FIFTEENTH Postdoctoral Data & Dine Symposium

MAY 25, 2022 | 4-6:30 PM
CONNOLLY BALLROOM, ALUMNI HALL
May 25, 2022

Dear Colleagues,

Welcome to the Fifteenth Postdoctoral Data & Dine Symposium. This year’s event is dedicated to the postdoctoral fellows at the University of Pittsburgh, a highly talented and innovative pool of early stage investigators whose passion and dedication to science fuels new discoveries every day.

The goals of this annual symposium continue to be to provide a forum of support and networking for postdoctoral fellows across the University, as well as to serve as a showcase of the significant scientific accomplishments of our postdoctoral community.

We are delighted to continue our tradition of giving awards for best poster presentations. Thanks to the generous support of our academic community, the University of Pittsburgh Postdoctoral Association will be recognizing ten postdoctoral fellows with professional development awards.

This event would not have been possible without the help of the Office of Academic Career Development, and financial support from the Office of the Senior Vice Chancellor for the Health Sciences, the Office of the Provost, and academic sponsors. Special thanks are also extended to the poster judges, and other volunteers contributing to tonight’s success.

And finally, we thank fellows, faculty, and administrators for sharing this wonderful evening with us!

--2022 UPPDA Executive Board
# Executive Board Officers 2021-2022

<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
<th>School or Department</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>President</td>
<td>David Gau, PhD</td>
<td>School of Medicine, Department of Medicine</td>
<td><a href="mailto:dave.gau@pitt.edu">dave.gau@pitt.edu</a></td>
</tr>
<tr>
<td>Advocacy Committee Chair</td>
<td>Vaishali Aggarwal, PhD</td>
<td>School of Medicine, Department of Immunology</td>
<td><a href="mailto:vaa30@pitt.edu">vaa30@pitt.edu</a></td>
</tr>
<tr>
<td>Career Development Chair</td>
<td>Stephanie Mutchler, PhD</td>
<td>School of Medicine, Department of Medicine</td>
<td><a href="mailto:smm226@pitt.edu">smm226@pitt.edu</a></td>
</tr>
<tr>
<td>Communication Chair</td>
<td>Mariarosaria De Rosa, PhD</td>
<td>School of Public Health, Department of Environmental and Occupational Health</td>
<td><a href="mailto:mad373@pitt.edu">mad373@pitt.edu</a></td>
</tr>
<tr>
<td>Diversity and Inclusion Chair</td>
<td>Santiago Panesso, PhD</td>
<td>School of Medicine, Department of Ob/Gyn and Reproductive Sciences</td>
<td><a href="mailto:panesso@pitt.edu">panesso@pitt.edu</a></td>
</tr>
<tr>
<td>International Committee Chair</td>
<td>Ramanujan Srinath, PhD</td>
<td>School of Arts &amp; Sciences, Department of Neuroscience</td>
<td><a href="mailto:ramanujan@pitt.edu">ramanujan@pitt.edu</a></td>
</tr>
<tr>
<td>Networking Chair</td>
<td>Cristina Espinosa Diez, PhD</td>
<td>School of Medicine, Department of Cardiology and Vascular Medicine</td>
<td><a href="mailto:espinosa@pitt.edu">espinosa@pitt.edu</a></td>
</tr>
</tbody>
</table>
AGENDA
May 25, 2022 | 4:00-6:30 PM
CONNOLLY BALLROOM, ALUMNI HALL

2:00-4:00 PM  Poster Set-Up

3:30-5:30PM  Registration

4:00-4:45 PM  Poster Session 1

4:45-5:30 PM  Poster Session 2

5:30-6:30 PM  Networking & Awards Reception
POSTER PRESENTATION AWARD GUIDELINES

Posters will be judged from 4 PM to 5:30 PM. Presenters with even numbered posters will be judged from 4 PM to 4:45 PM. Presenters with odd numbered posters will be judged from 4:45 PM to 5:30 PM. Presenters are to be present at their posters at their assigned times.

Ten poster presenters will each receive a $750 Professional Development Award. These awards will provide funds for professional development, including participation in a scientific conference, and are to be used by recipients between July 2022 and May 2023. The award categories include:

- Basic Biomedical and Pharmacological Sciences
- Behavioral Sciences
- Cellular and Molecular Biology
- Clinical and Surgical Sciences
- Engineering, Physical, and Computational Sciences

All posters have been assigned one of these categories for judging purposes. One or more posters in each category will be recognized.

Posters will be judged by a group of faculty and postdoctoral fellows knowledgeable in the above fields. The judges will not be identified during the poster sessions. Scoring will be based upon creativity, style, content, impact, presentation, and overall impressions.

Award winners will be announced during the reception following the poster sessions.
POSTER PRESENTATION AWARD SPONSORS

GOLD SPONSORS
$501 - $1,000

Department of Neurobiology
School of Medicine

Department of Pharmacology and Chemical Biology
School of Medicine

Swanson School of Engineering

Department of Pharmaceutical Sciences
School of Pharmacy

SILVER SPONSORS
$251 - $500

Department of Microbiology and Molecular Genetics
School of Medicine

Department of Psychiatry
School of Medicine

Innovation Institute

Vascular Medicine Institute

BRONZE SPONSORS
$0 - $250

Department of Computation and Systems Biology
School of Medicine

Department of Neurology
School of Medicine
<table>
<thead>
<tr>
<th>Page</th>
<th>Name</th>
<th>Category</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Aggarwal, Vaishali</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:vaa30@pitt.edu">vaa30@pitt.edu</a></td>
</tr>
<tr>
<td>12</td>
<td>Anzell, Anthony</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:ara126@pitt.edu">ara126@pitt.edu</a></td>
</tr>
<tr>
<td>13</td>
<td>Atiya, Huda</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:hua20@pitt.edu">hua20@pitt.edu</a></td>
</tr>
<tr>
<td>14</td>
<td>Ballance, Heather</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:ballance@pitt.edu">ballance@pitt.edu</a></td>
</tr>
<tr>
<td>15</td>
<td>Banerjee, Srijon</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:skbanerjee@pitt.edu">skbanerjee@pitt.edu</a></td>
</tr>
<tr>
<td>16</td>
<td>Bansal, Manik</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:mbansal@pitt.edu">mbansal@pitt.edu</a></td>
</tr>
<tr>
<td>17</td>
<td>Barak, Oren</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:barako@upmc.edu">barako@upmc.edu</a></td>
</tr>
<tr>
<td>18</td>
<td>Barbosa, Anne</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:acs173@pitt.edu">acs173@pitt.edu</a></td>
</tr>
<tr>
<td>19</td>
<td>Basu, Paramita</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:pab171@pitt.edu">pab171@pitt.edu</a></td>
</tr>
<tr>
<td>20</td>
<td>Bengur, Fuat Baris</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:fub2@pitt.edu">fub2@pitt.edu</a></td>
</tr>
<tr>
<td>21</td>
<td>Bengur, Fuat Baris</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:fub2@pitt.edu">fub2@pitt.edu</a></td>
</tr>
<tr>
<td>22</td>
<td>Bian, Fuyun</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:fuyunb@pitt.edu">fuyunb@pitt.edu</a></td>
</tr>
<tr>
<td>23</td>
<td>Bugga, Parameshya</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:buggap@pitt.edu">buggap@pitt.edu</a></td>
</tr>
<tr>
<td>24</td>
<td>Capella-Monsonis, Hector</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:hec71@pitt.edu">hec71@pitt.edu</a></td>
</tr>
<tr>
<td>25</td>
<td>Cuevas, Rolando</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:rac218@pitt.edu">rac218@pitt.edu</a></td>
</tr>
<tr>
<td>26</td>
<td>Dewey, Marley</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:mjd173@pitt.edu">mjd173@pitt.edu</a></td>
</tr>
<tr>
<td>27</td>
<td>Didwischus, Nadine</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:NDidwischus@pitt.edu">NDidwischus@pitt.edu</a></td>
</tr>
<tr>
<td>28</td>
<td>Duvall, Samuel</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:swd11@pitt.edu">swd11@pitt.edu</a></td>
</tr>
<tr>
<td>29</td>
<td>Espinosa-Diez, Cristina</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:espinosa@pitt.edu">espinosa@pitt.edu</a></td>
</tr>
<tr>
<td>30</td>
<td>Feng, Wei</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:WEIFENG@pitt.edu">WEIFENG@pitt.edu</a></td>
</tr>
<tr>
<td>31</td>
<td>Feturi, Firuz</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:fgf3@pitt.edu">fgf3@pitt.edu</a></td>
</tr>
<tr>
<td>32</td>
<td>Fleres, Giuseppe</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:gif18@pitt.edu">gif18@pitt.edu</a></td>
</tr>
<tr>
<td>33</td>
<td>Gabe, Claire</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:cmg172@pitt.edu">cmg172@pitt.edu</a></td>
</tr>
<tr>
<td>34</td>
<td>Ghosh, Sayan</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:SAYANG@pitt.edu">SAYANG@pitt.edu</a></td>
</tr>
<tr>
<td>35</td>
<td>Gocher-Demske, Angela</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:agocher@pitt.edu">agocher@pitt.edu</a></td>
</tr>
<tr>
<td>36</td>
<td>Goz, Roman</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:roman.go@pitt.edu">roman.go@pitt.edu</a></td>
</tr>
<tr>
<td></td>
<td>Name</td>
<td>Department</td>
<td>Email</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------</td>
<td>-------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>37</td>
<td>Grigsby, Erinn</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:emg90@pitt.edu">emg90@pitt.edu</a></td>
</tr>
<tr>
<td>38</td>
<td>Hardy, Jimmaline</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:jjh115@pitt.edu">jjh115@pitt.edu</a></td>
</tr>
<tr>
<td>39</td>
<td>Hildebrandt, Britny</td>
<td>Behavioral Sciences</td>
<td><a href="mailto:bah101@pitt.edu">bah101@pitt.edu</a></td>
</tr>
<tr>
<td>40</td>
<td>Hossain, Md Belayat</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:hossainbm@pitt.edu">hossainbm@pitt.edu</a></td>
</tr>
<tr>
<td>41</td>
<td>Ibrahim, Mohammed Nasar</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:moi13@pitt.edu">moi13@pitt.edu</a></td>
</tr>
<tr>
<td>42</td>
<td>Jia-Richards, Meilin</td>
<td>Behavioral Sciences</td>
<td><a href="mailto:meilin.jr@pitt.edu">meilin.jr@pitt.edu</a></td>
</tr>
<tr>
<td>43</td>
<td>Joshi, Supriya</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:supriyaj@pitt.edu">supriyaj@pitt.edu</a></td>
</tr>
<tr>
<td>44</td>
<td>Kaminski, Tomasz</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:kamins1@pitt.edu">kamins1@pitt.edu</a></td>
</tr>
<tr>
<td>45</td>
<td>Khouja, Tumader</td>
<td>Behavioral Sciences</td>
<td><a href="mailto:tuk4@pitt.edu">tuk4@pitt.edu</a></td>
</tr>
<tr>
<td>46</td>
<td>Kochetov, Bogdan</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:bok25@pitt.edu">bok25@pitt.edu</a></td>
</tr>
<tr>
<td>47</td>
<td>Koerner, Frederick</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:fsk8@pitt.edu">fsk8@pitt.edu</a></td>
</tr>
<tr>
<td>48</td>
<td>Koltun, Kristen</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:kjk116@pitt.edu">kjk116@pitt.edu</a></td>
</tr>
<tr>
<td>49</td>
<td>Lee, Sanghoon</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:sal170@pitt.edu">sal170@pitt.edu</a></td>
</tr>
<tr>
<td>50</td>
<td>Leiker, Emily</td>
<td>Behavioral Sciences</td>
<td><a href="mailto:ekl33@pitt.edu">ekl33@pitt.edu</a></td>
</tr>
<tr>
<td>51</td>
<td>Liu, Haitao</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:hal140@pitt.edu">hal140@pitt.edu</a></td>
</tr>
<tr>
<td>52</td>
<td>Lopez Caballero, Francisco Jose</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:lopezcaballero@upmc.edu">lopezcaballero@upmc.edu</a></td>
</tr>
<tr>
<td>53</td>
<td>Lu, Yuankai</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:yul363@pitt.edu">yul363@pitt.edu</a></td>
</tr>
<tr>
<td>54</td>
<td>MacFawn, Ian</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:macfawni@pitt.edu">macfawni@pitt.edu</a></td>
</tr>
<tr>
<td>55</td>
<td>Mahlke, Megan</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:mahlkem@upmc.edu">mahlkem@upmc.edu</a></td>
</tr>
<tr>
<td>56</td>
<td>Makeen, Mutasim</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:makeemm@upmc.edu">makeemm@upmc.edu</a></td>
</tr>
<tr>
<td>57</td>
<td>Matela, Abigail</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:arm208@pitt.edu">arm208@pitt.edu</a></td>
</tr>
<tr>
<td>58</td>
<td>Mihalko, Emily</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:epm35@pitt.edu">epm35@pitt.edu</a></td>
</tr>
<tr>
<td>59</td>
<td>Mitchell-Miland, Chantele</td>
<td>Behavioral Sciences</td>
<td><a href="mailto:cem54@pitt.edu">cem54@pitt.edu</a></td>
</tr>
<tr>
<td>60</td>
<td>Munyoki, Sarah</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:skm53@pitt.edu">skm53@pitt.edu</a></td>
</tr>
<tr>
<td>61</td>
<td>Muoio, Daniela</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:dam387@pitt.edu">dam387@pitt.edu</a></td>
</tr>
<tr>
<td>62</td>
<td>Narain, Apoorva</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:narain@pitt.edu">narain@pitt.edu</a></td>
</tr>
<tr>
<td>63</td>
<td>Natarajan, Niranjana</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:nataraj1@pitt.edu">nataraj1@pitt.edu</a></td>
</tr>
<tr>
<td>64</td>
<td>Nath, Poulomi</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:pon6@pitt.edu">pon6@pitt.edu</a></td>
</tr>
<tr>
<td>#</td>
<td>Name</td>
<td>Department</td>
<td>Email</td>
</tr>
<tr>
<td>----</td>
<td>------------------</td>
<td>---------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>95</td>
<td>Nuwer, Jessica</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:jln78@pitt.edu">jln78@pitt.edu</a></td>
</tr>
<tr>
<td>96</td>
<td>O’Brien, Julia</td>
<td>Behavioral Sciences</td>
<td><a href="mailto:jao99@pitt.edu">jao99@pitt.edu</a></td>
</tr>
<tr>
<td>97</td>
<td>Orczyk, John</td>
<td>Behavioral Sciences</td>
<td><a href="mailto:jjo56@pitt.edu">jjo56@pitt.edu</a></td>
</tr>
<tr>
<td>98</td>
<td>Panesso, Santiago</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:pannessos@upmc.edu">pannessos@upmc.edu</a></td>
</tr>
<tr>
<td>99</td>
<td>Parr, Ashley</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:acp73@pitt.edu">acp73@pitt.edu</a></td>
</tr>
<tr>
<td>100</td>
<td>Pathak, Trayambak</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:pathak@pitt.edu">pathak@pitt.edu</a></td>
</tr>
<tr>
<td>101</td>
<td>Pelletier, Jonathan</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:pelletierjh@upmc.edu">pelletierjh@upmc.edu</a></td>
</tr>
<tr>
<td>102</td>
<td>Perez Cortes, Luis</td>
<td>Behavioral Sciences</td>
<td><a href="mailto:lep101@pitt.edu">lep101@pitt.edu</a></td>
</tr>
<tr>
<td>103</td>
<td>Pfister, Katherine</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:kep103@pitt.edu">kep103@pitt.edu</a></td>
</tr>
<tr>
<td>104</td>
<td>Poploski, Kathleen</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:kmp174@pitt.edu">kmp174@pitt.edu</a></td>
</tr>
<tr>
<td>105</td>
<td>Pulgarin, Andres</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:jrocha@pitt.edu">jrocha@pitt.edu</a></td>
</tr>
<tr>
<td>106</td>
<td>Rankine, Jacquelin</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:jmr274@pitt.edu">jmr274@pitt.edu</a></td>
</tr>
<tr>
<td>107</td>
<td>Rice, Gavin</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:grr24@pitt.edu">grr24@pitt.edu</a></td>
</tr>
<tr>
<td>108</td>
<td>Riden, Katherine</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:katieriden@pitt.edu">katieriden@pitt.edu</a></td>
</tr>
<tr>
<td>109</td>
<td>Rosato, Teresa</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:tlc53@pitt.edu">tlc53@pitt.edu</a></td>
</tr>
<tr>
<td>110</td>
<td>Roth, Alexa</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:rothan@upmc.edu">rothan@upmc.edu</a></td>
</tr>
<tr>
<td>111</td>
<td>Ruffo, Elisa</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:eruffo@pitt.edu">eruffo@pitt.edu</a></td>
</tr>
<tr>
<td>112</td>
<td>Sagan, April</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:aps111@pitt.edu">aps111@pitt.edu</a></td>
</tr>
<tr>
<td>113</td>
<td>Santra, Mithun</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:santram@pitt.edu">santram@pitt.edu</a></td>
</tr>
<tr>
<td>114</td>
<td>Scott, Jewel</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:jls471@pitt.edu">jls471@pitt.edu</a></td>
</tr>
<tr>
<td>115</td>
<td>Scott, Madeline</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:mrs215@pitt.edu">mrs215@pitt.edu</a></td>
</tr>
<tr>
<td>116</td>
<td>Shang, Peng</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:shangp1@pitt.edu">shangp1@pitt.edu</a></td>
</tr>
<tr>
<td>117</td>
<td>Shang, Pengchen</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:pes69@pitt.edu">pes69@pitt.edu</a></td>
</tr>
<tr>
<td>118</td>
<td>Sheahan, Tayler</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:tayler.sheahan@pitt.edu">tayler.sheahan@pitt.edu</a></td>
</tr>
<tr>
<td>119</td>
<td>Smith, Kelly</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:ksmith5@pitt.edu">ksmith5@pitt.edu</a></td>
</tr>
<tr>
<td>120</td>
<td>Socorro, Mairoby</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:mas624@pitt.edu">mas624@pitt.edu</a></td>
</tr>
<tr>
<td>121</td>
<td>Sridar, Soumya</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:sos57@pitt.edu">sos57@pitt.edu</a></td>
</tr>
<tr>
<td>122</td>
<td>Strizhakova, Anastasia</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:strizhak@pitt.edu">strizhak@pitt.edu</a></td>
</tr>
<tr>
<td>123</td>
<td>Styler, Breelyn</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:brs251@pitt.edu">brs251@pitt.edu</a></td>
</tr>
<tr>
<td>Student ID</td>
<td>Name</td>
<td>Department</td>
<td>Email</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------</td>
<td>-------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>94</td>
<td>Szczupak, Diego</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:dis60@pitt.edu">dis60@pitt.edu</a></td>
</tr>
<tr>
<td>95</td>
<td>Tangudu, Naveen Kumar</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:nkt13@pitt.edu">nkt13@pitt.edu</a></td>
</tr>
<tr>
<td>96</td>
<td>Tian, Xiaoguang</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:XIT41@pitt.edu">XIT41@pitt.edu</a></td>
</tr>
<tr>
<td>97</td>
<td>Tracy, Eunjin</td>
<td>Clinical and Surgical Sciences</td>
<td><a href="mailto:tracyel@upmc.edu">tracyel@upmc.edu</a></td>
</tr>
<tr>
<td>98</td>
<td>Uboteja, Apoorva</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:apu7@pitt.edu">apu7@pitt.edu</a></td>
</tr>
<tr>
<td>99</td>
<td>Valle, Vincente</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:valvarez@pitt.edu">valvarez@pitt.edu</a></td>
</tr>
<tr>
<td>100</td>
<td>Vasametti, Sathish Babu</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:sathishv@pitt.edu">sathishv@pitt.edu</a></td>
</tr>
<tr>
<td>101</td>
<td>Vasylyeva, Iaroslavna</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:iav7@pitt.edu">iav7@pitt.edu</a></td>
</tr>
<tr>
<td>102</td>
<td>Vats, Abishek</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:avats@pitt.edu">avats@pitt.edu</a></td>
</tr>
<tr>
<td>103</td>
<td>Vats, Kavita</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:kav68@pitt.edu">kav68@pitt.edu</a></td>
</tr>
<tr>
<td>104</td>
<td>Verbaarschot, Ceci</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:verbaar@pitt.edu">verbaar@pitt.edu</a></td>
</tr>
<tr>
<td>105</td>
<td>Vieira Neto, Eduardo</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:EDV9@pitt.edu">EDV9@pitt.edu</a></td>
</tr>
<tr>
<td>106</td>
<td>Vincent, Ben</td>
<td>Cellular and Molecular Biology</td>
<td><a href="mailto:bjb22@pitt.edu">bjb22@pitt.edu</a></td>
</tr>
<tr>
<td>107</td>
<td>Wang, Bingrui</td>
<td>Engineering, Physical, and Computational Sciences</td>
<td><a href="mailto:biw29@pitt.edu">biw29@pitt.edu</a></td>
</tr>
<tr>
<td>108</td>
<td>Wilson, Tyla</td>
<td>Behavioral Sciences</td>
<td><a href="mailto:tkw13@pitt.edu">tkw13@pitt.edu</a></td>
</tr>
<tr>
<td>109</td>
<td>Woodcock, Chen-Shan</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:jcswh17@pitt.edu">jcswh17@pitt.edu</a></td>
</tr>
<tr>
<td>110</td>
<td>Woods Acevedo, Mikal</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:MAW372@pitt.edu">MAW372@pitt.edu</a></td>
</tr>
<tr>
<td>111</td>
<td>Wu, Limei</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:wul7@upmc.edu">wul7@upmc.edu</a></td>
</tr>
<tr>
<td>112</td>
<td>Zhang, Yue</td>
<td>Basic Biomedical and Pharmacological Sciences</td>
<td><a href="mailto:YUZ181@PITT.EDU">YUZ181@PITT.EDU</a></td>
</tr>
</tbody>
</table>
Interferon-γ, a master regulator driving immune response and T-cell exhaustion

Vaishali Aggarwal1,2; Chang Liu1,2; Erin A Brunazzi1,2; Kate Vignali1,2; Creg J Workman1,2 and Dario A A Vignali1,2,3

1Department of Immunology, School of Medicine, University of Pittsburgh;
2Tumor Microenvironment Center, UPMC Hillman Cancer Center;
3Cancer Immunology and Immunotherapy Program, UPMC Hillman Cancer Center

Immune checkpoint therapy has revolutionized therapeutic response in cancer patients. However, a subset of patients still do not respond to immunotherapy, which highlights the importance of understanding therapeutic resistance in non-responders. Interferon-gamma (Ifng), a key molecule, which drives protective T cell responses, augments anti-tumor response as well as promotes T cell exhaustion during tumor progression. This dynamic effect of Ifng impacts response to immune-checkpoint therapies.

Our lab is extensively studying the lineage plasticity of lymphocyte activating 3 (LAG3), an exhaustion marker, in the context of anti-tumor response. Our lab has generated a novel murine model that allows for restricted deletion of Ifng on Lag3 expressing cells (Lag3CreERT2IfngL/LRosa26LSL-tdT). This model was used to assess the effect of time-dependent deletion (D5~D7, D8~D10, D11~D13) of Ifng on Lag3 cells and correlate its effect on tumor growth, T cell exhaustion (T_ex) and response to immunotherapy. Preliminary results suggest that Ifng is an important mediator of effective anti-tumor responses and its deletion potentiates tumor growth in adenocarcinoma model. This suggests Ifng is necessary for reinvigoration of anti-tumor response and deletion of Ifng augments the T cell exhaustion and immunosuppressive tumor microenvironment (TME). This unique murine model will further help us in understanding the temporal role of Ifng through tumor progression in vivo. It will allow us to examine the pleiotropic effects of Ifng in early immune responses as well as T cell exhaustion (early vs terminal).

Elucidating the time-dependent expression profile of Ifng in the TME with respect to Lag3-T_ex will help provide us with mechanistic insights into the immune response to immunotherapy.
Blood flow regulates acvrl1 transcription via ligand-dependent Alk1 activity

Anzell, Anthony1; Kunz, Amy1; Donovon, J.P.1; Rochon, Elizabeth1; Rosato, Teresa1; Yang, J.1; Roman, Beth1

1Department of Human Genetics, School of Public Health, University of Pittsburgh

Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disease characterized by the development of arteriovenous malformations (AVMs) that can result in fatal complications. HHT is caused by mutations in ACVRL1/ALK1 or endoglin (ENG); overexpression of Acvrl1 prevents AVM development in Eng null mice, suggesting that enhancing ACVRL1 expression may be a promising approach to development of targeted therapies for HHT. Therefore, we seek to understand the molecular mechanism of acvrl1 regulation. We previously demonstrated in zebrafish embryos that acvrl1 is expressed in arterial endothelial cells proximal to the heart and that expression requires blood flow. Here, we document the time course of this response, demonstrating that acvrl1 is decreased within 1 hr after stopping heartbeat and re-expressed within 1 hr after restarting flow. Using a transgenic acvrl1:egfp reporter line, we find that flow-mediated acvrl1 regulation is at the level of transcription. Based on these results, we hypothesized that blood flow may be required for distributing the circulating Alk1 ligand, Bmp10, and that Bmp10/Alk1 activity may regulate acvrl1 expression by a positive feedback mechanism. In support of this hypothesis, we find that acvrl1 expression is significantly decreased in bmp10/bmp10-like double mutants, and that acvrl1 recovery after restarting flow largely depends on Bmp10. Notably, there is striking regional heterogeneity in the dependence of acvrl1 expression on flow and bmp10. Additionally, we find that bmp10 is sufficient for maintaining acvrl1 expression in the absence of blood flow. These data suggest that bmp10 acts downstream of blood flow to maintain acvrl1 expression and that ALK1 activating therapeutics may have dual functionality by increasing both ALK1 signaling flux and ACVRL1 expression in some but not all vascular beds.
CD10 negative Endometriosis Mesenchymal Stem Cells Promote Ovarian Clear Cell Carcinoma Tumorigenesis Through Altered Iron Regulation

Atiya, Huda¹; Frisbie, Leonard²; Goldfeld, Ester³; Orellana, Taylor⁴; Coffman, Lan¹,⁴.

¹Division of Hematology/Oncology, Department of Medicine, Hillman Cancer Center, ²Department of Integrative Systems Biology ³University of Pittsburgh School of Medicine ⁴Division of Gynecologic Oncology, Department of Obstetrics, Gynecology, and Reproductive Sciences, Magee Women’s Research Institute

Ovarian clear cell carcinoma (OCCC) is one of the most deadly and treatment resistant gynecologic cancers and is thought to arise within the unique microenvironment of endometriosis. However, the mechanisms underlying how endometriosis contributes to OCCC tumorigenesis is unclear. We identified a subset of endometriosis derived mesenchymal stem cells (enMSCs) that specifically support OCCC growth, chemotherapy resistance and metastasis. These enMSCs are characterized by loss of CD10 expression. Gain and loss of function experiments verified the functional importance of CD10 expression with enMSC CD10 knock down conveying tumor supportive properties while enMSC CD10 overexpression prevented pro-tumorigenic functions. RNA sequencing identified alterations in iron export in CD10 negative vs CD10 positive enMSCs and changes in metal transport and handling in OCCC cells grown with CD10 negative vs CD10 positive enMSCs. Independent verification confirmed that CD10 negative enMSCs express increased iron export proteins hephaestin and ferroportin. Functionally, CD10 negative enMSCs increased the levels of labile intracellular iron in associated OCCC cells. Importantly, the CD10 negative enMSC-mediated increase in tumor cell iron resulted in a unique sensitivity of these tumor cells to ferroptosis. Treatment with the ferroptosis inducer, Erastin, resulted in significant tumor cell death when grown with CD10 negative enMSCs both in vitro and in vivo. Collectively, this work identifies a subset of enMSCs with CD10 loss which support OCCC growth through altered iron regulation. We also identify a unique therapeutic vulnerability of OCCC to ferroptosis, dependent on the stromal phenotype. This work provides key insights into the role of endometriosis in OCCC tumorigenesis, emphasizes the importance of iron regulation within the tumor microenvironment and presents a potentially powerful new therapeutic target for this treatment-resistant disease.
Four-dimensional nuclear speckle phase separation dynamics regulate proteostasis

Dion, William¹; Ballance, Heather¹; Lee, Jane¹; Pan, Yinghong²; Irfan, Saad¹; Edwards, Casey¹; Sun, Michelle¹; Jing Zhang¹; Xin Zhang³; Liu, Silvia⁴,⁵; Zhu, Bokai¹,⁴,⁶

¹Aging Institute of UPMC, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
²UPMC Genome Center, Pittsburgh, PA, USA.
³Department of Chemistry, The Pennsylvania State University, University Park, PA, USA.
⁴Pittsburgh Liver Research Center, University of Pittsburgh, Pittsburgh, PA, USA.
⁵Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
⁶Division of Endocrinology and Metabolism, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

Phase separation and biorhythms control biological processes in the spatial and temporal dimensions, respectively, but mechanisms of four-dimensional integration remain elusive. Here, we identified an evolutionarily conserved XBP1s-SON axis that establishes a cell-autonomous mammalian 12-hour ultradian rhythm of nuclear speckle liquid-liquid phase separation (LLPS) dynamics, separate from both the 24-hour circadian clock and the cell cycle. Higher expression of nuclear speckle scaffolding protein SON, observed at early morning/early afternoon, generates diffuse and fluid nuclear speckles, increases their interactions with chromatin proactively, transcriptionally amplifies the unfolded protein response, and protects against proteome stress, whereas the opposites are observed following reduced SON level at early evening/late morning. Correlative Son and proteostasis gene expression dynamics are further observed across the entire mouse life span. Our results suggest that by modulating the temporal dynamics of proteostasis, the nuclear speckle LLPS may represent a previously unidentified (chrono)-therapeutic target for pathologies associated with dysregulated proteostasis.
Enhancing efferocytosis to promote resolution of MRSA SSTIs

Banerjee Srijon K¹, Thurlow Lance R² and Richardson Anthony R¹

¹ Microbiology and Molecular Genetics, School of Medicine, University of Pittsburgh
² Microbiology and Immunology, University of North Carolina, Chapel Hill

Community acquired (CA) Methicillin resistant *S. aureus* (MRSA) skin and soft tissue infections (SSTIs) are a major healthcare burden in the United States often left untreated leading to other fatal systemic diseases like sepsis. Therefore, the need to discover therapeutic interventions is urgent. The mouse model of CA MRSA SSTIs shows a distinct inflammatory phase followed by a resolution phase. The inflammatory phase is necessary but not sufficient for initial clearance of the pathogen. Instead, the resolution phase ensures bacterial clearance and healing of the abscess. Wound resolution in mammalian systems is largely dependent on efferocytosis of dead/dying cells by macrophages. Previously, our lab demonstrated that myeloid host factors like peroxisome proliferator-activated receptor gamma (PPARγ) are essential for the onset of the resolution phase in MRSA SSTIs. PPARγ activation also induces polyamine production by activating Arg-1 and polyamine turnover is crucial for efferocytosis. Here, we hypothesize that enhancing efferocytosis in MRSA SSTIs through external use of therapeutics would improve healing of the infected wound. We expect that such treatment would likely work by activating PPARγ and facilitating polyamine turnover.

Lipidomics analyses of murine MRSA abscesses revealed a temporal increase in abundance of mono- and poly-unsaturated fatty acids (MUFAs and PUFAs) in the absence of myeloid PPARγ. Further, in our attempt to understand the signals triggering PPARγ response in efferocytic macrophages, we find evidence that nitrated MUFAs like 10-nitro-oleic acid and poly-unsaturated fatty acids PUFAs like 10-nitro-linoleic acid not only promote digestion of apoptotic cells by macrophages but also induce the expression of the PPARγ regulon including certain polyamine synthesis genes. Finally, treatment of MRSA abscesses with conjugated linoleic acid in a murine model of skin infection reduces bacterial burden and abscess size. Our results point towards a promising method of enhancing the resolution phase of MRSA SSTIs through treatment with MUFAs and PUFAs.
Estimation of insult to retinal ganglion cell axons induced by elevated intraocular pressure: An axon-centric approach

Bansal, Manik\textsuperscript{1}; Zhong, Fuqiang\textsuperscript{1}; Hua, Yi\textsuperscript{1}; Reynaud, Juan\textsuperscript{3}; Fortune, Brad\textsuperscript{3}; Sigal, Ian A.\textsuperscript{1,2}

\textsuperscript{1}Department of Ophthalmology, University of Pittsburgh, Pittsburgh PA; \textsuperscript{2}Department of Bioengineering, University of Pittsburgh, Pittsburgh PA; \textsuperscript{3}Discoveries in Sight Research Laboratories, Devers Eye Institute Legacy Health Research, Portland OR

Purpose. Intraocular pressure (IOP)-induced insult to retinal ganglion cell axons contributes to progressive visual field loss in glaucoma. Current experimental and computational methods estimate axon insult without considering the spatial characteristics of axons. We developed an axon-centric approach to approximate 3D axon paths and quantify mechanical insult considering axon orientation in the optic nerve head.

Methods. Optical coherence tomography (OCT) images of a normal monkey eye were acquired at IOPs of 10 mmHg (normal) and 40 mmHg (elevated). Stretch and compression field maps were obtained by tracking IOP-induced deformations using digital volume correlation. The eye-specific non-collagenous tissue volume incorporating lamina cribrosa (LC) pore details was formed by combining manual reconstructions from OCT images (at normal IOP) and polarized light microscopy of cryosections. The large blood vessels were removed from the volume. Axon paths from the retinal nerve fiber layer to the optic nerve were approximated by a custom fluid tracing technique. The stretch and compression data obtained from the tracking were mapped on the 3D axon paths. The mapped values at each point on the axon path were then resolved in the directions transverse and longitudinal to the axons.

Results. Maximum (97.5\textsuperscript{th} percentile) stretch longitudinal and transverse to the axon was 11\% and 7\%, respectively. Maximum (97.5\textsuperscript{th} percentile) compression longitudinal and transverse to the axon was 14\% and 4\%, respectively. Axons in the retinal rim region experienced high longitudinal stretch and transverse compression. However, axons in the LC region experienced high longitudinal compression and transverse stretch.

Conclusions. The direction of stretch and compression represents a distinct mechanism of potential axon damage. The presented approach has the potential to help identify the link between IOP-induced deformation and neural tissue damage and glaucoma.
A Multiomics Approach To Placental Dysfunction In Common Obstetrical Syndromes
Barak, Oren\textsuperscript{1,2}; Lovelace, Tyler\textsuperscript{3,4}; Chu, Tianjiao\textsuperscript{1,2}; Sadovsky, Elena\textsuperscript{1}; Moulliet, Jean-Francois\textsuperscript{1,2}, Ouyang, Yingshi\textsuperscript{1,2}; Benos, Panayiotis\textsuperscript{3,4}; Sadovsky, Yoel\textsuperscript{1,2}

\textsuperscript{1}Magee Womens Research Institute; \\
\textsuperscript{2}University of Pittsburgh; \\
\textsuperscript{3}Department of Computational and Systems Biology, University of Pittsburgh; \\
\textsuperscript{4}Joint CMU-Pitt PhD Program in Computational Biology

Introduction:

Despite its high prevalence, the pathogenesis of human placental dysfunction (PD) and related clinical complications remains unknown. We used a high throughput multiomics approach to better define the molecular phenotype of PD and conditions related to it, including preeclampsia (PE), fetal growth restriction (FGR), and spontaneous preterm delivery (sPTD).

Methods:

Using Magee Obstetrical Maternal Infant Database and Biobank, we identified women with the: severe PE (n=75), FGR (birthweight<3rd centile; n=37), FGR with a hypertensive disorder (FGR+HDP; n=29), sPTD (n=72), and two control groups: 1) uncomplicated, term deliveries, birthweight >10th centile (n=113) 2) Induced PTD or elective cesarean section without PE, FGR or preterm labor (n=16). Placental biopsies, snap-frozen in liquid nitrogen, were used for transcriptomics, targeted proteomics, and untargeted metabolomics. We deployed an unsupervised dimensionality reduction algorithm, hierarchical clustering, differential expression analysis, and machine learning models to evaluate differences between the groups.

Results:

While there were no clear patterns between using the dimensionality reduction algorithm, applying hierarchical clustering to each data type displayed a noticeable separation of the cohorts from the controls, most significantly the FGR+HDP group. Among the differentially expressed molecules, we identified some known to play a role in placental dysfunction (sFLT-1, PGF, VEGF, endoglin) and some molecules that can contribute to our understanding (ADM, CXCL-1, IL-33). Features selection and causal models identified leptin and IGBP1 as noteworthy molecules.

Conclusions:

Multiomics analyses of the placental tissue provide new insights into the biological pathways underlying PD and identify potential biomarkers and biosignatures. Future integration of this multiomics approach with clinical and histopathological data may improve our ability to define and treat clinical syndromes that emanate from PD.
Dicarboxylic acid supplementation protects mice from AKI via increased renal peroxisomal activity

Barbosa, Anne\(^1\); Pfister, Katherine\(^1\); Chiba, Takuto\(^1\); Schilling, Birgit\(^2\); Young, Victoria\(^1\); Zhang, Bob\(^1\); Goetzman, Eric\(^1\); Sims-Lucas, Sunder\(^1\)

\(^1\) Department of Pediatrics, Children’s Hospital of Pittsburgh, University of Pittsburgh; \(^2\) The Buck Institute

Lysine succinylation is a posttranslational modification associated with the control of several diseases, including acute kidney injury (AKI), a condition associated with high morbidity and mortality. Hypersuccinylation favors peroxisomal fatty acid oxidation (FAO) instead of mitochondrial. In addition, the medium-chain fatty acids dodecanedioic acid (DC\(_{12}\)) and octanedioic acid (DC\(_{8}\)), upon FAO, originate succinyl-CoA, resulting in hypersuccinylation. Mice were fed mice with a control, a 10% w/w DC\(_{12}\), or 5-10% w/w DC\(_{8}\) diet, and subjected to unilateral renal ischemia-reperfusion (IRI), a classic AKI mouse model. Interestingly, DC\(_{12}\) prevented the rise of renal injury markers. However, DC\(_{8}\) was even more protective against AKI than DC\(_{12}\). Morphological features were also more preserved in DC\(_{8}\)-treated mice, showing that a DC\(_{8}\)-enriched diet mitigated the effects of ischemic AKI even at a 5% w/w supplementation. Intriguingly, while DC\(_{8}\) promotes succinylation in the kidneys only, DC\(_{12}\) promotes it in both liver and kidneys. Finally, a lysine succinylome-based mass spectrometry evidenced that, regardless of surgical status, the kidneys of DC\(_{12}\)- and DC\(_{8}\)-fed mice showed, respectively, a mild and an extensive upregulation of a myriad of peroxisomal activity-related peptides, and a decline in mitochondrial FAO, in comparison to control-fed mice. DC\(_{8}\) or DC\(_{12}\) supplementation drives renal hypersuccinylation, promoting a shift from mitochondrial to peroxisomal FAO, and protecting against AKI. Dicarboxylic acid supplementation is convenient, inexpensive, easily administered, and efficient. We believe this study could be translated in the future to the clinical setting, which would highly benefit the high-risk population.
Neuropeptide Y Y2 receptors in sensory neurons tonically suppress nociception and itch, but facilitate behavioral signs of neuropathic and postsurgical pain

Basu P\textsuperscript{1}, Maddula A\textsuperscript{1}, Nelson TS\textsuperscript{1}, Prasoon P\textsuperscript{1}, Taylor BK\textsuperscript{1}

\textsuperscript{1}Dept. of Anesthesiology and Perioperative Medicine, Pittsburgh Center for Pain Research, and Pittsburgh Project to end Opioid Misuse, University of Pittsburgh School of Medicine, Pittsburgh, PA

Exogenous or endogenous neuropeptide Y (NPY) acts at its cognate Y1 receptor in dorsal horn to tonically inhibit signs of inflammatory and neuropathic pain. The actions of NPY at the Y2 receptor (Y2R) on thinly myelinated primary afferent neurons are not as clear. To address this gap, we first asked whether intrathecal administration of the Y2R selective agonist, PYY\textsubscript{3-36}, would reduce behavioral signs of persistent pain after plantar incision or spared nerve injury (SNI) in male and female C57BL/6 mice. PYY\textsubscript{3-36} had minimal effect on either mechanical or thermal hypersensitivity when tested 2 days after incision or 14 days after SNI. This unexpected result could be attributed to endogenous NPY release and saturation of Y2Rs leading to unavailability of Y2Rs to bind to exogenous Y2 agonists. To begin to test this idea, we intrathecally administered the Y2R antagonist BIIE0246 in naïve mice. BIIE0246 did not change heat hypersensitivity or motor coordination, but dose-dependently (0.01-3\mu g) elicited mechanical and cold hypersensitivity. BIIE024 also elicited noifensive and itch-like behaviors. To determine whether these effects were mediated by Y2Rs in dorsal root ganglion (DRG) neurons, we crossed Pirt\textsuperscript{cre} mice with Npy2r\textsuperscript{lox/lox} mice to create Npy2R\textsuperscript{DRG-/-} conditional knockout mice. Npy2R\textsuperscript{DRG-/-} mice expressed Y2 protein in hippocampus but not DRG. BIIE0246 (3\mu g, i.t.) induced pain- and itch-like behaviors in Npy2r\textsuperscript{lox/lox} controls but not in Npy2R\textsuperscript{DRG-/-} mice. Npy2R\textsuperscript{DRG-/-} mice exhibited less mechanical and thermal hypersensitivity as compared to Npy2r\textsuperscript{lox/lox} controls in the SNI model of neuropathic pain and the plantar incision latent sensitization model of chronic postoperative pain. Together, our findings suggest that Y2Rs on primary afferent neurons exert two functions: 1) tonic inhibition of nociception and itch in the absence of injury; and 2) facilitate persistent pain in the setting of nerve or tissue injury. These results support that Y2R antagonists could serve as promising therapeutic agents for treatment of chronic neuropathic and/or postsurgical pain.
Isolated Support with an Extracorporeal Perfusion System Deters Ischemia-related Metabolic Derangement of a Rat Fasciocutaneous Free Flap

Fuat Baris Bengur 1, Ryan A. Orizondo 1,2, Chiaki Komatsu 1, Kelly R. Strong 2, Mario G. Solari, MD 1,3

1 Department of Plastic Surgery
2 Department of Bioengineering
3 McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Background: Machine perfusion of vascularized tissues can be used in microsurgery by enabling isolated perfusion of composite tissues such as free flaps. The perfusion can be used to temporarily perfuse tissues until fully supported by neovascularization. Rodent free flaps have been employed with limited success in this setting due to challenges with small vessels. This study aimed to establish a rodent model of machine perfusion in a fasciocutaneous free flap to serve as an affordable testbed and determine the potential of the developed protocol to deter ischemia-related metabolic derangement.

Methods: A 2x3 cm rat epigastric free flap was harvested and the vessels were cannulated. The flap was transferred to a closed circuit that provides circulatory support via a peristaltic pump and respiratory support via a custom gas exchanger. Whole rat blood was used as the perfusate. Outflow from the flap vein was recirculated during 8 hours of support. Blood samples were collected. Extracellular tissue lactate and glucose levels were characterized with a custom microdialysis probe placed in the flap tissue. Lactate to glucose ratio (L/G) was used as an indicator of tissue metabolism and compared with warm ischemic, cold ischemic and anastomosed free flap controls at the same timepoints.

Results: Maintenance of physiologic arterial pressures (85-100 mmHg) resulted in average pump flow rates of 300-450 uL/min with minimal flap bleeding. Blood-based measurements showed maintained glucose and oxygen consumption throughout support, indicating sustained metabolic activity. Average normalized L/G for the perfused flaps was 5- to 32-fold lower than that for the warm ischemic flap controls during hours 2-8 (p<0.05).

Conclusion: We developed a rat model of extended machine perfusion of a fasciocutaneous free flap. Ex vivo machine perfusion maintained stable perfusion and tissue metabolic activity out to 8 hours of support. This model can be used to further assess critical elements of support in this setting as well as explore other novel therapies and technologies to improve free tissue transfer.
Tissue Engineered Nerve Guide Containing GDNF Microspheres Improves Recovery After Facial Nerve Injury in Rats

Fuat Baris Bengur¹, Chiaki Komatsu¹, Jocelyn S. Baker¹, Caroline Nadia Fedor¹, Aanchal Totwani¹, Shawn Loder¹, Zhazira Irgebay¹, W. Vincent Nerone¹, Mario G. Solari¹,² Kacey G. Marra¹,²,³

¹Department of Plastic Surgery
²McGowan Institute for Regenerative Medicine
³Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA

Background: Injury to the facial nerve and the resulting facial nerve palsy leads to devastating functional, psychological, and cosmetic challenges. Rapid functional recovery after facial nerve injury is critical to prevent muscle atrophy and restore expression. Bioengineering plays an important role to create artificial scaffolds that can enhance the recovery. This can be improved by addition of exogenous neuro-supportive agents such as glial-derived neurotrophic factor (GDNF). In this study, we evaluated efficacy of a biodegradable nerve guide containing double-walled GDNF microspheres on outcomes in a rat facial nerve injury model.

Methods: GDNF was encapsulated within double-walled poly(lactic-co-glycolic acid)/poly(lactide) microspheres and embedded in the walls of poly(caprolactone) nerve guides. Lewis rats underwent transection and primary repair of the buccal branch of the facial nerve and divided as: a) transection and repair only, b) empty guide, c) GDNF-guide. Weekly measurements of the whisking movements were recorded. At the endpoint of 12-weeks, compound muscle action potentials at the whisker pad were assessed and nerve, muscle, and whisker pad were collected for histomorphometric analysis.

Results: GDNF-guide treated rats displayed earliest peak and achieved the highest whisking amplitude compared to the baseline. Compound muscle action potentials were significantly higher after GDNF-guide placement versus all others (p<0.001). Mean muscle fiber surface area at the levator labii superioris muscle was the highest (p<0.01). The axonal integrity loss was less prominent within the GDNF-guides and GDNF-guide group had the highest Schwann cell count. Gross morphology of the whisker pad was not different across the groups.

Conclusion: The novel tissue engineered nerve guide containing double-walled GDNF microspheres enhances recovery after facial nerve transection. Results support the clinical viability of these guides to enhance recovery after nerve injury and hold promise to facilitate recovery in defects with larger gaps.
Functional dissection of Vsx2 cis-regulatory elements during retinal development

Bian, Fuyun; Daghsni, Marwa; Lu, Fangfang; Liu, Silvia; Gross, Jeffery; Al Diri, Issam

Department of Ophthalmology, University of Pittsburgh

The roles of retinal cis-regulatory landscape in controlling the expression of gene regulatory networks important for retinogenesis remain poorly understood. Vsx2 is a transcription factor essential for retinal proliferation and bipolar cell differentiation. During early development, Vsx2 is expressed in retinal progenitor cells (RPCs) while later it is exclusively expressed in bipolar neurons and at low levels in Müller glia. A regulatory super-enhancer (SE) upstream of Vsx2 was identified previously. However, the molecular mechanisms underlying Vsx2 function and the functional roles of Vsx2 super-enhancer components are far from elucidated. Here, we profiled VSX2 genomic occupancy during mouse retinogenesis, revealing extensive retinal genetic programs associated with VSX2 during development. We defined an autoregulatory loop in which VSX2 binds and transactivates its own enhancer in association with the transcription factor PAX6. We identified VSX2 elements that drive robust reporter expression within the developing retina. Genetic deletion of a Vsx2 enhancer element leads to microphthalmia and reduced proliferation process, recapitulating retinal defects observed in Vsx2-null mutant mice. However, different from Vsx2-null mutant mice, these knock out mice still have bipolar cells. Taken together, our analyses illuminate important mechanistic insights on how VSX2 is engaged with gene regulatory networks that are essential for retinal development. Our results also revealed a cell specific cis-regulatory element which is responsible for the expression of Vsx2 and proliferation of retinal progenitor cells in retinal development. Thus, providing insights onto gene regulatory networks that govern Vsx2 expression during retinal development.
Cardioprotective mechanism of GCN5L1 in cardiac ischemia-reperfusion injury

Paramesha Bugga¹, Janet Manning¹, Michael Stoner¹, Bellina Mushala¹, Iain Scott¹

¹Vascular Medicine Institute, Department of Medicine, University of Pittsburgh

Ischemia-reperfusion (I/R) injury remains the leading cause of heart failure. Reduced expression of the acetyltransferase enzyme GCN5L1 is commonly observed in ischemic heart disease, and its reduction is hypothesized to reduce protection from ischemic injury. The purpose of this study is to understand the molecular mechanisms underlying GCN5L1 cardio protection from ischemic injury, and identify potential therapeutic targets in this pathway. In our first study, we induced cardiac I/R injury in WT and GCN5L1 KO mice using an ex-vivo working heart system, and evaluated cardiac function and infarct size by TTC staining. GCN5L1 KO mice were more prone to cardiac I/R injury when compared with WT mice.

To identify the cardioprotective mechanisms whose function is dependent on GCN5L1 expression, we performed in-vitro hypoxia-reoxygenation (H/R) experiments in control and GCN5L1 knockdown cardiac AC16 cells, followed by measurement of mitochondrial ROS and the activation of proteins responsible for cardiomyocyte cytoprotection. We found a significant increase in mitochondrial ROS in GCN5L1 KD AC16 cells at baseline. H/R injury further amplified this mitochondrial-derived ROS, due to a perturbation in the expression and function of antioxidant enzyme proteins in GCN5L1 KD AC16 cells.

Finally, we examined whether GCN5L1 regulates the AKT protective pathway in cardiac cells. AKT is activated in response to stress in cardiac cells, and this activation is required to induce cardioprotective signaling. AKT is activated by the mTORC2 kinase complex, and we found that GCN5L1 interacts with, and acetylates, the mTORC2 regulatory protein Rictor. Loss of GCN5L1 expression reduced Rictor expression, which correlated with reduced AKT activation. Our future studies will examine how the GCN5L1:Rictor interaction may be targeted for novel therapeutic purposes.
Matrix-bound nanovesicles as selective modulators of the immune response

Hector Capella-Monsonis\textsuperscript{1,2}, Raphael J Crum\textsuperscript{1,2}, Stephen F Badylak\textsuperscript{1,2,3}

\textsuperscript{1}McGowan Institute for Regenerative Medicine
\textsuperscript{2}Department of Surgery
\textsuperscript{3}Department of Bioengineering

Matrix bound nanovesicles (MBV) have recently been identified as an inherent component of the extracellular matrix (ECM) and possess the ability to mitigate the proinflammatory activation state of macrophages. While the “anti-inflammatory” properties of MBV have several potential clinical applications, it is unknown if there is an associated compromise of the broader immune system. Stated differently, the systemic effects of MBV, and more specifically the effects of MBV upon the adaptive immune system and the ability to mount a protective immune response to pathogens is unknown and has not been explored.

Mice were vaccinated with the pneumococcal vaccine PneumoVax™23 at day 0. Then, for 5 weeks, MBV (10\textsuperscript{11}/mouse) were injected IP weekly, while a weekly dose of methotrexate was used as immunosuppressor control. Anti-pneumococcal polysaccharide IgG and IgM antibody titers were measured at days 7 and 28. At week 5 mice were infected with \textit{Streptococcus pneumoniae} either in an infection (intranasal) or sepsis (IP) model, and the survival of the animals was recorded over 2 weeks.

Pneumococcal vaccine-immunized mice with and without MBV treatment presented similar anti-\textit{S. pneumoniae} polysaccharide IgG and IgM titers at days 7 and 28; that is, there was no compromise of the adaptative immune response following treatment with MBV. In contrast, immunized mice treated with methotrexate showed lower IgG and IgM titers. The intact functionality of the humoral immune response in MBV treated animals was further confirmed with the higher titer levels in vaccinated mice with and without MBV treatment after infection. Interestingly, when infected IP with a lethal dose of \textit{S. pneumoniae} (10\textsuperscript{10} CFU/mouse), 50\% of mice treated with MBV after vaccination showed complete recovery after 14 days, whereas the rest of the groups showed no survival after 2 days of infection. These results suggest a boosting effect on the adaptative immune response elicited by MBV.

Overall, the anti-inflammatory properties of MBV do not compromise the adaptative immune system.
Telomerase Reverse Transcriptase and Signal Transducer and Activator of Transcription 5A Mediates Osteogenesis in Calcific Aortic Valve Disease

Rolando A. Cuevas¹, Claire Chu¹, Ryan Wong¹, Alex Crane¹, John Sembrat², Ibrahim Sultan³, and Cynthia St. Hilaire¹,⁴

¹Division of Cardiology, Department of Medicine, Pittsburgh Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.
²Division of Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
³Division of Cardiac Surgery, Department of Cardiothoracic Surgery, Heart and Vascular Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.
⁴Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.

Cardiovascular calcification is a highly prevalent pathological process found in the vessels and valves in the elderly and patients with diabetes, hypertension, and renal disease. Calcific aortic valve disease (CAVD) stiffens and remodels the valve leaflets and leads to valve dysfunction, cardiac failure, and increased stroke risk. Vascular calcification can occur in the necrotic core of atherosclerotic plaques or in the medial layer of arteries. However, the initial steps dictating the onset of calcification remain ill-defined. Multiple studies have revealed that the protein telomerase reverse transcriptase (TERT), the catalytic subunit of the enzymatic complex required for telomere length maintenance, is a co-factor to stimulate gene transcription, and its overexpression primes mesenchymal stem cells to differentiate into osteoblasts. We determined that TERT is required for the calcification valve interstitial cells (VICs) and coronary smooth muscle cells (SMCs); TERT expression and protein are increased in calcified tissues and cell lines, independent of changes in telomere length. Genetic deletion of Tert in murine VICs and SMCs prevented calcification. Upon osteogenic stimulation, TERT binds to Signal Transducer and Activator of Transcription 5A/B (STAT5) to translocate into the nucleus where the complex binds to the RUNX2 promoter to induce expression. We found that VICs cultured under inflammatory conditions calcified and upregulated STAT5, suggesting that inflammatory signaling may promote calcification in VICs. Lastly, testing several STAT5 inhibitors, we determined that STAT5A is required for the calcification of VICs and SMCs. These findings are the first to suggest that TERT and STAT5 are involved in driving the osteogenic switch and calcification of vascular cells and constitute potential therapeutic targets for the treatment of CAVD.
Biogenesis of Matrix-Bound Nanovesicles

Dewey, Marley¹, Badylak, Stephen¹

Department of Surgery, University of Pittsburgh

Extracellular vesicles are key components of cell-signaling, as these lipid vesicles can direct cell fate. Matrix-bound nanovesicles (MBV) are a recently discovered class of extracellular vesicle, which are found residing in the extracellular matrix (ECM) and are distinctly different from other vesicle types, such as exosomes. To better understand and utilize MBV as a therapy and diagnostic, there remains a significant question of interest: how exactly are MBV produced?

My work aims to understand the biogenesis of MBV, or the cellular biology behind their production. To accomplish this, I have broken this large question into three: (1) Where are MBV produced in the cell? (2) How do MBV bind to the ECM? (3) What are the mechanisms used to produce MBV? Answering these questions will lead to a better understanding of the differences between extracellular vesicle types and effective use of MBV. Fluorescent lipid dyes specific to various regions of the cell are used to examine differences in MBV and exosome staining to determine MBV origins. Western blotting is then used to probe for various ECM-binding related proteins to determine if MBV have a higher binding affinity to matrix components. High-resolution imaging is used to visualize whether MBV bind to the ECM before or after their production. Finally, I block the endosomal sorting complex required for transport machinery (ESCRT), which influences exosome production, and evaluate whether blocking this pathway also inhibits MBV release and collagen formation.

In answering these questions about how MBV are produced we can elucidate more of the differences between MBV and exosomes, and then we can better apply these vesicles in regenerative medicine.
Bioscaffold-induced brain tissue regeneration after stroke

Nadine Didwischus¹,² PhD, Michel Modo PhD¹,²,³

¹McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA 15260, USA
²Department of Radiology, University of Pittsburgh, Pittsburgh, PA 15260, USA
³Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15260, USA

Stroke is an ischemic event during which parts of the brain experience interruption of blood flow. The resulting lack of oxygen and nutrient supply causes affected brain cells to die in minutes. Even when eligible, in patients who receive a thrombectomy treatment within the recommended treatment window, the lost brain tissue cannot be restored. Up to 1 year after stroke, tissue repair is ongoing, which is indicated by proliferating neural progenitor cells (NPCs) invading peri-infarct tissue. However, formation of new neural tissue in the cavity does not occur, potentially due to the lack of a physical substrate for cell positioning. In previous studies, we showed that implantation of bioscaffolds, such as extracellular matrix (ECM) hydrogels, provide structural support for NSCs to invade the cavity and generate de novo tissue. This is followed by neovascularization of the cavity as host cells infiltrate and degrade the bioscaffold. To form new brain tissue, the bioscaffold milieu needs to be prepared for target organ cell invasion, which is facilitated through M2-polarization of macrophages. In previous studies we reported the infiltration of macrophages into the bioscaffold, but observed a significantly lower neuron content of <5% 90 days after implantation versus 42% in healthy brain tissue. To enhance neural tissue recovery to a level that potentially allows behavioral recovery, we hypothesize that an increase of the endogenous NPC population will improve the neuron density in the tissue cavity. Our preliminary data showed that the epidermal growth factor family member betacellulin (BTC) stimulates neurogenesis and increases the number of NPCs in the peri-infarct area and in the ECM bioscaffold. We aim to investigate (i) if a dose of 6.6 µg/mL BTC for 4 weeks will be optimal to enhance neurogenesis and promote the invasion of NPC into the bioscaffold. Further, we will examine (ii) if stroke animals that received ECM bioscaffold implantation and BTC administration will results in behavioral improvements.
Methods to detect and target mitochondrial DNA G-quadruplexes

Duvall S¹, Johnson M¹, Redding K¹, Kaufman B¹

¹ University of Pittsburgh, Department of Medicine, Vascular Medicine Institute (VMI)

Introduction: Mitochondrial DNA (mtDNA) contains guanine rich stretches that likely form secondary structures called G-quadruplexes (G4s). G4s are strong secondary structures that require intervention by helicases to unwind for transcription or replication to occur. Few mtDNA G4s have direct evidence of formation and the biological roles these G4s play is not well understood. G4 sequences in cells can be targeted by single-chain variable fragments (scFv), which consist of a fusion of the variable heavy and variable light domains of an antibody. G4-binding scFv can be used to identify mtDNA G4s using chromatin immunoprecipitation (ChIP). scFv are also a potential therapeutic for treating mtDNA heteroplasmy by binding the pathogenic G4 sequences and blocking replication. One G4 forming sequence is m.10191T>C, which results in Leigh Syndrome. Leigh Syndrome occurs when the heteroplasmy level, the ratio of wild-type and pathogenic mtDNA, reaches a disease threshold. The m.10191T>C sequence takes on a specific conformation, S2, that is inaccessible to m.10191T. This specific conformation presents a unique target to reduce heteroplasmy in cells by blocking replication.

Methods: scFv expressed as intrabodies in cells are targeted to mitochondria using a mitochondrial targeting sequence referred to as cox8l (mt-intrabodies). Two scFv, BG4 and HF1, are general G4 binders and are being used to map mtDNA G4s using ChIP-seq approaches. Impact on mitochondrial replication and transcription is being measured using qPCR. A bioinformatics pipeline we developed using Python and R has been used to identify candidate sequences for future biochemical studies.

Results/Conclusions: BG4 and HF1 have been expressed as mt-intrabodies in 143B cells and have colocalization with mitochondria by immunostaining. HF1 has an impact on mtDNA and transcription compared to controls. ChIP enrichment is also higher for HF1. Phage-display screening has generated enriched scFv libraries. Using a bioinformatics pipeline we developed, candidate scFv sequences have been identified.
The Smooth-Muscle-Cell-Angiotensin II-sensitive lncRNA controls cell division fidelity and mitochondrial organization

Cristina Espinosa-Diez¹, Mingjun Liu¹², Sidney Mahan¹, Mingyuan Du¹, Wenxi An¹, Scott Hahn¹, Adam C Straub¹, Sruti Shiva¹, Delphine A Gomez¹²

¹Pittsburgh Heart, Lung, Blood, and Vascular Medicine Institute, University of Pittsburgh; ²Division of Cardiology, Department of Medicine, University of Pittsburgh

Reactivation of the cell cycle and increase in proliferation rate (hyperplasia) is a common response of vascular smooth muscle cells (SMC) to modifications of their environment during remodeling. Although SMC hyperplasia is a predominant feature of many vascular diseases, SMC can also increase their mass within the remodeled vessel wall by enlarging their size and becoming hypertrophic. Hypertrophy is usually accompanied by cell cycle defects, cell polyploidy and binucleation, and senescence. However, the molecular mechanisms favoring SMC hypertrophy and their repercussion on SMC phenotype are not fully understood. Long-non-coding-RNAs (LncRNAs) are epigenetic regulators of gene expression, and they have been identified as modulators of cell division. We recently discovered a novel lncRNA, SAS (SMC-Angiotensin II-Sensitive), whose expression was markedly decreased in multiple models of SMC dedifferentiation. Publicly available transcriptional datasets revealed that SAS is preferentially expressed in SMC-rich tissues, including the aorta, in humans and mice. Knockdown of SAS reduces proliferation, cell arrest and migration in aortic and renal artery-derived SMC treated with Platelet Derived Growth Factor. SAS knockdown was also associated with distinct SMC morphological changes including increase in cell size and binucleation. Together, these observations suggest that decrease in SAS causes SMC hypertrophy due to defects in cell cycle completion. Interestingly, SAS expression is decreased in response to Angiotensin-II in cultured VSMC and in the aorta of hypertensive mice (2 Kidney-1 Clip model), suggesting a role in mediating hypertension induced SMC hypertrophy. Similarly, to Angiotensin-II treatment, SAS knockdown promoted senescence. Furthermore, SAS deficient cells present mitochondria hyperfusion and increased oxygen consumption that correlates with the observed exacerbated senescence. Altogether, our results indicate that SAS is a potent regulator of VSMC morphology and is required for proper cell division and mitochondria organization.
Ebstein’s anomaly (EA) is a rare but serious congenital heart defect accounting for about 1 in 200,000 live births. EA is featured with atrialized right ventricle and abnormal tricuspid valve. Heart surgery to repair the tricuspid valve is typically used in conjunction with medical therapy for heart failure to improve patient survival. However, the fact that the causes of the disease are still largely unclear makes it challenging to cure the disease by medical intervention at early embryonic stages and medical treatments at later life stages. In this study we have used human induced Pluripotent Stem Cell (hiPSC) to understand the cellular and molecular mechanisms of EA pathogenesis. Using CRISPR/Cas9 we first generated an isogenic line by introducing an EA associated mutation to the myocardial gene Nkx2-5. Then we differentiated the line into heart organoids to understand its ventricular and atrial lineage specifications. Meanwhile, we generated multiple iPSC lines from EA patients through somatic reprogramming. Our preliminary analysis of the cardiomyocytes from these patient lines indicated that they had abnormal cell proliferation and misspecification of cardiac lineages. Next, we are planning to analyze the genetic variants in these patient cells using exome sequencing and to investigate the valve development defect using in vitro valve cell differentiation assays. These research together are expected to give a systematic understanding of the disease causes in EA.
Evaluation of a Model-Based Precision Dosing Platform using Bayesian Forecasting Method for Personalized Busulfan Therapy in the Adult Hematopoietic Stem Cell Transplantation Population with Limited Sampling

Feturi, Firuz1; Seton, Jack1; Hughes, Maria-Stephanie2; Pilla, John2; L'Altrelli, Alfred1,3; Keizer, Ron2; Venkataramanan, Raman1

1 Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, USA
2 InsightRX, San Francisco, CA, USA
3 UPMC Presbyterian-Shadyside Hospital, Pittsburgh, PA, USA

The purpose of this study was to evaluate the ability of a model-based precision dosing platform utilizing Bayesian forecasting (BF) to reliably estimate busulfan exposure and recommended doses in comparison to the current clinical practice and to determine a limited sampling strategy (LSS) to minimize the number of blood samples necessary and the inconvenience to patients.

Data from patients who received intravenous busulfan as part of conditioning regimen prior to hematopoietic stem cell transplantation (HSCT) were entered into BF software, InsightRX Nova. Estimation of busulfan exposure and dose recommendations was compared to the clinical practice values, aiming for the predefined target exposure of approximately 4X4800 µmol*min/L. Estimation performance was tested using several LSSs.

Fifty-one adult patients (23 to 65 years) provided exposure measurements estimated using 7 blood samples collected following the first dose administration. BF provided acceptable bias and precision of exposure estimations under all tested sampling strategies (<15%). The bias and precision of AUC estimates ranged from MAE of 0.7–8.5% and rRMSE of 9–14%, respectively. Two LSSs including LSS-VI (with 3, 4, 5, and 6h) and LSS-VII (with 3, 4, and 6h) predict AUC-ISS with good precision and minimal bias. These LSSs performed similarly well in an independent external validation, where the AUC and recommended doses predicted by BF using these LSSs have acceptable agreement, with those calculated by NCA using ISS (MAE ≤15%) in >80% of the cases.

Model-based precision dosing platform utilizing BF with only 3 or 4 plasma busulfan concentrations can be used to reliably estimate busulfan exposure after intravenous administration in adults undergoing HSCT. This study provides supporting evidence for the utility of Bayesian forecasting-based platforms in clinical practice as it will simplify and standardize the hospital service.
Genotypic and Phenotypic Diversity of Contemporaneous Carbapenem Resistant *Klebsiella pneumoniae* from Blood Cultures of Individual Patients.

**Fleres, Giuseppe**¹; **Cheng, Shaoji**¹; **Chen, Liang**²; **Liu, Guojun**¹; **Hao, Binghua**³; **Newbrough, Anthony**¹; **Shields, Ryan K**¹,³; **Kreiswirth, Barry**²; **Nguyen, M. Hong**¹,³; **Clancy, Cornelius J**¹

¹Infectious Diseases Division, Department of Medicine, University of Pittsburgh; ²Hackensack Meridian School of Medicine & Hackensack Meridian Health Center for Discovery and Innovation, Nutley, NJ; ³University of Pittsburgh Medical Center, Pittsburgh, PA

**Background:** The longstanding paradigm is that almost all bloodstream infections (BSIs) stem from a single, clonal organism. We hypothesized that carbapenem resistant *K. pneumoniae* (CRKP) from individual patients (pts) with BSIs are genetically diverse and manifest phenotypic differences that are not typically recognized by the clinical microbiology laboratory at time of diagnosis.

**Methods:** We streaked blood culture (BC) broth from 6 pts with CRKP BSI onto blood agar plates, and randomly picked 9 colonies (strains) for HiSeq (Illumina) whole genome sequence. Single BSI isolates recovered by the clinical micro lab from individual pts underwent short- and long-read MinION (ONT) sequencing.

**Results:** 2 pts were infected with clade 1 (B, G), and 4 with clade 2 (A, D, F, J) ST258 KPC-producing CRKP. BC bottles from each pt harbored genetically heterogenous CRKP populations, with strains differing from each other by cgSNPs, presence/absence of specific antibiotic resistance genes, mutations of capsular genes and at other loci involved in host interactions, and/or loss of plasmids or plasmid-borne genes. Differences in capsular gene composition were observed in KL107 capsule type strains from pts A, D and J. Pangenome analyses showed accessory gene composition diversity among strains from all pts. Within-host genetically diverse strains exhibited phenotypic differences in antibiotic resistance, viscosity and mucosity, capsular content, and resistance to serum and macrophage killing. Various strains from pts A and J differed in ability to cause target organ infections or mortality in a mouse model of intravenous disseminated infection.

**Conclusion:** We identified genotypic and phenotypic variant strains of ST258 KP from BSIs of individual patients that were not recognized at time of diagnosis. Our data suggest a new, population-based paradigm for BSIs by CRKP. The findings potentially have profound implications for medical, microbiology laboratory and infection prevention practices, and for understanding emergence of antibiotic resistance and pathogenesis.
Lack of Amelogenin Phosphorylation Leads to Acidification of the Forming Enamel

Claire M. Gabe1,2, Ai Thu Bui1,2, Brent P. Vasquez1,2, Elia Beniash1,2, Henry C. Margolis1,2,3

1 Department of Oral and Craniofacial Sciences, Center for Craniofacial Regeneration, University of Pittsburgh School of Dental Medicine, Pittsburgh, PA, USA
2 Center for Craniofacial Regeneration, Pittsburgh, PA, USA
3 Department of Preventive Dentistry and Periodontics, Pittsburgh, PA, USA.

Introduction: Enamel is the most mineralized tissue of our organism. It consists of well-arranged hydroxyapatite (HA) crystals. While forming, it is composed predominantly of organic material. The roles of enamel proteins have not yet been fully elucidated, although they are crucial to reaching enamel’s thickness and unique crystalline structure. Amelogenin (AMELX) is the major protein of the forming enamel. Carrying a phosphorylation on Serine 16, it is necessary in controlling enamel mineralization. Earlier in vitro studies showed that phosphorylated AMELX helps stabilizing calcium phosphate phase and the environmental pH [1,2]. To study effects of AMELX phosphorylation in vivo, we developed a AMELX<sup>Ser16Ala</sup> knock-in (KI) mouse model, which lacks AMLEX phosphorylation. KI mice showed a severe phenotype and an accelerated rate of HA formation [3].

We hypothesize that accelerated enamel mineralization in AMELX<sup>Ser16Ala</sup> KI mice leads to local acidification that results in structural changes the forming enamel.

Methods: We isolated incisors from wild-type (WT), and KI male mice mandibles and immersed them in pH indicators. The differences in color patterns associated with pH changes were analyzed with ImageJ. To obtain structural information on organic and mineral phases, we also conducted Fourier-transform infrared spectroscopy (FTIR) microspectroscopy.

Results: pH-indicator studies revealed that the initial forming enamel was more acidic in KI vs. WT incisors. Furthermore, a pronounced pattern of alternating low and high pH, typical of WT enamel, was absent in KI mice. FTIR spectra revealed higher rates of mineralization of KI enamel. Marked differences in protein Amide I peak were also observed, indicating conformational differences in WT and KI enamel matrices.

Conclusion: Together, our observations demonstrate that lack of amelogenin phosphorylation leads to acidification of secretory enamel that affects its mineral and organic phases.
Crosstalk between metabolism and inflammation in dry age-related macular degeneration

Ghosh, Sayan¹; Shang, Peng¹; Stepicheva, Nadezda A¹; Liu, Haitao¹; Chowdhury, Olivia¹; Koontz, Victoria¹; Strizhakova, Anastasiia¹; Daley, Rachel¹; Hose, Stacey¹; J. Zigler, Samuel Jr.²; Sinha, Debasish¹,²

¹Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
²The Wilmer Eye Institute, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Purpose: The dry form of age-related macular degeneration (AMD) is one of the leading causes of blindness in the elderly. Metabolic alterations and chronic inflammation have been identified as critical factors that contribute to the progression of dry AMD. This study evaluated the crosstalk between metabolic changes and inflammation induction in a mouse model that shows a dry AMD-like phenotype, with the intent to target specific molecules in these pathways to delay the progression of the disease.

Methods: RNAseq analysis was performed on retinal pigmented epithelial (RPE) cells from wild type RPE explants overexpressing βA1-, βA3- or βA3/A1-crystallin. To compare the metabolic gene profile/intermediates in the RPE cells at the early and advanced stages of disease progression in a mouse model, scRNAseq (cells from the sub-retinal space) and metabolomics/lipidomics (RPE cells) analyses were performed on 3, 10 and 15 month old Cryba1-floxed and cKO mice. βA3 KO mice were fed a high fat diet (HFD) to induce metabolic stress and the resulting changes in signaling mediators and histopathology were assessed.

Results: Metabolomics studies revealed a significant increase in the levels of known inducers of mTORC1 signaling, like aspartate and betaine, in cKO RPE relative to controls. Lipidomic profiling showed elevated fatty acids (FAs) in cKO RPE, owing to the activation of the mTORC1-dependent FA synthesis pathway. We identified IL-17 signaling pathway activation as the link between these metabolic changes and chronic inflammation induction in the RPE of Cryba1 cKO mice during disease progression. Further, HFD treatment in βA3 KO mice exacerbated the alterations in IL-17 and FA levels and induced lipid droplet accumulation in RPE cells.

Conclusions: We show that IL-17 mediated inflammatory signaling as the link between cellular metabolic alterations and chronic inflammation in a mouse model of dry AMD. Hence, targeting IL-17 and/or molecules of this signaling pathway could be an avenue for future therapeutic intervention to treat dry AMD.
Interferon Gamma Induction of T\textsubscript{H}1-like T\textsubscript{regs} Controls Anti-viral Responses

Gocher-Demske, Angela M.\textsuperscript{1,2}, Cui, Jian\textsuperscript{1,2}, Szymczak-Workman, Andrea L.\textsuperscript{1}, Vignali, Kate M.\textsuperscript{1,2}, Latini, Julianna N.\textsuperscript{1,2}, Pieko, Gwenth P.\textsuperscript{1,2}, Avery, Lyndsay\textsuperscript{1}, Cipolla, Ellyse L.\textsuperscript{3}, Huckestein, Brydie R.\textsuperscript{3}, Hedden, Lee\textsuperscript{1}, Meisel, Marlies\textsuperscript{1,4}, Alcorn, John F.\textsuperscript{3}, Kane, Lawrence P.\textsuperscript{1,4}, Workman, Creg J.\textsuperscript{1,2}, Vignali, Dario A. A.\textsuperscript{1,2,4,*}

\textsuperscript{1}Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA.
\textsuperscript{2}Tumor Microenvironment Center, University of Pittsburgh Cancer Institute, Pittsburgh, PA.
\textsuperscript{3}Department of Pediatrics, Children’s Hospital of Pittsburgh of UPMC, Pittsburgh, PA.
\textsuperscript{4}Cancer Immunology and Immunotherapy Program, UPMC Hillman Cancer Center, Pittsburgh, PA 15232, USA.

Regulatory T cells (T\textsubscript{regs}) are an immunosuppressive cell population that inhibits immune cell function to maintain peripheral tolerance and homeostasis. We have previously shown that T\textsubscript{reg} response to interferon gamma (IFN\textgamma) is required for response to cancer immunotherapy. We aimed to broaden these findings and determine the impact of T\textsubscript{reg} response to IFN\textgamma and the IFN\textgamma-inducing cytokine IL12, during viral infection. Mice, with T\textsubscript{reg}-restricted deletion of the IFN\textgamma receptor (IFNGR1, Ifngr\textsubscript{1}\textsuperscript{L/L}-Foxp\textsuperscript{3Crc-YFP}) or the IL12 receptor \textbeta\textsubscript{2} subunit (IL12R\textbeta\textsubscript{2}, Il12b\textsubscript{2}\textsuperscript{L/L}-Foxp\textsuperscript{3Crc-YFP}), were infected with the acute (Armstrong), or chronic (Clone 13), strain of lymphocytic choriomeningitis virus (LCMV). Surprisingly, T\textsubscript{reg} response to IFN\textgamma but not IL12, induced helper T cell 1 (T\textsubscript{H}1)-like polarization (expression of T-bet, CXCR3 and IFN\textgamma) of T\textsubscript{regs} in mice with chronic LCMV infection. Importantly, this effector-like T\textsubscript{H}1 T\textsubscript{reg} state was required for adequate suppression of effector T cells during LCMV infection. Additionally, during LCMV infection, T\textsubscript{reg}-restricted deletion of IFNGR1 not only prevented T\textsubscript{H}1-like polarization but also reverted these T\textsubscript{regs} to a T\textsubscript{H}2-like state (expression of GATA-3, CCR4 and IL-4). scRNAseq of viral antigen-specific CD8\textsuperscript{+} T cells from Clone 13 infected mice showed that Ifngr\textsubscript{1}-deficient T\textsubscript{regs} favored CD8\textsuperscript{+} T cell memory. These findings were confirmed in an Armstrong/Listeria monocytogenes-GP33 memory model. Further, Ifngr\textsubscript{1} deletion from T\textsubscript{regs} enhanced response to CD8\textsuperscript{+} T cell-mediated vaccination when challenged with Clone 13. These findings provide fundamental insight on how T\textsubscript{regs} sense inflammatory cues from the environment (IFN\textgamma) during viral infection to prevent overt tissue damage and shape the memory response.
Somatosensory and thalamic inputs correlative in pairs of parvalbumin positive inhibitory and pyramidal excitatory neurons in mouse motor cortex.

Goz$^1$ R. U.; Schneider$^2$ N. A.; Arnold$^2$ M. P.; Williamson$^2$ R. S.; Hooks$^1$ B. M

$^1$Department of Neurobiology, University of Pittsburgh School of Medicine
$^2$Otolaryngology, University of Pittsburgh School of Medicine

In mammalian cortex, feedforward excitatory connections invariably recruit feedforward inhibition. This is often carried by fast-spiking (parvalbumin, PV+) interneurons, which potentially connect densely to local pyramidal (Pyr) neurons. Whether this inhibition generically inhibits all local excitatory cells or is targeted to specific subnetworks is unknown. Here, we test how feedforward inhibition is recruited by cortical and thalamic afferents by using 2-channel circuit mapping to excite (S1 and PO) inputs to PV+ interneurons and pyramidal neurons of mouse motor cortex. We find that connected pairs of PV+ interneurons and excitatory pyramidal neurons receive correlated cortical and thalamic inputs. This suggests that excitatory inputs to M1 target inhibitory networks in a specific pattern which permits recruitment of feedforward inhibition to specific subnetworks within the cortical column.
Towards neuromodulation for stroke motor symptoms - direct electrical stimulation of the motor thalamus to augment speech production

Erinn M. Grigsby1,2, Jonathan C. Ho2,3, Arianna Damiani1,2, J. Raouf Belkhir3, Jessica Barrios-Martinez4, Brad Z. Mahon4,5,6, Marco Capogrosso2,4,7, Jorge A. Gonzalez-Martinez4, Elvira Pirondini1,2

1Physical Medicine & Rehabilitation, University of Pittsburgh
2Rehabilitation and Neural Engineering Labs, University of Pittsburgh
3School of Medicine, University of Pittsburgh
4Department of Neurological Surgery, University of Pittsburgh
5Neuroscience Institute, Carnegie Mellon University
6Department of Psychology, Carnegie Mellon University
7Department of Bioengineering, Univ. of Pittsburgh, Pittsburgh

Stroke is a leading cause of permanent disability in the United States, commonly associated with severe motor deficits. An equally detrimental impact is the loss or impairment of speech production through muscle weakness or speech arrest, affecting about 50% of acute stage and a third of chronic stage stroke patients. This inability to effectively communicate often results in patients suffering greater isolation and accelerated deterioration of health. While there are promising developments in neurostimulation for motor deficits, speech therapy remains the gold standard for language deficits despite its limited impact on moderate to severe cases. Interestingly, previous studies noted an array of secondary effects on speech when applying deep brain stimulation (DBS) to the motor thalamus to improve essential tremor. We posit that thalamic stimulation improves speech output by potentiating inputs to motor cortex. We present our results using targeted DBS of motor thalamus to improve speech and vocalization.

We performed acute stimulation studies in participants (n=3) implanted with stereoelectroencephalography (SEEG) electrodes in the thalamus for seizure monitoring. Subjects performed speech-therapy exercises to measure their face muscle strength and articulation. In the facial muscle task, subjects were asked to move between instructed and neutral facial expressions. In the speech task, subjects would repeat two word “tongue-twister” phrases. These tasks were performed with and without thalamic stimulation. To quantify the effects, we collected video and audio recordings of the tasks, and surface muscle signals on the jaw, cheek, and neck.

We observe larger and more consistent muscle movements during stimulation. With the speech task, we observed a significant decrease in the number of articulation mistakes when stimulating. Furthermore, the articulation was cleaner and clearer between syllables and there was an increase in speech volume. Overall, these results provide promising evidence that we could restore speech functionality through thalamic stimulation.
Transgenic Mouse Models of Human Gene Variants Associated with Non-Obstructive Azoospermia

J. Hardy¹, N. Pollock¹, C. Doungkamchan¹, J. Kuong¹, K. Tran¹, Y. Sheng¹, S. Yatsenko¹, T. Jaffe², M. Olszewsk¹, M. Kurpisz³, K. Orwig¹, M. Brieño-Enríquez¹, F. Tüttelmann⁴, A. Yatsenko¹

¹Department of Obstetrics, Gynecology, and Reproductive Sciences, Magee-Womens Research Institute, School of Medicine, University of Pittsburgh, Pittsburgh, USA, ²Department of Urology, School of Medicine, West Virginia University, Morgantown, USA, ³Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, ⁴Institute of Reproductive Genetics, University of Münster, Münster, Germany

Approximately 1% of all men and 10% of infertile men suffer from azoospermia or absence of spermatozoa in the ejaculate. Our group previously identified a large deletion in TEX11, a testis specific, meiotic gene associated with non-obstructive (without physical impairment to the reproductive tract) azoospermia (NOA). Subsequent analyses identified TEX11 single nucleotide variants and insertion/deletions (indels) presumed to result in NOA. To date, TEX11 variants are posited to account for ~2% of NOA cases yet validation of pathogenicity and functional mechanism have yet to be shown. Given the limited availability of testicular tissue from affected individuals, we utilized a transgenic mouse model approach to demonstrate causality of TEX11 point mutations and indels.

CRISPR/Cas9 technology was used to introduce 3 likely pathogenic variants, one missense, one splice site, and one frameshift leading to a premature stop, into the homologous gene of DBA/2 mice. Fertility status, testis morphology, seminiferous tubule histology, and prophase I chromosome analysis were assessed using standard and advanced molecular techniques.

In 12 week old, F1 mice, each of the 3 variants resulted in testicular phenotypes that at least partially mimicked the human condition. The frameshift variant which caused infertility due to complete meiotic arrest most strongly resembled the human phenotype. Both missense mutations did not result in infertility at this age/generation though chromosomal abnormalities were noted. In the F3 generation, mice harboring the splice site variant had a significantly reduced epididymal sperm count.

The disparity in spermatozoa production observed with human TEX11 variants versus the mouse complement could be attributed to the 15% of the genome that is not shared. Alternatively, TEX11 induced NOA may be a progressive disorder with increasing severity due to age and/or transgenerational effect. Long term analysis of germ cell dynamics in mouse may provide additional insight into the consequence and mechanism of identified variants.
Neural dynamics of the dorsolateral striatum underlying loss-of-control-relevant behaviors in mice with binge-like eating

Hildebrandt, Britny1,4, Fisher, Hayley1, LaPalombara, Zoe1, Young, Michael2, Ahmari, Susanne1,3,4

1Department of Psychiatry, University of Pittsburgh School of Medicine
2Department of Psychological Sciences, Kansas State University
3Center for Neuroscience, University of Pittsburgh
4Translational Neuroscience Program, Department of Psychiatry, University of Pittsburgh

Binge eating (BE) is a primary feeding behavior present across nearly all eating disorder diagnoses. Importantly, loss of control (LOC) over eating (i.e., being unable to control the quantity of food consumed) is the core feature of BE and predictor of weight gain and obesity. However, clinical research is currently limited by methodological barriers to examining LOC during BE. This has resulted in no mechanistic investigations of in vivo neural activity patterns underlying LOC, limiting the development of new treatments. LOC is associated with the excessive initiation of eating (feeding onset) and/or difficulties disengaging from eating (feeding offset); which can be precisely measured using pre-clinical approaches. Therefore, the aim of this study was to longitudinally examine in vivo neural activity associated with feeding onset/offset within the dorsolateral striatum (DLS), a key region involved in behavior cessation, in a robust pre-clinical model for BE. Female C57BL/6 mice (N=32) were randomized to receive intermittent (daily, 2-hour) binge-like access to palatable food (BE mice), or continuous, non-intermittent (24-hour) access to palatable food (control mice). Neural activity was captured using fiber photometry at baseline and after 4 weeks of engagement in the model for BE. Feeding behaviors were assessed using contact lickometers that generated TTL outputs for precise alignment of behavior to neural data. Multilevel spine regressions were used to examine neural activity 3 seconds prior to and after feeding onset/offset. While there were no differences between groups at baseline, after 4 weeks, BE animals had reduced activity -2.5 to -1.0 seconds prior to feeding onset (z=-4.46 to -2.72, ps<.01), and a trend toward reduced activity at -0.5 and 0 seconds prior to feeding offset (z=-1.76 to 1.80, ps<.09) versus controls. Results suggest that reduced recruitment of DLS, particularly during feeding onset, is specific to animals with a history of binge-like eating, highlighting a neural mechanism in the DLS as a potential target for future BE treatment intervention.
A Sample-efficient framework for breast lesion detection on Digital Breast Tomosynthesis using Ensemble with multi-depth level convolutional models

Md Belayat Hossain¹, Robert M Nishikawa¹, and Juhun Lee¹

¹Department of Radiology, University of Pittsburgh School of Medicine

We report a sample-efficient breast lesion detection framework based on single-stage convolutional models using a set of limited biopsied digital breast Tomosynthesis (DBT) lesions. We used the non-biopsied (actionable) false positive (FP) lesions to augment the small DBTex training set, instead of using a large external lesion dataset that only a few can access. We utilized the various lesion detection models by changing the depth in the convolution layers, as well as an ensemble approach to combine the multi-depth level detection models. Using the DBTex’s independent validation set, we showed that actionable FP findings are useful for lesion algorithm development, and ensembling with multi-depth models improved the algorithm’s performance.
Quantification of choroidal Haller’s sublayer vasculature in 3D based on wide-field SS-OCT scans using 3D tensor voting and geometric modeling

Mohammed N. Ibrahim1; Sumit R. Singh2; Anindya Samanta3; Amrish Selvam1; Shan Suthaharan4; Jose-Alain Sahel1; Soumya Jana6; Jay Chhablani7; Kiran K. Vupparaboina1

1Department of Ophthalmology, University of Pittsburgh School of Medicine, USA; 2Nilima Sinha Medical College & Hospital, India; 3Department of Ophthalmology, Texas Tech University Health Sciences Center, USA; 4Department of Computer Science, University of North Carolina at Greensboro, USA; 5Department of Electrical Engineering, Indian Institute of Technology Hyderabad, India

The choroid is the dense vascular layer present posterior to the outer retina, serving various metabolic functions such as supplying oxygen and nutrients to the retina layers. Studies indicate that choroidal structural changes are related to several vision-threatening posterior segment disorders. Clinicians hypothesize that early manifestations of retinal diseases may be localized to specific regions of choroidal vasculature. However, studies so far were based on gross choroidal biomarkers, including the thickness, volume, and vascularity index, but not on finer vasculature quantification. In this regard, clinicians seek to have biomarkers at the level of blood vessels in 3D to enable early diagnosis. In response, we proposed and validated an end-to-end methodology to quantify choroidal Haller’s sublayer vasculature in 3D using wide-field SS-OCT volumes.

This retrospective study was performed using healthy and diseased wide-field SS-OCT volume scans taken from the Carl Zeiss Plex Elite 9000 device. We proposed an end-to-end algorithm to quantify Haller’s sublayer vasculature. Firstly, we employ previously validated 3D residual U-Net, binarization based on exponential and non-linear enhancement, and 3D smoothing to segment the vasculature. Secondly, we employ TEASAR, 3D tensor voting, and geometrical modeling to estimate centerline (CL) and cross-sectional (CS) radius. The accuracy of CL and CS estimates was validated based on subjective grading performed on both synthetic and choroidal (of one healthy and two diseased eyes) vasculature. In each session (total of three sessions), the grader graded CS at five randomly picked CL points to measure various parameters.

For each of the four parameters, we achieved an average grading score above 92%, demonstrating the efficacy of the proposed methodology. 3D heatmaps depicting relative CS radius and histograms of the representative healthy and diseased eyes facilitate quantitative visualization of the vasculature. The proposed methodology is accurate and robust to quantifying choroidal Haller’s sublayer vasculature in 3D.
Differential effects of UPPS-P impulsivity factors on externalizing trajectories from childhood to adolescence

Jia-Richards, Meilin¹; Wang, Frances¹; Bachrach, Rachel L.²; Versace, Amelia¹

¹Department of Psychiatry, University of Pittsburgh
²Center for Health Equity and Research Promotion, VA Pittsburgh Healthcare System
³Mental Illness Research, Education, and Clinical Center, VA Pittsburgh Healthcare System

Trait impulsivity has been linked to greater externalizing behaviors in children and adolescents, however the differential effects of distinct factors of trait impulsivity on developmental changes in externalizing behaviors across childhood to adolescence are largely unknown. Therefore, the current study examined the unique effects of specific factors of trait impulsivity in childhood on externalizing trajectories over time.

Participants (N = 11,331; 48% female; 53% White, 20% Hispanic, 14% Black, 2% Asian, 10% Other racial/ethnic group) were drawn from a large, demographically diverse sample of youth enrolled in the Adolescent Brain Cognitive Development (ABCD) study. Externalizing was measured annually via the parent-reported Child Behavior Checklist (CBCL) from 9-10 years old (baseline) to 11-12 years old. Child ratings at baseline on the Abbreviated Child UPPS-P Impulsive Behavior Scale were used to measure five factors of impulsivity: negative urgency, (lack of) perseveration, (lack of) premeditation, sensation seeking, and positive urgency. Latent growth curve modeling was then used to assess the differential effects of UPPS-P factors on externalizing trajectories.

Overall, externalizing decreased over time (b = -.91, SE = .20, p < .001). Negative urgency (b = 1.28, SE = .13, p < .001), premeditation (b = 1.16, SE = .11, p < .001), perseveration (b = .54, SE = .12, p < .001), and sensation seeking (b = .20, SE = .10, p = .042) were associated with higher externalizing at baseline. Only negative urgency (b = -.14, SE = .05, p = .001) significantly predicted externalizing trajectories.

Our findings suggest that high negative urgency in childhood predicts greater reduction of externalizing over time. Children high in negative urgency may “catch up” to their peers developmentally as they transition to adolescence, a time when the ability to regulate emotions is typically improving. To better understand the maturational processes from childhood to adolescence, future work should examine how longitudinal changes in negative urgency also relate to externalizing trajectories.
Metabolic reprogramming of therapeutic T cells using small molecule modulators

Joshi, Supriya1,2; Rivadeneira, Dayana1,2; Klapholz, Catherine3; Delgoffe, Greg1,2

1Tumor Microenvironment Center, University of Pittsburgh
2Department of Immunology, University of Pittsburgh
3Nanna Therapeutics, UK

Adoptive cell therapies (ACT) have revolutionized the landscape of cancer treatment. These therapies enhance a patient’s immune response to tumor cells, with major success in treating hematologic malignancies. However, the efficacy of cellular therapies is limited by barriers like limited persistence, poor tumor infiltration, and an immunosuppressive tumor microenvironment (TME), often leading to treatment failure or relapse. T cell effector functions require high energy. However, the TME has limited availability of nutrients leading to metabolically dysfunctional T cells with limited cytotoxicity. There is great interest in enhancing the metabolism of therapeutic T cells genetically or pharmacologically to enhance metabolic features during the in vitro expansion phase. In this study, we assess the efficacy of novel small molecule compounds on T cell function and their subsequent therapeutic efficacy, when treated during in vitro expansion. Here, we show that treatment with these novel small molecules can increase the mitochondrial capacity of human T cells and transduced CD19 Chimeric Antigen Receptor (CAR) T cells. We also show that boosting the cells 48 hrs before the end of expansion is sufficient to induce the effect on the mitochondrial capacity of T cells. CD19 expressing CAR T cells boosted with the small molecules can also successfully kill both liquid and solid tumor cells in vitro. It is well known that the mitochondrial respiratory capacity is strongly correlated with immune persistence in the tumor microenvironment. We hypothesize that boosting CD19 CAR T cells with small molecule compounds can increase their persistence and subsequent therapeutic efficacy, through metabolic reprogramming.
Neutrophil-platelet aggregates contribute to cigarette smoke induced flu severity.

Tomasz W. Kaminski¹, Tomasz Brzoska¹, Keven Robinson², Toru Nyunoya², Prithu Sundd¹²

¹Pittsburgh Heart, Lung and Blood Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA
²Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA

Rationale: Epidemiological evidence suggests that prior exposure to cigarette smoke (CS) or habitual smoking increases the risk of influenza A virus (IAV)-triggered respiratory failure (severe flu). Although emerging evidence supports the role of thrombo-inflammation in the development of CS and IAV-triggered lung injury, the innate immune mechanism that contributes to this morbidity remains poorly understood.

Materials and methods: We have developed a two-hit model of CS-induced severe flu in mice. Mice were exposed to four weeks of room air (air) or CS followed by intra-nasal administration of A/PR/8/34 (H1N1) IAV. The body weight was measured every day for two weeks after IAV administration followed by assessment of lung injury at day 7 and 14. Lungs were harvested for histological assessment of lung injury and estimation of viral titer by RT-PCR. Quantitative fluorescence intravital lung microscopy (qFILM) was conducted at 2-, 3- and 4-days post IAV-infection to visualize dynamics of neutrophil and platelet recruitment in the lung of mice IV administered with fluorescent dextran, anti-Ly6G Ab and anti-CD49Ab.

Results: Mice exposed to CS+IAV manifested significantly more weight loss, lung injury, lung congestion and alveolar hemorrhage compared to mice administered air+IAV. QFILM revealed that severity of lung injury was associated with significantly more entrapment of neutrophil-platelet aggregates within the pulmonary microcirculation and air spaces of CS+IAV than air+IAV administered mice.

Conclusion: These initial results suggest that CS primes pro-thrombo-inflammatory signaling in neutrophils and platelets to promote severe lung injury following flu.
Objective: To describe opioid prescribing trends among oral surgeons (OMFS).

Methods: Opioids prescribed by OMFS were identified from 2016-2019. OMFS-based, patient-based and population-based prescribing rates and changes in high-risk opioid prescribing were calculated annually overall, by agent, and by state. We used linear regression to describe trends.

Results: There were 13.9M opioid prescriptions among 12.5M patients (627 prescriptions/OMFS/year). Hydrocodone and oxycodone decreased by 20.9% and 39.2% (p<0.05), respectively, while tramadol and codeine increased by 24.3% and 6.1% (p<0.05), respectively. Opioid prescribing rates significantly decreased by 27 prescriptions/OMFS/year, 18.6 patients/OMFS/year and by 0.9 prescriptions/100,000 population/year (p<0.05 for all). From 2016-2019, the proportion of opioids >3 days decreased by 54.2% (p<0.05) and prescriptions >50 MME/day decreased by 66.3% (p<0.05). Although the number of opioid prescriptions by OMFS decreased in most states, 12% of states experienced increases.

Conclusion: Opioid prescribing, especially high-risk prescribing, by OMFS has decreased. However, targeted interventions are warranted in some areas.
Unsupervised segmentation of cells nuclei in fluorescence microscopy images of complex biological tissues

Kochetov, Bogdan\textsuperscript{1,7}; Bell, Phoenix\textsuperscript{2}; Raphael, Rebecca\textsuperscript{1,7}; Raymond, Benjamin\textsuperscript{3}; Leibowitz, Brian J.\textsuperscript{4,7}; Tong, Jingshan\textsuperscript{4}; Shalaby, Akram\textsuperscript{2}; Garcia, Paulo S.\textsuperscript{2}; Diergaard Brenda\textsuperscript{5,7}; Yu, Jian\textsuperscript{2,7}; Pai, Reet\textsuperscript{2,6}; Schoen, Robert E.\textsuperscript{6,7}; Zhang, Lin\textsuperscript{4,7}; Singhi, Aatur\textsuperscript{2,7}; Uttam, Shikhar\textsuperscript{1,7}

\textsuperscript{1} Department of Computational and Systems Biology, University of Pittsburgh; \textsuperscript{2} Department of Pathology, University of Pittsburgh; \textsuperscript{3} Department of Bioengineering, University of Pittsburgh; \textsuperscript{4} Department of Pharmacology and Chemical Biology, University of Pittsburgh; \textsuperscript{5} Department of Human Genetics, University of Pittsburgh; \textsuperscript{6} Department of Medicine, University of Pittsburgh; \textsuperscript{7} UPMC Hillman Cancer Center

The ability to segment cells and their nuclei with their diverse shapes and sizes in complex tissue samples has opened new avenues in profiling spatial cellular distributions within tissue microenvironments. In particular, it enables us to model spatial intercellular interactions in biological tissues including tumor microenvironments. Modern approaches to cell segmentation are primarily based on deep learning architectures, such as convolutional neural networks using the U-Net architecture. The most recent of these approaches are Cellpose and Mesmer, two deep learning models for segmentation of cells and nuclei in microscopy images trained on large manually labeled datasets. However, despite demonstrating accurate segmentation of many types of cells and nuclei, it can be difficult to adapt these methods in many real-world cases as it is difficult to ascertain the cause of their failure, when they do fail. Failure usually requires retraining which is not always usually logistically, computationally, and economically feasible. To avoid the retraining of deep neural networks that require ground truth data, we have developed a new unsupervised learning approach to segment both cells and their nuclei in complex biological tissues. Our approach assumes two-channel images have been acquired using fluorescence imaging, where the first (second) channel contains nucleus (cell membrane) \textit{a priori} knowledge. These independent channels are processed together within a Bayesian framework that includes watershed method and convexity analysis. We demonstrate that the proposed unsupervised approach achieves highly accurate segmentation and its efficiency is comparable with the state-of-the-art deep learning algorithms.
Linking scales of auditory neural activity through resource governed repetition suppression.

Koerner, F. Spencer¹; Teichert, Tobias¹,²

¹Department of Psychiatry, University of Pittsburgh; ²Department of Bioengineering, University of Pittsburgh

Auditory evoked responses are strongly modulated by stimulus repetition, presumably via short-term presynaptic plasticity, i.e., by depleting the pool of readily releasable vesicles in responsive neurons. The effect can be observed using non-invasive electroencephalographic recordings (EEG) or intracranial recordings, e.g., local field potentials, current source densities and multi-unit activity (MUA) in primary auditory cortex (A1). In monkey EEG recordings, the effect strongly modulates specific event-related potentials (ERPs). While some ERPs are believed to originate in A1, it is unclear if their modulation by repetition measures the same phenomenon as the modulation of intracranial signals and whether any of them can be linked to the presumed cause – the dynamics of vesicle depletion and recovery.

To quantify how modulatory effects are related across neural signals, we simultaneously recorded all four modalities in macaque monkeys passively listening to series of auditory clicks that varied in intensity and inter-click delay. Responses were strongest for the loudest clicks and clicks presented after the longest delays. To each modality of single-trial data, averaged across different time-bins, we fit a model of feedforward short-term synaptic depression assuming auditory processing of a click expends a set fraction of currently available resource, and that the neural response is proportional to that expended amount. We found that the modulation depth of MUA and EEG components covaried most prominently at similar latencies. Expanding the model to accommodate multiple resources evidenced the presence of three resources with distinct recovery time-constants. In both EEG and MUA, the resource with the shortest time-constant boosted responses and likely corresponds to short-term pre-synaptic facilitation, while the remaining two components attenuated responses, and are consistent with short-term depression. Ongoing work is aimed at estimating properties of short-term pre-synaptic plasticity in A1 solely from non-invasive EEG recordings.
Sex-differences in Parameters of Bone Health following 10-weeks of Military Officer Training

Koltun, Kristen¹; Bird, Matthew¹; Sekel, Nicole¹; Lovalekar, Mita¹; Mi, Qi¹; Martin, Brian¹; Nindl, Bradley¹

¹Neuromuscular Research Laboratory, Department of Sports Medicine and Nutrition, School of Health and Rehabilitation Sciences University of Pittsburgh, Pittsburgh, PA

Mechanical loading (e.g. physical activity) is associated with changes in bone density and structure; however, few investigations have examined the adaptive bone response to arduous military training in men and women. This investigation examined the effects of military training on volumetric bone density (vBMD), geometry, and strength in men and women who completed Marine Corps Officer Candidates School (OCS).

Male and female candidates (n=267) completed a tibial peripheral quantitative computed tomography (pQCT) scan before and after a 10-week military training course. vBMD, geometry, and estimated bone strength were assessed at the metaphysis, mid-shaft, and proximal diaphysis. Wilcoxon signed-rank tests assessed changes across training. Data are mean±SEM, α=0.05.

Subjects were aged 19-35 (25.3±0.2 yrs) with a BMI 25.5±0.1 kg/m². At the distal (4%) tibial metaphysis, increases in total vBMD (pre: 354.5±2.7, post: 356.3±2.7 mg/cm³), trabecular vBMD (294.3±2.2, 295.6±2.2 mg/cm³), and estimated compression strength (BSI; 154.7±2.2, 156.2±2.1 mg²/mm⁴) were observed in men (n=222, p<0.001). In women (n=39), total vBMD (324.2±5.1, 326.5±5.2 mg/cm³ p=0.03), trabecular vBMD (262.7±4.8, 264.4±4.9 mg/cm³ p=0.01), and BSI (105.9±3.3, 107.4±3.4 mm³ p<0.01) also increased. At the midshaft (38%) tibia, total vBMD (938.1±3.7, 938.9±3.7 mg/cm³ p=0.03), cortical thickness (6.8±0.1, 6.8±0.1 mm, p<0.01), periosteal circumference (77.0±0.3, 77.1±0.3 mm p<0.01) and estimated bending strength (SSI; 2182.7±25.9, 2193.8±25.1 mm³ p=0.02) increased in men (n=208). In women (n=40), only periosteal circumference increased (70.0±0.6, 70.1±0.6 mm p=0.05). At the proximal (66%) tibia, no significant changes were observed in men; in women (n=38), total vBMD decreased (735.9±9.0, 732.7±8.8 mg/cm³ p=0.04) and periosteal circumference increased (82.5±0.9, 82.8±0.9 mm p<0.01).

Bone adaptations following 10 weeks OCS are slight (≤1.5%), but may be sufficient to improve estimates of bone strength, and are further dependent on biological sex and anatomical location.

Funding Acknowledgement: ONR N00014-20-C-2020, the views presented are those of the authors and do not necessarily represent the views of DoD or its components
Decoding the regulatory landscape in macrophage-rich breast tumor

Lee, Sanghoon1,2; Lee, Adrian3,4; Oesterreich, Steffi3,4; Zervantonakis, Ioannis4; Osmanbeyoglu, Hatice1,2,5

1Department of Biomedical Informatics, School of Medicine, University of Pittsburgh, 2UPMC Hillman Cancer Center, University of Pittsburgh 3Women's Cancer Research Center, University of Pittsburgh 4Department of Pharmacology and Chemical Biology, School of Medicine, University of Pittsburgh 5Department of Bioengineering, School of Medicine, University of Pittsburgh

The tumor microenvironment (TME) plays a critical role in tumor progression and includes heterogeneous cell types that can have pro-tumorigenic (e.g., immunosuppressive macrophages) and anti-tumorigenic (e.g., cytotoxic T cells). Among the most abundant cell types in the TME, an inflammatory cell population such as tumor-associated macrophage (TAM), is an influential component for tumor growth and immune-therapeutic response, which has potential to provide diagnostic and prognostic biomarkers. However, TAM-based biomarkers that predict tumor aggressiveness and the complex interaction network with cancer cells and other immune cells in breast TME remain unexplored. We characterized TAM density in breast tumors and gene signatures associated with clinical characteristics using an integrative scRNA-seq analysis of ER-positive tumors (n=33) from two independent public datasets. We found that TAM-rich tumors have a high fraction of cancer epithelial cells, but a low fraction of T-cells compared to TAM-poor tumors, as well as a distinct transcriptomic landscape. Specifically, we found that TAM-rich tumors exhibited lower expression of the cytokine receptor interaction and secretome signatures. Next, we examined associations between TAMs and cancer cell derived factors. Our cell-cell interaction analyses show that cancer epithelial cell-secreted MDK (Midkine growth factor) interacts with its receptor LRPI or SORLI on macrophage, which is known to promote immunosuppressive macrophage differentiation. Indeed, MDK was found overexpressed in cancer epithelial cells of TAM-poor tumors. This study reveals the relationship between TAM density and clinicopathological parameters in human breast tumor and elucidates the interaction between TAMs and cancer cells or other immune cells. In our ongoing studies, we are exploring TAM-targeted interventions as a promising novel strategy to reverse immunosuppression in the era of precision oncology.
Reduced amygdala activity to positive memories in young adults with high familial risk for depression

Leiker, Emily¹; Compère, Laurie¹; Barb, Scott²; Lazzaro, Sarah²; Canovali, Gia²; Riley, Emma²; Krawczak, Rebecca²; Siegle, Greg¹; Young, Kymberly¹

¹Department of Psychiatry, University of Pittsburgh School of Medicine; ²University of Pittsburgh Medical Center

Background: Depression is associated with a tendency to recall fewer specific and fewer positive autobiographical memories (AMs), and reduced amygdala hemodynamic activity when recalling positive AMs. This amygdala response may be a causal mechanism underlying depression, yet whether it is a precursor to disease onset is unclear. This study evaluates if blunted amygdala activity during positive AM recall is detectable in healthy individuals at risk for major depressive disorder (MDD), to inform its utility as an early therapeutic target aimed at disease prevention.

Methods: Healthy young adults (ages 18-25) with high familial risk for MDD (high-risk; N=14) and those without familial risk (low-risk; N=31) completed an emotional AM recall task during fMRI. All participants completed a diagnostic interview confirming they did not meet diagnostic criteria for MDD as a condition of enrollment.

Results: Healthy individuals at high risk for MDD exhibited reduced amygdala hemodynamic activity during positive AM recall compared to low-risk individuals. This was accompanied by reduced activity in regions implicated in self-referential and interoceptive processing (medial prefrontal, inferior frontal, insula), but elevated activity in regions implicated in memory and valuation (hippocampus, striatum, cingulate cortex).

Discussion: We show for the first time that young adults at high risk for depression show a reduced amygdala response during positive AM recall, similar to effects consistently observed in patients with MDD. These results support further investigation of reduced amygdala reactivity to positive AM recall as a precursor to depression onset, as well as its potential utility as an early intervention therapeutic target.
Reducing Akt2 in RPE cells causes a compensatory increase in Akt1 and attenuates diabetic retinopathy

Haitao Liu1, Nadezda A. Stepicheva1, Sayan Ghosh1, Peng Shang1, Olivia Chowdhury1, Rachel A. Daley1, Meysam Yazdankhah1, Urvi Gupta1, Stacey L. Hose1, Mallika Valapala2, Christopher Scott Fitting1, Anastasia Strizhakova1, Yang Shan3, Derrick Feenstra4, José-Alain Sahel1,5, Ashwath Jayagopal6, James T. Handa7, J. Samuel Zigler Jr.7, Patrice E. Fort3, Akrit Sodhi7, Debasish Sinha1,7

1Department of Ophthalmology, University of Pittsburgh School of Medicine; 2School of Optometry, Indiana University; 3Kellogg Eye Center, University of Michigan School of Medicine; 4Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche; 5Institut de la Vision, INSERM, CNRS, Sorbonne Université; 6Opus Genetics; 7The Wilmer Eye Institute, The Johns Hopkins University School of Medicine

Purpose: Evidence has emerged to suggest that retinal pigment epithelium (RPE) injury contributes to the development of diabetic retinopathy (DR), the leading cause of blindness among working-age adults. However, the role of the RPE in DR remains poorly understood. Since Akt2 signaling has been implicated in diabetes and is integral to both RPE homeostasis and glucose metabolism, we investigated whether Akt2 in the RPE could influence DR.

Methods: Best1-Cre generated RPE-specific Akt2 conditional knockout (cKO) mice were used. Diabetes was induced in Aktr2fl/fl and Akt2 cKO mice by intraperitoneal injection of streptozotocin for 5 consecutive days. Akt2 and Akt1 activities were examined in retinas from both DR patients and diabetic mice. Retinal expression of inflammatory proteins, reactive oxygen species, and electroretinograms (ERG) were measured at a 2 month duration of diabetes (4 months of age). The GSK3β/NF-κB signaling pathway was also examined. Mice at an 8 month duration of diabetes (10 months of age) were used to evaluate retinal capillary degeneration and vascular permeability.

Results: We found that Akt2 and Akt1 activities were reciprocally regulated in the RPE of DR patients and diabetic mice; Akt2 cKO inhibits a diabetes-induced increase in retinal leukostasis, inflammatory proteins, and production of ROS. Retinal capillary degeneration, vascular leakage and ERG abnormalities caused by diabetes were also attenuated in Aktr2 cKO mice. The protective effect found in Aktr2 cKO mice appears to be the result of a compensatory upregulation of phospho-Akt1, leading to inhibition of inflammatory proteins mediated by the GSK3β/NF-κB signaling pathway.

Conclusions: Our results suggest that targeting Akt1 activity in the RPE may be a novel therapeutic strategy for treating DR.
Subcortical contributions to auditory perceptual deficits in first episode psychosis as assessed by the Frequency-Following Response: Preliminary results from pilot data

López-Caballero, Fran¹; Seebold, Dylan¹; Brown, Christopher²; Salisbury, Dean F¹

¹Clinical Neurophysiology Research Laboratory, Western Psychiatric Hospital, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
²Department of Communication Science and Disorders, School of Health and Rehabilitation Sciences, University of Pittsburgh, Pittsburgh, PA, USA

Individuals with psychotic disorders, including schizophrenia (SZ), often experience difficulties in the discrimination of basic acoustic stimulus features, such as pitch or intensity, that contribute to higher-order cognitive symptoms, particularly social cognition. Impairments in subcortical structures within the auditory brainstem, largely unexplored in early psychosis, may contribute to these deficits, and can be assessed by using a neurophysiological index of subcortical speech sound encoding, the Frequency-Following Response (FFR).

We measured electroencephalographic (EEG) FFR to a consonant-vowel /ba/ in 7 individuals with first episode psychosis (FEP) and 7 healthy controls (C). Participants also underwent psychophysical testing to determine hearing thresholds (HT), frequency discrimination (FD), amplitude modulation (AM) and inter-aural time differences (ITD) thresholds and completed the MATRICS neuropsychological battery of social cognition.

Preliminary results from EEG suggest that the FFRs from FEP followed the periodicity of the stimulus less precisely than that of controls. FFR spectral power at the stimulus’ F0 were visibly reduced in FEP as compared to C, suggesting a reduced encoding of the stimulus F0. In the psychoacoustic tests, FEP individuals had overall higher HT, FD, and AM thresholds than C. Higher FFR spectral signal-to-noise ratio values (better representation of stimulus’ F0) correlated with lower FD \((r = -.57)\) and AM \((r = -.9)\) thresholds (smaller differences detected) in FEP. Moreover, MATRICS battery’s social cognition t scores for 5 FEP individuals were higher (better mental operations underlying social behavior) when FD thresholds were smaller (smaller pitch differences detected) \((r = -.60)\).

Our results suggest basic auditory perceptual deficits may be present already in individuals that experience psychotic symptoms for the first time and may contribute to social cognition difficulties. The pathophysiology of such deficits may involve an impaired subcortical representation of the incoming sounds as assessed with the FFR.
The lamina cribrosa vascular network is heavily interconnected with that of adjacent regions, not just the periphery, likely improving perfusion resilience

Lu, Yuankai; Hua, Yi; Lee, Po-Yi; Quinn, Marissa; Waxman, Susannah; Sigal, Ian A.

1Department of Ophthalmology, University of Pittsburgh School of Medicine; 2Department of Bioengineering, University of Pittsburgh

Purpose. A reliable blood supply to the optic nerve neural tissues is essential to prevent damage and vision loss. At the lamina cribrosa (LC) this is achieved by a complex network of capillaries intertwined with collagen beams. Conventional understanding has been that LC blood perfusion originates from the posterior ciliary artery (PCA) at the canal periphery, draining at the central retinal vein (CRV). To better understand the LC perfusion and potential challenges to perfusion that could lead to ischemia we map the LC 3D vasculature.

Methods. The vasculatures of six normal monkey eyes were labelled post-mortem by perfusion of lipophilic carbocyanine dyes through the carotid arteries. After enucleation serial coronal cryosections 16um thick through the LC were imaged using fluorescence and polarized light microscopy to visualize the labeled vessels and label-free collagen, respectively. The collagen structures were used to identify the LC and form an image stack from which the fluorescence was segmented to reconstruct the 3D vascular network. We did several 3D analyses of the LC vascular networks.

Results. Three of the six eyes exhibited vessel branches directly from the CRA to the LC. A watershed analysis revealed distinct perfusion regions for the initial branching levels, after which the vessels interconnected with the rest of the LC vascular network. The LC vessels were heavily interconnected with the adjacent vasculature. The largest number of anastomoses was with the retro-laminar region (479), followed by the periphery (409), the pre-laminar region (159) and the central vessels (53).

Conclusions. The LC vascular network is heavily interconnected with the PCA, but also with the CRA and with the pre-laminar and retro-laminar regions. We postulate that multiple potential perfusion sources provide redundancy that can help protect the tissues from compromised perfusion and ischemia, at all levels of IOP, particularly for tissues further away from the vasculature. More work is necessary to map more eyes, and to develop the technology to measure LC blood flow and perfusion.
The impact of stromal factors on B cell infiltrate and tertiary lymphoid structure (TLS) development in ovarian cancer.

MacFawn Ian¹,², Ruffin, Ayana¹,², Atiya, Huda³, Kunning Sheryl¹,², Lampenfeld, Caleb¹,², Joy, Marion¹,⁵, Coffman, Lan¹,³,⁴, Bruno, Tullia¹,²

¹UPMC Hillman Cancer Center, Pittsburgh, PA
²Department of Immunology, University of Pittsburgh
³Magee Women’s Research Institute, Pittsburgh, PA
⁴Division of Hematology/Oncology, University of Pittsburgh
⁵NSABP Foundation Inc.

Abstract: B cell infiltrate is a common feature of high grade serous ovarian cancer (HGSOC) and approximately 15% of patients contain B and T cell-rich tertiary lymphoid structures (TLS). Further, B cells, plasma cells, and TLS correlate with improved prognosis in HGSOC. This research aims to identify stromal and tumor factors that can impact TLS development. We hypothesize that pro-tumorigenic cancer associated mesenchymal stem cells (CA-MSC) hinder TLS development, while BRCA mutant tumor cells promote TLS formation via DNA damage response and/or type I interferon signaling. Using serially sectioned HGSOC clinical samples we surveyed the frequency, size, and location of TLS in the tumor microenvironment. Further, using multispectral imaging, we dissected TLS composition and surrounding cellular neighborhoods to confirm associations between TLS formation and maturity and CA-MSC presence. Second, we correlated TLS presence and maturity with the BRCA mutational status of the tumors. This spatial analysis is complemented by high dimensional flow cytometric analysis of B cell infiltrate in a cohort of HGSOC tumors, supplying unprecedented analysis of the phenotype and potential function of B cells in HGSOC. Ultimately, we aim to trace the origins of TLS in HGSOC, thereby providing new therapeutic strategies to promote the development of these potent anti-tumor structures.
Human centromeres drift through cellular proliferation


1 Department of Pharmacology and Chemical Biology, Hillman Cancer Center, University of Pittsburgh Medical Center
2 Department of Pathology, University of Texas Southwestern Medical Center

CENP-A is a heritable epigenetic mark that determines centromere identity and is essential for centromere function. Centromeres are the central genetic element responsible for accurate chromosome segregation during cell division, and as such, they are anticipated to be evolutionarily stable. How centromeres evolved to allow faithful chromosome inheritance on an evolutionary timescale despite their epigenetic maintenance is unclear. Here we sought to determine whether CENP-A is capable of precisely and stably specifying human centromere position throughout cellular proliferation. To investigate the positional stability of human centromeres as cells proliferate, we used the PD-NC4 fibroblast cell line that harbors a neocentromere (epigenetic stable acquisition of a new centromere at a new chromosomal site) on chromosome 4. This non-repetitive neocentromere provides an advantage for centromere analysis by allowing precise mapping of CENP-A-bound DNA molecules to resolve CENP-A distribution at high, base pair resolution. CENP-A ChIP-sequencing in the parental PD-NC4 cell line and in nine single cell-derived clones reveals significant differences in patterns of CENP-A deposition. While parental cells show three major peaks of CENP-A binding at the neocentromere, three distinct patterns of CENP-A deposition are seen in the single-cell derived clones. Thus, the neocentromere position on chromosome 4 in PD-NC4 cells varies within a population. Our preliminary data reveals that each pattern of CENP-A deposition is relatively stable over cellular proliferation, i.e., the number of major CENP-A peaks is stable. However, significant drift for CENP-A binding is observed over cellular proliferation within each clone but the total neocentromere length does not change significantly. Our results suggest that while the deposition pattern of CENP-A may change, the number of CENP-A-containing nucleosomes remains constant over cellular proliferation, which is important for preserving centromere function.
Acute Postpartum Pain and Anxiety Influence Long-term Postpartum Pain, Maternal-Infant Attachment and Parenting Self-Efficacy

Makeen, Mutasim¹; Farrell, Lia¹; Kenkre, Tanya¹; Lim, Grace ¹²

¹ Department of Anesthesiology & Perioperative Medicine, University of Pittsburgh
² Department of Obstetrics, Gynecology, & Reproductive Sciences, University of Pittsburgh

Pain and depression are bi-directionally related in chronic pain settings, and worse labor pain has been linked to postpartum depression symptoms. However, few studies have examined the relationships between postpartum pain and negative mood, and their effects on parent-infant relationship outcomes. We aimed to assess the relationships between postpartum pain, depression, parent-infant attachment, and parenting self-efficacy, as we hypothesized that new mothers who have lower pain intensity and unpleasantness during the labor and delivery period will have a reduced risk for postpartum depression, and will have improved maternal-infant attachment, higher parenting self-efficacy, lower perceived stress, and improved child development.

This was a prospective longitudinal observational study of healthy, adult, nulliparous women, at term gestation presenting for labor and delivery at ≥38 weeks gestational age. Baseline self-reported assessments included validated inventories of depression (Edinburgh postnatal depression screen, EPDS), anxiety (state-trait inventory, STAI), and pain (brief pain inventory short, BPI). Demographic and labor variables were recorded. At 6 weeks and 3 months postpartum, self-reported assessments included EPDS, STAI, BPI, maternal-infant attachment (MPAS), and parenting self-efficacy (PMPSE).

Data from 87 subjects who completed the study assessments revealed that worse postpartum anxiety scores were associated with lower PMPSE scores. Higher pain severity at 3 months was associated with lower MPAS and PMPSE scores. Pain severity scores at 6 weeks postpartum were significantly associated with pain severity at 3 months.
EvolvingSTEM: an authentic classroom research curriculum that improves understanding of key life science topics and inspires an enduring interest in science

Abigail Matela¹ & Vaughn Cooper¹,²

¹Department of Microbiology and Molecular Genetics, University of Pittsburgh, School of Medicine
²Center for Evolutionary Biology and Medicine, University of Pittsburgh

Achieving equity in the STEM career pipeline must start for youths in the classroom, where all students encounter science. Further, the science we teach should involve inquiry, productive uncertainty, and ideally, authentic experimentation. Authentic research experiences help students see themselves as scientists and motivate them to pursue higher education and STEM careers, yet they are rarely provided in secondary schools and almost never in introductory classes serving large populations of URM students. These educational disparities contribute to the persistent lack of diversity in the STEM workforce. To address this issue, we developed EvolvingSTEM (https://evolvingstem.org/), a secondary school curriculum where students conduct their own microbial evolution experiment. Typical evolution curricula rely on abstract narratives requiring little student participation, yet the experience of witnessing evolution occur in real time can be transformative. The microbiology methods also provide many essential skills for careers in biotechnology and enable productive dialogue about microbes, infections, and antibiotics.

EvolvingSTEM has already reached >3000 students from 15 high schools and 10 colleges in 13 states. In introductory high school biology classes assessed by a delayed intervention method, our curriculum produced significantly greater subject-matter test scores than the established curriculum (Cooper et al. Evolution, Education, and Outreach 2019). In addition, surveys of student attitudes and motivation demonstrate that our program encourages students from varied backgrounds to form a sense of agency as scientists and motivates their interest in related careers. Widespread implementation of programs like ours that use engaging and inclusive teaching practices will grow a diverse workforce capable of tackling significant threats to human health and our planet, such as antimicrobial resistance, cancer, and climate change.
Arterial thrombosis simulated with small volume stenotic microfluidic devices for evaluating platelet dose response

Mihalko, Emily; Hoteit, Lara; Rahn, Katelin; Neal, Matthew; Shea, Susan

Trauma and Tranfusion Medicine Research Center, Department of Surgery, University of Pittsburgh

Microfluidics incorporate physiologically relevant substrates and flows that mimic the vasculature, and are therefore a valuable tool for studying thrombosis. Utilizing devices that allow for small sample volume (<500 µL) can additionally aid in evaluating a single donor’s response to various pharmacological agents at a wide range of doses by quantifying thrombosis capacity *ex vivo*. The study objective was to evaluate dose response of Ticagrelor, a P2Y₁₂ receptor antagonist, using a small volume stenotic microfluidic device.

Polydimethylsiloxane device channels were 137 ± 4 µm high and 462 ± 3 µm wide with a narrowed center (40 ± 3 µm high), resulting in 71 ± 2% stenosis. A 3 mm diameter reservoir and withdrawal perfusion allowed for very small sample volumes (~200 µL). Channels were collagen-coated to mimic plaque rupture (*in vivo* thrombosis). Citrated whole blood from healthy volunteer donors (n=5; IRB21100141) was stained with anti-CD41 (platelet label), dosed with Ticagrelor (0-50 µM), added to the upstream reservoir, and withdrawn via syringe pump (27 µL/min, initial wall shear rate 3500 s⁻¹). Fluorescent images were collected for 5 minutes (0.29 s⁻¹) and analyzed using MATLAB. Fold change (FC) in mean fluorescent intensity (MFI) and area under the MFI curve (AUC) were determined.

This device allowed for platelet-rich thrombus formation at the stenosis throat, which was inhibited with increasing Ticagrelor. AUC was reduced from 103 ± 47 to 9 ± 7, and a 64% reduction in final FC MFI was observed at 50 µM compared to vehicle. Additionally, ~50% reduction in platelet response was observed at 2.4 µM. In comparison, impedance aggregometry with ADP agonist resulted in ~77% reduction in platelet response at 0.2 µM. Therefore, the use of this small volume stenotic microfluidic device allows for the study of antiplatelet dose responses in a physiologically-relevant high shear arterial environment, which will facilitate directing anti-thrombotic therapies in a high-throughput biofidelic fashion and improve fidelity for detection in a range closer to therapeutic dosing.
Assessing Perceptions of Belongingness and Institutional Inclusivity among Underrepresented Postdoctoral Fellows and Early Career Faculty

Mitchell-Miland CE¹, White GE¹, Morone NE²,³, Murrell AJ¹, Rubio DM¹

¹Department of Medicine, Division of General Internal Medicine, Institute for Clinical Research Education, University of Pittsburgh
²Department of Medicine, General Internal Medicine, Boston University
³General Adult Primary Care Department, Boston Medical Center
⁴School of Business, University of Pittsburgh

Introduction: Diversity increases research productivity and creativity, yet the scientific workforce lacks sufficient diversity. Underrepresented (UR) individuals leave institutions that are not inclusive. We developed a survey to examine how Building Up participants perceive belongingness and inclusivity at their institution.

Methods: Building Up includes 217 UR post-doctoral fellows and early-career faculty from 25 institutions who completed a 28-item institutional inclusivity survey. The survey included questions with 5-point Likert scale responses to represent six domains: 1. Inclusive Leaders; 2. Authenticity; 3. Networking and Visibility; 4. Clear Career Paths; 5. Intergroup Conversations; and 6. Presence of Policy and Practices. We report the proportion agreement (agree or strongly agree) questions; high or low agreement (≥ 50% or ≤25%, respectively). Participant characteristics (age, race/ethnicity, gender) are reported as means and SD for continuous data and percentages for categorical data.

Results: A total of 144 participants completed ≥1 inclusivity survey question. The mean age was 39 years (SD = 6), 81% were female, 38% identified as non-Hispanic Black, and 35% identified as Hispanic. Most participants reported feeling a sense of belonging at their institution (59%), having a supervisor that demonstrates commitment to and support of diversity (74%) or handles diversity matters appropriately (63%), and feeling respected by colleagues (79%), supported in career growth at their institution (69%), or that their institution’s policies or procedures encourage diversity, equity, and inclusion (58%). High agreement questions were from Inclusive Leaders, Networking and Visibility, and Presence of Policies and Practices domains. Fewer respondents felt that it is harder for LGBTQIA+ faculty to get ahead [at their institution] than cis-gendered heterosexual faculty (20%; from the Clear Career Paths domain).

Discussion: These findings identify several areas for improvement regarding institutional inclusion and sense of belongingness.
The microbiome synchronizes diurnal rhythms in innate immunity in female mice: Implications for the circadian control of immunity during pregnancy.

Sarah K. Munyoki PhD¹, Julie P. Goff PhD¹, Eldin Jašarević PhD¹

¹Department of Obstetrics, Gynecology and Reproductive Sciences, Department of Computational and Systems Biology, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA

The microbiome is involved in the education of the immune system and breaking down diet-derived nutrients to synthesize metabolites that support various homeostatic processes. Recent work suggests that the circadian clock and diurnal rhythms potently regulate this vast functional capacity of the microbiome. However, while the gut microbiome, circadian rhythms, and immune function are all dynamically regulated during pregnancy, the exact mechanisms through which diurnal rhythms and the microbiome may converge to influence immune function across the reproductive span of females remain unknown.

To examine the hypothesis that rhythmic functions of the microbiome and its metabolites may influence pregnancy and fetal developmental outcomes, we employ an integrative analytical approach combining shotgun metagenomics, metatranscriptomics, metabolomics, and single-cell immunophenotyping using mass cytometry.

We compared the expression of genes encoding vital antimicrobial peptides in the distal ileum from nonpregnant female mice with a simple microbiome (JAX) or a complex microbiome (TAC). Analysis showed that intestinal innate immune effector proteins Lcn2, S100a8, S1009a, and Reg3g showed rhythmic expression in TAC but not JAX females, suggesting that key microbial taxa present within the diverse microbiome are necessary to drive diurnal rhythms in innate immune effector function. The focus of ongoing experiments is to confirm the identity, function, and metabolic output of these rhythmic microbiota.

These preliminary results validate our mouse model and set the stage to determine whether similar processes are conserved during pregnancy and how diet and nutritional changes influence this delicate balance between circadian rhythms, gut microbiome, maternal immunity, and fetal development.
PARP1 and PARP2 cooperation in the prevention of oxidative stress-mediated telomere crisis

Muoio D¹, Werner N¹, Darkoa-Larbi S¹, Uttam S², Fouquerel E¹

¹Department of Biochemistry and Molecular Biology, Thomas Jefferson University, 233 S. 10th street, Philadelphia, PA 19107, USA.
²Department of Computational and Systems Biology, UPMC Hillman Cancer Center, University of Pittsburgh, 5117 Centre Avenue, Pittsburgh, PA 15213.

Telomeres are key nucleoprotein structures that cap and protect linear chromosome ends. Due to their inability to be fully replicated, telomeres shorten progressively, which limits the number of divisions cells can undergo. Critically short telomeres trigger cellular senescence in normal cells but also genomic instability in pre-malignant cells leading to several age-related diseases, including cancer. Numerous studies have shown that excessive telomere attrition and dysfunction can arise from exposure to various environmental agents, including oxidative stress. However, the mechanisms underlying telomere shortening mediated oxidative damage are still poorly understood. Oxidative DNA lesions are primarily repaired by the Base Excision Repair (BER) pathway, in which Poly(ADP-ribose) polymerase (PARP) family members PARP1 and PARP2 are key players. Their poly(ADP-ribosyl)ation (PARylation) activity is triggered by DNA single-strand breaks (SSBs) that are generated as repair intermediates during BER. We propose that unrepaired BER SSBs intermediates are responsible for excessive telomere shortening due to their conversion into DSBs during replication. To test this hypothesis, we depleted both PARP1 and PARP2 in HeLa cells expressing the fluorogen activated peptide (FAP) that induces the oxidation of guanines into 8oxoguanines (8oxoG), specifically at telomeres (Fouquerel et al., 2019). Our data reveal cooperation between PARP1 and PARP2 and a significant involvement of PARP2 enzyme in the repair of 8oxoG at telomeres, despite its low PARylation activity.
The curious case of lipid metabolism in atherosclerotic Tregs

Narain Apoorva¹, Bustos Martha¹, Kohan Alison B¹

¹Department of Medicine, Division of Endocrinology and Metabolism, University of Pittsburgh, Pittsburgh, PA

Regulatory T-cells (Tregs) have emerged in recent years as key regulators of metabolic inflammation. Tregs are well-known consumers of lipids when compared to other immune cells. FOXP3 is the master regulator of CD4+ Treg phenotype and CD4+Foxp3+Tregs are almost exclusively anti-inflammatory (with a sustained CD25 expression and TGF-β and IL-10 secretion). This anti-inflammatory activity is a known modulator of atherosclerosis progression, though it is unclear whether Tregs are protective due to IL-10 secretion or their suppression of inflammatory effector cells like T-cells and macrophages. Atherosclerosis is an inflammatory cardiovascular disease (CVD) characterized by lipid deposition in the aorta, ultimately leading to an ischemic event. The role of lipid receptors on the surface of effector immune cells, particularly macrophages, has been a major focus of studies of atherosclerotic mechanisms. LDLr and SRB1, for example, help internalize plasma lipids and drive macrophage phenotypes (ranging from anti-inflammatory M1 to pro-inflammatory M2 macrophage phenotypes). Despite the importance of both plasma lipids and immune lipid receptors in this disease, the role of lipid receptors in Treg activity in atherosclerosis is totally unknown. We propose to characterize lipid receptor function and lipid accumulation in Tregs, and further, to determine how this mechanism changes the inflammatory macrophage profile in murine atherosclerosis.
The enigmatic role of VCAM-1 expressed by macrophages in mitochondrial metabolism and atherosclerosis

Natarajan, Niranjana, Florentin, Jonathan, O’Neil, Scott Patrick, Ohayon, Lee, Partha Dutta

Vascular Medicine Institute, Division of Cardiology, Department of Medicine, University of Pittsburgh

Background:
Atherosclerosis is a primary underlying cause of most cardiovascular diseases. Endothelial vascular cell adhesion protein 1 (VCAM-1) has been shown to mediate rolling and adhesion of monocytes to endothelial cells under atherosclerotic conditions. However, the role of VCAM1 expressed by monocyte and macrophage in atherosclerosis has not been explored. Here, we show that VCAM1 in macrophages signals via CMPK2 and STING to modulate mitochondrial biogenesis and promote atherosclerosis.

Results:
Atherosclerotic plaque macrophages expressed high levels of VCAM-1 in humans and mice. Concomitantly, plaque macrophages had increased mitochondrial volume, mitochondrial DNA (mtDNA) synthesis, oxidized mtDNA and oxidative phosphorylation. We observed that mice lacking Vcam1 in macrophages had reduced atherosclerotic plaque and necrotic core areas. Additionally, Vcam1 silencing in macrophages diminished inflammation, oxidative phosphorylation, mitochondrial biogenesis and downregulated mitochondrial DNA synthesis genes including Cmpk2. Cmpk2 knock down in macrophages after oxidized LDL treatment reduced inflammatory mediators that aggravate atherosclerosis. Transcriptomic analysis of Vcam-1-deficient plaque macrophages identified Fcor and Lyz1 as the target genes of Vcam1 and Cmpk2. Interestingly, atherosclerotic plaque macrophages deficient of Sting, which mediates inflammatory signaling in response to oxidized mitochondrial DNA, had increased levels of Fcor and Lyz1.

Conclusion:
Macrophage-specific VCAM-1 augments mitochondrial biogenesis and DNA oxidation via Cmpk2. Oxidized mtDNA in plaque macrophages increases inflammation via Sting, resulting in exacerbation of atherosclerosis.
Mechanisms of CENP-A overexpression induced genomic instability

Nath, Poulomi1,2; Nechemia-Arbely, Yael 1,2*

1 UPMC Hillman Cancer Center, Pittsburgh, PA 15213, USA
2 Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA 15261, USA

CENP-A, the centromeric histone H3 variant, is the major epigenetic mark that determines centromere identity and is essential for faithful chromosome segregation during mitosis. CENP-A is highly expressed in several cancers, serving as a marker of poor prognosis. When overexpressed, CENP-A is ectopically loaded onto non-centromeric transcriptionally active sites. Ectopic CENP-A sites are removed during DNA replication to restrict CENP-A to the centromeres only, ensuring faithful chromosome segregation during mitosis and maintenance of genome stability. Induced overexpression of CENP-A in cancerous cells has been shown to lead to chromosome segregation defects and micronuclei formation. Whether the sole overexpression of CENP-A in non-transformed near-diploid cells can induce genomic instability that can drive tumor formation remain poorly understood. Here we aim to determine if persistently overexpressed CENP-A at clinically relevant levels in two non-transformed near-diploid cell lines, hTERT-RPE-1 and H6c7 is sufficient to induce chromosome segregation defects, including lagging chromosomes and micronuclei formation using microscopy and cytogenetics approaches. To generate lines capable of CENP-A overexpression at different levels, we transduced hTERT-RPE-1 and H6c7 cells with lentivirus expressing Doxycycline (Dox)-induced CENP-A1AP. Our preliminary data demonstrate that 30-fold or 10-fold CENP-A overexpression over the course of 14 days in hTERT-RPE1 or H6c7 cells, respectively, is sufficient to induce ectopic CENP-A deposition and micronuclei formation within 2 days, suggesting a driving role for CENP-A overexpression in tumor formation and/or progression. With whole genome sequencing (WGS) we plan to determine whether a link be made between CENP-A overexpression and chromothripsis, the catastrophic shattering of one or few chromosomes, that can fuel tumorigenesis. Our study may reveal novel pathways used by certain tumor types to become progressively genetically unstable through increased chromosome segregation errors.
Prolonged α5 GABA<sub>A</sub> receptor negative allosteric modulation – effects on inhibitory and excitatory hippocampal neurotransmission

Nuwer, Jessica L<sup>1</sup>; Brady, Megan L.<sup>1</sup>; Povysheva, Nadya V.<sup>2</sup>; Coyne, Amanda<sup>1</sup>; Jacob, Tija C.<sup>1</sup>

<sup>1</sup>Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine
<sup>2</sup>Department of Neuroscience and Center for Neuroscience, University of Pittsburgh

In the central nervous system, GABA type A receptors (GABA<sub>A</sub>Rs) generate fast inhibitory signals to dampen and control neuronal activity. Thus, GABAergic dysfunction plays a key role in the symptomology of epilepsy, neuropsychiatric disorders, and neurodevelopmental disorders. GABA<sub>A</sub> function and pharmacology depends on subunit composition and arrangement within a given receptor, which is regulated by the spatial, temporal, and subcellular expression pattern of the 19 GABA<sub>A</sub> subunits. α5 subunit containing GABA<sub>A</sub>Rs are of particular interest due to their enriched hippocampal expression and critical roles in development, synaptic plasticity, cognition, and memory. Negative allosteric modulators (NAMs) that target α5 GABA<sub>A</sub>Rs enhance cognition and have shown promise in preclinical studies to mitigate cognitive impairment in Down syndrome, schizophrenia, and post-anesthesia. Despite in vivo efficacy in both rodents and humans, no study has examined the effects of sustained α5 NAM treatment on inhibitory and excitatory synapse plasticity to identify mechanisms of action. Here we used L-655,708, an imidazobenzodiazepine that acts as a highly selective but weak α5 NAM, in conjunction with microscopy, biochemical, and electrophysiological techniques to better understand the functional effects of reducing α5 GABA<sub>A</sub>R signaling for 2 or 7 days. We show that 2-day α5 NAM treatment in mature neurons (DIV21) enhances surface synaptic GluN2A and reduces synaptic GluN2B without altering the total surface levels or distribution of α5 GABA<sub>A</sub>Rs or the gephyrin inhibitory synaptic scaffold. Conversely, 7-day α5 NAM treatment reduces surface levels of both GluN2A and GluN2B and increases the surface synaptic levels of α5 GABA<sub>A</sub>R and gephyrin. Functionally, 2-day α5 NAM treatment does not change mEPSC, mIPSC, or tonic inhibition, while 7-day treatment increases basal intracellular Ca<sup>2+</sup> and reduces activity-induced Ca<sup>2+</sup> transients. Together, these results provide initial mechanistic insight into α5 GABA<sub>A</sub>R regulation of the mature hippocampal circuit via an NMDAR-dependent mechanism.
Influence of sickle cell stigmatization on self-efficacy and health outcomes

Julia A. O’Brien, PhD, RN1, Ronald Hickman, Jr., PhD, RN, ACNP-BC, FAAN2

1Health & Community Systems, School of Nursing, University of Pittsburgh
2Frances Payne Bolton School of Nursing, Case Western Reserve University

Aims
Everyday discrimination and perceptions of stigma influence health promoting behaviors among racial minorities. Among persons living with Sickle Cell Disease (SCD), stigma can influence self-efficacy and health outcomes. Therefore, the aim of this study was to examine whether self-efficacy mediated the relationship between perceived stigma and health outcomes (operationalized as HRQOL and pain interference) among persons with SCD.

Methods
A cohort of 60 adults with SCD was enrolled in a descriptive study employing a cross-sectional design with convenience sampling. Electronic survey methodology was used to administer the Sickle Cell Disease Health-related Stigma Scale, Sickle Cell Self-efficacy Scale, PROMIS Global Health, and PROMIS Pain Interference 4a. Descriptive statistics, correlation coefficients, and a series of linear regression models were used to examine the relationships among the study variables.

Results
Stigma was associated with self-efficacy (r= -.332, p = .010) and health outcomes (mental [r= -.382, p = .003] and physical [r= - .505, p < .001] HRQOL and pain interference [r= .430, p = .001]). Self-efficacy was also correlated with two of the three health outcomes (physical HRQOL [r= .449, p < .001] and pain interference [r= -.353, p = .006]). Additionally, self-efficacy partially mediated the association between stigma and physical HRQOL.

Conclusions
Perceptions of stigma negatively influence SCD self-efficacy, HRQOL, and pain interference in adults with SCD. Future research should consider sociocultural and de-biasing strategies to reduce stigma, which can influence an individual’s self-efficacy, health promoting behaviors and HRQOL. Additionally, increased SCD self-efficacy may be protective against the deleterious effects of stigmatization on health outcomes and may be a strong candidate for future intervention development to promote self-care and improve health outcomes.
Evidence for the Negative Trace Hypothesis of Echoic Memory from Decoding Tone Frequencies

Orczyk, John1; Teichert, Tobias1,2

1Department of Psychiatry, University of Pittsburgh
2Department of Bioengineering, University of Pittsburgh

Echoic memory (EM) is a pre-categorical form of auditory short-term memory which is important for the temporal integration of complex sounds and used in comprehension of conspecific vocalizations. Despite its importance, the neural substrate of EM is unclear. The most promising hypothesis holds that EM stores information about past sounds in the form of a ‘negative trace’ of depleted vesicles. However, it remains unclear if and how the information encoded in such a negative trace can be read out. Here we test whether the negative trace can be reactivated and read out by the subsequent presentation of a non-informative sound.

To test this hypothesis, we performed single-trial decoding of sound-evoked local field potentials and multi-unit activity that were recorded from a semi-chronically implanted 96-channel electrode array which covered the entire tonotopic map in primary auditory cortex in one macaque monkey. Each trial consisted of one of 21 pure tone pips of different frequency followed by an identical white noise burst 350-450 ms after the tone. Decoding used a support vector machine classifier with a linear kernel. Decoding was either time-resolved using 10-ms sliding windows, or combined activity across multiple time windows.

To validate the approach, we first decoded tone identity from the tone-evoked responses themselves. As expected, decoding accuracy was high, and reached a peak of 32% correct responses in the time window 25-35 ms after tone onset. Decoding accuracy increased to over 40% when combining activity from multiple time windows. We then tested if it is possible to decode the identity of the previous tone using responses evoked by the subsequent white noise-burst. Decoding was at chance levels (~4.5%) for single time points but increased to almost 7% when combining multiple time windows. This above-chance decoding of previous sounds from responses to a subsequent non-informative white noise burst is favorable to the negative trace hypothesis of EM. Continuing research aims to optimize properties of the neutral reactivation stimulus to enhance decoding accuracy.
Quiescent stem-like cancer cells, in response to chemotherapy, secrete follistatin to induce chemotherapy resistance in surrounding cells.

**Santiago Panesso-Gómez, MD, 1 Alex Cole, PhD, 1 Ronald J. Buckanovich, MD, PhD**

1 Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA, USA.

Ovarian cancer (OvCa) remains one of the deadliest cancers with a high mortality:incidence ratio. High mortality relates in part to high rates of the development of chemotherapy-resistant disease. We recently reported that the transcription factor NFATC4, in response to chemotherapy, induces a reversible cellular quiescence to drive OvCa chemoresistance; however, its mechanism of action remains undefined. We conducted RNA-seq on OvCa cell lines overexpressing NFATC4. The ovarian hormone Follistatin (FST), a critical regulator of ovarian biology, was found to be upregulated 7-fold in NFATC4 driven quiescent cells. FST was similarly upregulated in quiescent cells isolated from patient cancer cells. Furthermore, we found FST gene expression and secretion is upregulated following cancer cell exposure to chemotherapy. FST treatment of OvCa cells reduced cellular proliferation and increased chemotherapy resistance. Indicating a direct role for FST, antibody neutralization of FST-sensitized OvCa cells to chemotherapy and CRISPR FST-KO in OvCa cells significantly increased sensitivity to chemotherapy in vitro, and in vivo, allowing for chemotherapy mediated tumor eradication in an otherwise chemoresistant OvCa model. Importantly, supporting a role for FST in chemoresistance in patients, we found FST protein is in elevated levels in the abdominal fluid of patients with OvCa; FST levels increased in fluid taken immediately after chemotherapy exposure and then declined to baseline levels in patients no longer receiving chemotherapy. Together, this suggests follistatin is induced in quiescent cells in response to chemotherapy, induces chemotherapy resistance in an autocrine/paracrine manner and represents a therapeutic target to improve outcomes for patients with chemoresistant OvCa.
Variation in Striatal Dopamine-Related Neurophysiology Supports Age-Related Changes in Glutamate through Human Adolescence

Parr AC1, Perica MI1, Calabro F1,2, Tervo-Clemmens B3, Foran W1, Yushmanov V4, Hetherington H5, Luna B1

1Department of Psychiatry, University of Pittsburgh, 2Department of Bioengineering, University of Pittsburgh, 3Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, 4Department of Radiology, University of Pittsburgh, 5Department of Radiology, University of Missouri Columbia

Recent research from our laboratory has identified changes in prefrontal cortex (PFC) glutamate (Glu), gamma-aminobutyric acid (GABA), and GABA/Glu balance in adolescence (Perica et al., Flux 2021/22), potentially reflecting critical period plasticity that supports developmental specialization of PFC-dependent cognitive functions. The mechanisms mediating the engagement of this process remain unknown. Emerging evidence implicates dopamine (DA) in regulating changes in prefrontal E/I balance through adolescence (Reynolds & Flores, 2021); here, we assess the role of DA in supporting developmental changes in PFC Glu and GABA from adolescence to adulthood.

Indices of cortical Glu and GABA were obtained in 143 10-30 year olds (73F) using 7T Magnetic Resonance Spectroscopic imaging (MRSI). An oblique MRSI slice of 24x24 voxels (1.0x0.9x0.9mm) using a J-refocused spectroscopic imaging sequence (TE/TR=35/1500ms) facilitated data collection across multiple cortical regions. MR-based indices of striatal tissue-iron (time-averaged and normalized T2*; nT2*) provided an indirect measure of DA-related striatal neurophysiology.

Increased striatal nT2* was associated with higher Glu in anterior cingulate cortex ($\beta=-.17$, $p=.04$), medial PFC ($\beta=-.21$, $p=.02$), and anterior insula (Ins; $\beta=-.23$, $p=.005$). In dorsolateral PFC (DLPFC) and Ins, we observed nT2* by age interactions on Glu (DLPFC: $\beta=-.29$, $p=.007$, Ins: $\beta=-.22$, $p=.02$), and follow-up tests revealed that age-related decreases in Glu were driven by individuals with high levels of nT2* (DLPFC: $\beta=.36$, $p=.004$, Ins: $\beta=.32$, $p=.005$) relative to low (DLPFC: $\beta=-.13$, $p=.33$, Ins: $\beta=.22$, $p=.07$).

This study provides novel in vivo evidence linking DA processes to age-related changes in PFC GABA/Glu. Models applied at future timepoints will identify longitudinal associations between DA and shifts in GABA/Glu through adolescence. Understanding developmental mechanisms underlying regulation of E/I transmission can inform the emergence of psychopathologies, such as schizophrenia, that involve changes in DA, Glu, and GABA.

This study provides novel in vivo evidence linking DA processes to age-related changes in PFC GABA/Glu. Models applied at future timepoints will identify longitudinal associations between DA and shifts in GABA/Glu through adolescence. Understanding developmental mechanisms underlying regulation of E/I transmission can inform the emergence of psychopathologies, such as schizophrenia, that involve changes in DA, Glu, and GABA.
CRAC independent role of STIM2 in colorectal cancer metabolism and progression

Pathak, Trayambak1; Trebak, Mohamed1

1Vascular Medicine Institute, Department of Medicine and Department of Pharmacology and Chemical Biology, University of Pittsburgh

Colorectal cancer (CRC) is the third most common type of cancer, accounting for 10% of all cases. Most cancer cells undergo a metabolic transformation to support uncontrolled growth and metastasis. To undergo a metabolic transformation, cancer cells must produce ATP and biomolecules, which requires a healthy mitochondrion. Recent studies indicate that STIM, a stromal interaction molecule present in the endoplasmic reticular membrane, can regulate mitochondrial function. TCGA data analysis shows that the reduced STIM2 mRNA level significantly correlates with the reduced survival of CRC patients. Therefore, to study the role of STIM1 and STIM2 in CRC progression, we have generated multiple clones of STIM1, STIM2, and STIM1/2 double KO HCT116 and DLD1 CRC cells through CRISPR/Cas9.

In contrast to STIM1 KO, loss of STIM2 resulted in increased OCAR, ECAR, ATP generation, and mitochondrial biomass. However, the mitochondria were significantly smaller in STIM2 KO cells. STIM2 KO cells also showed a significantly increased proliferation. Metabolic profiling of STIM2 KO cells showed an increased glucose dependency. Transcriptomic analysis of STIM2 KO cells showed increased expression of glycolysis enzymes, suggesting that STIM2 transcriptionally regulates glycolysis. Xenograft tumor modeling in mice resulted in significantly larger tumor size generation by the STIM2 KO cells compared to control and STIM1 KO cells. The mice injected with STIM2 KO cells also showed significantly enhanced metastasis to the liver and colon, resulting in higher lethality in STIM2 KO injected mice. These results indicate STIM2, not STIM1, is critical for CRC cell viability and function. The specific STIM2-dependent pathways that control these distinct phenotypes are currently under investigation.
Association Between Mask Recommendations and Bronchiolitis Hospital Admissions in the United States

Pelletier, Jonathan H1; Rakkar, Jaskaran1; Au, Alicia K1,4; Fuhrman, Dana Y2; Aldewereld, Zachary1,2; Clark, Robert SB1,4; Kochanek, Patrick M1,4; Horvat, Christopher M1,5

1Department Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
2Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
3Brain Care Institute, UPMC Children’s Hospital of Pittsburgh, Pittsburgh, Pennsylvania
4Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
5Division of Health Informatics, UPMC Children’s Hospital of Pittsburgh; Pittsburgh, Pennsylvania

Importance: Bronchiolitis represents 18% of hospital admissions in children under two. The US CDC issued two recommendations for masking during 2020-2022. The effects of masking on bronchiolitis hospitalizations are unknown.

Objective: To analyze temporal associations between mask recommendations and bronchiolitis admissions.

Methods: Retrospective cross-sectional study of 33 US hospitals contributing to the Pediatric Health Information Systems database. All admissions with for viral bronchiolitis between 1/12010 and 12/31/2021 were included. Historic bronchiolitis admissions were used to train ensemble time series forecasting models. Monthly admissions for bronchiolitis during the COVID-19 pandemic were examined relative to forecast predictions and mask recommendations.

Results: During the first CDC mask recommendation, there were 1,508 admissions for bronchiolitis, compared to 19,426 (95% CI: 11,500-31,047) predicted (92.2% reduction). After the CDC masking recommendation ended, bronchiolitis admissions increased leading to an out-of-season peak in August 2021 with 2,641 admissions compared to 578 (95% CI: 267-1,067) predicted. After masking recommendations resumed, admissions again decreased. There were 2,257 admissions in December 2021 compared to 4,155 (95% CI: 2,668-6,041) predicted. Of 15 states with a mask mandate, 15/15 (100%) had fewer-than-forecasted admissions during their mandate period. 8/15 (53.3%) states had higher-than-forecasted bronchiolitis admissions after mask mandates ended (p = 0.013).

Conclusions and Relevance: There was a 92% reduction in bronchiolitis admissions during mask recommendations, followed by an out-of-season peak. Masking may be useful in increasing hospital surge capacity in future epidemics.
“Cuéntame Cuentos”: Incorporating Elaborative Reminiscing into a Dialogic Reading Technology for Latino Child Literacy

Pérez Cortés, Luis E.; Leyva, Diana; Walker, Erin

1Learning Research and Development Center, University of Pittsburgh

This project seeks to co-design and pilot test an intervention program that promotes literacy practices among Latino families and their preschool children (3- to 5-year-olds). This project combines theoretical and practical insights about learning and literacy within the context of an interactive reading comprehension app that members of our research team have previously developed. We have collected pilot test data to develop an improved intervention, reading comprehension app, and related curriculum materials. We collected this data through participant-logged in-app information, focus group interviews, and design thinking activities that all form part of our co-design efforts with seven participating Latino families.

Analyses of these data are aiding in our goal of developing culturally responsive and technology-enhanced approaches to promoting literacy among Latino families. After using the app for one month, parents reported wide variability in children’s initial and sustained interests during repeated readings. While two participants mentioned that their 5-year-old children showed higher interest when reading within the app than with print books, two other participants mentioned that their 3-year-old children thought that the ways of interacting with the current in-app stories were “boring” and “difficult”. Though further data analyses may yield more nuanced interpretations for such differences, an initial explanation based on the children’s ages may suggest that the current version of the app should be revised so that it is more developmentally appropriate for younger preschool children. In addition, participants commented on specifics of certain stories as being too “unfamiliar” to a Latino context. For instance, one of the stories shows a farmer feeding apples to a horse to help clean its teeth. This was considered as unfamiliar by participants, in part, because they never thought apples served such a purpose. Such unfamiliarity points to how some in-app stories might be revised to include more easily recognizable Latino knowledges and practices.
**Succinylation of Park7 Activates Protective Metabolic Response to Acute Kidney Injury**

Katherine Pfister; Victoria Young; Anne Barbosa; Takuto Chiba; Birgit Schilling; Eric Goetzman; and Sunder Sims-Lucas

1Department of Pediatrics, Children’s Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA
2Buck Institute for Research on Aging, Novato, CA

Acute Kidney Injury (AKI) is extremely prevalent among hospitalizations and presents a significant risk for the development of chronic kidney disease and increased mortality. Ischemia caused by shock, trauma, and transplant are common causes of AKI. To attenuate AKI occurrence therapeutically we need a better understanding of the physiological and cellular mechanisms underlying damage. The most pronounced effect of AKI is on the Proximal Tubule Epithelial Cells (PTECs) which have the highest metabolic activity and are therefore most reliant on undisturbed blood flow and oxygen content. Instances of Ischemia and Reperfusion Injury (IRI) result in hypoxia signaling and, more rapidly, posttranslational modifications (PTMs) on proteins. A growing list of PTMs have been identified and their effects described in AKI but among the understudied are succinylation and glycation. We have previously shown a protective effect on PTECs after depletion of the desuccinylase Sirtuin 5, however Sirtuin 5 is not a druggable target. Mass spectrometry analysis of Sirtuin 5 knockout PTECs revealed changes in mitochondrial and peroxisomal activity and we suggest that this activity is modulated in part by the protective effects of the deglycase Park7. Park7 expression is decreased after IRI but increased in Sirtuin 5 knockout cells. These data in combination with published results of Park7’s protective role in cardiovascular damage and chronic kidney disease lead us to hypothesize that Park7 may ameliorate oxidative damage resulting from AKI and prevent disease progression. We hope to harness this mechanism to develop novel therapies for AKI.
Information Retrieval from ACL Reconstruction Operative Reports: A Pilot Study

Poploski, Kathleen¹; Mao, Haiyi²; Hughes, Jonathan³; Boyce, Richard⁴; Irrgang, James¹,³; Musahl, Volker³

¹ Department of Physical Therapy, University of Pittsburgh;
² School of Computing and Information, University of Pittsburgh;
³ Department of Orthopaedic Surgery, University of Pittsburgh;
⁴ Department of Biomedical Informatics, University of Pittsburgh

**Introduction:** Rates of reinjury and adverse outcomes following anterior cruciate ligament reconstructions (ACLR) remain high. Over 100,000 patients undergo ACLR each year, providing rich electronic medical record (EMR) data to improve outcomes, but manual chart review can be time-consuming and expensive. We pilot tested information retrieval techniques to extract variables of interest from ACLR operative reports and evaluated relevancy metrics to determine feasibility of scaling the approach for larger studies.

**Methods:** Fifty operative reports were randomly selected from patients (≥14 years) who underwent ACLR by a University of Pittsburgh Medical Center (UPMC) orthopaedic surgeon between 2012-2017. We used the CLAMP natural language processing toolkit to annotate 20 operative reports for variables related to ACLR procedures (training sample). Regular expression and keyword matching were then used to extract values for each variable from 30 additional operative reports (testing sample). Relevancy metrics, including recall, precision, and the balanced F-measure were calculated using clinician-identified values as the gold standard.

**Results:** Several variables of interest, including *pre-operative diagnoses*, *side of surgery*, *tourniquet time/pressure*, *surgeon*, *graft type*, and *ACLR procedure* were reliably identified with all relevancy metrics between 0.9-1. Other variables, including *articular cartilage involvement* and *procedure*, and *meniscus procedure* had lower metrics (F-measure: 0-0.5) due to heterogeneity in documentation and limited prevalence in the training sample.

**Conclusions:** Relatively simple natural language processing techniques show promise to extract variables from ACLR operative notes accurately. This pilot work will inform a larger study in which we will annotate ≥ 200 operative reports and iteratively evaluate performance until relevancy metrics plateau. With refinement, efficient and reliable data extraction from clinical notes for individuals that underwent orthopaedic surgery will make large, EMR-based studies more feasible.
Microbiome-derived Short-Chain Fatty Acid Butyrate Attenuates Pulmonary Vascular Endothelial Inflammatory Activation and Pulmonary Hypertension

J. Andres Pulgarin¹, Jacob Dubner¹, Imad Al Ghouleh¹

¹ University of Pittsburgh Division of Medicine, Division of Cardiology and the Pittsburgh Heart, Lung and Vascular Medicine Institute

Pulmonary hypertension (PH) is a progressive, severe disease characterized by high blood pressure in the pulmonary circulation, inflammatory cells infiltration and excessive pulmonary vascular remodeling. Endothelial cells (EC) dysfunction is increasingly recognized as a precipitating event for this remodeling. Short chain fatty acids (SCFAs) present in circulating blood and produced by host microbiome (MB) have been associated with benefit in cardiovascular diseases and hypertension. However, there is a dearth of knowledge on the role of MB-derived molecules in PH-EC. We hypothesized that butyrate (a bacterial SCFA) plays a protective role on ECs under PH. In our results, mice supplementation with butyrate (sodium butyrate, NaB) attenuated hypoxia-induced right ventricular (RV) pressure and hypertrophy in vivo. NaB also lowered hypoxia-induced circulating monocyte numbers and reduced gene expression in hypoxic lung CD31+ ECs of inflammatory adhesion molecule VCAM-1 as well as increase expression of ERM binding Phosphoprotein 50 (EBP50), a protein we have shown to modulate endothelial reprogramming in PH (qRT-PCR). In vitro, NaB attenuated PH-related inflammatory cytokine IL1β-induced human pulmonary arterial EC (HPAEC) migration at 48 hrs. Consistent with in vivo results, butyrate also reversed IL1β-induced increase in VCAM-1 expression at the mRNA and protein level in HPAEC (qRT-PCR and Western blot, respectively). Moreover, butyrate rescued IL1β induced reduction in integrin α3 and EBP50 expression. Collectively our results demonstrate that butyrate attenuates PH potentially via protecting against myeloid cell induction and pathophysiological proinflammatory, promigratory pulmonary vascular EC activation. Butyrate may also reduce EC reprogramming through its effects on EBP50. These findings support the potential for therapeutic benefits of SCFAs in PH and the need for further investigation of the role of microbiome-derived metabolites in this devastating disease.
Chronic Absenteeism among Middle School Students with High Exposure to Violence

Rankine, Jacquelin¹; Fuhrman, Barbara¹; Copperman, Ethan¹; Miller, Elizabeth¹; Culyba, Alison¹

¹Division of Adolescent and Young Adult Medicine, Department of Pediatrics, University of Pittsburgh

Purpose: Chronic absenteeism, defined as missing 10% of school days, impacts over 8 million U.S. students. Chronic absenteeism in middle school predicts failure to graduate high school, which subsequently predicts poor health and increased overall mortality in adulthood. Contextual factors associated with absenteeism may be underrecognized in school and clinical settings. We examined the prevalence of absenteeism and violence exposure and tested their associations among middle school students with identified risk of trauma.

Methods: We analyzed baseline data from an ongoing cluster-randomized trial of a school-based teen dating violence and sexual violence prevention program in Pittsburgh, PA. Participants completed surveys identifying lifetime exposure to 10 types of violence and past 30-day absence. Violence exposure and absenteeism were summarized and compared across demographic groups. Generalized linear models examined associations between 1) any history of violence exposure, 2) each type of violence exposure, and 3) summed exposures to different types of violence, and frequent absenteeism (≥2 absences in past 30 days).

Results: 45.5% of participants (overall n = 499) reported frequent absenteeism and 71.5% reported violence exposure. Any violence exposure was associated with absenteeism (aRR = 1.43, 95% CI: 1.06-1.92). However, no specific type of violence exposure predicted absenteeism. Comparing summed exposures to different types of violence to no violence exposure, exposure to 1 type of violence was associated with absenteeism (aRR = 1.59, 95% CI: 1.15-2.20), with no evidence of stronger associations with greater exposure (2-3 types: aRR = 1.37, 95% CI: 1.00-1.88; ≥4 types: aRR = 1.31, 95% CI: 0.98-1.74).

Conclusions: Youth in our sample experienced both high rates of violence exposure and absenteeism. Prior violence exposure was associated with absenteeism. Resources and contextual support for youth exposed to family or community violence may play a role in school attendance, emphasizing need for trauma-sensitive approaches to absenteeism.
Investigating the evolution of new body parts in the rapidly evolving genitalia of Drosophila

Rice, Gavin1; Charles-Obi, Kenechukwu1; David, Jean2; Gompel, Nicolas3; Yassin, Amir4; Zeitlinger, Julia5,6; Rebeiz, Mark1

Department of Biology, University of Pittsburgh, Pittsburgh, PA1; Laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE), UMR 9191, CNRS,IRD, Univ.Paris-Sud, Université Paris-Saclay, cedex, France2; Ludwig-Maximilians Universität München, Fakultät für Biologie, Biozentrum, Grosshaderner Strasse 2, 82152 Planegg-Martinsried, Germany3; Institut de Systématique, Evolution et Biodiversité, UMR7205, Centre National de la Recherche Scientifique, MNHN, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, 75005 Paris, France4; Stowers Institute for Medical Research, Kansas City, MO5; Department of Pathology and Laboratory Medicine, Kansas University Medical Center, Kansas City, MO6

Recently evolved traits i.e., novelties, often represent key features that allow animals to exploit new ecological niches (e.g. feathers in birds) and can even help them find a mate (e.g. bioluminescence in fireflies). The rapidly evolving genitalia of Drosophila provides a powerful system to study the developmental basis of qualitative changes in morphology. However, this high morphological diversity also poses a distinct challenge, since it can be difficult to disentangle which structures are homologous and which represent novelties.

To determine whether genital structures of different species were homologous or novel, we compared the development of phallic outgrowths in the Oriental lineage, which contains Drosophila melanogaster. We found that most phallic outgrowths are formed by multicellular projections. However, several multicellular outgrowths that were thought to be homologous, are in fact formed by different tissues of the phallus in different species, indicating that they are heterologous structures. Furthermore, we uncovered evidence that phallic outgrowths found in the Drosophila eugracilis phallus are formed by large unicellular projections, suggesting that they have evolved convergently to the previously described multicellular outgrowths. These unicellular outgrowths are likely novel as they are absent the homologous tissue of five other species of the Oriental lineage. We found that the trichome genetic network is expressed in these unicellular phallic outgrowths, suggesting that the co-option of this network underlies this dramatic phenotype. In fact, activation of the trichome genetic network in the phallus of Drosophila melanogaster induces a phenocopy of the unicellular outgrowths found in Drosophila eugracilis.

Our work highlights that understanding development can be key to discern between homology vs heterology, allowing us to formulate testable evolutionary models (i.e. co-option vs convergence).
Preparing for PGx results to routinely be available in the EHR

Katherine A. Riden¹; Lucas A. Berenbrok¹; James C. Coons¹; Philip E. Empey¹

¹ Pharmacy and Therapeutics, School of Pharmacy, University of Pittsburgh

Pharmacogenomics (PGx) is increasingly being utilized for precision prescribing. PGx results will be placed in UPMC EHRs with clinical decision support (CDS). Previous literature has surveyed clinicians’ knowledge, perceptions, and attitudes toward PGx testing, but has not focused on preparedness for utilizing PGx results when they are already in the EHR. This study aims to evaluate roles, multidisciplinary clinician preparedness, and needed education and institutional support.

An anonymous IRB-approved survey was developed utilizing two frameworks and contained 18 questions. It was piloted in three phases and launched in early 2022. Descriptive and comparative statistics were used to evaluate results.

Thus far, 1178 respondents [physicians (68%), pharmacists (10%), nurse practitioners (8%), physician assistants (10%), and other (3%)] have completed the survey. Perceived self-efficacy was different when participants were stratified for previous PGx education (p<0.00001). Participants reported that they absolutely must have: access to a PGx consult service (56%), clinical web applications (60%), and PGx CDS (62%). Participants (76%) also reported that they would either be very interested/somewhat interested in online PGx education.

Respondents believed multiple clinician roles have roles in PGx RoR, with pharmacist and physician involvement in all aspects. Preliminary findings of the survey also indicate that few clinicians are currently prepared to utilize PGx results. Results emphasize the desire for PGx education, consult services, and online resources for institutional support resources.
Identification of half-processed proBMP10/BMP9 GF, a novel circulating ALK1 ligand

Rosato, Teresa¹; Morosky, Stefanie¹; Fiore, Jack¹; Schwartze, Tristin²; Hinck, Cynthia²; Hinck, Andrew²; Roman, Beth L. ¹

¹Department of Human Genetics, University of Pittsburgh
²Department of Structural Biology, University of Pittsburgh

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular disorder characterized by the development of arteriovenous malformations. Though defective ALK1 signaling has been identified as causal for disease manifestation, no targeted therapies exist for HHT patients. We postulate that enhancing signaling flux via administration of ALK1 ligand may prevent AVMs in HHT. Accordingly, it is critical to understand the precise molecular nature of endogenous ALK1 ligands, BMP9 and BMP10. Using enzyme-linked immunosorbent assays (ELISAs) specific for each of these proteins, we detect BMP10 in human plasma only in an unprocessed, full length form—pro-BMP10—that is assumed to be latent. By contrast, we detect BMP9 in a processed form—BMP9 growth factor (GF)—that is assumed to be fully active. However, additional ELISAs that detect only heterodimers reveal a novel “half-processed” ligand, pro-BMP10/BMP9 GF, which predominates over all other ligand forms. To study the mechanism of pro-BMP10/BMP9 GF synthesis, we transfected a human hepatic stellate cell (HSC) line, LX2, with BMP9 and/or BMP10. Results suggest that BMP9 and BMP10 monomers may be retained in the ER and secreted only as dimers, with BMP9 but not BMP10 undergoing processing prior to or post-secretion. These findings suggest that HSCs may be the primary source of this predominant circulating ALK1 ligand, and that the BMP10 prodmain may play a key role in ligand stability or homing that should be considered in development of highly targeted ligand-based therapeutics for HHT.
Age-related restriction of reovirus in the intestine

Roth AN\textsuperscript{1,2}, Welsh OL\textsuperscript{1,2}, and Terence S. Dermody\textsuperscript{1,2,3}

\textsuperscript{1}Department of Pediatrics, University of Pittsburgh School of Medicine
\textsuperscript{2}Institute of Infection, Inflammation, and Immunity, UPMC Children’s Hospital of Pittsburgh
\textsuperscript{3}Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine

Viral infections are a significant cause of childhood morbidity and mortality. Mammalian orthoreovirus (reovirus) is a prototypical double-stranded RNA virus that infects most humans worldwide and is associated with a variety of diseases in young mammals. As reoviruses usually spread by fecal-oral transmission, the intestine is a major site of initial reovirus replication. In mice, reovirus replication and dissemination are limited in adults relative to neonates. We therefore sought to define host factors responsible for restricting reovirus replication in the adult mouse intestine. By inoculating mice of different age groups perorally and assessing viral loads in different intestinal regions, we discovered that restriction of viral replication in the mouse intestine occurs after weaning. Physiological changes to the intestine occur during the weaning period, including maturation of the epithelium, diversification of commensal microbiota, and expansion of the mucosal immune system. The changing intestinal environment may influence viral pathogenesis including viral tropism for discrete cells and tissues. To identify specific cell types targeted by reovirus, we conducted immunohistochemical analyses of infected tissue. Prior to weaning, reovirus strain T1L primarily infects the intestinal mucosa, with viral replication factories observed in e-cadherin-positive intestinal epithelial cells. After weaning, a greater percentage of detectable virus infects gut-associated lymphoid tissues, including mesenteric lymph nodes and Peyer’s patches, suggesting a redistribution of virus to immune cells in mature animals. These data indicate a complex tropism for reovirus in the intestine, including epithelial and immune cells, that is influenced by age.
Universal SNAP CAR T cells show HER2-directed anti-tumor function.

Ruffo E', Kvorjak M', Adams E' and Lohmueller J'

'Division of Surgical Oncology, Departments of Surgery and Immunology, University of Pittsburgh, Pittsburgh, PA

Universal chimeric antigen receptors (CARs) are engineered T cell receptors that instead of directly binding to a target antigen, recognize one or more adaptor molecules that bind to a target antigen. Universal CARs are of high clinical interest due to their tunable activity antibody by adaptor dose and the capabilities of targeting multiple antigens on the same cancer or different cancers by combining with different adaptors. We previously developed a unique universal CAR, “SNAP-CAR,” that carries out a chemical reaction to covalently fuse to antibody adaptors with a benzylguanine tag. Our previous experiments showed potent SNAP-CAR function with co-administered antibody adaptor. We now expand on these findings evaluating the function of the SNAP-CAR when pre-assembled with the adaptor prior to therapy. Primary human SNAP-CAR T cells were pre-incubated with different amounts of antibody adaptor, showing a titratable level of receptor assembly. When challenged with antigen positive target cells, SNAP-CAR T cells showed potent activation and killing activity that correlated with the amount of preloaded adaptor in a dose-dependent manner. The activity of pre-assembled SNAP-CAR T cells was then tested in vivo in a human tumor xenograft NSG mouse model targeting the HER2 antigen. A single dose of preloaded SNAP CAR T cells showed significant tumor regression that could be further enhanced by additional injections of the preloaded SNAP CAR T cells. Administration of adaptor along with preloaded CAR T cells further improved tumor regression. In conclusion, preloading of SNAP CAR T cells prior to therapeutic administration can deliver a potent pulse of anti-tumor activity with the capability of further programming by additional adaptor.
Utilizing spatial transcriptomics data to identify cell context-specific regulatory programs
Sagan A¹; Osmanbeyoglu H U¹²

¹Department of Biomedical Informatics, University of Pittsburgh
²Department of Bioengineering, University of Pittsburgh

Elaborately orchestrated transcriptional programs distinguish specialized cell types and define their functionality. Combinations of transcription factors (TFs) drive these transcriptional programs and control cellular identity and functional state. Other types of cells in close proximity are also critical for instructing context-specific transcriptional programs. Rapid advances in spatial technologies offer highly multiplexed profiling of RNAs, while preserving spatial context of the tissue. For example, spatial transcriptomics (ST) measure genome-wide mRNA expression across thousands of spots on a tissue slice while preserving information about the spatial location of spots. 10X Visium ST measures up to 5000 spots, each spot containing up to 10 cells. Multiple computational methods have been introduced to analyse ST data, including the identification of spatial patterns of gene expression, spatially distributed differentially expressed genes, and spatial cell-cell communication patterns. However, it is not yet clear how to best leverage these datasets to systematically estimate TF activity influencing cell states related to human health and disease.

We develop a linear mixed effect model for integrating gene expression, cis-regulatory information, spatial data, and imaging data to reveal cell context-specific transcriptional programs. Combining the features at each spot and its neighbouring spots, we inferred spatially resolved spot-specific transcription factor activities. We apply our method to a publicly available ER⁺ breast cancer spatial gene expression datasets to identify spatially variable transcription factors, cluster spots into regions with distinct regulatory features, and quantify the spatial relationship between TF activities and cell types.
Corneal stromal stem cell-derived extracellular vesicles transport TGFβ3 to mediate anti-fibrotic effect on corneal scarring

Santra, Mithun1; Weng, Lin1; Geary, Moira1; Yang, Tianbing1; Jhanji, Vishal1; Yam, Gary1

1Department of Ophthalmology, School of Medicine, University of Pittsburgh, Pittsburgh

Transparent cornea is paramount for vision. Corneal scarring is one of the top leading causes of global blindness. Conventional corneal transplantation has been challenging due to the scarcity of donor tissue. Alternative corneal stromal stem cell (CSSC) therapy successfully prevented scarring development. The paracrine action of CSSC, mediated by extracellular vesicles (EVs), was shown to block corneal fibrosis and stimulate native tissue regeneration. This study investigated the role of anti-fibrotic transforming growth factor (TGF) β3 transported in CSSC-EVs in the scarless regeneration of mouse corneas after injury.

The current study showed that TGFβ3 was upregulated in CSSCs after stimulation with M1 RAW cells in both co-culture and paracrine conditions. More importantly, EVs derived from the stimulated CSSCs contained increasing levels of TGFβ3 mRNA transcripts. Naïve CSSC or TGFβ3 knockdown CSSCs were applied to mouse corneas after wounding by stromal ablation using Algerbrush. After CSSC application to injured mouse corneas, TGFβ3 was significantly upregulated, in conjunction with the inhibition of mouse fibrotic genes. At day 14 post-injury, the treated corneas remained clear and the expression of fibronectin, hyaluronan synthase 2, SPARC, tenascin C, collagen 3a1, and α-smooth muscle actin were significantly reduced. CSSCs with TGFβ3 knockdown by specific siRNAs lost these therapeutic effects.

Our study revealed the paracrine action of human CSSCs on corneal scar prevention through EV-mediated delivery of TGFβ3 mRNA transcripts. The elevated TGFβ3 expression in injured corneas could redirect the wound healing process to a reduced scarring or scar-free tissue regeneration, resulting in clear corneas.
Characterizing changes in health and resiliency behaviors during the COVID-19 pandemic among postpartum women with a recent hypertensive disorder in pregnancy

Jewel Scott1,2, Alisse Hauspurg1,2, Esa Davis1,3,4, Janet Catov1,5

University of Pittsburgh1, Department of Psychiatry2, Department of Medicine3, Department of Clinical and Translational Science4, Department of Obstetrics, Gynecology, and Reproductive Sciences5

COVID-19 presented many challenges to maintaining a healthy lifestyle. These challenges may be especially detrimental for people at high risk for cardiovascular disease, such as women with a recent hypertensive disorder in pregnancy (HDP).

Participants were approximately 18 months postpartum and all had an HDP in their most recent pregnancy (N=82). Change in health behaviors was assessed with the Coronavirus Perinatal Experiences - Impact Survey. The research team created an 8-item measure to assess resilience behaviors such as finding new purpose and gratitude (alpha = 0.89). We measured depression and PTSD symptoms with the PHQ9 and Breslau scales, respectively. To describe the impact of the Covid-19 pandemic on health behaviors we used chi-square for categorical variables and one-way ANOVA for continuous variables.

Most participants were married or partnered (80%), college graduates (57%), and first-time parents (55%). One-fourth of respondents reported worse sleep and 39% reported worse diet and physical activity. Compared to people without changes in physical activity, those with the most disruption was more likely to have probable depression and PTSD, more disruption in work, more housework, and less support from extended family (all p < .05). The pattern was similar for people with worse sleep and diet, compared to those with no change or improved behavior. There were no differences in resiliency behaviors, except people with worse diet demonstrated a trend toward lower resiliency behaviors (F 2.59, p =.083).

Our findings highlight the importance of assessing COVID-19 related health impacts among people with HDP. If these findings are confirmed in larger samples, clinicians should consider targeted approaches to improve adherence to lifestyle recommendations for women with an HDP, with a focus on those with depressive and PTSD symptoms.
12 h Rhythm Abnormalities in the Human Dorsolateral Prefrontal Cortex of Subjects with Schizophrenia

Scott, Madeline R. ¹, PhD, Zong, Wei², Ketchesin, Kyle D. ¹, PhD, Seney, Marianne L. ¹, PhD, Tseng, George C. ², PhD, Zhu, Bokai ³, PhD, and McClung, Colleen A. ¹, PhD

¹Translational Neuroscience Program, Department of Psychiatry, UPMC
²Department of Bioinformatics, University of Pittsburgh
³Aging Institute, Division of Endocrinology and Metabolism, Department of Medicine, UPMC

Twelve-hour (12 h) ultradian rhythms are a well-known phenomenon in coastal marine organisms. While 12 h cycles are observed in human behavior and physiology, no study has measured 12 h rhythms in the human brain. Here we used RNA sequencing data collected by the Common Mind Consortium to identify 12 h rhythms in human postmortem dorsolateral prefrontal cortex (DLPFC), a region associated with executive function. In subjects with no psychiatric illness (NP; n = 104), we identified transcripts with 12 h rhythms that peak either at sleep/wake transitions (~ 9 AM/PM) or static times (~ 3 PM/AM). In subjects with schizophrenia (SZ; n = 46), a chronic neuropsychiatric illness associated with DLPFC dysfunction, we found fewer 12 h rhythms, which was associated with the unfolded protein response and neuronal structural maintenance. Moreover, genes involved in mitochondrial function and protein translation, which normally peak at sleep/wake transitions, peaked instead at static times in SZ, suggesting suboptimal timing of these essential processes that may contribute to cognitive deficits commonly found in SZ.
HDAC11 is a crucial regulator for visual cycle genes and retinal function


Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

It has been reported that retinal pigment epithelial (RPE) cells isolated from age-related macular degeneration (AMD) patients present decreased chromatin accessibility and reduced expression of RPE signature genes. In this study, we aim to dissect the role of histone deacetylase 11 (HDAC11) in regulating RPE genes and RPE function.

Best1-Hdac11 constitutive knock-in (KI) mice were generated for this study. Histological results revealed decreased pigmentation of RPE cells and abnormal photoreceptor outer segments in Hdac11 KI mice. Immunofluorescence experiments showed reduced opsin and rhodopsin staining in 12-month-old Hdac11 KI mice. Electroretinography (ERG) showed decreased a, b and c wave amplitudes in 12-month-old Hdac11 KI mice indicating impaired retina function. Decreased expression of visual cycle genes such as Rpe65 and Lrat were found in Hdac11 KI mice relative to WT mice. RPE flatmounts overexpressing Hdac11 also showed significantly decreased expression of visual cycle genes (Rpe65, Lrat, Rdh5, Rbp1, Rgr), but not lysosomal genes (Atp6v0, Lamp1), or genes involved in other processes such as Il1b, Bcl-2, and Sox9. No significant changes in the expression of Hdac1, Hdac2, Hdac6, Hdac7, Hdac8 were found in RPE flatmounts overexpressing Hdac11. We also found that both increasing and decreasing Hdac11 levels in human fetal RPE cells (fRPE) inhibited cell growth. fRPE cells with knocked-down Hdac11 were more sensitive to cigarette smoke extract (CSE) treatment.

Our results suggest that HDAC11 may control the chromatin accessibility for RPE specific genes, such as visual cycle genes, and thereby normalize physiological functions of the RPE/retina. Our in vitro data also suggested a role of HDAC11 in oxidative stress, possibly by its non-histone deacetylation activity. The current study provides mechanistic insight as to the upregulation of HDAC11 reported in patients with dry AMD and thereby provides a novel therapeutic avenue for delaying the progression of the disease.
Paired immunoglobulin-like receptor B is an internalization receptor for mammalian orthoreovirus

Shang P,1,2 Simpson JD,3 Taylor GM,1,2 Sutherland DM,1,2 Welsh OL,1,2 Aravamudhan P,1,2 Michel JJ,1 Rajasundaram D,1 Köhler M,3 Alsteens D,3 Dermody TS1,2,4

1Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, USA
2Institute of Infection, Inflammation, and Immunity, University of Pittsburgh School of Medicine, Pittsburgh, USA
3Louvain Institute of Biomolecular Science and Technology, Université Catholique de Louvain, Louvain-la-Neuve, Belgium
4Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, USA

Mammalian orthoreovirus (reovirus) causes age-restricted illness in many mammals and is associated with celiac disease in humans. In mice, reovirus infects intestine and disseminates systemically to cause serotype-specific neurologic disease. Serotype 1 (T1) reovirus infects ependymal cells and causes hydrocephalus, whereas serotype 3 (T3) reovirus infects neurons and causes lethal encephalitis. Receptors used by reovirus to infect intestine and brain are unknown. To identify T3 reovirus receptors, we conducted a genome-wide CRISPR activation screen, in which the top candidate that promoted T3 reovirus binding was the murine homolog of the leukocyte immunoglobulin-like receptor (LILR) family, paired immunoglobulin-like receptor B (PirB). PirB is expressed in immune cells and neurons. Ectopic expression of PirB in non-susceptible cells promotes reovirus binding and infection, which can be blocked by PirB-specific antibody and recombinant PirB extracellular region proteins. We defined reovirus-PirB interaction using atomic force microscopy and found that reovirus binds PirB with high affinity. Reciprocal exchange of extracellular domains of PirB and the paralog PirA, which does not bind reovirus, indicate that PirB D3D4 region is required for reovirus attachment and infectivity. Reovirus transcription and replication are diminished following binding to cells expressing PirB constructs with altered intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs), suggesting that PirB signaling contributes to reovirus internalization. Yields of T3 reovirus, but not T1 reovirus, are diminished in the brain of PirB−/− mice relative to those in WT mice. Moreover, PirB−/− mice showed improved survival following T3 reovirus infection. In primary neuron cultures, PirB-specific antibody diminishes T3 reovirus infection, and reovirus infectivity is reduced in PirB−/− neurons. Collectively, these data suggest that PirB functions as a binding and internalization receptor for reovirus and promotes T3 reovirus replication and pathogenesis in murine brain.
Identification of itch spinal neuron networks and their inhibition by kappa opioid signaling

Sheahan TD¹, Manalo AP¹, Perry VJ¹, Fanien LG¹, Ross SE¹

¹Pittsburgh Center for Pain Research, Department of Neurobiology, University of Pittsburgh School of Medicine

Itch is a prevalent health problem in need of safe, effective treatments, yet our limited understanding of how itch stimuli are transmitted within the nervous system has left it poorly managed. In particular, little is known about how itch stimuli are integrated in the spinal cord. Thus, we sought to identify which neurons in the spinal cord convey itch for the first time, and to test the hypothesis that compounds that cause itch behavior in mice activate a common spinal neuron network. We are addressing this fundamental question using a combination of multiphoton imaging, behavioral pharmacology, genetics, and molecular approaches.

First, we leveraged an *ex vivo* spinal cord preparation in combination with two-photon (2P) Ca²⁺ imaging to visualize activity of excitatory spinal cord dorsal horn neurons in response to itch spinal neuropeptides. We show that while pruritogens (e.g. gastrin-releasing peptide, somatostatin, substance P) act directly on different spinal neurons, they engage a common downstream spinal neuron network. Next, we investigated whether kappa opioid receptor (KOR) agonists, which are currently in the development for the treatment of chronic itch, act on this itch spinal neuron network to inhibit itch. We demonstrate that spinal delivery of KOR agonists blocks scratching evoked by the same centrally acting pruritogens. Moreover, using 2P Ca²⁺ imaging of the spinal cord, we visualize where KOR agonists act within spinal itch networks to inhibit the spinal transmission of itch. Finally, we present a molecular characterization of KOR-expressing spinal neurons using a combination of viral tracing and fluorescent in situ hybridization. In sum, these data provide both novel basic insights into the spinal coding of itch, as well as mechanistic understanding of KOR agonists that are in clinical development for the treatment of chronic itch. Supported by NIH grants F32NS110155 (TDS), R01NS0967905 (SER).
Using two-photon Ca^{2+} imaging to reveal spinal cord dorsal horn networks

Smith Kelly M1, Warwick Charles A1, Sheahan Tayler D1, Koerber H Richard1 and Ross Sarah E1

1Pittsburgh Center for Pain Research, Department of Medicine, University of Pittsburgh, Pittsburgh, PA.

The spinal cord dorsal horn is a key area for the processing of sensory information such as pain, heat, cold, touch and itch. Our understanding of spinal cord microcircuitry has proved difficult, in part, due to a lack of effective tools to allow identification of dorsal horn neuron populations and how they are connected. A recent technique developed in our lab using pharmacology-based profiling (termed CICADA) has allowed us to visualize eight populations of excitatory neurons in the superficial dorsal horn. Here, we use two-photon Ca^{2+} imaging and CICADA to tease apart connectivity across these CICADA-defined populations. Through back labeling spinoparabrachial projection neurons, we also identify spinal output neurons, and the spinal networks that provide input onto these cells. These experiments reveal the network connectivity, including the spinal output neurons, that are downstream of each CICADA-defined population. Together, these findings provide new insight into the connectivity in the superficial dorsal horn.
Odontoblast-specific Trps1 Deficiency Compromises Quality of Enamel and Dentin Increasing Susceptibility to Dental Caries

Socorro Mairobys1, Hoskere Priyanka1, Roberts Catherine1, Lukashova Lyudmila1, Verdelis Kostas1, Beniash Elia1,2, Napierala Dobrawa1,2

1 Department of Oral and Craniofacial Sciences, Center for Craniofacial Regeneration, University of Pittsburgh School of Dental Medicine, Pittsburgh, PA, United States
2 McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, United States

Objectives: Dental Caries is a multifactorial disease and a major public health problem. The complex etiology of dental caries includes environmental factors and host genetics, which together contribute to inter-individual variation in susceptibility. Mutations in the TRPS1 gene cause tricho-rhino-phalangeal syndrome (TRPS). Interestingly, TRPS patients present extensive dental caries. Studies in mice demonstrated that Trps1 gene is highly expressed in odontoblasts progenitors and odontoblasts-responsible to produce dentin. Considering that developing dentin affects formation of enamel, the purpose of this study was to determine the consequences of odontoblast-specific Trps1 deficiency on the quality of dental tissues, and its impact on susceptibility to caries.

Methods: We generated a tamoxifen-inducible conditional knockout mouse with targeted deletion of Trps1 gene in odontoblasts (2.3kbCol1a1-CreERT2;Trps1floflo aka Trps1Col1acKO). Mandibular first molars of 4wk old male and female Trps1Col1acKO mice were analyzed by micro-computed tomography (µCT) and histology. Mechanical properties of enamel and dentin were analyzed by Vickers microhardness test. The susceptibility to caries was compared between WT and Trps1Col1acKO using an ex vivo artificial caries approach.

Results: µCT and microhardness results demonstrate that odontoblast-specific Trps1 deficiency results in compromised quality of dentin and indirectly affects enamel formation, making these tissues more susceptible to acid demineralization. This effect of Trps1 deficiency is particularly strong in pits, which are the sites highly susceptible to dental caries in human teeth. Interestingly, Trps1Col1acKO males demonstrate a stronger phenotype than females.

Conclusion: We identified that Trps1 is important for formation of sound enamel and dentin, as well as it may constitute a causal factor contributing to sex differences in dental caries experience. This study suggests that compromised quality of dental tissues contributes to the high prevalence of dental caries in TRPS patients.
Additive Manufacturing of Copper-Bearing High-Strength Low-Alloy Steels using Laser Powder Bed Fusion with Post-Heat Treatments

Sridar, Soumya¹; Wang, ZhangWei², Xiong, Wei¹

¹ Physical Metallurgy and Materials Design Laboratory, Department of Mechanical Engineering and Materials Science, University of Pittsburgh, USA; ² Department Microstructure Physics and Alloy design, Max-Planck-Institut für Eisenforschung, Germany.

Copper-bearing high-strength low-alloy (HSLA) steels possess high strength, excellent low-temperature toughness, and good weldability. The superior properties achieved due to the synergistic effects from strengthening precipitates and alloy composition facilitate these steels to be suitable for naval applications. In this work, HSLA steels were fabricated using laser powder bed fusion process. Pre-alloyed powders are manufactured based on uncertainty quantification of alloy composition using ICME framework. The critical printing parameters were optimized, and the optimum processing window for achieving least porosity was identified. The porosity was found to be ~0.5% for the optimized builds. The post-heat treatment was guided by thermodynamic calculations after verification by key experiments. The optimized post-heat treatment is different from the one applied on traditionally manufactured HSLA steels. Atom probe tomography results showed that the fraction of Cu and M₂C was the highest after 5 hours of aging. The mechanical properties of the builds printed with optimized parameters and those printed with factory-default parameters for SS316L (porosity~3%) were compared. Improved tensile properties such as yield strength and ductility were obtained for the builds printed with optimized parameters. The low-temperature toughness was found to be anisotropic, although it reached the design target. The ductile-to-brittle transition temperature was below -40°C for samples with a notch in the XZ plane, while it was between -20 and -40°C for samples with a notch in the XY plane.
The circadian nature of lysosomal clearance in the retinal pigment epithelium may be mediated by βA3/A1-crystallin

Strizhakova, Anastasia¹, Koontz, Victoria¹, Shang, Peng¹, Liu, Haitao¹, Ghosh, Sayan¹, Chowdhury, Olivia¹, Hose, Stacey¹, Stepicheva, Nadezda¹, Sinha, Debasish¹²

¹Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; ²Wilmer Eye Institute, The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Age-related macular degeneration (AMD) is one of the leading causes of blindness in the elderly. Impairment of circadian clock has been identified as a major pathological factor that contributes to the progression of AMD. In this research, we hypothesize that βA3/A1-crystallin, a protein known to be important for maintaining lysosomal homeostasis in the RPE, may provide an important link between lysosomal clearance and the circadian rhythm in the RPE.

6-8 week old C57BL/6J mice (WT) were maintained at light-dark (LD 12:12) cycle, and RPE samples were collected at different timepoints during a day. To distinguish circadian rhythm from the biological rhythm, a second set of mice was exposed to complete darkness for 3 days prior to sacrificing. We observed oscillations of Cryba1 expression on mRNA and protein levels during the day in the RPE from WT mice and protein expression peaks coincided with spikes of lysosomal activity. fRPE cells entrained with serum shock supported that Cryba1 expression is consistent with the circadian regulation of Cryba1 transcription in the RPE.

Bioinformatic analysis of the Cryba1 gene sequence was performed with MoLoTool and miRDB and revealed potential regulation of Cryba1 expression by the circadian machinery – transcriptionally through NR1D1 and translationally through miR-206*. To confirm the regulation by miR-206, we first performed qPCR to detect the presence of miR-206 and miR-206* and found that both strands are expressed in the RPE from WT mice, suggesting that miR-206* can indeed regulate Cryba1 expression. We are currently cloning Cryba1 3’UTR to experimentally support direct binding and translational repression.

Overall, we have demonstrated that Cryba1 expression is regulated by circadian rhythm. This work contributes to a more complete understanding of circadian rhythm entrainment in the RPE cells and introduces a novel, RPE-specific member into the circadian network. Targeting Cryba1 could be a potential avenue for future therapeutic interventions to delay the progression of AMD.

Styler B\textsuperscript{1,2}; Chung C\textsuperscript{2}; Ding D\textsuperscript{1,2}

\textsuperscript{1}Human Engineering Research Laboratory, Department of Veteran Affairs; \\
\textsuperscript{2}Department of Rehabilitation Science and Technology, University of Pittsburgh

We describe the pilot testing and development of a vision guided (VGS) assistive robotic manipulator (ARM) for electric power wheelchair (EPW) users that eases joystick control burdens. For those with arm impairments, basic tasks like eating or drinking are nearly impossible. The ability to reach and manipulate objects is consistently rated as an important challenge. In some cases, family or hired caregivers help, but, ideally, users could access technology to empower them to engage independently in self-care. ARMs facilitate this; however, currently available wheelchair-mounted ARMs are cumbersome to control. ARMs come with a 2-axis joystick that requires mode switches for moving the end effector forward/backward, up/down, rotating the wrist, and open/closing the gripper. This control is cognitively difficult and increases operation time. The VGS pre-assigns control authority (user control or robot software control) to actions within a kitchen task. User control is assigned to gross motions that move the end effector towards interaction objects and actions that require unknown time durations (e.g., drinking, filling water). The autonomous robot software control automatically moves the arm through finer manipulation actions (e.g., aligning gripper, grasping). Transitions between user and robot control occur when QRCode-like tags, fiducial markers, are selected via a touchscreen. Our initial evaluation compares VGS, with pre-assigned control authority, to the user’s manual joystick operation. VGS controlled tasks are completed more efficiently with less mode switching but are not always more successful. Pilot studies suggest that users have different joystick control expertise which lead to different control authority preferences. Therefore, this work also describes the system’s extension to a hierarchical modular software architecture to facilitate future investigation of the discretization of control authority assignment among more general subtasks (i.e push, pick, place) that combine into a kitchen task (i.e. make popcorn, fill and drink cup).
The importance of heterotopic fibers of the Mammalian Brain: Most Callosal Axonal Fibers are Heterotopic, Not Homotopic

Szczupak, Diego¹, Iack, Pamela Meneses², Rayée, Danielle³, Liu, Cirong³, Lent, Roberto²,⁵, Tovar-Moll, Fernanda⁴, and Silva, Afonso C.¹

University of Pittsburgh Brain¹, Federal University of Rio de Janeiro², Albert Einstein College of Medicine³, Center for Excellence in Brain Science and Intelligence Technology⁴, D'Or Institute Research and Education (IDOR)⁵

Abstract

The corpus callosum is the largest white matter structure of the brain and the primary pathway for interhemispheric communication. Previous anatomical studies have characterized the bulk of callosal axonal fibers as connecting homotopic cortical areas. Whenever detected, the much fewer heterotopic callosal connections were attributed to plastic adaptations altering sprouting and pruning mechanisms in neurodevelopmental diseases such as corpus callosum dysgenesis. We hypothesized that heterotopic callosal fibers had been grossly overlooked and underestimated due to their complex nature and methodological limitations. Contrary to previous belief, we show that callosal connections in the mammalian brain are predominantly heterotopic. Using the Allen Brain Institute Mouse Brain Connectivity Atlas, we determined that ~63% of the overall interhemispheric callosal fibers in the C57BL/6J wild-type mouse brain are heterotopic. Furthermore, we used diffusion-weighted magnetic resonance imaging to show that ~75% of interhemispheric callosal connections in mice, monkeys, and humans are heterotopic. In all three species, heterotopic projections are the primary constituent of interhemispheric cortical networks, presenting distinct and complementary topographies to homotopic connections. These findings reshape the view of the corpus callosum and its role as the primary hub for interhemispheric brain connectivity, directly impacting multiple neuroscience fields that investigate cortical connectivity and brain development.
Non-canonical roles of p16 in nucleotide metabolism

Naveen Kumar Tangudu¹, Raquel Buj¹, Nathaniel W. Snyder², Katherine M. Aird¹

¹Hillman Cancer Center, Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
²Center for Metabolic Disease Research, Department of Microbiology and Immunology, Temple University, Lewis Katz School of Medicine, Philadelphia, PA, USA

Alterations in nucleotide synthesis are associated with various pathologies including cancer. p16 is an well-known tumor suppressor and cell cycle regulator whose expression is frequently lost in many cancers. We previously published that loss of p16 non-canonically activates mTORC1-mediated translation of a nucleotide metabolism enzyme to upregulated nucleotide biosynthesis through the de novo pathway. However, how mTORC1 is activated downstream of p16 loss has yet to be explored. We found that melanoma patient samples that have low p16 expression have an increase in the “DNA repair pathway” signature. Consistently, p16-low cells have an increase in DNA replication stress, DNA damage, and activation of the ATR and ATM DNA damage response pathways. Inhibition of ATR, but not ATM, decreased mTORC1 signaling, suggesting that ATR may be upstream of mTORC1 and its associated activity on nucleotide metabolism. Surprisingly, inhibition of the ATR substrate Chk1 did not affect downstream mTORC1 signaling, suggesting a direct or indirect regulation of mTORC1 by ATR independently of Chk1. Excitingly, immunoprecipitation experiments show a direct interaction between mTOR and ATR, and experiments are ongoing to assess whether mTOR is a direct substrate of ATR’s kinase activity. To determine the nucleotide metabolism enzymes that are critical for p16-low cells downstream of the ATR-mTORC1 axis, we performed a knockout screen with a nucleotide metabolism CRISPR library and found multiple hits associated with nucleotide salvage that were depleted only in p16-low cells. Future directions are to explore the contribution of these nucleotide metabolic genes on the proliferation and viability of p16-low cancer cells to determine whether this pathway is a novel therapeutic strategy for p16-low cancers. Together, our data provide a novel mechanism of mTORC1 activation, which is highly relevant in multiple cancers and other pathologies.
An integrated resource for functional and structural connectivity of the marmoset brain

Tian, Xiaoguang¹; Deco, Gustavo⁴; Rosa, Marcello GP³; Liu, Cirong²; Silva, Afonso C.¹

¹Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261, USA
²Institute of Neuroscience, CAS Key Laboratory of Primate Neurobiology, Chinese Academy of Sciences, Shanghai, China
³Department of Physiology and Neuroscience Program, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia
⁴Center for Brain and Cognition, Computational Neuroscience Group, Department of Information and Communication Technologies, Universitat Pompeu Fabra, Roc Boronat 138, Barcelona, 08018, Spain

The neuroscience research in non-human primates (NHP) bridges the knowledge gaps across species from the non-invasive neuroimaging approach. Also, it overcomes the constraint of human neuroimaging by invasively accessing "ground truth" in cellular resolution. Therefore, in this study, we acquired the largest awake NHP resting-state functional MRI dataset to date across two research institutions, the National Institutes of Health, USA, and the Institute of Neuroscience, China (13 from ION; 26 from NIH; 12053 mins) and sampled the most extensive collection of NHP marmoset neuronal tracing data (52 marmosets and 143 injections) in the same MRI space to a mapping of NHP marmoset brain architecture by integrating both invasive and non-invasive ways. To further enhance the capacity of our resource, we integrated extra high-resolution ex-vivo diffusion MRI and in-vivo diffusion MRI data obtained from the same cohort.

Based on this comprehensive resource, we first created a functional mapping of resting-state brain networks; Then developed a fine-grained cortical functional parcellation based on resting-state connectivity. This functional cortical parcellation is a function-related template that provides complementary information about the organization that cannot be observed via anatomy. We also developed a deep-learning-based generator to accurately map the proposed functional cortical parcellation onto every brain to reflect individual characteristics. Finally, based on the comprehensive structural connectivity from diffusion MRI and neuronal tracing datasets, we used whole-brain computational modeling to link the structural and functional connectivity for investigating the relationship behind the proposed functional parcellation.

In alignment with the development of the scientific community, this dataset and associated tools will be open-access, enabling testing hypotheses about the NHP brain's functional and structural organization. We hope our study will shine new light on brain evolution and facilitate comparative and translational brain research.
Dysregulation of the homeostatic sleep drive and circadian rhythmicity are related to the development or maintenance of insomnia. Age-dependent alterations in homeostatic sleep and circadian regulatory processes may also contribute to insomnia among older adults. We examined whether homeostatic sleep drive and circadian rhythmicity differ in older adults with insomnia (OAI) compared to older good sleepers (GS).

OAI (n=37) and GS (n=30) participated in a 60-hour in-lab study with sleep deprivation and constant routine paradigms. Homeostatic sleep drive was assessed by examining the effect of sleep deprivation on delta EEG power and theta EEG power, and repeated sleep latency tests. Circadian rhythm was assessed with salivary melatonin (phase and amplitude), core body temperature (phase, amplitude, and mesor), and sleep latency during a constant routine paradigm. Mixed models were used to assess interactions of group (OAS vs GS) with homeostatic sleep and circadian effects.

Compared to GS, OAI showed a greater linear increase in waking theta power during sleep deprivation, but the two groups did not show differential responses to sleep deprivation in delta EEG, or in repeated sleep latency tests. The two groups did not differ in circadian phase or amplitude of melatonin or core body temperature rhythms. OAI had a significantly elevated core body temperature mesor compared to GS.

Homeostatic response to sleep deprivation was intact in OAI compared to GS; theta EEG power suggested a greater homeostatic response in OAI. Circadian rhythm amplitude and phase were similar in OAI compared to GS. Elevated body temperature mesor in OAI may indicate elevated physiological arousal. These findings suggest that effective treatments for insomnia in older adults may leverage intact sleep and circadian regulatory mechanisms, rather than repair defective sleep and circadian regulation.
Tumor cells reprogram cellular metabolism to sustain their uncontrolled growth. Epigenetic dysregulation is as a major consequence of altered metabolism in cancer cells. For instance, altered alpha ketoglutarate (αKG) changes methylation patterns as it is a co-substrate for demethylases. We previously published that ovarian cancers preferentially utilize glucose in the TCA cycle compared to normal cells. Ovarian cancers have high expression of the TCA cycle enzyme wildtype isocitrate dehydrogenase 1 (wtIDH1), which increases αKG and decreases histone methylation. Preliminary data suggest that wtIDH1 is transcriptionally upregulated and localizes to the nucleus specifically in cyclin E-high ovarian cancers, corresponding with increased αKG abundance. ~20% of ovarian cancers have amplification or overexpression of cyclin E, and these patients respond poorly to standard-of-care therapies cisplatin and poly (ADP-ribose) polymerase inhibitors (PARPi) in part due to HR proficiency. Whether increased wtIDH1 is required for cyclin E-high cells to maintain HR proficiency and the mechanism underlying this is unknown. We found that knockdown of wtIDH1 in cyclin E-high cells decreased expression of HR genes, which was rescued by supplementation with αKG. Additionally, cyclin E-high cells displayed decreased methylation of multiple histone marks associated with promoting the non-homologous end joining (NHEJ) DNA repair pathway, which suppresses HR. Knockdown of wtIDH1 markedly increased markers of DNA double strand breaks and induced a senescence-associated cell cycle arrest. Finally, the combination of an IDH1 inhibitor and cisplatin or PARPi was highly synergistic only in cyclin E-high cells. Together, these studies suggest that wtIDH1 is critical for DNA repair in cyclin E-high ovarian cancers and provide proof-of-principle to target IDH1-mediated metabolism to induce HR-deficiency and sensitivity to DNA damaging agents. As αKG is also decreased during aging, our data also provide new evidence for this metabolite in epigenetic reprogramming in other pathologies.
Longitudinal characterization of gangliogenesis in the chick retina: peeking at the HAA

Valle, Vicente¹; Iannotta, John¹; da Silva, Susana ¹

¹ School of Medicine, Department of Ophthalmology, University of Pittsburgh

The visual high acuity area (HAA) is a specialized retinal region present in species highly reliant on their sense of vision. It is usually located in the center of the retina and presents a series of unique cellular features governing the gift of sharp vision, such as an absence of rod photoreceptors and substantial increased ganglion cells (GCs) density. The presence of an anatomical pit that minimizes the scattering of light makes the human fovea a highly sophisticated HAA.

The chick retina possesses two unique attributes that are absent in most animal models commonly used in visual research: the presence of an HAA and an exceptional embryonic accessibility during retinogenesis period, resulting in a powerful model system extremely useful to study fovea development. Using the chick retina, we have previously described how retinoic acid (RA), a classic signaling pathway regulating many aspects of embryogenesis, is modulated in the developing HAA. Specifically, the absence of RA due to localized expression of its catabolizing enzymes defines the limits of the presumptive HAA and is essential for proper HAA development.

To better understand the molecular and cellular mechanisms underlying HAA development, we manipulated RA signaling by in ovo pharmacological injection of all-trans RA (atRA) during the period of foveogenesis and performed bulk RNAseq of central retina 18 hours after. Initial analysis comparing atRA- and DMSO-injected retinas revealed differentially expressed genes mostly related to GCs differentiation. These results motivated us to carry out a comprehensive longitudinal study based on flat-mount retinal preparations to evaluate GCs genesis, maturation and distribution across the entire retina. The use of unreported antibodies in chick retina labeling GCs populations in combination with advanced imaging analysis of flat-mounts and regional areas, allows the possibility of identifying HAA-specific attributes regarding this retinal cell type and provides insights into mechanisms regulating acquisition of increased GCs density in the HAA.
The global prevalence of heart failure (HF) has become a crucial medical problem and is a major cause of morbidity and mortality. The presence of HF predicts the development of insulin resistance (IR), which is reported to be 18-22% higher in HF patients compared to healthy control patients. Although elevated serum cholesterol levels and high body mass index (BMI) represent a risk factor of HF in the Framingham study, other clinical studies also suggest that low lipid levels and higher BMI are associated with lower mortality rate in HF patients. This phenomenon is called “obesity paradox, but the underlying mechanisms are not fully understood. Although there is a plethora of evidence that HF affects the heart, kidney, brain, and intestine, the studies addressing the impact of HF on adipose tissue and clinical outcomes are largely lacking. In the present study we observed that VAT weight was markedly diminished in the HF mice compared to controls. Adipose cells in VAT of HF mice were also fewer and smaller compared to age-matched control mice. Moreover, serum concentrations of triglycerides, free glycerol, free fatty acids and adipocytokines like adiponectin and leptin were significantly lower in HF mouse. Consistent with reduced amount of VAT and decreased serum lipid profile in mice with HF, the expression of the genes responsible for adipogenesis, adipose expansion and fatty acid synthesis was significantly reduced in VAT of patients and mice with HF. Mice deficient of adiponectin had worsened glucose intolerance and elevated insulin levels, suggesting the role of diminished systemic adiponectin levels in HF-associated IR. We conclude that HF affects development of adipocytes, and their differentiation and expansion, resulting in reduced VAT weight. This results in reduced adipokine production and increased systemic glucose intolerance.
Towards fully automatic analysis of neuroimaging data acquired by ribbon-scanning confocal microscopy

Vasylieva, I.¹; Smith, M. C.²; Watson, A. M.¹

¹Center for Biologic Imaging, Dept. of Cell Biology, University of Pittsburgh

Recent advances in microscopy, tissue clearing and computation have been game changing for neuroimaging, enabling whole brains to be imaged with subcellular resolution. This leads to an unprecedented flow of data generated by high-speed robotized microscopes, like ribbon-scanning confocal microscopes (RSCM). These data need to be analyzed in a reasonable time frame. To address the data deluge, the neuroscience community created various software tools that facilitate registration and analysis of volumetric imaging datasets. Unfortunately, most of these tools require substantial expertise in programming to be installed and used, which limits their audience. We present a fully automatic pipeline that combines tools for cleaning, registering, cell counting and statistically analyzing RSCM data, which eliminates the need for manual inputs, thus making the tools usable by a larger audience. The pipeline first gets rid of imaging artifacts, by using a combination of filtering in Fourier space, contrast stretching, denoising and background removal. Then it registers brain images to the corresponding brain atlas, using brainreg. Eventually, it counts activated cells in the brain, using cellfinder, generates a pandas dataframe to store information on all detected spots for further statistical analysis, and visualizes cells in the standard coordinate space using brainrender.

The pipeline was developed for images of whole mouse brains, acquired by RSCM, but it can be generalized to work with other imaging modalities and other types of samples. It has a simple graphical interface, and soon will be available as an executable file for Windows, Mac and Ubuntu operating systems. It is a free open-source tool written solely in Python programming language.
Pharmacological chaperone of rhodopsin (YC-001 and F5257-0462) improves rhodopsin homeostasis and protects photoreceptors in the P23H mouse model of retinitis pigmentosa.


1. Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA, 15213 USA.
2. Department of Pharmacology and Chemical Biology, University of Pittsburgh, PA, 15213 USA.
3. McGowan Institute of Regenerative Medicine, University of Pittsburgh, PA, 15213 USA.
4. Department of Chemistry, Case Western Reserve University, Cleveland, OH 44106
5. Sorbonne Université, INSERM, CNRS, Institut de la Vision, 17 rue Moreau, Paris, 75012 France

Purpose: Mutations in RHODOPSIN (RHO) accounts for ~25-30% of autosomal dominant retinitis pigmentosa (adRP). Most of these RHO mutants are structurally unstable and triggers dominant negative effect which disrupt RHO homeostasis. This leads to the death of rod photoreceptors and cause vision loss. The purpose of this study to restore the RHO homeostasis and rescue rods in RHO-associated adRP, using non-retinoid chaperone of RHO (YC-001 and F5257-0462) using in vitro, ex vivo and in vivo models of adRP.

Methods: We used high content imaging (HCI) to test the chaperone activities of YC-001 and F5257-0462 in NIH3T3 cells. AutodockVina was used for docking calculation. RHO homeostasis was studied using retinal explant culture. Intravitreal injection (IVI) of YC-001 and F5257-0462 microparticles was done to study their chaperone effect in vivo using Rho<sub>P23H/+</sub> adRP mice model.

Results: HCI of 27 RHO mutants shows that YC-001 and F5257-0462 significantly increasing the RHO cell surface level and rescued 9 and 11 mutants, respectively. The docked YC-001 sat in β-ionone ring pocket while F5257-0462 fits in a larger portion of chromophore pocket. Further treated with the chaperones, Rho<sub>P23H/+</sub> retinal explant showed higher RHO level, improved RHO glycosylation, less ubiquitinated RHO, longer OS length, and thicker ONL, suggesting an improved RHO homeostasis and supported photoreceptor survival. Additionally, YC-001 treatment reduces the number of microglia-derived macrophages. Furthermore, the IVI of chaperones microparticles significantly increased photoreceptor function and ONL thickness in Rho<sub>P23H/+</sub> mice.

Conclusion: We showed that the YC-001 and F5257-0462 chaperones increased RHO transport, improved RHO homeostasis and protected photoreceptors. Collectively, we provide a strong proof-of-principle that non-retinoid chaperones are promising drug candidates in treating RHO-associated RP.
UVB initiates skin inflammation by promoting keratinocyte ferroptosis

Vats K¹, Kruglov O¹, Mizes A², Samovich SN³, Amoscato AA³, Tyurin VA³, Tyurina YY³, Kagan VE³, Bunimovich YL¹, 4

1 Department of Dermatology, University of Pittsburgh, Pittsburgh, PA, 15213, USA
2 School of Medicine, University of Pittsburgh, Pittsburgh, PA, 15213, USA
3 Center for Free Radical and Antioxidant Health, Department of Environmental Health and Occupational Health, University of Pittsburgh, Pittsburgh, PA, 15213, USA
4 Hillman Cancer Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, 15213, USA

Epidermis, composed primarily of the keratinocytes, is subjected to ultraviolet radiation (UVR)-induced oxidative and genotoxic stresses. That the keratinocytes are the initial source of pro-inflammatory mediators in the skin after UVR exposure has been well established. Ultraviolet B radiation (UVB) is a strong initiator of cutaneous inflammation, and contributes to the etiology and the exacerbation of several cutaneous diseases, such as lupus erythematosus. However, the mechanism of UVB-induced inflammation in the skin remains unclear. Utilizing primary human keratinocytes and human epidermal explants, we found that ferroptosis, a type of non-apoptotic programmed cell death associated with an excessive phospholipid peroxidation, is activated in the keratinocytes after UVB exposure. We further found that keratinocyte susceptibility to UVB-induced ferroptosis is dictated by the extent of lipid peroxidation and the dysregulation of glutathione system. Ferroptosis inhibition prevented HMGB1 release from the keratinocytes and protected UVB-irradiated skin from inflammation. While apoptosis and pyroptosis were also detectable in the keratinocytes after UVB exposure, we determined that ferroptosis of the keratinocytes plays a dominant role in initiating UVB-induced cutaneous inflammation. Our findings have significant implications for the prevention and treatment of a wide range of skin diseases fostered by UVB-induced inflammation.
Conveying object characteristics through customized intracortical microstimulation of the human somatosensory cortex

Verbaarschot, Ceci1,5; Karapetyan, Vahagn1,2; Greenspon, Charles4; Boninger, Michael1,2,3; Sorger, Bettina5; Gaunt, Robert1,2,3,6

1Rehab Neural Engineering Labs, University of Pittsburgh
2Bioengineering, University of Pittsburgh
3Physical Medicine and Rehabilitation, University of Pittsburgh
4Department of Organismal Biology and Anatomy, University of Chicago
5Psychology and Neuroscience, Maastricht University
6Blackrock Microsystems, Salt Lake City

Intracortical microstimulation (ICMS) of the somatosensory cortex is one approach to enable intuitive tactile feedback in prosthetics, and the only feasible option in individuals with spinal cord injury. To date, the effects of ICMS have been primarily explored using experimenter-driven parameter manipulations. In this study, we use a participant-driven approach to (1) test whether ICMS can convey object characteristics of compliance, structure, texture, and temperature, (2) identify a set of meaningful stimulus parameters for targeted object interactions, and (3) test whether these parameter selections are unique and identifiable. Three people with tetraplegia participated in this study and have microelectrode arrays implanted in their somatosensory cortex that generate tactile sensations localized to their right hand. Using a tablet interface, participants were able to actively control four stimulation parameters in a blinded fashion: stimulation amplitude, pulse frequency, stimulus onset/offset transients, and the degree of stimulus overlap between consecutive electrodes. We asked the participants to select parameters that best represented each of five objects over many repetitions. Results indicate that all three participants could create distinct object characteristics. Using this method, users can create desirable sensations in an efficient and engaging way, potentially allowing us to identify parameters that represent informative object characteristics.
Correlation of genotype and molecular phenotype in mitochondrial trifunctional protein deficiency

Vieira Neto, Eduardo1,2; Ferraro, Lethicia1,2; Wang, Meicheng1,2; Wang, Yudong1,2; Van’t Land, Clinton1,2; Koppes, Erik1,2; Anthonymuthu, Tamil S.4,5; Bayır, Hülya4,5; Vockley, Jerry1,2

1 Division of Pediatric Genetic and Genomic Medicine, Department of Pediatrics, University of Pittsburgh;
2 UPMC Children's Hospital of Pittsburgh;
3 Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil;
4 Department of Critical Care Medicine, University of Pittsburgh;
5 Safar Center for Resuscitation Research, School of Medicine, University of Pittsburgh;

Background: Long-chain fatty acids are catabolized by the removal of successive two carbon units via β-oxidation, a cyclic process consisting of four enzymatic steps. Mitochondrial trifunctional protein (TFP) catalyzes the last three steps as well as an unrelated step in cardiolipin metabolism. It is a heteromeric enzyme composed of two α and two β-subunits, encoded by HADHA and HADHB genes, respectively. More than 150 mutations in HADHA and HADHB are reported, typically resulting in complete TFP deficiency, while a common HADHA missense mutation (p.E510Q) usually leads to an isolated LCHAD deficiency. We investigated the biochemical heterogeneity of fibroblasts from TFP/LCHAD deficiency patients.

Methods: Genotype was confirmed by sequencing of DNA from skin fibroblasts. α and β-TFP, and MLCL-AT proteins were analyzed by western blot (WB). Droplet digital PCR was used to evaluate mRNA expression. Mitochondrial bioenergetics were measured with a Seahorse Analyzer. Cardiolipins (CLs) and monolyso-CLs were quantified by LC-MS/MS. Acylcarnitine profiles were determined by ESI-MS/MS.

Results: WB of cells with splice site mutations in HADHA or a point deletion with frameshift in HADHB revealed significant decrease in levels of both subunits. Contrary to the HADHB frameshift, a HADHA splice site mutation did not affect mRNA levels. Cells homozygous for the common LCHAD mutation or compound heterozygous for HADHB missense mutations had protein levels comparable to controls. However, the former showed drastically impaired mitochondrial bioenergetics while the latter was close to controls. Significantly increased C16-OH levels were seen in cells with HADHA mutations. All patient cells showed a variable decrease in mature cardiolipins and an increase in immature species.

Conclusion: We show that null mutations cause a decrease in both TFP subunits, confirming that TFP tetramer formation and/or stability requires the presence of both. Bioenergetics are especially affected in cells with the common LCHAD mutation or null mutations, and both interfere with cardiolipin remodeling.
Reorganizations in the apical extracellular matrix underlie morphological diversification in *Drosophila* genital structures

Vincent, Ben J.¹, Davidson, Lance² and Rebeiz, Mark¹

¹Department of Biological Sciences and ²Department of Bioengineering, University of Pittsburgh

Identifying the genetic changes that cause morphological differences between species is a major goal of evolutionary and developmental biology. The posterior lobe in the *Drosophila melanogaster* clade is an ideal system to investigate morphological evolution – this genital structure exhibits staggering diversity between the sister species *Drosophila mauritiana* and *Drosophila simulans*, and we can track its development by dissecting and staining pupal terminalia. Previous work has shown that the posterior lobe develops in *Drosophila melanogaster* due to cell elongation, and posterior lobe morphology is controlled in part by the apical extracellular matrix (aECM) component Dumpy. We therefore tested whether the aECM also underlies posterior lobe diversification between *Drosophila simulans* and *Drosophila mauritiana*. By labeling the aECM with fluorescent lectins, we have found that it forms attachments to the posterior lobe and other genital structures during early development, and these attachments are more extensive in *Drosophila simulans*, the species with the larger lobe. We also found that the lobe-specific gene expression pattern for *dumpy* is expanded in *Drosophila simulans*, which suggests that these morphological changes are controlled at the level of transcriptional regulation. Finally, we have found that distinct combinations of aECM genes are expressed in distinct genital compartments, which suggests that the aECM may be a heterogeneous component underlying the evolution of multiple structures. Our results suggest that morphological diversity may be generated by alterations in extracellular matrix organization during development, and that we can find the genes controlling this process within quantitative trait loci associated with genital evolution.
Intraocular pressure-induced lamina cribrosa deformations are larger and more inhomogeneous between quadrants in an experimental glaucoma eye than in healthy eye

Wang, Bingrui 1, Zhong, Fuqiang 1, Wei, Junchao 1, Hua, Yi 1, Reynaud, Juan 3, Fortune, Brad 3, Sigal, Ian A. 1,2

1Department of Ophthalmology, University of Pittsburgh School of Medicine
2Department of Bioengineering, University of Pittsburgh
3Devers Eye Institute, Legacy Health Research

Elevated intraocular pressure (IOP) is a primary risk factor for the development and progression of glaucoma, but how elevated IOP causes glaucoma is not understood. A leading theory postulates that the IOP-induced lamina cribrosa (LC) deformations contribute to a cascade of effects that eventually lead to degeneration of the astrocytes and retinal ganglion cell axons at the LC, and thus loss of vision. Here, we measured and analyzed the IOP-induced LC deformations in-vivo in experimental glaucoma and healthy eyes in four anatomical quadrants of the LC.

The optic nerve head of a rhesus macaque monkey with unilateral experimental glaucoma was imaged using optical coherence tomography (OCT) at controlled IOPs of 10, 20, 30, 40, 50, and 60 mmHg. A newly reported high-accuracy digital volume correlation method was used to analyze the OCT image sets for displacement field (Zhong F et al, 2022). The LC region was divided into four quadrants: temporal, superior, nasal, and inferior. All the deformations in each quadrant were extracted and compared.

As IOP increased, the LC deformations of the glaucoma eye were larger than those of the healthy eye. For example, when IOP increased from 10 mmHg to 60 mmHg, shear deformation in the glaucoma eye doubled compared to that in the control eye (0.2 vs. 0.1). The LC strain distribution in the glaucoma eye was inhomogeneous across the four quadrants, whereas the healthy eye strain distribution was relatively homogeneous. In the glaucoma eye, the stretch in the nasal and superior quadrants was larger than in the temporal and inferior quadrants, especially at very high IOPs. When IOP increased from 10 to 60 mmHg, the stretch in nasal and superior quadrants is almost three times larger than those in the temporal and inferior quadrants.

The LC strain distribution in glaucoma eye was larger and more inhomogeneous across the four quadrants. It is possible that as glaucoma develops, tissue remodeling in the LC is not uniform, resulting in the non-uniform compliance in the LC. The results may help explain the regional pattern of axon damage progression in early glaucoma.
Exploring the Impact of Racism on Black Youth: A multidimensional examination of discriminatory experiences across place and time

Wilson, Tyia¹²; Riley, Alexander¹³; Khetarpal, Susheel¹³; Abernathy, Paul¹⁴; Booth, Jaime⁵; and Culyba, Alison¹³

¹ University of Pittsburgh School of Medicine
² Department of Psychiatry
³ Department of Pediatrics
⁴ Neighborhood Resilience Project
⁵ University of Pittsburgh School of Social Work

Background: Community violence disproportionally impacts Black youth. Experiences of racism and discrimination may create additional challenges for youth recovering from violence exposure. This study used ecological momentary assessment (EMA) to elucidate how perceptions of racism and social support influence health and safety outcomes among Black youth following violence exposure.

Methods/Approach: Twenty-five Black youth (14-19 years-old, 58% female) who had witnessed violence within the past three months completed a baseline survey that assessed discrimination experiences, social support, stress, post-traumatic stress symptoms (PTS), and perceived safety. Youth also completed EMAs three times daily for two weeks about their location, people they were with, their current emotional state, and in-the-moment racism perceptions. Multilevel models estimated the relationship between both overall and time-varying perceptions of racism and social support and stress, PTS, and perceived safety.

Results: Overall, 76% of youth reported at least one discrimination experience at baseline. Prior discrimination was associated with higher PTS ($B=1.86$, $p=.001$) and depressive symptoms ($B=.13$, $p=.013$). Youth who reported higher overall perceptions of racism in-the-moment reported higher stress ($B=.50$, $p=.002$), PTS ($B=.52$, $p=.002$), and lower perceived safety ($B=-.53$, $p=.000$). In-the-moment perceptions of racism were associated with lower perceived safety in that place ($B=-.09$, $p<.01$). Both emotional and instrumental support were associated with lower PTS and stress, and higher perceived safety ($p<0.05$).

Conclusions: Experiences of racism and being in discriminatory places impacted youth’s depressive symptoms, stress, PTS, and safety. Interventions attuned to in-the-moment experiences of racism, and that leverage social support, are needed to support Black youth exposed to violence and discrimination.
RNA Editing Controls Pulmonary Endothelial Apoptosis in PAH via Nocturnin Gene Signaling

Woodcock, Chen-Shan; Tang, Ying; Tai, Yi Yin; Zhao, Jingsi; Handen, Adam; Lafyatis, Robert; Chan, Stephen

1Center for Pulmonary Vascular Biology and Medicine, Pittsburgh Heart, Lung, Blood, and Vascular Medicine Institute, Division of Cardiology, Department of Medicine, University of Pittsburgh School of Medicine
2Division of Rheumatology and Clinical Immunology, Department of Medicine, University of Pittsburgh School of Medicine

Pulmonary arterial hypertension (PAH) is a lethal disease without a cure. Increased apoptosis of pulmonary artery endothelial cells (PAECs) is a key trigger to drive vascular remodeling in the pathogenesis of PAH, but its regulation in PAH is not fully unknown. Adenosine deaminase acting on RNA 1 (ADAR1) is an RNA editing enzyme that converts adenosine to inosine (A-to-I) in genome-encoded RNA transcripts and plays a vital role in gene regulation. While emerging evidence indicated cells lacking ADAR1 RNA editing stimulates aberrant cytosolic innate immunity, leading to apoptosis, the exact RNA editing targets in regulation of pulmonary endothelial survival is unknown. We hypothesized that specific gene dysregulation by ADAR1 RNA editing promotes PAEC apoptosis and drives PAH.

Our data indicate that ADAR1 knockdown in cultured PAECs induced caspase-3/7 activity and cytokine interferon beta 1 (IFNB1) levels. Via RNA-seq analysis, Nocturnin (NOCT) was identified as an ADAR1 target that contains two A-to-I RNA editing sites at the 3’UTR region. Immunoprecipitation with RT-qPCR (IP-qPCR) revealed that NOCT transcripts interacted with ADAR1. ADAR1 RNA editing deficiency upregulated and stabilized NOCT mRNA as a predominately unedited isoform. Forced expression of NOCT in PAECs displayed an increase in apoptosis and IFNB1 level. NOCT silencing restored the induction of PAEC apoptosis and innate immunity signaling by ADAR1 deficiency, thus demonstrating that ADAR1 depends upon NOCT for such activity. In human PAH lungs, ADAR1 was downregulated, accompanied by reduced A-to-I conversions at NOCT RNA editing site A2 and elevated NOCT levels. Hypoxic mice treated with ADAR1 inhibitor promoted RV pressure and hypertrophy index. Finally, genetic deletion of NOCT mitigated PAH in hypoxic IL-6 Tg mice.

These findings suggest that ADAR1 RNA editing deficiency promotes aberrant NOCT-IFNB1 axis signaling in PAECs, which in turn promotes apoptosis and PAH. These results provide substantial impetus to target the ADAR1-NOCT axis for more effective diagnostics and therapeutics for PAH.
Mechanisms of EVD68 Neurovirulence

Mikal A. Woods Acevedo,1,2 Megan Culler Freeman,1,2 and Terence S. Dermody1,2,3

1Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
2Institute of Infection, Inflammation, and Immunity, UPMC Children’s Hospital of Pittsburgh, Pittsburgh, PA, USA
3Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Enterovirus D68 (EV-D68) is an emerging, positive-sense, single-stranded RNA virus that is associated with neurological conditions such as acute flaccid myelitis (AFM), a poliomyelitis-like syndrome that results in paralysis and disability in infants and young children. Mechanisms by which EV-D68 leads to neuronal cell death are unknown. To better understand how EV-D68 infection leads to neuronal cell killing, we recovered two EV-D68 strains from infectious cDNA clones, one that is pathogenic in mice and another that is attenuated. Using primary cultures of cortical neurons, we developed an ex vivo model to study strain-specific differences in EV-D68 neuropathogenesis. We infected primary cortical neurons derived from wild-type and type 1 interferon receptor knockout (IFNAR−/−) mice and monitored viral replication and cell death. We discovered that the pathogenic strain produced significantly higher viral yields and enhanced cytotoxicity in neurons from both mouse strains, suggesting that the increased viral yield and neuronal cytotoxicity of the virulent strain occurs independently of the type 1 interferon response. These results demonstrate that EV-D68 strains recovered from infectious cDNA clones are capable of infecting and inducing cell death in primary cortical neurons. This research establishes the foundation for studies of EV-D68 using primary cortical neurons to identify cellular factors that contribute to neuronal cell death.
Persistent DNA damage and oncogenic stress-induced Trem1 promotes leukemia in mice

Wu, Limei 1,2; Li, Xue 1,3,4; Chatla, Srinivas 5; Wilson, Andrew F. 3; Atale, Neha1,2; Du, Wei 1,2

1Division of Hematology and Oncology, University of Pittsburgh School of Medicine; 2UPMC Hillman Cancer Center; 3Cincinnati Children’s Hospital Medical Center, Cincinnati; 4Institute for Brain Research and Rehabilitation, South China Normal University, Guangzhou, China; 5Fels Cancer Institute for Personalized Medicine, Lewis Katz School of Medicine at Temple University, Philadelphia;

The immune receptor TREM1 (Triggering receptor expressed on myeloid cells 1) is a master regulator of inflammatory response. Compelling evidence suggests important pathological roles for TREM1 in various types of solid tumors. However, the role of TREM1 in hematologic malignancies is not known. Our previous study demonstrates that TREM1 cooperates with diminished DNA damage response to induce expansion of pre-leukemic hematopoietic stem cells (HSCs) in mice deficient for the Fanconi anemia gene Fanca. Here we investigate TREM1 in leukemogenesis using mouse models of the DNA repair-deficient Fanca−/− and the oncogenic MLL-AF9 or KrasG12D. We found that Trem1 was highly expressed in pre-leukemic HSCs and leukemia stem cells (LSCs). By selective deletion of the Trem1 gene in the hematopoietic compartment, we showed that ablation of Trem1 reduced leukemogenic activity of the pre-leukemic HSCs and LSCs in mice. Trem1 was required for the proliferation of the pre-leukemic HSCs and LSCs. Further analysis revealed that Trem1 expression in pre-leukemic HSCs and LSCs was associated with persistent DNA damage, prolonged oncogenic stress, and a strong inflammatory signature. Targeting several top Trem1 inflammatory signatures inhibits the proliferation of pre-leukemic HSCs and LSCs. Collectively, our observations uncover previously unknown expression and function of TREM1 in malignant stem cells and identify TREM1 as a driver of leukemogenesis.
Improved antitumor activity against prostate cancer via synergistic targeting of Myc and GFAT-1

Yue Zhang¹, Jiang Li¹, Yixian Huang¹, Yuang Chen¹, Zhangyi Luo¹, Haozhe Huang¹, Song Li¹

¹Center for Pharmacogenetics, Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy

Myc activation is considered to be a hallmark of cancer initiation and progression, and inhibition of Myc activity promotes the regression of many types of Myc-driven tumors including prostate cancer. However, the success of anti-Myc therapy is hampered by the lack of a strategy to effectively deliver the inhibitors to the tumor site, and also by our limited understanding of the feedback mechanisms that cancer cells use to adapt the metabolic reprogramming following Myc inhibition. Here we report that treatment with Myc inhibitor 10074-G4 or 10058 led to significant induction of glutamine: fructose-6-phosphate amidotransferase-1 (GFAT1), the rate limiting enzyme of hexosamine biosynthesis pathway (HBP), and enhanced protein glycosylation. Mechanistically, Myc inhibition triggered GFAT1 induction through IREα-Xbp1s pathway. Combination of Myc inhibition with 6-Diazo-5-oxo-L-norleucine (DON, a GFAT1 inhibitor) led to synergistic effect in inhibiting the proliferation and cell migration of cultured prostate cancer cells. To facilitate the therapeutic application of this novel finding, a prodrug conjugate of 10074-G4 and DON was developed, which could be effectively loaded into a polysaccharide-based nanocarrier (PS). Enhanced in vivo delivery of 10074-DON via the PS nanocarrier led to significant inhibition of tumor growth along with improvement in tumor immune microenvironment in several PCa models. Simultaneous targeting of Myc and GFAT-1 may represent a novel strategy for the treatment of prostate cancer.
PROGRAM SPONSORS

The UPPDA would like to thank the following co-sponsors:

Office of Academic Career Development, Health Sciences
oacd.pitt.edu

Office of the Provost
provost.pitt.edu