<td>0.71</td>
<td>100.68</td>
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<td>0.459</td>
<td>LV</td>
</tr>
<tr>
<td>Ponatinib</td>
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<td>3.70</td>
<td>0.54</td>
<td>4.30</td>
<td>0.14</td>
<td>0.483</td>
<td>**Vas, HF, LV, Hy</td>
</tr>
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<td>Ibrutinib</td>
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<td>10.01</td>
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<td>0.507</td>
<td>Afib</td>
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<td>Dasatinib</td>
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<td>42.00</td>
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<td>0.524</td>
<td>QT, PE, Hy</td>
</tr>
<tr>
<td>Erlotinib</td>
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<td>87.60</td>
<td>3.11</td>
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<td>Mi (rare)</td>
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<tr>
<td>Pazopanib</td>
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<td>73.86</td>
<td>1.19</td>
<td>N/A</td>
<td>103.08</td>
<td>0.671</td>
<td>#QT, LV (rare)</td>
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<td>Cabozantinib</td>
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<td>91.14</td>
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<td>4.43</td>
<td>0.769</td>
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<tr>
<td>Trametinib</td>
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<td>33.00</td>
<td>2.37</td>
<td>66.80</td>
<td>0.02</td>
<td>1.000</td>
<td>LV</td>
</tr>
<tr>
<td>Axitinib</td>
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<td>71.79</td>
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<td>N/A</td>
<td>0.07</td>
<td>1.000</td>
<td>HF (rare) Hy</td>
</tr>
<tr>
<td>DMSO</td>
<td>N/A</td>
<td>100.00</td>
<td>0.58</td>
<td>N/A</td>
<td>N/A</td>
<td>1.000</td>
<td>None</td>
</tr>
</tbody>
</table>
Results: TKI Toxicity Screening

- **Results:** Platform also used to assess for QT prolongation and calcium imaging of hiPSC-CM to determine electrophysiologically safe TKIs

- hiPSC-CFs and hiPSC-ECs also tested; similar to hiPSC-CMs, sorafenib, regorafenib, and ponatinib most cytotoxic

- hiPSCs differed with more toxicity to VEGFR2/PDGFR inhibition (axitinib)

- Additional experiments performed to understand downstream effects of TKIs and effects of rescue strategies
Conclusions and Perspective

- **Conclusions:** An improved understanding of mechanisms of toxicity and cardiac safety index was developed using hiPSC-CMs derived from healthy individuals and patients

- **Perspective:** Strategy of using human-derived, patient-derived tissue or blood seems like a viable opportunity to screen for toxicity from a drug development perspective

- Can we use this strategy to personalize the delivery of therapy and cardioprotection? What is the specificity, throughput/scalability, and cost? What is the most relevant cell type/mixture? Effect of load and other conditions?
Increased Afterload Augments Sunitinib-Induced Cardiotoxicity in an Engineered Cardiac Microtissue Model

Rachel Truitt, PhD, Anbin Mu, Elise A. Corbin, PhD, Alexia Vite, PhD, Jeffrey Brandimarto, PhD, Bonnie Ky, MD, MSCE, Kenneth B. Margulies, MD

- **Question:** What is the impact of afterload on sunitinib cardiotoxicity?

- **Methods:** Engineered microtissues of rat cardiomyocytes or human iPS-derived cardiomyocytes grown in 3D platform with pillars of varying degrees of stiffness

  - Caspase activation, cell viability, microtissue force generation, mitochondrial membrane potential and ATP assays performed
Sunitinib Results in Dose Dependent Toxicity

• Results: Sunitinib induced dose- and duration dependent activation of apoptosis and decreases in CMT force generation, spontaneous beating, & mitochondrial membrane potential

• Increased afterload exacerbated cardiotoxicity, especially in human iPSC-CMTs
Conclusions and Perspective

• **Conclusions**: Sunitinib results in a dose dependent toxicity, that are worsened by afterload

• 3D cardiac microtissue model can be used as a preclinical cardiomyocyte model for testing for toxicity

• Findings independent of endothelial cell or pericytes

• **Perspective**: Cardiac microtissue platform with human iPSC may serve as a preclinical screening tool for drug toxicity; adaptability and scalability, effects of other cell types, and robust strategies to alter afterload remain important questions
Question: What is the therapeutic potential of the small tetrapeptide N-acetyl-Ser-Asp-lys-Pro (Ac-SDKP) in inhibiting radiation-induced cardiotoxicity?

Methods: Rats received 30Gy radiation or sham therapy to left hemithorax

Three treatment groups: 1) age matched controls; 2) thoracic radiation (RT); 3) RT+ Ac-SDKP (3.2 mg/kg/day Ac-SDKP delivered SQ x 18 weeks)

CMR, immunohistochemistry, tissue morphometry, cardiomyocyte nuclear density, cell imaging, ELISA, and fibroblast isolations performed
Ac-SDKP and Decreased Extracellular Volume and Collagen Volume

• Results: In controls, RT resulted in increased myocardial ECV; this was not seen in RT+Ac-SDKP rats

• No change in parameters of cardiac remodeling: volumes, LVEF, dimensions; there was improvement in diastolic and systolic velocities with Ac-SDKP

• Less collagen in RT+Ac-SDKP rats compared to RT alone
Ac-SDKP Results in Less Cell Death, Inflammation and Macrophage Expression

- Ac-SDKP therapy significantly decreased number of TUNEL-positive cells; CD68 cell infiltration

- Demonstrated uptake of Ac-SDKP into macrophages; inhibition of Mac-2 (galectin-3) expression with Ac-SDKP

- Attenuation of the RT induced profibrotic expression in Mac-2⁻/⁻ mice
Conclusions and Perspective

• **Conclusions:** RT therapy induces increased fibrosis, increased macrophage expression

• **Ac-SDKP** results in a decrease in fibrosis and macrophage expression in RT treated animals

• **Perspective:** This work provides new mechanistic insight, opportunities for diagnostic and therapeutic targets

• **What is the therapeutic efficacy?** Pharmacokinetics? Safety in humans?
Phosphoinositide 3-Kinase Gamma Inhibition Protects From Anthracycline Cardiotoxicity and Reduces Tumor Growth

**Question:** What is the role of PI3Kγ in Dox-CTX?

**Does PI3Kγ inhibition have both cardioprotective and anticancer effects?**

**Perspective:** Potential role of autophagy in Dox-CTX

**Ongoing trials of PI3Kγ blockade will inform clinical impact**
Summary

- A number of provocative studies published over the past year suggestive of mechanistic links between cancer and cardiovascular disease; novel pre-clinical tools, mechanisms, diagnostic and therapeutic tools

- Additional work necessary to advance the field:
  - Improving Outcomes in Cancer Treatment Related CTX (PA 18-003, 18-013)
  - NHGRI Serious Adverse Drug Reactions (PAR 16-274, 16-275)
  - Cancer Moonshot (PAR-16-238)
  - Provocative Questions 8 & 12 (RFA-CA-17-017, 17-018)
  - NHLBI Clinical Ancillary Studies (PAR-18-643)
THANK YOU!
bonnie.ky@uphs.upenn.edu
**A**

Healthy donor

DOX-induced cardiomyopathy

**B**

4T1 tumor growth

Vehicle

DOX

AS605240

**C**

8 Vehicle

8 IPI 145

**D**

Her2fN uTumor growth

Vehicle

- DOX

- AS605240

**E**

Vv vCl8

AS60 240

**F**

Her2/NtuT tumor mice

Survival (%) by Fractions

Survival (%)

0 1 2 3 4 5 6 7

Weeks post treatment

0 1 2 3 4 5 6 7

**G**

Heart

Cardiomyocyte

Macrophage

Tumor

Pro-Tumoral Inflammation

DOX

TLR 9

ncs

1 2 3 4 5 6 7

Weeks post treatment