BMT & MED RESEARCH DAY
26.10.2017

ABSTRACTS

University of Tampere, Kauppi campus, Arvo building
<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The Role of Regulatory T Cells in Unexpected Exacerbated Gluten-induced Immune Response after CCR9 Antagonist Treatment</td>
<td>Airaksinen, Laura</td>
</tr>
<tr>
<td>2</td>
<td>Proprotein convertase Furin1 expression in the Drosophila fat body is essential for a normal antimicrobial peptide response and bacterial host defense</td>
<td>Aittomäki, Saara</td>
</tr>
<tr>
<td>3</td>
<td>Application of iPSC-hepatocytes to coronary artery disease modelling</td>
<td>Alexanova, Anna</td>
</tr>
<tr>
<td>4</td>
<td>Circulating tumor DNA predicts resistance to abiraterone and enzalutamide in prostate cancer</td>
<td>Annala, Matti</td>
</tr>
<tr>
<td>5</td>
<td>Dithiocarbamate fc14-584b a β-ca specific inhibitor: a novel antimycobacterial agent with potential to treat drug-resistant tuberculosis</td>
<td>Aspatwar, Ashok</td>
</tr>
<tr>
<td>6</td>
<td>Investigating talin rod’s mutation in cancer database</td>
<td>Azizi, Latifeh</td>
</tr>
<tr>
<td>7</td>
<td>Expression of the alternative NADH dehydrogenase promotes the selective degradation of ROS-producing mitochondria in Hek293T cells</td>
<td>Dufour, Eric</td>
</tr>
<tr>
<td>8</td>
<td>Pim kinases promote prostate cancer cell migration and invasion by regulating NFATc activity</td>
<td>Eerola, Sini</td>
</tr>
<tr>
<td>9</td>
<td>Delayed celiac disease diagnosis predisposes to reduced quality of life and incremental use of health-care services and medicines: a prospective nationwide study</td>
<td>Fuchs, Valma</td>
</tr>
<tr>
<td>10</td>
<td>Response of the astrocyte-neural network INEXA to different electrical stimuli</td>
<td>Genocchi, Barbara</td>
</tr>
<tr>
<td>11</td>
<td>AOX SNIM RNA: A POTENTIAL THERAPY TO OVERCOME MITOCHONDRIAL DISFUNCTIONS</td>
<td>Giordano, Luca</td>
</tr>
<tr>
<td>12</td>
<td>RNase H1 role in mtDNA maintenance</td>
<td>González de Cózar, Jose M.</td>
</tr>
<tr>
<td>13</td>
<td>Sox11 expression in pediatric acute lymphoblastic leukemia</td>
<td>Grönroos, Toni</td>
</tr>
<tr>
<td>14</td>
<td>Porous poly(butylene succinate) films as promising candidates for retinal tissue engineering</td>
<td>Haapala, Anne</td>
</tr>
<tr>
<td>15</td>
<td>Immunohistochemical fluoro-chromogenic triple staining and digital image analysis for accurate detection of PD-L1 and PD-1 in NSCLC</td>
<td>Haapaniemi, Teppo</td>
</tr>
<tr>
<td>16</td>
<td>Prostate cancer biomarkers in blood samples</td>
<td>Haflidadóttir, Benedikta</td>
</tr>
<tr>
<td>17</td>
<td>High-resolution live cell imaging during mechanical vibration loading</td>
<td>Halonen, Heidi</td>
</tr>
<tr>
<td>18</td>
<td>Bioimpedance-measurement from biopsy needle can provide tissue type information</td>
<td>Halonen, Sanna</td>
</tr>
<tr>
<td>19</td>
<td>A Coxsackievirus B vaccine protects Against Virus-Induced Diabetes in an Experimental Mouse Model for Type 1 Diabetes</td>
<td>Hankaniemi, Minna</td>
</tr>
<tr>
<td>20</td>
<td>Interleukin 10 mutant zebrafish have an enhanced Th1 response and improved resistance against a Mycobacterium marinum infection</td>
<td>Harjula, Sanna-Kaisa</td>
</tr>
<tr>
<td>21</td>
<td>Ex vivo culture of duodenal biopsies from dermatitis herpetiformis patients suggests that epidermal transglutaminase antibody production occurs in the gut</td>
<td>Hietikko, Minna</td>
</tr>
</tbody>
</table>
22. Holmström, Kira: Mitochondrial dysfunction in a model of spinocerebellar ataxia (Poster)...... 29

23. Honkimaa, Anni: Ability of Type 1 Diabetes associated enteroviruses to cause chronic infection in pancreatic cells and use of antiviral drugs to eradicate such infection (Poster) ......................... 30

24. Hyväri, Laura: Molecular Mechanisms of Bioactive Glass -Induced Cell Adhesion and Osteogenic Differentiation in Human Adipose Stem Cells (Poster) ................................................................. 31

25. Häkli, Martta: Maturation of human induced pluripotent stem cell-derived cardiomyocytes grown on polyethylene terephthalate textiles (Poster)................................................................. 32

26. Hämäläinen, Mari: MKP-1 as a protective factor and novel drug targetin scleroderma: MKP-1 deficient mice develop more severe dermal fibrosis in a widely used experimental model of scleroderma (Poster)................................................................................................................. 33

27. Hämäläinen, Santeri: USE OF INHALED SHORT ACTING β2-AGONIST DURING PEAK FLOW MONITORING DOES NOT INDUCE BETA-AGONIST TOLERANCE - A RETROSPECTIVE ANALYSIS OF REAL LIFE DATA (Poster) .................................................................................................................... 34

28. Hänninen, Aleksi: A wireless bioresorbable pressure sensor (Pitch & Poster)........................ 35

29. Högnäs, Gunilla: Tracing the evolution of metastatic prostate cancer (Poster) ....................... 36

30. Ilmarinen, Tanja: Xeno- and Feeder-free Human Pluripotent Stem Cell –Derived Retinal Pigment Epithelial Cells for Ocular Cell Therapy (Talk)............................................................................. 37

31. Jacobs, Howy: Mitochondria are hot (Poster)........................................................................ 38

32. Jacome, Dafne: Role of CD4-Furin-deficient-T-cells in Gut Inflammation (Poster)............ 39

33. Jongprasitkul, Hatai: Heparin Nanoparticles with Sustained Drug Release: Overcoming Doxorubicin side effects (Poster)...................................................................................................................... 40

34. Juntunen, Miia: The effect of donor BMI on adipose stem cell characteristics - obesity-discordant and weight-concordant monozygotic twin study (Poster) ................................................................. 41

35. Kallio, Heini: Androgen receptor splice variants AR-V3, AR-V7 and AR-V9 are co-expressed in castration resistant prostate cancer metastases (Poster)................................................................................. 42

36. Kalliokoski, Suvi: Circulating TG3-IgA immune complexes in dermatitis herpetiformis (Poster) ........................................................................................................................................ 43

37. Kananen, Laura: The trajectory of the blood DNA methylome ageing rate is largely set before adulthood: evidence from two longitudinal studies (Poster)................................................................. 44

38. Kandavalli, Vinodh: Sources and regulatory mechanisms of lineage-to-lineage variability in transcription rates at the single gene level (Poster)......................................................................................... 45


40. Karvinen, Jennika: Evaluation of the microstructure of hydrazone crosslinked hydrogels (Poster)........................................................................................................................................ 47

41. Karvonen, Hanna: Targeting ROR1 pseudokinase as a therapeutic strategy for mantle cell lymphoma (Poster) ........................................................................................................................................ 48

42. Karvonen, Tuomas: Comparison of feasibility and estimates of central and peripheral nitric oxide parameters by different mathematical models (Pitch & Poster) ................................................. 49

43. Kaukoniemi, Kirsi: Methylation Profiling of Prostate Cancer Tumors (Poster)....................... 50
44. Kemppi, Hanna: Hyaluronic acid based hydrogel with inter-penetrating network for culturing human mesenchymal stem cells (Poster) ................................................................. 51

45. Khan, Muhammad Waqas Ahmad: Impact of Coating Thickness and Temperature on the Performance of an Implantable Intracranial Pressure Sensing System (Poster) .................. 52

46. Kiamehr, Mostafa: Investigating lipidomics of patient-specific iPSC-derived hepatocytes (Poster) .................................................................................................................. 53

47. Kivelä, Laura: Long-term health and treatment outcomes in adult celiac disease patients diagnosed in childhood by screening (Pitch & Poster) ....................................................... 54

48. Kohvakka, Annika: Expression of a novel androgen-regulated long noncoding RNA correlates with progression-free survival in prostate cancer patients (Talk) .................................. 55

49. Koivisto, Janne: Gelatin functionalized gellan gum hydrogel for 3D culturing of cardiomyocytes (Poster) ............................................................................................................. 56

50. Koivusalo, Laura: Hydrazone crosslinked hyaluronan-based hydrogels for therapeutic delivery of adipose stem cells to treat corneal defects (Poster) ................................................................. 57

51. Kontro, Heidi: Impaired metabolism by DAPIT over-expression induces stem like characteristics in Hek293T cells (Poster) ..................................................................................... 58

52. Korkka, Iina: Electrophysiological Characterization of Chloride Channels in Stem Cell-Derived Retinal Pigment Epithelium (Poster) .................................................................................... 59

53. Kreutzer, Joose: Portable platform for hypoxia studies outside an incubator (Poster) ........ 60

54. Kukkonen, Konsta: In vitro model of prostate carcinogenesis (Poster) ........................................... 61

55. Kukkurainen, Sampo: Molecular dynamics study of talin head domain (Poster) .......................... 62

56. Kummola, Laura: IL-7Rα Expression Regulates Murine Dendritic Cell Sensitivity to Thymic Stromal Lymphopoietin (Pitch & Poster) .................................................................................. 63

57. Laakkonen, Johanna: Proprotein convertase Furin in maturation of Gnt-VA (Poster) .................. 64

58. Latonen, Leena: Integrative analysis of the proteome in prostate cancer (Poster) ...................... 65

59. Laukkonen, Saara: Targeted therapy with dasatinib for T-cell acute leukemias: studies in a zebrafish model (Poster) ............................................................................................................. 66

60. Laurikka, Pilvi: Dietary Factors and Mucosal Immune Response in Celiac Disease Patients Having Persistent Symptoms Despite a Gluten-free Diet (Poster) .................................................. 67

61. Liimatainen, Kaisa: Cell counting from bright field z-stack with fully convolutional neural network (Pitch & Poster) .............................................................................................................. 68

62. Luoto, Kaisa: Behavioral activation in a real-life sample of depressed patients with non-psychotic comorbidities (Talk) .................................................................................................. 69

63. Lyyra, Inari: Biocompatibility of Li, Sr and B-doped bioactive silicate glasses (Poster) ............ 70

64. Martinez Cordova, Zuzet: Characterization of Furin-dependent secretome in macrophages (Poster) ................................................................................................................................. 71

65. Marttila, Saara: Immortal Kombat - Or in other words, how to cure ageing? (Poster) .............. 72

66. Mattila, Pirkko: Insights into AML Heterogeneity at Single Cell Level (Poster) .......................... 73
67. Mishra, Ayush: Albumin and Fibronectin attachment on silicate and phosphate bioactive glasses (Poster) ............................................................... 74

68. Mustata, Alina: miR-32 promotes Myc-driven prostate cancer in vivo (Poster) ....................... 75

69. Mykuliak, Vasyl: Stable 3-helix intermediates in mechanoreregulated proteins revealed by SMD and AFM (Talk) ............................................................................................................................ 76

70. Mäkynmäki, Henna: Identification of novel protective post-exposure mycobacterial vaccine antigens using an immunosuppression-based reactivation model in the adult zebrafish (Poster). 77

71. Mäki, Anti-Juhana: Modular cell cultivation platform (Poster) ................................................ 78

72. Mäkit-Opas, Ilari: PYRAZINE-FUSED TRITERPENOIDS BLOCK TRPA1 ION CHANNEL IN VITRO AND INHIBIT TRPA1-MEDIATED INFLAMMATION IN VIVO (Talk) ................................ 79

73. Määttä, Juha: Biochemical characterization of recombinant Enterovirus proteases with model FRET peptides (Poster) ............................................................................................................... 80

74. Nikkilä, Atte: Radiation exposure from CT imaging and childhood leukemia: a nationwide case-control study (Talk) ........................................ 81

75. Nommeots-Nomm, Amy: Oxyfluoride phosphate glasses: thermal and dissolution characteristics (Poster) ................................................................................................................ 82

76. Nummenmaa, Elina: Transient receptor potential ankyrin 1 (TRPA1) expression is downregulated by dexamethasone and aurothiomalate in human chondrocytes (Poster) ........ 83

77. Nurminen, Anssi: StructureMapper: a high-throughput algorithm for analyzing protein sequence locations in structural data (Poster) .................................................................................. 84

78. Ojanen, Markus: Intelectins in the immunity against mycobacteria in zebrafish (Danio rerio) (Poster) ........................................................................................................................................ 85

79. Ojansivu, Miina: Optimizing extrusion-based 3D bioprinting for bone tissue engineering applications (Poster) ............................................................. 86

80. Oksa, Laura: Analysis of DNA structural variations in ALL (Poster) ........................................ 87

81. Ortutay, Zsuzsanna: Studying the role of FURIN in the determination of T cell fate (Poster) .. 88

82. Paavilainen, Tanja: Characterization of human iPSC-derived reactive astrocyte phenotype (Poster) ........................................................................................................................................ 89

83. Partinen, Jenni: Knock-down of Tousled-like kinase triggers a strong blood cell activation phenotype in Drosophila melanogaster (Poster) .................................................................................. 90

84. Pasternack, Camilla: Self-reported fractures in dermatitis herpetiformis and coeliac disease (Poster) ........................................................................................................................................ 91

85. Pekki, Henna: Long-term Follow-up in Patients with Celiac Disease: Predictors and Effect on Health Outcomes (Poster) ............................................................................................................... 92

86. Pelkonen, Anssi: PDMS Tunnel Devices on Microelectrode Arrays Increase the Measurable Activity of Human Stem Cell-Derived Neuronal Networks (Talk) .................................................. 93

87. Peltokangas, Mikko: Areas under arterial pulse waves as a marker of peripheral arterial disease (Poster) .......................................................................................................................... 94

88. Pelttari, Saku: Application of international aortic noninflammatory pathology consensus nomenclature in a clinical setting (Poster) .......................................................................................... 95
89. Pemmari, Antti: Widespread Regulation of Gene Expression by Glucocorticoids in Chondrocytes from OA Patients as Determined by RNA-Seq-based Genome Wide Expression Analysis (Poster) ................................................................. 96

90. Pirhonen, Mikko: Recursive, Bayesian tracking of intrinsic modes in combination of spectral reassignment methods for respiratory rate assessment (Poster) ......................................................... 97

91. Pölönen, Risto-Pekka: Antiarrhythmic effects of carvedilol and flecainide in cardiomyocytes derived from catecholaminergic polymorphic ventricular tachycardia patients (Talk) .................. 98

92. Rahikainen, Iida: Kynurenine pathway related genetic polymorphisms and inflammation induced depression (Poster) ........................................................................................................ 99

93. Rahikainen, Rolle: Mechosensing via talin rod domain is indispensable for cell polarization (Poster) ...................................................................................................................................... 100

94. Rauhala, Hanna: Feasibility of CRISPR-Cas9 based in vitro drug target identification for personalized prostate cancer medicine (Pitch & Poster) ............................................................ 101

95. Repo, Marleena: Celiac disease is the most common endoscopic diagnosis in children presenting with anemia irrespective of other symptoms (Poster) .................................................. 102

96. Ryynänen, Tomi: Single cell level microelectrode array for cardiomyocytes (Poster) ........ 103

97. Saari, Sina: Dietary Restriction Affects the Development of Drosophila melanogaster Expressing the Ciona intestinalis Alternative Oxidase (Poster) ................................................................. 104

98. Saarinen, Niila: Antibody response against viral proteases in enterovirus infections (Pitch & Poster) ....................................................................................................................................... 105

99. Saralahti, Anni: A forward genetic screen for zebrafish genes involved in Streptococcus pneumoniae infection (Poster) ................................................................................................... 106

100. Sariola, Veikko: Acoustically-actuated Droplet Microfluidics (Poster) ...................... 107

101. Saxén, Heikki: Personalized Medicine Challenges Boundaries Between Research and Care: Could Ethics Consultation Provide Answers? (Poster) ................................................................. 108

102. Shah, Disheet: Modeling dilated cardiomyopathy due to Lamin A/C gene mutation using human induced pluripotent stem cells (Poster) ........................................................................ 109

103. Sipilä, Veli: Image-based Cell Cycle Analysis at Single Cell Level (Poster) .................. 110

104. Skogberg, Anne: Cell Growth and Alignment on Nanocellulose Surfaces (Poster) ...... 111

105. Sutinen, Maiju Reetta: Identifying breast tumors and healthy breast tissue by differential ion mobility (DMS) spectrometry analysis of diathermy smoke (Talk) .............................................. 112

106. Szibor, Marten: TESTING THE THERAPEUTIC POTENTIAL OF AOX IN DIVERSE DISEASE MODELS (Poster) ..................................................................................................... 113

107. Tainio, Jenna: In vitro properties of magnesium and strontium containing bioactive borosilicate glasses (Poster) ............................................................................................................ 114

108. Tanskanen, Jarno: In Vitro Neuronal Electrophysiology Signal Analysis Research in the Computational Biophysics and Imaging Group of TUT BMT (Poster) .................................................. 115

109. Teppo, Susanna: Molecular profiling of diagnostic and drug resistant clones in ETV6-RUNX1-positive pediatric leukemia (Poster) ......................................................................................... 116
110. Tervonen, Aapo: Computational model of stress wave propagation in epithelium (Poster). ................................................................. 117

111. Turunen, Sanna: Direct Laser Writing of Microtowers for 3D Culture of hPSC Derived Neuronal Cells (Poster) .................................................. 118

112. Tuukkanen, Sampo: Multi-material bio-printing facilities (Poster) .......................................................................................... 119

113. Tuure, Lauri: EXPRESSION OF INDUCIBLE PROSTAGLANDIN E SYNTHASE-1 (mPGES-1) IN CHONDROCYTES IS REGULATED BY MAP KINASE PHOSPHATASE-1 (Poster) .......................................................... 120

114. Uusi-Mäkelä, Meri: CRISPR-Cas9 efficiency in correlates with chromatin accessibility in zebrafish (Danio rerio) embryos (Pitch & Poster) ...................................................................................... 121

115. Valanne, Susanna: Osa-containing Brahma complex regulates the activity of the Toll pathway and the expression of the immune regulator lincRNA-IBIN in Drosophila (Poster) .... 122

116. Valkonen, Mira: Dual structured convolutional neural network with feature augmentation for quantitative characterization of tissue histology (Poster) ........................................................................ 123

117. Vattulainen, Meri: Marker expression during differentiation of human pluripotent stem cells towards limbal epithelial stem cells (Poster) ...................................................................... 124

118. Velagapudi, Vidya: Biomedical Applications of High-throughput Metabolomics and Lipidomics Analyses (Poster). ........................................................................ 125

119. Vesala, Laura: Foxo modulates the innate immune response against parasitoid wasps in Drosophila (Poster) ........................................................................................................ 126

120. Viheriälä, Taina: Primary cilia in the maturation of human pluripotent stem cell-derived RPE cells (Poster) .................................................................................. 127

121. Viitasalo, Liisa: ASCA is a possible new marker for nonresponsive celiac disease (Poster) .................................................................................................................. 128

122. Vistbakka, Julia: MiR-191-5p, miR-24-3p and miR-128-3p as potential biomarkers in multiple sclerosis (Poster) ................................................................................................. 129

123. von Essen, Magdalena: Computational Protein Modeling in Talin Mechanobiology (Poster). ................................................................................................. 130

124. Vuolteenaho, Katriina: High synovial fluid interleukin-6 levels are associated with increased matrix metalloproteinase levels and radiographic severity in osteoarthritis patients (Poster) ...... 131

125. Vuornos, Kaisa: Efficient osteogenic induction of human adipose stem cells encapsulated in 3D hydrogels (Talk) ................................................................. 132

126. Ylä-Outinen, Laura: Dual mesh hydrogel as a 3D growth scaffold for human pluripotent stem cell derived neuronal cells (Poster) ................................................................. 133

127. Zhurina, Anastasia: Investigation of novel long-chain diol derivatives as potential growth inhibitors of glioblastoma (Poster) .................................................................. 134
Background: In celiac disease (CeD), the ingestion of gluten in wheat, rye and barley leads to small bowel mucosal damage characterized by villous atrophy, crypt hyperplasia and inflammation in the lamina propria (LP). To date, the only treatment for CeD is life-long strict gluten-free diet. Due to the challenges in the diet, novel treatment modalities are called for. One potential drug candidate is CCX282-B, an orally active chemokine receptor antagonist targeting CCR9. CCR9 is expressed on lymphocytes and with its ligand, chemokine CCL25, mediates lymphocyte recruitment to the small intestinal mucosa. It was hypothetized that in CeD CCX282-B could prevent CCR9-dependent T cell homing to gut thus reducing gluten-induced inflammation and small bowel mucosal injury. However, in a randomized, placebo-controlled, double-blind clinical trial CCX282-B drug exacerbated the effects of gluten.

The objective of this project was to study whether the mechanism behind CCX282-B-induced aggravation involved blocking the entry of immune supressive regulatory T cells (Tregs) to the small intestine.

Materials and methods: The density of FOXP3+ Tregs in small intestinal LP was determined by immunohistochemical stainings in sections prepared from paraffin-embedded biopsy samples taken upon esophago-gastroduodenoscopy from patients participating in the clinical trial. The number of FOXP3+ cells per mm2 of LP was quantified utilizing ImageJ software.

Results: At baseline, the median densities of LP FOXP3+ cells were 69.2 cells/mm2 and 144.3 cells/mm2 in the placebo and CCX282-B group, respectively (p<0.001). After the gluten challenge, the density increased significantly (p<0.001) in both study groups being 165.0 cells/mm2 in placebo and 270.5 cells/mm2 in CCX282-B group. The increase of FOXP3+ cell density in LP appeared to be even more pronounced in CCX282-B group, although the difference to the placebo group was not statistically significant (p=0.1).

Conclusion and future aspects: CCR9 antagonist treatment did not inhibit the entry of FOXP3+ Tregs to small intestinal LP. Hence, the unexpected exacerbation of gluten-induced immune response was not due to prevented immune suppression by Tregs.
Invading pathogens provoke robust innate immune responses in Dipteran insects, such as Drosophila melanogaster. In a systemic bacterial infection, a humoral response is induced in the fat body, the fly equivalent of the mammalian liver. Gram-positive bacteria trigger the Toll signaling pathway, whereas Gram-negative bacterial infections induce the IMD pathway. We show here that the RNAi-mediated silencing of Furin1, a member of the proprotein convertase enzyme family, specifically in the fat body, results in a reduction in the expression of antimicrobial peptides. This in turn compromises the survival of adult fruit flies in systemic infections caused by both Gram-positive and negative bacteria. Furin1 plays a non-redundant role in regulating the immune responses, as silencing of Furin2, the other member of the enzyme family had no effect on survival or the expression of antimicrobial peptides upon a systemic infection. Furin1 does not directly affect the Toll or IMD signaling pathways, but the reduced expression of Furin1 upregulates stress response factors in the larval fat body. We also demonstrate that Furin1 is a negative regulator of the JAK/STAT signaling pathway, which is implicated in stress responses in the fly. In conclusion, our data identify Furin1 as a novel regulator of the humoral immunity and cellular stress responses in Drosophila.
Atherosclerosis can cause life-threatening myocardial or cerebral infarction and is the leading cause of death in Western world. Atherosclerosis occurring in the coronary arteries is referred to as coronary artery disease (CAD). The disease is caused by plaque formation in the artery wall that can rupture and cause an acute event. However, some of the patients never experience rupturing of the plaque and currently there is no reliable way to discriminate between patients with the risk of developing acute event and those whose plaque will remain stable. Many patients are exposed to unnecessary medication.

The mechanisms behind atherogenesis and rupturing of the plaque are still not fully characterized, especially from the lipidomics point-of-view, due to novelty of methods allowing detailed lipid analysis. Lipids are famously involved in atherogenesis, and liver, in turn, has an important role in lipid metabolism. The aim of this research is to utilize hepatocyte-like cells differentiated from induced pluripotent stem (iPS) cells to investigate CAD in vitro. The objective is to use 30 iPS cell lines derived from patients with either acute or stable CAD and healthy controls. Currently, we can produce functional hepatocytes reliably in vitro and the differentiation of the 30 cell lines is in progress. The hepatocyte-like cells are subjected to lipidomics, proteomics and microRNA analysis after the differentiation. We are hoping to discover differences between the cell lines of control and CAD patients that would shed light on mechanisms of atherogenesis and help us to find novel biomarkers to improve atherosclerosis diagnostics.
4. Annala, Matti: Circulating tumor DNA predicts resistance to abiraterone and enzalutamide in prostate cancer (Poster)

matti.annala@uta.fi, University of Tampere

List of all authors
Matti Annala 1,2, Gillian Vandekerkhove 2, Daniel Khalaf 3, Sinja Taavitsainen 1, Kevin Beja 2, Evan W Warner 2, Katherine Sunderland 3, Christian Kollmannsberger 3, Bernhard J Eigl 3, Daygen Finch 4, Conrad D Oja 5, Joanna Vergidis 6, Muhammad Zulfiqar 7, Arun A Azad 8, Matti Nykter 1, Martin E Gleave 2, Alexander W Wyatt 2, Kim N Chi 2,3

Primary resistance to androgen receptor (AR) directed therapies in metastatic castration resistant prostate cancer (mCRPC) is poorly understood. We randomized 202 treatment-naive mCRPC patients to abiraterone or enzalutamide, and performed whole exome and deep targeted 72-gene sequencing of plasma cell-free DNA prior to therapy. For these agents, which have never been directly compared, time to progression was similar. Defects in BRCA2 and ATM were strongly associated with poor clinical outcomes independently of clinical prognostic factors and circulating tumor DNA abundance. Somatic alterations in TP53 and the PI3K pathway, previously linked to reduced tumor dependency on AR signaling, were also predictive of rapid resistance. Although detection of AR amplifications did not outperform standard prognostic biomarkers, AR structural rearrangements truncating the ligand binding domain were identified in several patients with primary resistance. These findings establish the genomic drivers of resistance to first-line AR directed therapy in mCRPC.
Drugs targeting novel pathways of Mycobacterium tuberculosis (M. tuberculosis) offer alternative approaches for treating multi-drug resistant tuberculosis. In vitro studies have shown that dithiocarbamate derived beta-carbonic anhydrase (β-CA) inhibitors Fc14-594A and Fc14-584B effectively inhibit the activity of M. tuberculosis β-CA enzymes. With the purpose of developing dithiocarbamate derived β-CA inhibitors into safe and potent anti-tuberculosis drugs, we screened the two dithiocarbamates (Fc14-594A and Fc14-584B) for safety and toxicity, and studied in vivo inhibitory effect of the least toxic inhibitor on M. tuberculosis in a zebrafish larval model. In our safety screening, Fc14-584B emerged as a least toxic drug and showed no phenotypic defects in 5-day-old larvae at 300 µM concentration. In vitro inhibition of Mycobacterium marinum (M. marinum) in culture showed that both the compounds inhibited growth the growth of M. marinum at a concentration of 75 µM. In vivo inhibition studies using 300 µM Fc14-584B showed significant (P < 0.05) impairment of bacterial growth in zebrafish larvae at 6 days post infection. Our studies highlight the therapeutic potential of the dithiocarbamate Fc14-584B as a β-CA inhibitor against tuberculosis, and set the stage for subsequent preclinical characterization of the drug in an adult tuberculosis zebrafish model.
Talins are adaptor proteins that regulate focal adhesion assembly and signalling by connecting integrins to the cytoskeleton and acting as a docking site for several other proteins. The mechanisms for the binding and activating of integrins are not well understood. As integrin activation and overexpression of talins promote cancer metastasis, understanding the mechanism which activate integrins will clarify their role in cancer metastasis.

We studied Talin1 mutations from COSMIC (Catalogue of Somatic Mutations in Cancer) database. In total of 368 cancer-associated mutations in 27 tissue types were evaluated. After leaving the nonsense and the frameshift mutations out from the analysis, we ended up with 246 missense mutations, which were screened and ranked by bioinformatics tools.

Talin 1 mutants were selected based on multiple scoring coefficient combinations and we eventually ended up with top 10 mutants with highest score. To investigate the properties of these proteins, the mutants were overexpressed in talin-deficient fibroblast cells and the rate of cell migration was monitored. The preliminary data shows the morphology and migration rate differences in one or two talin mutant, which indicates their potential to promote cancerous phenotype. In addition to cell biology experiments, we also aim to conduct biophysical analysis of the most interesting mutants.

Our study aims to reveal association between mutations modulating the mechanical properties of talin and how these changes may translate to defects in mechanosignaling, eventually leading to disease. Our study will help to discover connections between mechanical properties of talin and cancer phenotype. Ultimately, this kind of understanding may help to develop novel treatments and therapeutic methods.
In Hek293T cells a secondary shutdown of the respiratory chain is activated in response to an acute respiratory chain dysfunctions. We will now present evidences that bypassing this secondary shutdown leads to enhanced mitochondrial degradation, a result consistent with a protective role for this secondary respiratory chain shutdown. We also demonstrate that the resulting enhanced mitochondrial degradation preferentially targets ROS-producing mitochondria and is specifically inhibited by mitochondrial-targeted antioxidant treatments. We identify and describe the interrelations between three different pathways involved altogether in this mitochondrial degradation. Finally we demonstrate that purifying selection of mitochondria is induced in dividing cells in the G2/M stage of the cycle through one of these pathways in particular.

Our findings depicts a complex, multilevel and coordinated quality control for mitochondria. This could have profound implication for the development of therapies targeting mitochondrial diseases. We present early evidences that other cell types, while also being capable to shut-down their respiratory chain activity in response to respiratory chain defects, have adapted slightly different regulatory mechanisms for this secondary shutdown.
NFATc (Nuclear Factor of Activated T cells) transcription factors are ubiquitously expressed in human tissues and regulate various cellular processes including immune responses and development. NFATc localization and activation are regulated by phosphorylation. We have previously shown that the oncogenic Pim-1 kinase directly interacts with NFATc1, phosphorylates it and enhances NFATc-dependent transactivation in both immune and neuronal cells. Interestingly, knock-out animals lacking all three pim family genes show defects in T-cell proliferation, suggesting a role for Pim kinases in NFATc regulation also in vivo. Since both Pim kinases and NFATc proteins have recently been linked to several functions important for cancer progression such as cell survival, migration and angiogenesis, we investigated whether NFATc1 protein could mediate the pro-migratory and invasive effects of Pim kinases in prostate cancer.

Here we show that PC-3 prostate cancer cells exhibit constitutive NFAT-activity. Moreover, the Pim kinase inhibitor DHPCC-9 as well as mutation of Pim target sites in NFATc1 significantly decrease the pro-migratory and invasive effects of NFATc1 in PC-3 cells. These results suggest that NFATc proteins are important players in the signaling pathways through which Pim kinases regulate cancer cell motility and invasion.
9. Fuchs, Valma: Delayed celiac disease diagnosis predisposes to reduced quality of life and incremental use of health-care services and medicines: a prospective nationwide study (Poster)
valma.fuchs@gmail.com, University of Tampere

List of all authors
Valma Fuchs1, Kalle Kurppa1,2, Heini Huhtala3, Markku Mäki2, Leila Kekkonen4, Katri Kaukinen1,5

Background. The diverse clinical picture of celiac disease is a challenge to physicians. Most affected individuals remain unrecognized and when they finally are diagnosed, a long diagnostic delay often occurs.

Objectives. Our aim here was to investigate associated socio-demographic risk factors and consequences of diagnostic delay in celiac disease.

Methods. Altogether, 611 adult celiac disease patients were surveyed at diagnosis and after one year on a gluten-free diet (GFD) regarding socio-demographic variables, well-being and use of medicines and health-care services. Quality of life was measured by a validated PGWB (Psychological General Well-Being) questionnaire. The findings in patients with (preceding symptoms ≥ 3 years) and without delayed diagnosis were compared.

Results. A diagnostic delay of at least three years was reported by 332 (54 %) of the subjects. Students and homemakers were at a reduced risk of delay, whereas gender, marital or occupational status, site of diagnosis and place of residence had no effect on diagnostic delay. Poor self-perceived health was associated with diagnostic delay at diagnosis. PGWB scores were poorer in the delay group, anxiety and general health even on GFD. Those with the delay also had more days of sickness and visits to physicians before and following the diagnosis. Use of analgesics, drugs for dyspepsia and antidepressants was increased in the delay group, the two latter still on a GFD.

Conclusion. A delay in celiac disease diagnosis of only 3 years or more predisposes to reduced well-being and incremental use of medicines and health-care services.
10. Genocchi, Barbara: Response of the astrocyte-neural network INEXA to different electrical stimuli (Poster)
barbara.genocchi@tut.fi, Tampere University of Technology

List of all authors
Barbara Genocchi, Jari Hyttinen

Epilepsy is the fourth most common neurological disorder, and there are increasing evidences that astrocytes have an important role in epilepsy; however, it has not been properly studied, in particular in the case of epilepsy. To study that the group developed an in-silico model called INEXA; the model reproduce the tripartite synapse with the presynaptic neuron, the postsynaptic neuron and the astrocytes, and it simulates the processes governing the communications between astrocytes and neurons, and also between astrocyte and astrocyte through gap junction. To simulate the function of the astrocytes in a neural network we ran simulations with different percentage of astrocytes in the network, i.e. 30%, 40% and 50%, and with different levels of noise in the presynaptic neurons, i.e. low, medium and high. Furthermore, we ran simulations, with the same three different percentage of astrocytes but this time with different custom patterns of electrical stimuli as presynaptic activity, with some hyperactivity areas, to see how the astrocytes respond to different stimuli and different frequencies of spikes. These last simulations are intended to reproduce epileptic seizure, where after a normal activity hyperactivity is presented to the synapses for several seconds. To validate these results we will culture neurons and astrocytes on microelectrode arrays, this will lead us to stimulate the population with the same stimuli we used for the simulations previously run.
Plants, fungi and lower organisms, but not vertebrates, express alternative oxidase (AOX), an enzyme that oxidizes ubiquinol to transfer electrons to oxygen thus bypassing complex III and IV. It prevents the over-reduction of the quinone pool, indirectly decreases the excess production of mitochondrial reactive oxygen species, and in turn retains Krebs cycle activity. Transient and constitutive expression of AOX from the sea squirt Ciona intestinalis in mammalian cells, flies and rodents revealed full catalytic activity without adverse biological effects. Of note, transgenic AOX expression was used to overcome lethality in mitochondrial disease models such as lipopolysaccharide-induced inflammation and systemic cyanide intoxication, making AOX a promising tool for future treatments.

To provide a base to implement AOX therapies, different designs of catalytically active and inactive AOX Stabilized Non-Immunogenic Messenger RNA (AOX SNIM RNA) were tested in vitro, to optimize their application for future in vivo studies.

Western blot analysis showed AOX protein expression as early as 3 hours after transfection in immortalized mouse embryonic fibroblasts. Furthermore, AOX expression and mitochondrial targeting was confirmed also in mouse primary neuroglial cells and human lung cancer epithelial cells. Finally, high resolution respirometry in permeabilized cells and cytotoxicity assay revealed that AOX protein encoded by SNIM RNA is enzymatically active and confers resistance to the complex III inhibitor, antimycin A, while mutant transcripts were stably expressed without showing activity.

Taken together, our data support to test the applicability of AOX SNIM RNA in animal models as a tool to develop RNA-based therapies for mitochondrial diseases.
Ribonuclease H (RNase H) family, which is widely present in all organisms, degrades RNA of RNA/DNA hybrids. Mutations in human rnaseh1 gene has been associated with chronic progressive external ophthalmoplegia; a disease triggered by mutations in proteins involved in mtDNA maintenance and/or replication. Human cells lacking RNase H1 shown permanent RNA/DNA hybrids in the non-coding region and at the lagging strand replication origin. We report a deep study of the impact at mitochondrial level of the misregulation of Drosophila melanogaster RNase H1. Lack of the ribonuclease causes depletion on mtDNA copy number and an accumulation of replication intermediates. In addition, lack of RNase H1 modifies the topology of mtDNA. On the other hand, we show that over-expression of RNase H1 causes a reduction of replication intermediates and a dramatic drop on the viability of cultured Drosophila S2 cells. Drosophila RNase H1 shows a dual localization on S2 cells: mitochondrial and/or nuclear localization. Depletion of the mitochondrial targeting sequence (MTS) or the nuclear localization signals (NLS) swap full localization into nucleus or mitochondrial, respectively. Our findings suggest that RNase h1 plays a key role in mtDNA replication and its misregulation affect mtDNA maintenance.
INTRODUCTION: Acute lymphoblastic leukemia (ALL) is the most common cancer in pediatric patients with good overall prognosis. New subgroup specific treatments are still needed. Sox11 is known to be a widely expressed biomarker in mantle cell lymphoma (MCL), but it is also found in ALL among other neoplasms. SOX11 knockdown has been shown to influence the proliferation rate of MCL. Sox11 expression has been shown to influence the patient survival in MCL.

AIM: The purpose of the study is to investigate the relationship between sox11 protein expression in bone marrow biopsies and clinical status and survival of pediatric patients with ALL to find out possible prognostic influence and connections with known genetic abnormalities and current subgroups. Also the effects of SOX11 knockdown in leukemia cell lines and the genetic targets of sox11 protein will be researched.

METHODS: Sox11 expression is analyzed by two pathologists from 126 primary FFPE bone marrow trephine biopsy samples and relapses. The results of the IHC studies will be correlated with the clinical statistics and ancillary studies. We will use previously combined and processed database containing microarray samples to study SOX11 expression levels in different leukemia subtypes and normal cells. Subgroup specific ALL cell lines will be used for SOX11 knockdown studies to explore changes in cell viability. RT-qPCR and Gro-seq will be performed for the SOX11 knockdown and overexpression cell lines to investigate the changes in possible target gene expression levels.
In the developed world, degenerative retinal diseases like age-related macular degeneration (AMD) are the leading cause of senior citizens’ vision loss. AMD is caused by degeneration of the retinal pigment epithelium (RPE), hence RPE transplantation is considered the most promising solution for reversing the degeneration and vision loss. The purpose of this study is to develop biodegradable scaffolds that have similar properties as the natural Bruch’s membrane. Particulate leaching and breath figure (BF) method were used to prepare porous films from poly(butylene succinate) (PBS). These films were characterized by their thickness, surface porosity and pore size, hydrophilicity, roughness, diffusion properties as well as their degradation behaviour. Films with honeycomb structured surface could be prepared with BF method by using 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) as a surfactant. These films were also found to be much more hydrophilic than any of the other films created without surfactant. Films prepared by particulate leaching with sucrose particles also showed a slightly higher hydrophilicity than the control films without sucrose or surfactant, but the pores on these films were not as organized as on the honeycomb films created with the BF method.

Even though the BF method produced a bit more homogeneous and organized films, films prepared by both methods may be promising carriers for RPE cells. Cell culture studies using human embryonic stem cell-derived RPE cells (hESC-RPE) will be carried out in a near future to confirm their potential as prosthetic Bruch’s membranes for hESC-RPE transplantation.
Background: A novel immunotherapy for non-small cell lung carcinoma (NSCLC) is based on blocking the signaling between the programmed death ligand 1 (PD-L1) and T-cell programmed death receptor 1 (PD-1). For therapy, demonstration of PD-L1 in malignant tumor cells is required. A major problem in immunohistochemical detection of PD-L1 expression in pulmonary carcinomas is PD-L1 expression in alveolar macrophages, which may lead to false positive staining interpretation. With fluoro-chromogenic staining, interpretation can be facilitated by exploiting cytokeratin expression of carcinoma cells. Also, ratio of PD-1 positive tumor-infiltrating lymphocytes (TILs) can be analyzed from the same section.

Methods: FFPE-samples of 40 NSCLCs were stained using fluoro-chromogenic staining method with PD-L1, PD-1, and cytokeratin antibodies on Autostainer platform. PD-L1 and PD-1 were sequentially detected with HRP and AP conjugated polymers and visualized with DAB and Permanent Red. Cytokeratin was demonstrated with Cy2 conjugated IgG. Samples were analyzed using virtual slide scans of stacked bright field and fluorescence images with developed image analysis software. Results: Improved accuracy of PD-L1 positive cancer cell count was observed, especially in tumors with low/negative PD-L1 expression and in low grade carcinomas. PD-1 positive TILs were typically seen as single cells in intra-tumoral and as aggregates in peri-tumoral areas.

Conclusion: Accurate and reproducible detection of PD-L1 expression was achieved with the described methods. Stacked virtual slides enable digital image analysis of PD-L1 and PD-1 and facilitates differentiation between PD-L1 expression in carcinoma cells, macrophages and necrotic tissue. Examples of the virtual slides can be found at http://wsiserver.jilab.fi/list/sites/satuteppo/
The aim of this project is to develop novel non-invasive tools for prostate cancer (PCa) management in order to meet the clinical needs in early detection and monitoring of therapeutic response, disease recurrence, and metastatic spread of PCa. Despite recent advances from the uptake in PSA-testing leading to reduced cancer deaths and the development of several novel therapies for castrate-resistant PCa (CRPC), PCa remains the leading cause of male cancer deaths with metastatic disease accounting for >90% of PCa deaths. Circulating tumour cells (CTCs) found in the peripheral blood originate from metastatic lesions sites and CTC-counts in peripheral blood are predictive of survival in CRPC. The aim is to isolate the CTCs cells from the sample and analyze the CTCs in terms of disease progression and treatment response.

We have tested a panel of PCa specific biomarkers on a PCa patient cohort and analyzed their correlation to disease progression and treatment outcome. The biomarkers tested are FOXA1, GRHL2, HOXB13, KLK2, KLK3, KLK15, and the long non-coding genes PCA3 and PCAT-2. An internal RNA control, KLK3-IC, was spiked into the blood samples during RNA isolation to be used for normalization of the results. Following cDNA synthesis the samples were run on Fluidigm Biomark HD. The cohort consists of 167 PCa patients and 30 healthy controls. Our preliminary results show that FOXA1 levels are higher in men with more progressed disease and patients with higher expression of FOXA1, HOXB13, KLK2, KLK3 and/or PCAT-2 have shorter survival compared to other patients.
The essence of mechanobiology in coregulating of cell and tissue physiology in tissue development and regeneration has been recognized already. However, currently there are only limited number of tools for studying mechanotransduction-based cell responses during dynamic mechanical stimulation. We have developed a device enabling simultaneous live imaging and mechanical vibration.

Our device consists of a 3D printed frame fitting on a standard inverted light microscope system and a commercial speaker (Partco Oy) producing horizontal vibration stimulation. A LabVIEW-program is used to control the system and to monitor real-time accelerations of the sample (3-axis, Analog Devices). The mechanical design allows imaging of the cell responses with high-resolution microscopy (63x water immersion, Zeiss LSM 780 LSCM, Carl Zeiss Microscopy GmbH).

Our system produces vibration stimulation from low magnitudes (LM, 1<Gpeak; 30 Hz, 100-300 Hz) to high magnitudes (HM, 1≥Gpeak; 40-90 Hz). Real-time imaging of gold-printed sample movement showed minimal sample drift and preserved field of view. Our pilot studies with epithelial cells (MDCKII) suggested that concentration of cell-cell junction protein occludin increases in the cytoplasm and nucleus morphology changes with increasing duration of the LM (0.35 Gpeak, 30 Hz, 10-40 min) vibration.

Our user-friendly system provides a possibility for simultaneous vibration stimulation and high-resolution microscopy with high performance and wide parameter range. Our initial findings on local structural changes of the epithelial cells in response to the vibration suggest rapid cellular responses to the mechanical stimulation. Our method provides an inexpensive tool for mechanobiology research in different cellular biomechanical applications.
Physicians need to take tissue samples, biopsies, often without accurate information of the needle tip location. We have developed core-type biopsy needle with real-time bioimpedance measurement from the very tip of the biopsy needle. Based on the bioimpedance measurement, tissue type is classified. The aim is to provide information about the needle tip location during the clinical procedure and by these means enable more accurate sample site.

This study assessed the feasibility of the method with porcine model in vivo (ESAVI/4389 /04.10.07/2015) and evaluated the tissue classifier performance to correctly classify different tissue types. Tissue data was gathered from adipose, muscle, spleen, liver and blood of anesthetized porcine. Each tissue was punctured multiple times with the created biopsy needles and bioimpedance data recorded from moving needle. Correct needle placement was ensured with visual control. Tissue classifier was created and tested based on the gathered tissue data using needle-specific hold out method. Maximum likelihood method was utilized for classification. Tissue classification accuracy of the method to differentiate tissue types was really good: Over 94% total accuracy was achieved with this data set.

The method was feasible with living tissue and tissue classification performance of the method was high. Based on these results, local bioimpedance-based measurement can provide accurate tissue information.
Coxsackie B viruses are among the most common enteroviruses, causing a wide range of diseases. Recent studies have also suggested that they may contribute to the development of type 1 diabetes. So far, no CVB vaccine has been developed for human use and clinical trials indicating that such a vaccine would prevent diabetes development are lacking. Here, we have produced a CVB vaccine to test whether vaccination against CVB can prevent virus-induced diabetes in an experimental SOCS-1-tg mice model for virus-induced diabetes.

CVB-vaccine induced strong neutralizing antibody responses and protected vaccinated mice against viremia and the dissemination of virus to the pancreas. Half of the non-vaccinated SOCS-1-tg mice developed diabetes upon infection with CVB, with a loss of the insulin positive beta-cells and pancreatic damage. In contrast, all vaccinated SOCS-1-tg mice were protected from virus-induced diabetes and showed no signs of beta-cell loss or pancreas destruction. Therefore, CVB vaccine can efficiently protect the mice against both CVB infection and CVB-induced diabetes. This pre-clinical proof-of-concept study provides a base for further studies aimed at developing a vaccine for the use in elucidating the role of enteroviruses in human T1D.
Tuberculosis ranks as one of the world’s deadliest infectious diseases causing more than a million casualties annually. Th1 cells have been demonstrated to be indispensable for the immune defense against Mycobacterium tuberculosis. IL10, in turn, is an anti-inflammatory cytokine, which inhibits the function of Th1 type cells, and IL10 deficiency has been associated with an improved resistance against a M. tuberculosis infection in a mouse model. Here, we utilized a Mycobacterium marinum infection in the zebrafish (Danio rerio) as a model for studying Il10 in the host response against mycobacteria. Unchallenged, nonsense il10e46/e46 mutant zebrafish were fertile, phenotypically normal and the expression levels of proinflammatory cytokine genes, such as tnfa, tnfb and il1b, were similar to those in wild type control fish. Following a chronic mycobacterial infection, il10e46/e46 mutants showed enhanced survival compared to the controls. Improved survival of the il10e46/e46 zebrafish associated with an increased expression of the Th cell marker cd4-1 and a shift towards a Th1 type immune response, which was demonstrated by the upregulated expression of tbx21 and ifng1-2, as well as the down-regulation of gata3. In addition, at 8 weeks post infection il10e46/e46 mutant zebrafish had reduced expression levels of tnfb and il1b, presumably indicating slower progress of the infection. Altogether, our data show that Il10 can weaken the immune defense against a M. marinum infection in zebrafish by restricting the Th1 cell mediated response. Importantly, our findings support the relevance of a M. marinum infection in zebrafish as a model for tuberculosis.
Background: Coeliac disease and its cutaneous manifestation dermatitis herpetiformis (DH) are characterised by antibody responses against the major autoantigens transglutaminase (TG) 2 and its epidermal isoform, TG3, respectively. Previous studies have shown that TG2 antibodies are produced in the gut and that they can be assessed in the organ culture medium of coeliac disease patient-derived small-intestinal biopsies. However, thus far no studies have investigated the TG3 antibody response in the organ culture system or exploited the method in DH overall.

Methods: Small intestinal mucosal biopsies derived from DH patients with active disease (n=5) and in remission (n=12) as well as coeliac disease patients with active disease (n=10) and in remission (n=10) were cultured for 24 h. Thereafter, mucosal autoantibody response was assessed by measuring TG2- and TG3-autoantibody secretion into the organ culture medium by enzyme-linked immunosorbent assay (ELISA) as well as by staining biopsies for the presence of TG2- and TG3-antibody-positive cells using immunofluorescence staining. In addition, TG2 and TG3 antibody levels in the serum of patients were determined.

Results: The majority of DH patients were negative for both serum and organ culture medium TG2-targeting antibodies, as opposed to active coeliac disease patients. Surprisingly, active DH patients secreted TG3 antibodies into the organ culture medium despite being negative for serum TG3 antibodies. In patients secreting high levels of TG3 antibodies into the culture medium, we also detected TG3-antibody-positive cells in the small intestinal mucosa.

Conclusions: Our findings show that similarly to TG2 antibodies, TG3 antibodies can be investigated in the small bowel mucosal organ culture system and that their secretion occurs in the small intestinal level, especially in active DH.
SCA28 is a rare autosomal dominant form of ataxia that stems from mutations in the mitochondrial protein ATPase Family Gene 3 Like 2 (Afg3L2), the principal subunit of the mitochondrial-AAA protease. Human mutations cause juvenile-onset ataxia. Disease causing mutations in the protein are known to lead to cerebellar atrophy as a result of degeneration of Purkinje cells. The phenotype is thought to be due to impaired mitochondrial respiration and calcium mishandling, although the exact disease mechanism is unknown. Mice devoid of Afg3L2 in the Purkinje cells (Afg3L2PC-/-) recapitulate the features of human SCA28 providing a great model to investigate our hypothesis both in vitro and in vivo. To study the respiratory defect in these mice we have used the alternative oxidase (AOX) from Ciona intestinalis, which is capable of bypasses complexes III and IV, by branching the electron transport chain by accepting electrons directly from quinones and reducing oxygen. Interestingly, our preliminary results suggest that the Afg3L2 pathology can be ameliorated by the addition of AOX.
Type 1 Diabetes (T1D) is a disease in which insulin producing pancreatic beta-cells are selectively destructed. Genetics has a strong role in T1D; however, genes cannot explain the pathogenesis of the T1D alone, since only a few of the genetically susceptible individuals develop T1D. Enteroviruses have been linked to T1D in studies and the most plausible mechanism seems to be a chronic infection in pancreatic beta cells being responsible at least partially for the disease. Most commonly Coxsackie B group viruses have been linked to the disease, and Coxsackievirus B1 (CVB1) seems to be associated with the increased risk of T1D.

We have established a chronic infection models, by four strains of CVB1, in pancreatic epithelial cell line named PANC-1 and by three stains of CVB1 in a beta cell line named 1.1B4. The effect of antiviral drugs was identified in acute infection model. The chronic infection model in pancreatic cell line was then employed to identify the effect of antiviral strategies. Pleconaril, a well known pocket factor replacing agent of Picornaviruses, was shown to be serotype and strain specific. We showed that it prevents the ATCC strain of CVB1 cytopathic effect in A549 cells in an acute infection model. The drug was also effective against the same strain in our chronic infection model in PANC-1 cells, with 1 μM concentration.

The study crates new knowledge about chronic enterovirus infection and its eradication with antiviral drugs. The antiviral drug test results can be applied in clinical trials on pre-diabetic patients.
Bioactive glasses (BaGs) have been extensively used in bone tissue engineering (TE) applications but the molecular response to the BaGs is not yet fully understood. This study set out to analyze the mechanisms of cell attachment on two discs of different BaG compositions, and the adhesion-mediated early osteogenic differentiation induced by BaGs of human adipose stem cells (hASCs). Human ASCs were cultured on two silica-based BaG discs: S53P4 (23.0Na2O-20.0CaO-4.0P2O5-53.0SiO2 (wt-%)) and 1-06 (5.9Na2O-12.0K2O-5.3MgO-22.6CaO-4.0P2O5-0.2B2O3-50.0SiO2) in the absence of osteogenic supplements. We discovered that the BaG discs supported cell adhesion (enhanced integrin $\beta_1$ and vinculin production) and that the mature focal adhesions were smaller but more dispersed than on cell culture plastic (polystyrene). Based on our results, both BaG-types enhanced early osteogenic differentiation (alkaline phosphatase activity (ALP) and the expression of osteogenic marker genes RUNX2a and OSTERIX). The BaG composition 1-06 with lower reaction rate was discovered as a stronger osteoinducer. We also analyzed the cell signaling response to BaG interaction and found out that Focal adhesion kinase (FAK), extracellular signal-regulated kinase (ERK1/2) and c-Jun N-terminal kinase (JNK)-induced c-Jun phosphorylations were upregulated by glass contact. Additionally, inhibition of FAK, ERK1/2 and JNK reduced the BaG-induced early osteogenesis indicating the significance of these pathways in the osteogenic course of hASCs. This study enhances our understanding of the molecular mechanisms of BaG-induced early osteogenesis in hASCs and provides tools for future biomaterial designing for bone TE.
25. Häkli, Martta: Maturation of human induced pluripotent stem cell-derived cardiomyocytes grown on polyethylene terephthalate textiles (Poster)
martta.hakli@uta.fi, University of Tampere

List of all authors
Martta Häkli

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) enable patient- and disease specific models for cardiovascular diseases, but have a disadvantage of being developmentally immature. This complicates the utilization of the collected data in disease modeling. Our method to improve the maturation is to grow the hiPSC-CMs polyethylene terephthalate (PET) textiles, which improves the maturity on structural level as the cells and their sarcomere structures align according the PET fibers. As structural properties are not the only aspect regarding maturation, also gene expression and functionality of the hiPSC-CMs should be assessed to be able to evaluate the effectiveness of the method.

hiPSC-CMs were cultured on PET textiles (PET hiPSC-CMs) and coverslips (control) for 11 days, after which their maturation state was evaluated. We evaluated cell alignment and sarcomere length with immunocytochemical staining and fluorescence imaging. The images were analyzed with CytoSpectre software. We evaluated the expression of one cardiac maturation marker, TNNT2, between the PET hiPSC-CMs and control using qPCR. Calcium-imaging was used to evaluate the functionality of hiPSC-CMs cultured on PET textiles and coverslips for 12 days.

The orientation of PET hiPSC-CMs is higher compared to the control but there is no significant difference in sarcomere length between the groups. Also, the length to width ratio is significantly higher with PET hiPSC-CMs. In addition, our results indicate that TNNT2 expression of PET hiPSC-CMs is higher than that of the control, which would further support the effectiveness of this method to maturate hiPSC-CMs.
26. Hämäläinen, Mari: MKP-1 as a protective factor and novel drug target in scleroderma: MKP-1 deficient mice develop more severe dermal fibrosis in a widely used experimental model of scleroderma (Poster)
mari.j.hamalainen@uta.fi, University of Tampere

List of all authors
Morena Scotece, Mari Hämäläinen, Tiina Leppänen and Eeva Moilanen

Scleroderma is a chronic connective tissue disease of unknown aetiology. In early stages, vascular injury and inflammation lead to fibrosis, resulting in irreversible damage in various organs. Inflammation is believed to be necessary in order to activate fibroblasts to over-produce extracellular matrix components. At the present, there is no effective standard treatment to reverse or slow down the progression of scleroderma but one of the feasible approaches is to target key inflammatory pathways that are involved in the pathogenesis of the disease. MKP-1 (Mitogen-Activated Protein Kinase Phosphatase-1) is a nuclear phosphatase present in most cell types and tissues. Studies with MKP-1 deficient mice have undoubtedly shown that MKP-1 is an important regulator of innate and adaptive immune responses to limit and suppress inflammation but its role in fibrosing diseases has not been studied.

In the present study, we aimed to investigate the potential protective role of MKP-1 in the pathogenesis of scleroderma by using MKP-1 deficient mice and a widely studied experimental model of scleroderma, namely the bleomycin-induced dermal fibrosis in the mouse.

Wild type (WT) and MKP-1 deficient mice were injected subcutaneously with bleomycin every other day for 28 days. Dermal thickness and collagen accumulation were determined by histological analyses. The expression of several inflammatory and pro-fibrotic mediators were measured by quantitative RT-PCR.

We found that bleomycin-induced dermal thickness and lipodystrophy were increased in MKP-1 deficient mice. Collagen accumulation in the dermis and mRNA expression of collagens 1A1 and 3A1 were enhanced in the skin from MKP-1 deficient mice as compared to the skin from WT animals. Affected skin from MKP-1 deficient mice presented increased expression of factors related to inflammation and fibrosis, namely IL-6, TGF-b1, fibronectin-1 and YKL-40 as well as chemokines MCP-1, MIP-1α and MIP-2.

This study demonstrates, for the first time, that MKP-1 deficient mice develop more severe bleomycin-induced dermal fibrosis than their WT counterparts, indicating that MKP-1 regulates the inflammatory and fibrotic processes typical for experimentally-induced scleroderma. These findings suggest that compounds which enhance expression / activity of MKP-1 have potential as novel drugs for the stage-specific modulation of the pathogenesis of scleroderma.
International guidelines recommend that diagnosis of asthma is grounded on objective measurements of lung function. Peak expiratory flow (PEF) monitoring is widely available in primary care but there are concerns whether regular use of short-acting β2-agonist (SABA) during the monitoring induces tolerance and affects neural mechanisms regulating bronchial tone. This could decrease bronchodilator response, impair PEF level or even cause false positive diagnosis by inducing airway hyperresponsiveness.

We retrospectively collected 165 pre-diagnostic PEF monitorings from primary and secondary health care in 127 subjects with adult-onset asthma. Two different two-week protocols had been used: one with regular use of SABA only on the second week and other with regular use of SABA on both weeks. We analysed PEF level, diurnal variation of PEF and bronchodilator response in all the PEF monitorings.

In the protocol 1 where SABA was used on the second week only, there was a statistically significant but clinically insignificant minor decrease in PEF level from first week to second week (-2.4 l/min, p=0.020, 95 % CI: -7.3 – -0.3 l/min). There were no statistically significant differences in diurnal variation of PEF or bronchodilation response between the first and second weeks in either of the two PEF monitoring protocols.

In conclusion, regular use of SABA for either one or two weeks during PEF monitoring does not induce clinical signs of beta-agonist tolerance such as increased airway variability, changes in absolute PEF levels or decreased bronchodilation effect in subjects with untreated asthma.
Bioresorbable implantable sensors could be used to monitor acute physiological conditions for a short period of time, after which they resorb into the body. Embedding such sensors into bioresorbable orthopedic implants would provide in situ data from the healing site without a need for additional operations. One possible clinical application would be to monitor intracranial pressure (ICP) after a traumatic head injury, because ICP might lead to a brain injury days after the actual trauma.

This study encompasses the fabrication and characterization of a bioresorbable wirelessly readable pressure sensor. The passive resonance sensor was based on resorbable poly(desamino tyrosyl-tyrosine ethyl ester carbonate) (PDTEC) substrates with evaporated magnesium patterns. A laser-cut PDTEC spacer was attached between two substrates using polycaprolactone as an adhesive. The cavities limited by the substrates and the spacer enabled capacitive pressure sensing. The sensor was capable of detecting pressure changes within the physiological pressure range from 0 to 200 mmHg.
Prostate cancer is one of the most common forms of cancer in men worldwide. Despite advances in this field, it remains difficult to determine whether a prostate cancer will develop into metastatic lethal disease or stay indolent and confined to the prostate. On one hand, this results in many cases in unnecessary surgery and overtreatment of patients with indolent disease, and on the other hand we continue to be unable to effectively manage metastatic disease. The goal of this study is to trace the genetic evolution of metastatic prostate cancer by sequencing DNA and RNA from primary tumors and matching lymph node metastases. This will be done by collecting whole mount radical prostatectomy samples with positive lymph nodes and fixing them using PAXgene fixative, which preserves biomolecule integrity. All tissue block face sections will be imaged using whole mount images on slides. Using laser capture microdissection, we will isolate DNA and RNA from regions of interest containing cancer, dysplastic regions, benign prostatic hyperplasia and non-cancerous cells. The sequencing data will be used to construct a phylogenetic tree based on shared and unshared genetic alterations. The results from this study will link genomic information to morphologic and histologic features of the cancer, which will ultimately facilitate improved disease categorization and treatment options.
Human pluripotent stem cell -derived retinal pigment epithelium (hPSC-RPE) provides a promising source to treat retinal degeneration. For cell therapy, standardized and xeno-free culture and differentiation protocols, as well as RPE of uniform quality, are required with potential implications to safety, functional cell integration and graft survival after transplantation. We aimed to develop a clinically compatible production and culture system for hPSC-RPE and identify the role of RPE basement membrane (BM) proteins to the maturation and quality of hPSC-RPE. The in vivo functionality of the hPSC-RPE was further evaluated in animal models. We successfully maintained several hPSC lines in a defined, xeno- and feeder-free culture system, and thereafter differentiated them to RPE, which formed functional epithelial monolayers in vitro. We also established xeno-free cryobanking protocols for both the pluripotent hPSCs and hPSC-RPE. Collagen IV and laminin are typically used individually for coating cell culture surfaces for hPSC-RPE culture. However, our results indicate, that it would be advantageous to combine these BM proteins and use extracellular matrix linker molecules such as nidogen to obtain high quality hPSC-RPE. After cryopreservation of hPSC-RPE, the culture substrate is even more relevant, with possible implications to clinical use of these cells after cryobanking. Altogether, these methods generate high-quality cells and can be applied for clinical use. When xenografted into animal models, hPSC-RPE monolayers can survive and support at least some retinal functions for at least four weeks.
Mitochondria are major bioenergetic organelles of eukaryotic cells, but their energy-conserving chemistry operates at far below 100% thermodynamic efficiency. This means that, whilst energy released by the oxidation of respiratory substrates drives ATP synthesis and metabolite transport, a significant and variable proportion is released as heat. Using a temperature-sensitive fluorescent probe targeted to mitochondria, we measured mitochondrial temperature under different physiological conditions. At a constant external temperature of 38 °C, mitochondria were more than 10 °C warmer when the respiratory chain was fully functional, both in HEK cells and primary skin fibroblasts. This differential was abolished by respiratory inhibitors or in cells lacking mitochondrial DNA, but enhanced with respect to metabolic flux, by thermogenic enzymes such as the uncoupling protein UCP1 or the alternative oxidase AOX. The activity of various respiratory chain enzymes was maximal at, or slightly above, 50 °C. Our findings prompts a re-examination of the literature on mitochondria, taking account of the inferred high temperature.

Acknowledgments: I acknowledge the contribution of members of my group, notably Eric Dufour, and the team of Pierre Rustin in Paris, who carried out almost all of the experimental work that enabled us to reach these conclusions.
Inflammatory bowel disease (IBD) stands for two conditions, Crohn’s disease and ulcerative colitis, affecting about 0.1% of western population. It is characterized by chronic inflammation of the gastrointestinal tract, with symptoms as diarrhea and weight loss. The cause remains unknown, but IBD often is the result of an unbalance between immune system and gut microbiota.

Murine models of colitis have been used to identify the cause and pathogenesis of IBD, as well as to develop novel targeted therapeutics. Several mouse models are available to assess the contribution, either being cause or consequence, of T-cells in IBD.

It is known that Furin is required for proper regulatory function of CD4+ T cells, to maintain intestinal homeostasis by preventing inappropriate innate and adaptive immune responses. Upon aging, T-cell-Furin-deficient animals (CD4cre-furflox/flox mice) develop IBD-like disease such impaired peripheral immune tolerance resulting in a systemic autoimmune disease.

To further investigate the role of Furin in gut inflammation, we quantify the gut wall cells cytokines and T-helper-cell-subtype transcription factors gene expression levels. From the mice stool, we evaluate the levels of six short-chain fatty acids and the 16S rRNA sequences from microbes, in order to get a detailed information of microbiome’s conforming communities.

Our aim is to investigate if dysregulated gut microbiota of the KO Furin T-cells is similar to other IBDs or does it have specific features against the wild-type littermate controls, aged between 3 and 5 months old. The results of this experiment could lead us towards the identification of novel immunoregulatory microbiomes.
Over the past few decades, the major thrust of anticancer research is aimed at developing drug delivery systems that could target the malignant cells. The efforts lead to the discovery of targeted drug delivery systems such as drug-loaded nanoparticles (NPs) and antibody drug conjugates. However, mounting evidence suggests that different nanoformulations adversely activate complement and coagulation cascades (J. Controlled Rel. 2014, 190, 556). Heparin is a natural extracellular matrix polymer that possess a unique anticancer property as it inhibits angiogenesis, tumor metastasis and suppress complement and coagulation cascade. Therefore, exploiting heparin to engineer nanoparticles for delivering chemotherapeutic agents offers enormous promise. In this study, we have designed a novel heparin based nanoparticles by conjugating fluorescein a hydrophobic fluorescent molecule. The degree of conjugating was tuned such that we could achieve a uniform nanoparticle below 150 nm with monomodal distribution. This design enabled efficient doxorubicin loading (≈ 100%) and stabilized the aromatic drug resulting in sustained drug release. Thereafter, we incubated the free drug and drug loaded nanoparticle with human blood to evaluate the activation of complement and coagulation cascade. Unlike the free doxorubicin (which significantly triggered the innate immune response), the drug loaded nanoparticles mitigated such effects. Such heparin based drug delivery system is valuable for delivering diverse drugs for anticancer applications.
Adipose tissue (AT) derived multipotent cells, known as adipose stem cells (ASCs) are promising candidates for clinical applications because of their regenerative capacity, low immunogenicity and their ability for immunomodulation. The success of the future allogeneic cell-based therapies depends on the appropriate selection of ASC donors. Several factors including donor age, sex and harvest location affect the ASC characteristics. These also depend on body mass index (BMI). In this study, ASCs were isolated from AT of obesity-discordant monozygotic twin pairs (n=5) and weight-concordant control twin pairs (n=2) selected from population-based twin cohorts. ASCs proliferation, multipotency and immunophenotype were studied with CCK8 cell proliferation assay, adipogenic and osteogenic differentiation and flow cytometry, respectively. Adipogenic differentiation was confirmed with Oil Red O –staining and analysis of adipogenic marker genes. Osteogenic differentiation was confirmed with quantitative alkaline phosphatase activity analysis, Alizarin Red -staining and analysis of osteogenic marker genes. The effect of BMI on ASC immunogenicity and immunosuppression potential was studied using one-way mixed lymphocyte reaction (MLR), and direct and indirect two-way MLR, respectively. ASC proliferation, immunogenicity or immunosuppression capacity were not related to BMI. ASCs derived from both low and high BMI donors showed low immunogenicity and were equally effective in immunosuppression. ASCs derived from both low and high BMI donors showed similar immunophenotype. Differentiation efficacy toward osteogenic and adipogenic lineages was not related to BMI. Our data suggest that under the same genetic background, BMI does not seem to have an effect on ASC proliferation, immunophenotype, immunogenicity, immunomodulation capacity and differentiation.
Treatment options for patients suffering from advanced prostate cancer (PC) have improved, but castration-resistant form of the disease (CRPC) still remains a lethal disease. Unfortunately, a significant subset of patients show primary resistance to AR-targeting drugs developed against CRPC. One explanation to this could be the expression of constitutively active androgen receptor splice variants (AR-Vs). The aim of this work was to study AR-Vs but also AR rearrangements, mutations and copy number variations (CNVs) to better understand the emergence of CRPC. This was done by analyzing specimens from different stages of prostate cancer by next-generation sequencing methods. Our sample cohorts included hormone-naïve PCs and lymph node metastases as well as locally recurrent and metastatic CRPCs. AR mutations and CNVs were detected only in locally recurrent and metastatic CRPC specimens. AR-GSRs were observed in 5/30 metastatic CRPC patients but they were not associated with the expression of previously known AR-Vs. The main AR-Vs detected were AR-V3, AR-V7 and AR-V9, whose expression levels were higher in metastatic CRPC cases in comparison to prostatectomies. The differences were statistically significant for either variant alone or when their expression fractions were combined (p=0.0006). The expression of these AR-Vs was strongly associated with the levels of full-length AR. Out of 25 CRPC metastases that expressed any AR variant, 17 cases harbored expression of all these three AR-Vs. In conclusion, AR-V3, AR-V7 and AR-V9 appear to be co-expressed in metastatic CRPC highlighting the fact that inhibiting one AR-V might not be sufficient to achieve a treatment response.
Coeliac disease (CD) and its skin manifestation, dermatitis herpetiformis (DH), are characterized by disease-specific autoantibodies targeted against transglutaminase (TG) 2 in the serum and small intestine. In addition, DH patients evince IgA response targeted against TG3 in the dermis, and often also in the serum. Since the localization of TG3 in papillary dermis is different compared to healthy skin, it has been suggested that dermal TG3 bound by IgA might be originated from circulating TG3-IgA immune complexes. The aim was to set up a sandwich ELISA to measure levels of such circulating immune complexes (CIC) in DH and CD. Further aim was to identify the characteristics of the TG3-IgA CIC in DH.

The TG3-IgA CIC levels were investigated from the CD (n=99) and DH patient (n=31) sera at the time of diagnosis and after one year on gluten-free diet and from controls (disease controls, n=40; healthy controls, n=30) by a sandwich ELISA. The TG3-IgA containing CIC will also be purified by affinity chromatography from the sera of the DH patients and controls and further subjected to mass spectrometry for the identification of the proteins in the CIC.

At diagnosis, the DH and adult CD patients had significantly higher levels of TG3-IgA CIC compared to controls. Patients’ CIC levels decreased significantly on gluten-free diet. In addition, TG3 autoantibody positive individuals had higher levels of TG3-IgA CIC compared to autoantibody negative individuals. These results together with the forthcoming knowledge about the components of the CIC will broaden the understanding of DH and might be applicable for developing novel serum-based diagnostic methods for DH.
The epigenetic clock, defined as the DNA methylome age (DNAmAge), is a candidate biomarker of ageing. In this study, we aimed to characterize the behaviour of this marker during the human lifespan in more detail using two follow-up cohorts (the Young Finns study, calendar age i.e. cAge range at baseline 15-24 years, 25-year-follow-up, N = 183; The Vitality 90+ study, cAge range at baseline 19-90 years, 4-year-follow-up, N = 48). We also aimed to assess the relationship between DNAmAge estimate and the blood cell distributions, as both of these measures are known to change as a function of age. The subjects' DNAmAges were determined using Horvath's calculator of epigenetic cAge. The estimate of the DNA methylome age acceleration (Δ-cAge-DNAmAge) demonstrated remarkable stability in both cohorts: the individual rank orders of the DNAmAges remained largely unchanged during the follow-ups. The blood cell distributions also demonstrated significant intra-individual correlation between the baseline and follow-up time points. Interestingly, the immunosenescence-associated features (CD8+CD28- and CD4+CD28- cell proportions and the CD4/CD8 cell ratio) were tightly associated with the estimate of the DNA methylome age. In summary, our data demonstrate that the general level of Δ-cAge-DNAmAge is fixed before adulthood and appears to be quite stationary thereafter, even in the oldest-old ages. Moreover, the blood DNAmAge estimate seems to be tightly associated with ageing-associated shifts in blood cell composition, especially with those that are the hallmarks of immunosenescence. Overall, these observations contribute to the understanding of the longitudinal aspects of the DNAmAge estimate.
Variability in cellular components among clonal cells emerges due to, e.g., asymmetric partitioning of component in division, contributing to different gene expression rates in sister cells. Consequently, this is expected to result in lineage-to-lineage variability in transcription rates. To show this, we tracked cell lineages and measured transcription at the single RNA level. We demonstrate the existence of lineage-to-lineage variability in RNA numbers and further show that there are two distinct sources of variability. One is the effect of variability of components in the transcription process, which acts as a constant source of noise. Meanwhile, the other is the effect of variability of uptake molecules in the intake process of gene expression inducers from the media, which acts as a transient source. As expected, the latter is the main source of variability early in induction but, as more cells initiate transcription, the former becomes the main source of lineage-to-lineage variability in RNA numbers. Finally, we show that the lineage variability resulting from these sources differs with both the inducer and between promoters. From additional experiments, we show that these differences can be explained by the different kinetics of the rate-limiting steps in transcription initiation of each promoter. As this phenomenon is both inducer and promoter-sequence dependent, it is subject to regulation and is evolvable.
Visual examination of biopsies by a pathologist under a microscope is a crucial part of the current diagnostic practice for prostate cancer. This involves assigning the sample a Gleason score, which is based on the appearance of the tissue, and is an important marker for prognosis and treatment decisions. However, there is a shortage of trained uropathologists and the variance in Gleason scoring even among experienced pathologists is considerable. These problems could potentially be tackled by developing decision support systems based on automated image analysis techniques enabled by digital pathology. The deployment of such systems could lead to reduced inter-pathologist variance, a more manageable workload for the pathologists and also reduced costs for the health care system.

In this study, we will develop image analysis and machine learning methods to automatically pinpoint potentially cancerous tissue from prostate biopsies and to estimate the Gleason score of the tissue. The dataset currently available as the basis of the study includes approximately 6000 scanned H&E-stained whole slides corresponding to more than 500 patients from the Stockholm region. In addition to per-biopsy Gleason scoring performed by a pathologist, the images include annotations of cancerous tissue, which can be used as labels for machine learning. During the project, we will evaluate machine learning systems based both on classical feature engineering approaches and deep learning techniques such as convolutional neural networks. We will also focus on developing visualization techniques for assessing the performance and interpreting the output of the machine learning systems in an intuitive manner.
Microstructure has an essential role in the control of hydrogel properties. It is an important factor to be considered especially when drugs or cells are encapsulated inside the hydrogel (3D). Indeed, the structure greatly determines how suitable hydrogels are as biomedical material and how well they perform. Structural parameters, such as crosslinking density and mesh size, dictate the material modulus and diffusional properties. Therefore, mesh size has been used to correlate the diffusivity of molecules inside the hydrogel. In this study, the microstructures of hydrazone crosslinked hyaluronan-, gellan gum- and alginate- based hydrogels were evaluated using rheological and diffusion (fluorescence recovery after photobleaching, FRAP) -based methods. The effect of gel parameters (degree of substitution and molecular weight of gel components, ratio of gel components, polymer concentration of hydrogel) on hydrogels’ viscoelastic and diffusion properties, and further to their structural parameters were studied. The results showed an equivalence between the mesh sizes of hydrogels (rheological method), and the dextran molecule sizes lowering the diffusivity inside the hydrogel (FRAP method). The mesh sizes were comparable with many other hydrogels. This size range allows the transportation of smaller molecules, whereas the penetration or release of larger molecules may be hindered. The results also showed a proportionality between the structural parameters and storage moduli (and second order elastic constants). To conclude, these methods enable the evaluation of hydrogels’ microstructure. Furthermore, hydrazone crosslinking offers an easy way to produce hydrogels with variable microstructures, but also variable viscoelastic and diffusion properties, by altering the gel parameters.
41. Karvonen, Hanna: Targeting ROR1 pseudokinase as a therapeutic strategy for mantle cell lymphoma (Poster)

hanna.karvonen@uta.fi, University of Tampere

List of all authors
Hanna Karvonen (1), David Chiron (2), Wilhelmiina Niininen (1), Sara Ek (3), Mats Jerkeman (4), Caroline A. Heckman (5), Astrid Murumägi (5) and Daniela Ungureanu (1)

A subset of tyrosine kinases have been neglected as potential drug targets because sequence analysis suggests that they contain inactive kinase domains, due to the absence of canonical motifs essential to catalytic reaction. These proteins have been termed pseudokinases and account for almost 10% of the human kinome. One of these proteins is receptor tyrosine kinase-like orphan receptor 1 (ROR1) which is primarily expressed during embryogenesis and to a lesser extent in healthy adult tissue. However, ROR1 expression is elevated in several types of cancer including multiple hematological malignancies such as B-cell chronic lymphocytic leukemia (B-CLL), mantle cell lymphoma (MCL) and t(1;19) acute lymphoblastic leukemia (B-ALL). Targeting ROR1 expression efficiently induced apoptosis in cancer cells, suggesting a critical role for ROR1 in maintaining tumor cell survival. We are investigating the regulatory mechanisms of ROR1 oncogenic signaling in MCL, an aggressive non-Hodgkin’s lymphoma largely incurable with current treatment strategies. MCL cells expresses ROR1 in almost 90% of B-cells isolated from blood or bone marrow patient samples. We show that targeting ROR1 expression resulted in downregulation of NF-κB p65 levels and that activation of the NF-κB pathway can antagonize ROR1-mediated apoptotic responses. High throughput drug sensitivity testing of MCL cells before and after ROR1 targeting revealed synergistic effects between co-targeting of ROR1 and B-cell antigen receptor (BCR) or Bcl-2 family, underlining high potential for ROR1 targeted therapies in overcoming MCL drug resistance. Our novel approaches to study ROR1 targeting in MCL may contribute to improve cancer therapy, especially for overcoming drug-resistance.
**Introduction:** Assessing central and peripheral nitric oxide (NO) dynamics of the lung provides information on the severity and anatomical site of pulmonary inflammation. Several mathematical methods to calculate alveolar and bronchial NO parameters have been introduced. Our aim was to compare these methods.

**Methods:** The study included 69 healthy adults, 66 healthy children, 73 asbestos-exposed subjects and 72 subjects with chronic obstructive pulmonary disease (COPD). Exhaled NO was measured at multiple flow rates and we used five mathematical methods (Tsoukias & George, Pietropaoli, Condorelli, Högman & Meriläinen, and Silkoff) to estimate alveolar and bronchial NO parameters.

**Results:** H&M method was less frequently feasible compared to other methods but it had the highest degree of agreement with the measured data. The methods were most often feasible in healthy or asbestos-exposed adults but distinctly more infrequently in children and adults with COPD, suggesting difficulties in NO measurements in these groups. The linear methods (T&G, Pietropaoli) yielded higher alveolar NO concentration and lower bronchial NO flux than the two non-linear methods (H&M, Silkoff) and linear method with correction for axial back-diffusion of NO (Condorelli).

**Conclusion:** In differentiating central and peripheral NO sources we recommend using the linear methods, as low flow rates are not needed and the feasibility of the methods is good. If bronchial wall NO concentration (CawNO) and diffusing capacity (DawNO) are of interest, non-linear methods are needed and we recommend using H&M method as only three flow rates are needed. However, the agreement between the model and measured data needs to be checked in real-time to ensure feasibility. If the subject has difficulties with the extremely low or high flow rates, we then recommend using the Silkoff method to improve feasibility, but more flow rates and measurements are then needed and the agreement between the model and the measured data may be poorer.
Epigenetic changes have been shown to be important in the development of prostate cancer, which is the second most common malignancy in men in Western countries. Here, we performed MeDIP-sequencing to profile the methylation pattern of 12 benign prostate hyperplasia (BPH), 28 untreated prostate cancer (PC) and 13 castration resistant prostate cancer (CRPC) samples. We identified distinct hyper- and hypomethylated regions in PC, CRPC and in both PC and CRPC samples. CpG islands harbored the strongest methylation, with the most differentially methylated regions (DMRs) locating within 2 kb of a CpG island and the majority of hypermethylated CpGs situating within 0-5kb from transcription start site (TSS). Our analysis revealed several previously unreported protein and miRNA coding genes to be methylation regulated. As expected, a significant fraction of genes underexpressed in PC were hypermethylated at their promoter region. Interestingly, we observed positive correlation between promoter DNA methylation and RNA expression in 56 protein coding genes and in 5 miRNAs. Furthermore, we found that DNA methylation and loss of H3K27me3 co-occur at the promoter DMRs of a number of genes, including the HOXC and miR-183~182~96 clusters. Moreover, using qPCR we detected reduction of HOXC4 expression in dihydrotestosterone (DHT) treated VCaP and modified LNCaP cells with high androgen receptor (AR) expression. In conclusions, our study characterizes common sites of differential methylation in hormone-naïve and CRPC tumors and found HOXC cluster as a methylation regulated prostate cancer associated locus.
Hyaluronic acid (HA) is an essential component of the extracellular matrix (ECM), which makes it a promising material for designing 3D scaffolds. The most common strategy for designing HA-based hydrogels involves hydrazone chemistry involving an aldehyde and hydrazide modified HA. However, the addition of proteins or growth factors to these gels compromises their stability as they interfere with the crosslinking density. We have designed a novel hydrazone crosslinked hydrogel that retains its stability and mechanical properties in the presence of proteins. As a model protein, we used gelatin as it would provide the integrin binding site for the cultured cells, which is generally absent in HA gels. We used gold chloride in our gels that generated gold nanoparticles in situ. This augmented the mechanical properties and stability of these gels by generating an interpenetrating network. We could modulate the viscoelasticity by changing the gold concentrations. Finally, we used this gel to culture human bone marrow derived mesenchymal stem cells (MSCs). We are currently evaluating the mechanotransduction and differentiating potential of these gels.
Intracranial Pressure (ICP) may rise due to an illness or injury and cause ischaemic events. Thus, long-term home monitoring would improve the safety of people predisposed to raised ICP. To achieve this, researchers have worked on both battery powered and fully passive wireless implantable pressure sensors. However, due to the limitations of these methods, we are developing an inductively powered battery-free implantable system that includes also an active radio transmitter.

Our system consists of three main modules: implant, on-body unit and off-body unit. It exploits two wireless links instead of one to overcome the limitations of the previously proposed approaches. The on-body unit powers the implant through inductive coupling. After activation, the implant monitors the pressure with a piezoresistive pressure element and transmits the pressure readout to an off-body unit. In this work, we have specifically focused on investigating the impact of the thickness of the biocompatible coating on both wireless links and the influence of temperature on the piezoresistive sensor’s output.
Coronary artery disease (CAD) remains asymptomatic for decades. Conventional laboratory tests such as LDL-cholesterol are known to be poor predictors of CAD and discovering novel biomarkers are highly needed. In this project, we aim to develop a patient-specific in vitro hepatic cell model using induced pluripotent stem cell (iPSC) technology to study in a personalized manner the lipids involved in the development of high risk CAD. iPSCs were derived from skin biopsies of CAD patients and then differentiated into hepatocyte like cells (HLCs). We first gained important knowledge on the lipid profile of iPSCs as well as HLCs while investigating the role of molecular lipids in differentiation and functionality of iPSC-HLCs. The alterations in cellular lipidome as well as the expression of lipid metabolism-related genes was monitored during the entire differentiation process using mass spectrometry and qPCR respectively. Furthermore, the transcriptome of HLCs was studied by Agilent human miRNA microarray as well as Global run-on sequencing (GRO-seq). Then, five hepatic differentiation methods were studied carefully to find the most efficient and robust protocol. We successfully generated HLCs with the expected morphology and cellular functions of actual primary human hepatocytes (PHHs) suitable for studying lipid metabolism and aberrations. In the last step, HLCs are differentiated from the iPSC of three groups of acute, stable CAD, and control patients (30 iPSC lines in total) with the selected protocol. The lipidomics and genomics of generated patient-specific-HLCs will be analyzed and compared between the patient groups and new lipid biomarkers as well as miRNAs will be investigated to find reliable predictor(s) for acute CAD events. The iPSC-derived hepatocytes can be also applied for new drug discovery.
Introduction: Most celiac disease patients are undiagnosed without active screening, but long-term effects of screening remain to be proven. To clarify this issue, we compared adult patients diagnosed in childhood either because of clinical suspicion or by risk-group screening.

Methods: Questionnaires about current health and lifestyle, quality of life, disease-related symptoms, adherence to gluten-free diet and follow-up were sent to 559 adults with a childhood diagnosis of celiac disease. Diagnostic and other relevant medical data were confirmed from patient records.

Results: Altogether 236 (42%) adults completed the questionnaires. Their median age was 27.0 years and 69% were women. Celiac disease in the family (79% vs 57%, p=0.005) and associated conditions (38% vs 10%, p<0.001) were more common and current smoking (4% vs 15%, p=0.042) and membership of celiac society (38% vs 57%, p=0.019) less common in screened patients (n=48) compared to clinically detected (n=188), whereas the groups did not differ in gender, current age, work situation, presence of children, other co-morbidities, physical exercise, anthropometric measures, self-experienced health or concerns about health, symptoms, daily life restrictions, quality of life, implementation of follow-up or dietary adherence. Screened patients without symptoms at diagnosis had currently more anxiety than symptomatic children, whereas the subgroups did not differ in other current characteristics.

Conclusions: Diagnostic approach of pediatric celiac disease does not affect long-term health and treatment outcomes in adulthood, supporting early risk-group screening. However, asymptomatic children may require special attention during follow-up and transition to adult care.
Prostate cancer (PC) is the second most frequently diagnosed cancer in men worldwide. 10-20% of the PC patients develop castration-resistant prostate cancer (CRPC) that has no curative therapies. There are also no effective prognostic markers to predict emergence of CRPCs. Long noncoding RNAs (lncRNAs) are a recently found group of RNAs that are not translated into proteins. Many of them are found to be differentially expressed in cancer, and shown to have a regulative role in tumorigenesis and tumor development. In addition, some lncRNAs have been associated with cancer progression and/or survival, making them potentially interesting as prognostic markers.

Previously, we performed RNA sequencing of 28 PC, 13 CRPC and 12 non-cancerous tissue samples, out of which 145 novel PC-associated lncRNAs (PCATs) were discovered. Subsequently, the expression of 39 PCATs were analyzed in 87 samples from prostatectomy-treated PCs by qRT-PCR on Fluidigm Biomark HD, and the results were associated with clinical data. Some of the PCATs had a significant correlation with progression-free survival. One of these PCATs was also found to be a target of androgen receptor (AR) regulation. An AR binding site was discovered in the transcription start site of this novel PCAT in PC, and the binding was further validated by AR-ChIP PCR and AR-siRNA studies in PC cells. Additional studies to reveal the role of the PCAT are under way.
Gellan gum (GG) is a polysaccharide hydrogel approved for use in food and pharmaceutical industry and is cheap, transparent, stable and has highly tunable mechanical properties. However, GG is relatively bioinert, even though the cytocompatibility has been proven good, there are not enough attachment sites for cells for optimal cell response. Another interesting biomolecule, gelatin, enables cell attachment already as a low concentration coating and has been used long in cell culture systems. Nevertheless, gelatin cannot make stable macroscopic hydrogels on its own. Here we combined these two biopolymers after chemical modification, creating macroscopic hydrogels via chemical crosslinking of aldehyde-hydrazide chemistry in mild conditions. The new hydrogel has been tested to be cytocompatible with human commercial fibroblast cell line and after very promising cell elongation and spreading results, never before seen in GG, also tested with human iPS-derived cardiomyocytes. The cardiomyocyte aggregates show typical spontaneous beating behavior when cultured both on top of and encapsulated inside the hydrogel, highly indicative of cells favoring their culture substrate. The beating can be studied via video recording and analysis, making this the first step towards moving cardiac disease modelling from 2D culture surfaces to 3D cultures.
Corneal blindness is a worldwide problem, plagued by insufficient amount of high-quality donor tissue. Cell therapy using human adipose stem cells (hASCs) has risen as an alternative to regenerate scarred corneal stromal tissue, the main structural and refractive layer of the cornea. In addition to their anti-inflammatory and antiangiogenic properties, hASCs have also been shown to differentiate towards keratocytes of the corneal stroma upon implantation. Herein we propose a method to deliver hASCs in hyaluronan (HA)-based hydrogels, which form rapidly in situ with hydrazone crosslinking. We fabricated two HA-based hydrazone-crosslinked hydrogels (HALD1-HACDH and HALD2-HAADH), and characterized their swelling, degradation, mechanical, rheological and optical properties and their ability to support hASC survival. Furthermore, we incorporated human collagen I (col I) into the more stable hydrogel, with the aim to promote hASC attachment and survival. We then used an organ culture model with excised porcine corneas to study the delivery of hASCs in these hydrogels for stromal defect repair. Although all hydrogels showed good hASC survival directly after encapsulation, only the collagen-containing HALD1-HACDH-col I hydrogel showed cells with elongated morphology, and significantly higher cell metabolic activity than the HALD1-HACDH gel. The addition of col I increased the stiffness and reduced the swelling ratio of the resulting hydrogel. Most importantly, the corneal organ culture model demonstrated these hydrogels as clinically feasible cell delivery vehicles to corneal defects, allowing efficient hASC integration to the corneal stroma and overgrowth of epithelial cells.
Various diseases implement metabolic defects due to impaired Oxidative Phosphorylation in mitochondria. Diabetes Associated Protein in Insulin-sensitive Tissues (DAPIT) is a membrane-imbedded subunit of fundamental ATP synthase, which forms dimer rows in mitochondrial inner membrane leading to its bending and current form called cristae. The dimerization of ATP synthase is another regulatory level of efficiency of energy production apart from the synthase enzymatic activity to produce ATP. Our cell model over-expressing DAPIT impairs Oxidative Phosphorylation by inactivating ATP synthase activity. This leads to increased formation of reactive oxygen species (ROS) and cells relying on glycolysis for fulfilling their energy demand. The cells underwent Epithelial to Mesenchymal Transition (EMT) but passivated in proliferation and migration.

In our understanding, the characteristics of DAPIT over-expressing cells resemble Cancer Stem Cells. Therefore, we studied pluripotency markers and cell differentiation in basic culture and on matrigel by immunofluorescence and microscope approach. The results showed that Oct3/4, Nanog, Sox2 and c-Myc were expressed in cell nuclei, and e.g. sphere, pigment, tube-like differentiation and an uncharacterized organoid-like structure were seen. In matrigel, DAPIT over-expressing cells implemented slowly growing, firm and definable colonies. We also studied Gene Omnibus (GEO) database and found up-regulated expression of Usmg5 (gene encoding DAPIT) in induced pluripotent cancer stem cells reprogrammed from prostate cancer associated stromal cells, EphB2low -population of intestinal stem cells, Non-small cell lung cancer H460-derived stem cells, in sphere vs. monolayer of neuroblastoma SKNAS induced stem cells and CD133- glioblastoma-derived stem cells.

Our results and GEO database suggests that DAPIT over-expression could be involved in metabolic regulation of a variety of cancer stem cells.
Retinal pigment epithelium (RPE) underlying the retina in the back of the eye is essential for our vision. Ion channels of the RPE play important roles for several retinal maintenance functions including phagocytosis, secretion, and epithelial transport. Disruption of the normal RPE functionality often leads to degenerative diseases of the retina causing visual impairment and even blindness. New transplantation therapies utilizing human embryonic stem cell (hESC)-derived RPE show great promise to treat these diseases. However, functionality of the RPE needs to be verified for the success and safety of the transplantation therapies. The presence of ion channels, and especially various chloride (Cl-) channels, in hESC-derived RPE is still poorly known. Therefore, we investigated the Cl-conductivity of hESC-derived RPE by whole-cell patch clamp recordings and the ion channel localization by immunolabeling the Cl- channels typical to RPE. Our patch clamp recordings revealed a diverse pattern of slowly inactivating currents with characteristics typical to voltage-dependent Cl-channels (CIC). Similar currents have previously been reported for cultured and native RPE. Furthermore, changes in intracellular calcium concentration modulated the peak value in some of the identified currents, indicating their calcium-dependency. The electrophysiological data, together with the immunolabeling, showed the presence of bestrophin-1, cystic fibrosis transmembrane regulator and CIC-2 channels in hESC-derived RPE. Importantly, our findings suggest the capability of hESC-derived RPE to mimic native Cl- physiology that is promising for the cell transplantation therapies.
Typically, living cells are cultured inside an incubator with atmospheric oxygen concentration (~20%). However, this oxygen concentration is not physiologically relevant for most of the human tissues in vivo (e.g. for brain tissue from 0.5% to 7%). Moreover, it has been shown that low oxygen conditions can promote growth and influence differentiation of stem cells in vitro. Therefore, it is important to develop tools to study cells in these biomimetic conditions. Here we demonstrate the functionality of our portable mini-incubator for hypoxia studies. Truly hypoxic conditions can be maintained similarly as inside a commercial hypoxia chamber, which was shown with HIF-1α induction of cells. The same system can also be utilized for prolonged MEA and microscopy experiments either in hypoxia conditions or on normal 5% CO2 conditions.
Prostate cancer (PCa) research suffers from lack of suitable cell models for primary cancer. Our goal is to develop sequential cell models mimicking natural prostate carcinogenesis using a normal epithelial cell line and the CRISPR method. So far, we have re-established androgen receptor (AR) expression to the prostate epithelial cell line RWPE-1 using lentiviruses. This is crucial as almost all primary PCas arise from the AR expressing epithelial cell populations and remain AR dependent and/or driven throughout the cancer progression. Next, we are planning to engineer TMPRSS2:ERG gene fusion to these cells with CRISPR by deleting the genomic region between the genes. TMPRSS2:ERG is the most common genetic alteration in primary PCa and occurs early on during the progression. As a consequence of the fusion, androgen responsive TMPRSS2 promoter drives overexpression of transcriptional regulator ERG that works in co-operation with other transcriptional master regulators remodeling their cistromes and driving changes to gene expression. The cancer signature and phenotype is further amplified by the deletion of tumor suppressor PTEN, which is the second alteration we are planning to make with CRISPR. With these models, we will be able to further study the biology of tumors with the most common genetic backgrounds and for example to screen for effective therapies in these genomic settings. In addition, we hope that these models could be adopted as a public resource for the functional studies of primary PCa in general.
The cytoplasmic protein talin cooperates with kindlin to activate integrins. The F3 subdomain of the talin head binds the integrin β cytoplasmic tail, and these interactions are already well characterized. How the rest of the talin head contributes to integrin activation is, however, less clear.

Talin head contains a flexible loop within its F1 subdomain. There is currently no structural evidence for how the loop is positioned in the complete talin head, and we therefore took to studying its structure and function by protein modeling. We utilized long, atomistic molecular dynamics simulations of the talin head, in complex with integrin αIIbβ3 embedded in a lipid bilayer, to analyze the protein-protein and protein-lipid interactions. Integrin clustering and activation was studied using cell assays, and specific structural features were studied by biophysical characterization.

Our results suggest a novel mechanism of integrin activation by talin, and highlight the importance of kindlin in the process.
Thymic stromal lymphopoietin (TSLP) and interleukin (IL)-7 are related cytokines that mediate growth and differentiation events in the immune system. TSLP and IL-7 signal through distinct receptors that share the IL-7Rα subunit. TSLP has been shown to have a role in allergic type 2 immune responses. Target cells of TSLP in these type 2 responses include CD4 T cells and dendritic cells (DCs). In this study, we show that freshly isolated murine splenic DCs are unresponsive to TSLP, indicated by their failure to phosphorylate STAT5, but in vitro overnight culture, especially in presence of IL-4, renders DCs responsive to both TSLP and IL-7. IL-4 is a key regulator of humoral and adaptive immunity during type 2 responses. The induced responsiveness to TSLP and IL-7 is accompanied by dramatic upregulation of IL-7Rα on DCs with little change in expression of TSLPR or of γc. In splenic DCs, the induction of IL-7Rα occurs mainly in CD8+ DCs. In vivo, we found that IL-4 has a differential regulatory role on expression of IL-7Rα depending on the cell type; IL-4 decreases IL-7Rα expression on CD4 T cells whereas it upregulates the expression on DCs. Our results indicate that the induction of IL-7Rα expression on DCs is critical for TSLP responsiveness and that IL-4 can upregulate IL-7Rα on DCs.
Post-translational modifications, like proteolysis, phosphorylation and glycosylation, are involved in maturation of wide variety of proteins. Many of them are cleaved into active form by proprotein convertases. Furin is one of the most ubiquitous proprotein convertases, having target proteins in nearly all cell types, including T cells, where its activity is essential e.g. for maintenance of immune tolerance. In T cells, enzymatic activity of glycosyltransferase Gnt-VA is rate-limiting for production of beta-1,6-GlcNAc-branched N-glycans. In case of normal T cells, galectin binding to Gnt-VA-modified N-glycans inhibits spontaneous TCR oligomerization. In Gnt-VA-deficient cells, TCRs tend to form clusters reducing the requirement for CD28 co-signalling, which in turn may lead to T-cell activation even in absence of antigen. This can further cause loss of immune tolerance and development of variety of autoimmune diseases.

The aim of this study is to investigate whether proprotein convertase Furin has a role in the cleavage and activation of Gnt-VA. To investigate if predicted cleavage site of Gnt-VA is target of Furin cleavage, lysates of Furin-deficient RPE.40 cells producing either transfected human Furin, Gnt-VA or both are analyzed with Western Blot. Flow cytometry is used to measure difference in L-PHA binding to cell surface glycoproteins of similarly transfected Jurkat T cells and will give information whether this cleavage is increasing or reducing the amount of Gnt-VA N-glycosylation products in cells. Findings of the study will give insight of molecular dynamics involved in T cell autoreactivity and in the mechanisms preceding development of autoimmune diseases.
To fully understand the output of alterations in cancer genomes and transcriptomes, we need to know how these aberrations are translated into the functional protein units in cells. We assessed proteomic changes during disease formation and progression in prostate cancer by performing high throughput mass spectrometry on clinical tissue samples of benign prostatic hyperplasia (BPH), untreated primary prostate cancer (PC) and castration resistant prostate cancer (CRPC). With SWATH-MS quantitation-based proteomics we found that each of these sample groups show a distinct protein profile. By integrative analysis of this mass spectrometry dataset with genetic, epigenetic, and transcriptional data from the same samples, we show that, especially in CRPC, gene copy number, DNA methylation, and RNA expression levels do not reliably predict proteomic changes. From our analysis, we have identified sets of novel expression changes occurring primarily at the protein level, in addition to identification of several miRNA - target correlations present at protein but not at mRNA level. We find novel expression changes in previously unrecognized pathways in prostate cancer that are likely to affect disease development and progression. For example, we identify two metabolic shifts in the citric acid cycle (TCA cycle), one occurring during primary cancer development and the second during castration resistance, having implications on drug targeting against cancer metabolism. Our proteogenomic analysis of prostate cancer uncovers robustness against genomic and transcriptomic aberrations during disease progression, reveals new disease mechanisms, and significantly extends understanding of prostate cancer biology.
Leukemia is the most common pediatric cancer with around 50 children diagnosed with leukemia in Finland every year. T-cell acute lymphoblastic leukemia (T-ALL) is rather rare type of leukemia in children but it has less favorable outcome and the prognosis of relapsed T-ALL is dismal.

By using in silico drug screen, cell lines and patient samples, we recently showed that 27% of T-ALL patients are sensitive to dasatinib, a tyrosine kinase inhibitor approved for treatment of BCR-ABL1-positive leukemias (Laukkanen et al. 2017). Our data also indicated that tyrosine kinase LCK is the main target of dasatinib in T-ALL, and that patients in TAL1 subgroup of T-ALL are most likely to respond to dasatinib treatment.

To gain better understanding about the mechanism of action of dasatinib and its effect in combination with traditional leukemia drugs, we have set up a zebrafish T-ALL model, originally published by Langenau et al. 2003, that is based on overexpression of a mouse MYC gene. This zebrafish model mimics human TAL1 subtype of T-ALL. Functional domains of LCK are highly similar between human and zebrafish, and LCK is highly expressed in this model.

Approximately 5% of the zebrafishes injected with mMyc at one cell stage developed leukemia. These leukemic zebrafishes were sacrificed, over 100 million leukemic cells were harvested and frozen in DMSO. We intend to transplant these cells into pigmentless, immunodeficient casper-prkdc-/- and casper-rag2-/- recipients, which are a courtesy of David Langenau from Harvard University. Zebrafishes with successful T-ALL engraftment will be exposed to dasatinib alone and in combinations with other drugs.
60. Laurikka, Pilvi: Dietary Factors and Mucosal Immune Response in Celiac Disease Patients Having Persistent Symptoms Despite a Gluten-free Diet (Poster)
laurikka.pilvi.l@student.uta.fi, University of Tampere

List of all authors
Pilvi Laurikka (a), Katri Lindfors (b), Mikko Oittinen (c), Heini Huhtala (d), Teea Salmi (e), Marja-Leena Lähdeaho (c), Tuire Ilus (f), Markku Mäki (c), Katri Kaukinen (g), Kalle Kurppa (c)

Introduction: For unexplained reasons, many treated celiac disease patients suffer from persistent gastrointestinal symptoms despite a strict gluten-free diet (GFD) and recovered intestinal mucosa. We investigated the role of dietary factors, distinct small-bowel mucosal immune cell types and epithelial integrity in the perpetuation of these symptoms.

Methods: We compared clinical and serological data and mucosal recovery in 25 symptomatic and 22 asymptomatic celiac patients on a long-term GFD. The density of CD3+ and γδ+ intraepithelial lymphocytes (IELs), CD25+ and FOXP3+ regulatory T cells and CD117+ mast cells, and the expression of tight junction proteins claudin-3 and occludin, heat shock protein 60 (HSP60), interleukin 15 (IL-15) and Toll-like receptors (TLR) 2 and 4 were evaluated in duodenal biopsies.

Results: All subjects kept a strict GFD and had negative celiac autoantibodies and recovered mucosal morphology. The patients with persistent symptoms had lower mean fiber intake (15.2 vs. 20.2 g/day, p=0.028) and density of CD3+ IELs (45.0 vs. 59.3 cell/mm, p=0.045) than asymptomatic patients. There was a similar but non-significant trend in γδ+ IELs (13.5 vs. 17.9, p=0.149). There were no differences between the groups in other parameters measured.

Conclusions: Low fiber intake may predispose to persistent symptoms in celiac disease. The results do not support the idea of innate immunity, epithelial stress or altered epithelial integrity having a marked role in the development of persistent symptoms. A higher number of IELs in asymptomatic subjects may indicate that the association between symptoms and mucosal inflammation is more complicated than previously thought.
With bright field microscopy we can acquire high-resolution images from cell cultures for automatic cell detection. Fluorescence imaging is commonly used in cell detection, but it is not well suited for imaging cell culture for consecutive days due to e.g. the phototoxic effect. It also requires special equipment and separate dark premises. Bright field imaging is a non-invasive method used by conventional cell culture laboratories. In this research we used fully convolutional neural networks to detect cells from bright field z-stacks. One z-stack consists of 25 focal planes with 10µm separation in focal distance and focal range of 240µm. We used only PC-3 cancer cell line for training various models and then tested their accuracy in detecting LNCaP, 22Rv1 and BT-474 cell lines. We used models that combine all or just a part of the focal planes with 3-dimensional convolution. We also trained a model for each focal plane separately so we could find out which planes have similarities between PC-3 and the cell line for testing. Our results show that PC-3 and LNCaP are quite similar and good results are acquired with z-stacks for both lines. BT-474 and 22Rv1 are much harder to detect. They grow in dense populations, and their profiles in z-stacks differ from PC-3 in images highly out-of-focus. However, they are similar in some of the focal planes, so we can acquire quite good results by training a model with PC-3 using only these planes.
Background: More systematic use of evidence-based brief therapies is needed in the treatment of depression within psychiatric care. The aim of this study was to explore the impact of behavioral activation (BA) therapy for patients with depressive symptoms and various comorbidities in a routine clinical setting of secondary psychiatric care.

Methods: The BA-treated intervention group (n = 242) comprised depressed patients with non-psychotic comorbidities. The Montgomery–Åsberg Depression Rating Scale was used to assess depressive symptoms in the intervention group. The control group (n = 205) patients received treatment as usual in the same catchment area. The groups were matched at baseline using Beck Depression Inventory scores, Alcohol Use Disorders Identification Test scores and inpatient/outpatient status. The groups were compared at 6-, 12- and 24-month follow-up points on functional outcome (Global Assessment of Functioning scale), service use, dropout and deaths.

Results: In the intervention group, depressive symptoms were systematically and significantly alleviated (pre–post effect sizes at three follow-up points: 1.02, 1.39 and 1.78, respectively). The intervention group showed a greater improvement in functional outcome (p < 0.001 at all follow-up points, effect sizes 0.27, 0.33 and 0.48, respectively). The rates of dropout, mortality and service use were similar between the groups.

Conclusions: BA is a useful treatment for heterogeneous groups of depressive patients in psychiatric secondary care and is superior to treatment as usual in terms of functional recovery. The systematic use of BA could provide more structured treatment for a greater number of patients.
Bioactive glasses are in clinical use in many bone-related applications. The main advantage of these materials comes from the ease of introducing any ions having potential therapeutic effects and controlling their release in the medium. However, little is known about their reactions with soft tissue. In this study, human adipose stem cells (hASC), human lung fibroblasts and urethral epithelium cells were cultured for 14 days in mediums based on 13-93 bioactive glass extracts doped with lithium, strontium or boron. The cell viability, proliferation and phenotype were studied and the ion concentrations in the extract-based mediums were quantified using ICP-OES. The initial results show that the hASC and the fibroblasts remain viable in the extracts and the 13-93 and Li-doped glasses perform as well as the basic medium with hASC. According to the live-dead images, the other glasses slow down the cells proliferation, but these results will be confirmed with the quantitative analysis. Culturing the hASC and fibroblasts in the boron-containing extract seems to increase the cell size. The extracts had a very distinct effect on the morphology of the urethral epithelium cells, especially the Li-containing extract resulted in a very different cell morphology compared to the reference medium. This is most likely due to the high calcium concentration in the extract, which is not well tolerated by epithelial cells.
FURIN is an ubiquitously expressed serine endoprotease which converts immature proproteins into functional mature end products. In cell, this enzyme is predominantly localized in the secretory pathway, but part of the FURIN pool circulates between TGN, endosomes and the cell surface. In vitro studies have suggested that FURIN can cleave several proteins known to be essential players in the regulation of the immune response. In addition, this enzyme is a key modulator of the secretory pathway, which is poorly understood in several cells types of the immune system including myeloid cells. Therefore, a better comprehension of the role of FURIN concerning the production of effector proteins in myeloid cells will contribute to uncover essential effector mechanisms involved in the immune response.

The aim of this study is to decipher the role of FURIN in the production of effector proteins in myeloid cells through a comprehensive characterization of the FURIN dependent secretome. To this end, we will apply Mass-Spectrometry technology to identify the proteins differentially secreted by a pro-monocytic human myeloid leukaemia cell line (U937) that have been in vitro differentiated into macrophage and stimulated with TLR ligands and cytokines in the presence or absense of FURIN inhibitor. In addition, using a mouse model with a conditional deletion for FURIN in myeloid cells, we will compare the ex vivo macrophage secretome profile of unstimulated and TLR ligands/cytokines stimulated FURIN deficient myeloid cells and Wild type myeloid cells. The putative proteins identified will be subjected to in vitro functional studies.
Should we cure malaria? It is unpleasant and eventually kills you. -Of course we should. Should we cure ageing? It is unpleasant and eventually kills you. -Of course not! Ageing is a natural part of life.

Research aimed at curing cardiovascular diseases, cancer and neurodegeneration is taken for granted. Curing ageing is a much more controversial topic, even though ageing is the greatest risk factor for the diseases mentioned. The molecular mechanisms that drive the ageing process are the same mechanisms that contribute to the development of malignant growth and dysfunction of tissues. From the perspective of an ageing researcher, it would be more efficient to manipulate the ageing process itself, not to focus on manipulating the individual manifestations of ageing one by one.

Should cancer, neurodegeneration and cardiovascular diseases be eradicated, the end results would be quite close to curing ageing, as no one has ever died of healthy ageing. This however is often forgotten when criticising research aimed at manipulating ageing or extending lifespan.

On the other hand, if we are able to decide whether we should manipulate ageing, the next question is can we? In order to answer this question, we must understand the proximal reason for ageing, i.e. why is the force of evolution driving species towards ageing instead of immortality.

I wish to stimulate discussion on these concepts and theories underlying biomedical ageing research, which are also of interest to all who age.
The rapidly growing field of single cell analytics seeks to answer questions about cellular heterogeneity in various diseases and developmental processes and can lead to the discovery of novel cell subtypes and stages. We studied the cellular heterogeneity of four acute myeloid leukemia (AML) bone marrow (BM) samples using the 10x Genomics Chromium 3’RNAseq analysis platform. The mononuclear fraction of each AML patient’s BM sample was cryopreserved enabling parallel analysis with the 10x Genomics Chromium platform with 4000 cells captured per sample. The Illumina ready sequencing libraries were prepared according to the 10x Genomics instructions and the sample libraries were sequenced in Illumina HiSeq 2500 platform aiming for sequencing depth of 50 000 reads per cell. The Cell Ranger v1.3 mkfastq and count analysis pipelines (10x Genomics) were used to demultiplex and convert Chromium single cell 3’ RNA-sequencing barcode and read data to FASTQ files and Seurat pipeline was used to generate align reads and gene-cell matrices. The estimated cell capture amounts ranged between 2562 and 4156 cells and median detected genes between 1362 up to 1992 genes per cell. Graph based clustering of the four AML samples showed clear separation between the blast populations originating from the different AML samples. Interestingly the T-cells from different patients seemed to cluster together indicating cell type specific overlap in cellular expression profiles. Single cell transcriptomics enables us to study cell-to-cell variation in transcriptional profiles and comparative RNA contents in AML.
Phosphate glasses within the composition 50P2O5-20CaO-20SrO-10Na2O (Sr50) react in biological media and form a dicalcium phosphate di-hydrate (DCPD) layer at their surface. They also promote gingival cell attachment and proliferation. Doping Sr50 with Fe, Cu and Ag helps in tailoring the glass dissolution rate while conferring for instance antimicrobial properties. However, the cell proliferation was much slower at the surface of these glasses compared to typical silicate bioactive glasses. Furthermore, when using human adipose stem cells, cells grew in the vicinity of the glass but not at its surface, due to the comparatively fast degradation rate.

It is expected that cells don’t interact with the biomaterials surface but rather with a dynamic protein (mono)layer attached on the surface. Therefore, to enhance the bioactivity of these glasses, surface washing followed by surface silanization was tested to promote protein adsorption. 3-aminopropyltriethoxysilane (APTS) was chosen for silanization as it has been shown to interact via ionic interactions with DNA.

The impact of surface treatment on the glass surface chemistry was studied with contact angle measurement and FTIR, and compared to modifications occurring at the surface of silicate bioactive glasses, used as reference. To confirm the presence of proteins (Albumin and Fibronectin), fluorescence microscopy was performed on the glass surface. Enhancement of protein adsorption was found to depend on the pH of the solution used to wash the glass surface, the presence of a APTS layer, and the protein type. Tailored protein-decorated bioglasses may allow more efficient biocompatible substrates for applications such as implanting.
The androgen receptor (AR) signaling pathway is central to the emergence of castration-resistant prostate cancer (CRPC). miR-32 is an androgen regulated miRNA which is differentially expressed in CRPC compared to benign prostatic hyperplasia (BPH) and able to provide a significant growth advantage to LNCaP cells.

To study how increased miR-32 expression contributes to prostate cancer formation and/or progression in vivo, we have established transgenic mice expressing miR-32 specifically in the prostate epithelium post-puberty. With this model we have found that miR-32 overexpression promotes replicative potential in prostate epithelium and increases incidence of goblet cell metaplasia in the prostates of aged mice.

To study the effect of miR-32 expression in prostate cancer, the miR-32 mice were cross-bred with mice overexpressing oncogenic Myc in the prostate. At 6 months, when the mice have developed adenocarcinoma, the prostates and tumors of Myc-overexpressing mice were significantly larger when miR-32 transgene was present. This indicates that miR-32 expression promotes tumor growth in high Myc expressing background. We have further analyzed markers for replication, mitosis, and apoptosis in the tumors, and the results of these experiments are presented.

Our data show that miR-32 is able to affect replication potential of prostate epithelium and promote prostate tumor growth in Myc-driven prostate cancer.
Mechanical signals are essential for biological function, however, the mechanisms associated with mechanosignaling are poorly understood. Mechanical stability is a key feature for the regulation of functions of structural scaffolding proteins. One such protein is talin, which physically connects the intracellular cytoskeleton with the extracellular matrix at focal adhesions. Mechanical stretching of talin regulates its function by exposing buried binding sites for certain binding partners. Talin is a large protein that consists of an N-terminal head domain, and a rod domain, which consists of amphipathic α-helixes arranged into 4- or 5-helix bundles. The rod domain contains 13 helix bundles (R1–R13) arranged into a chain, and a single helix at the C-terminal end of talin.

In this study, we used a combination of atomistic steered molecular dynamics (SMD) simulations and single-molecule atomic force microscopy (smAFM) to investigate unfolding mechanism of talin rod. SMD simulations revealed that talin rod bundles unfold through stable 3-helix intermediates. The 5-helix bundles were most stable in our experiments, and showed second stable conformation corresponding to 3-helix state. As it was shown in our previous study, 4-helix bundles are weaker than 5-helix, however they also were unfolded through 3-helix conformations. Similarly, smAFM experiments were carried out, and they support the SMD findings.

The biological significance of the 3-helix intermediate remains to be discovered in full extent, but previous studies indicate that it may serve as a binding epitope for protein partners at least in the case of talin R3.
Roughly one third of the human population carries a latent Mycobacterium tuberculosis infection with a 5-10 % lifetime risk of reactivation to active tuberculosis (TB). The mechanisms leading to the reactivation of a latent M. tuberculosis infection are insufficiently understood. We have used a natural fish pathogen Mycobacterium marinum to model the reactivation of a mycobacterial infection in the adult zebrafish (Danio rerio). A latent infection was reactivated by an oral treatment with the immunosuppressive drug, dexamethasone, which led to increase in the number of granulomas, disruption of the granuloma structures and dissemination of bacteria. This was associated with the depletion of lymphocytes, especially CD4+ T cells, which resembles the situation in TB-HIV-coinfected individuals. Using this model, we verified that ethambutol is effective against an active disease but not a latent infection. In addition, we screened 15 novel mycobacterial antigens as post-exposure DNA vaccines against reactivation of a latent mycobacterial infection. Of these, RpfB, MMAR_4207 and IprG reduced bacterial burdens upon reactivation, as did the Ag85-ESAT-6 combination. In conclusion, the adult zebrafish - M. marinum infection model provides a feasible tool for examining the mechanisms of reactivation in mycobacterial infections, and for screening vaccine and drug candidates that could improve the control of TB infection also in the immunocompromised.
We present here a modular cell cultivation platform that provide a stable, cell-friendly environment for long-term cell culturing outside an incubator. The platform includes online precise temperature control together with a cell imaging, image analysis, and oxygen measurements. Our platform can also provide hypoxia conditions to the cell cultures. We use beating cardiomyocytes to demonstrate the capability of the platform in two experiment, where we study the relationship between the beating rate and 1) culture temperature, 2) hypoxia conditions. For the further use, the modularity of the system provides flexibility to easily change system set-up to meet the requirements of the physiological study.
TRPA1 is a cation channel expressed mostly in non-myelinated nerve endings. TRPA1 has a significant role in sensing chemical and mechanical pain and, according to the more recent findings, also in inflammation. We have recently shown that pharmacological blockade and genetic deletion of TRPA1 alleviates inflammation and pain in murine models of gout and (osteo)arthritis. Furthermore, TRPA1 is expressed in human articular chondrocytes and synovial cells in inflammatory conditions, and mediates inflammatory and catabolic responses in vitro.

Triterpenoids are naturally occurring molecules, which have been discovered to have anti-inflammatory and anti-cancer properties. In the present study, we synthesized a series of derivatives of the triterpenoid betulin (which is a bioactive molecule from birch bark) and investigated their effects on TRPA1.

In the initial screening based on Fluo 3-AM intracellular Ca2+ measurements, five of the fourteen tested triterpenoids had a statistically significant blocking effect on TRPA1 at 10 µM concentration. In the further studies, the two most potent compounds (RHI30 and RHI170) were found to have dose-dependent, reversible and voltage-dependent blocking effects on TRPA1 at submicromolar concentrations based on whole-cell patch clamp recordings. Interestingly, the TRPA1 antagonistic activity of these two triterpenoid derivatives was also translated to in vivo, as RHI30 and RHI170 significantly attenuated TRPA1-mediated acute inflammatory paw edema in mice.

The results introduce pyrazine-fused triterpenoid derivatives as effective novel blockers of TRPA1 with lead potential for treatment of TRPA1 mediated adverse conditions, such as arthritis and arthritis-related pain.
Coxsackieviruses (CVs) are single-stranded RNA viruses belonging to the Enterovirus genus in the family Picornaviridae. Enterovirus infections are usually asymptomatic, but sometimes these viruses could cause even severe diseases like myocarditis and hepatitis. To find out molecular mechanisms how enteroviruses cause diseases, we have made recombinant proteins from two CVB3 enteroviral proteases 2A and 3C and studied their properties. These two proteases carry out the post-translational proteolytic processing of the viral polyprotein. Moreover, they also cleave several host-cell proteins which advance production of new virus particles, and also helps to avoid the cellular antiviral immune responses. We have optimized the protein expression of these proteases in E. coli and measured their cleavage kinetics with synthetic FRET peptide. In addition, we have also expressed several other 2A proteases from various enterovirus strains and compared how their kinetics differ. This system makes it possible to screen possible inhibitors for virus proteases, which could then be used in antiviral therapy.
Pediatric CT imaging offers significant benefits in clinical practice. However, children are more sensitive to carcinogenic effects of ionizing radiation than adults and red bone marrow is especially radiosensitive tissue type. There exists a need to assess the risks of low doses of ionizing radiation with a direct approach. We assessed the leukemia risk in children after computed tomography imaging studies with high-quality Finnish data.

We used nationwide, register-based case-control study design. We identified all childhood (0-15 years) leukemia cases from 1990 to 2011 (N=1093) in Finland and randomly selected thrice as many controls (N=3279) from the Population Registry. The cases were 81% (N=885) acute lymphoblastic leukemias and 13% (N=142) acute myeloid leukemias. We collected data on all pediatric CT scans from 1975–2011 from the databases of the ten largest hospitals in Finland. In total, we identified 49 CT scans to our subjects. We approximated that this approach covers 90% of pediatric CT scans performed in Finland from 1975 to 2011.

Overall, 10 cases (1.0%) and 10 controls (0.3%) had a record indicating at least one CT examination. In a conditional logistic regression analysis adjusted for birth weight, a significantly increased leukemia risk (OR=4.01, 95% CI 1.39, 11.5) was found for any CT examination (one or more) at least two years prior to leukemia diagnosis and for two or more CT examinations the OR was 14.0 (95% CI 1.62, 120). For one or more CT scans of the brain we observed an OR of 4.79 (95% CI 1.56, 14.7).
Fluoride containing glasses have been investigated since the 1970’s; they have become glasses of interest due to fluorides ability to enhance their optical properties allowing them to be suitable for a wide range of applications from lasers to fibre optics. The combination of oxygen and fluorine has gained interest in recent years, within the biomedical field, fluorine addition has shown to yield the precipitation of fluorapatite, which offers enhanced bioactive properties compared to fluoride free equivalents. Additionally, the combination of O and F alters the crystallisation and dissolution processes of the glass, while maintaining the optical properties of the glass within the UV spectral range.

Here, we have produced glass compositions in the range (80NaPO3-(20-x) CaO-xCaF2) (in mol%), with x=0, 5, 15 and 20. The transparency range of the glasses, as a function of fluoride content was assessed by UV-Vis-NIR spectroscopy. The thermal properties along with the crystallization kinetics of the glasses were assessed using non-isothermal DTA analysis and the activation energy for crystallization and Johnson Mehl Avrami exponent were quantified. The glasses degradation rate and ionic release in TRIS buffer solution were assessed and related to the materials bioactivity. Our results show that these glasses enable controlled precipitation of crystals within the glass bulk. These glass-ceramics are not only promising for medical applications but can also be employed within the photonics field.
List of all authors
E. Nummenmaa1, M. Hämäläinen1, LJ. Moilanen1, E-L. Paukkeri1, RM. Nieminen1, T. Moilanen1,2, K. Vuolteenaho1 and E. Moilanen1

Objectives: Transient receptor potential ankyrin 1 (TRPA1) is a membrane-associated cation channel which has primarily been studied in neurons, where it is known to be involved in nociception and neurogenic inflammation. We have recently shown that TRPA1 is also functionally expressed in human OA chondrocytes, where it mediates inflammatory and catabolic responses. In the present study we investigated the effects of anti-inflammatory drugs on the expression of TRPA1 in human chondrocytes.

Methods: Human chondrocytes were cultured with IL-1β alone and together with dexamethasone (0.01-10μM), aurothiomalate (3-30µM) or ammonium pyrrolidinedithiocarbamate (PDTC; NF-κB inhibitor, 100µM), after which the mRNA and protein expression of TRPA1 was measured by qRT-PCR and Western Blot, respectively, and the functional downregulation of TRPA1 was confirmed with Ca2+-influx measurements.

Results: Dexamethasone and aurothiomalate inhibited TRPA1 mRNA and protein expression in human chondrocytes while other tested anti-inflammatory compounds (methotrexate, sulfasalazine, hydroxychloroquine and ibuprofen) had no effect. The downregulation was functional: TRPA1-mediated Ca2+-influx was enhanced in chondrocytes cultured with IL-1β alone, and that effect was reversed in cells cultured with IL-1β and dexamethasone or aurothiomalate. NF-κB inhibitor PDTC also downregulated TRPA1 expression. This, together with previous reports showing that dexamethasone and aurothiomalate inhibit NF-κB, suggests that these drugs may exert their effects on TRPA1 expression via inhibition of NF-κB.

Conclusions: These results show a previously unknown mechanism of action for dexamethasone and aurothiomalate via functional downregulation of TRPA1 in human chondrocytes, and thus provide a novel concept for the development of drugs for (osteo)arthritis with analgesic and disease modifying properties.
StructureMapper is a high-throughput algorithm that enables automated mapping of protein primary amino sequence locations to existing three-dimensional protein structure data. The algorithm is intended for facilitating easy and efficient utilization of structural information in protein characterization and proteomics. StructureMapper provides an analysis of the identified structural locations that includes surface accessibility, flexibility, protein-protein interfacing, intrinsic disorder prediction, secondary structure assignment, biological assembly information, and sequence identity percentages, among other metrics. We have showcased the use of the algorithm by structurally profiling post-translational modification sites and identifying putative, novel phosphoswitches, as well as showing that different proteases have different preferences in terms of the structural properties of their substrate cleavage sites. The StructureMapper algorithm is available as a downloadable standalone Python implementation as well as an online service at http://structuremapper.uta.fi
Tuberculosis is an epidemic bacterial disease caused by Mycobacterium tuberculosis. Susceptibility to tuberculosis depends on the M. tuberculosis strain and on a number of host factors; environmental conditions, personal health status as well as genetic variation. Lectins are carbohydrate-binding proteins important in several biological processes such as host defense. Intelectins are a lectin sub-group shown to specifically bind microbial carbohydrates and to agglutinate bacterial species including Escherichia coli. Also, the expression of several intelectin genes is substantially modified in mycobacterial infection. In this study, we aim to reveal by fluorescent microscopy and by fluorescence-activated cell sorting if recombinant zebrafish Intelectins (Itln1, Itln2, Itln3) are able to bind mycobacteria in vitro. In addition, in order to study Intelectins in vivo, we use Mycobacterium marinum infection in zebrafish to model tuberculosis and combine it with two important reverse genetic tools; the CRISPR/Cas9 mutagenesis method and the morpholino oligonucleotide silencing. So far, we have mutated itln3 in zebrafish with the CRISPR/Cas9 system, and we are in the process of characterizing itln3-/- mutants in M. marinum infection. In these studies, the resistance to mycobacteria is determined with survival experiments and by quantifying mycobacterial loads with qRT-PCR after an infection. Host response is evaluated by measuring gene expression of important immunological genes such as tnfα and il1b and by comparing blood cell populations by flow cytometry. We believe that our work will provide important insights about the capability of Intelectins to bind mycobacteria and about their in vivo role in the immunity against mycobacterial infection.
Additive manufacturing is a powerful technique enabling the production of highly organized structures with exact precision and tailored components. It is becoming increasingly popular also in the field of tissue engineering (TE), providing an attractive strategy to engineer tissues for widespread applications. The aim of this study was to exploit the extrusion-based 3D bioprinting approach to create functional structures for the expanding needs of bone TE. Based on initial optimization of stability and printability, a hydrogel system based on 5 wt-% gelatin and 4 wt-% alginate was used in the experiments. In addition, 0.25 wt-% TEMPO-oxidized cellulose nanofibers and/or 1 wt-% bioactive glass particles (47.12SiO2-6.73B2O3-6.77CaO-22.66Na2O-1.72P2O5-5MgO-10SrO mol-%) were included to provide osteogenic cues for the cells. Human osteosarcoma cells (Saos-2) were mixed with the gels and 4-layer structures with parallel 0.51 µm diameter strands, in 90° angles between the layers, were printed, cross-linked with CaCl2 and cultured for 14 days. Cell viability, proliferation as well as alkaline phosphatase (ALP) activity in the printed structures were evaluated. Cells remained viable in the glass-free gel compositions throughout the culture. However, in the presence of bioactive glass, the viability of cells was decreased as indicated by live/dead staining and increased release of lactate dehydrogenase. Apart from the glass-containing gels, cell proliferation increased throughout the culturing period. There was also a constant increase in the ALP activity with all the gels, indicating good osteogenic activity of the cells. The proof-of-concept demonstrated in this study provides a good basis for further optimization of this bioprinting setup.
Acute lymphoblastic leukemia (ALL) is the most common childhood leukemia. It comprises of multiple subtypes with combinations of chromosomal rearrangements, indels and copy number variations. Furthermore, there is a significant number of somatic mutations located outside of protein coding regions of the genome.

To study these changes in DNA, we have assayed eight ALL patient samples, both diagnosis and remission states, using whole genome sequencing (WGS). Furthermore, we measured transcriptional activity from 12 patients and eight cell lines, using Global Run-on sequencing (Gro-seq).

So far, we have sequenced one T-ALL and seven pre-B ALL patient samples with different subtypes. In summary, we found 14 translocations, 55 deletions and one inversion across the all pre-B ALL samples. Of these, eight translocations, 28 deletions and the inversion hit some gene. The rest of the variations occurred in intergenomic regions of the genome. On further analysis of T-ALL sample we found that structural variations activated oncogenic transcription factors (LMO2, TAL1) by changing the regulatory context of the gene loci. We also found that patient had a deletion at the TAL1-STIL locus from one parental chromosome, complete deletion at the CDKN2A/2B locus and a complex translocation involving the LMO2 locus (chr11), TCR gene region (chr7) and a centromeric region of chr22.

We are developing a genome-wide approach to link structural variation events with gene regulatory features in ALL cells. This extends previous approaches that have mainly focused on genetic changes at coding regions.
Antigen emergence rapidly stimulates T cells leading to changes in cytokine production, cell proliferation, and differentiation. Some of the key molecules involved in these events, such as TGF-b1 and NOTCH1, are synthesized initially as inactive precursors and are proteolytically activated during T cell activation. Proprotein maturation is regulated by enzymes catalyzing the proteolytic cleavage of their substrates. The prototype proprotein convertase FURIN is induced upon TCR activation, and its expression is upregulated by the Th1 hallmark cytokine IL-12. FURIN is critical for the maintenance of peripheral immune tolerance.

Recently, Vahedi et al. investigated super-enhancers in mouse CD4+ T cells to identify genes associated with the different T helper cell subgroups. Interestingly, in Th1 cells, FURIN was one among the genes showing the highest association to super-enhancer architecture referring to a prominent role of this enzyme in the determination of Th1 cell fate. Our work is focusing on this aspect of FURIN; we investigate the effect of this super-enhancer region in T cell physiology.

To understand the role of the super-enhancer, we employ the CRISPR/Cas9 genome editing technique: super-enhancer and promoter-specific guideRNAs were cloned to the Cas9-expression vector px330. Transfecting T cells with these constructs result in the directed deletion of the super-enhancer (and promoter) DNA region of FURIN. Comparison of the physiology of these cells will provide new insight into the regulation of FURIN via its super-enhancer region. Since FURIN plays a prominent role in several physiologic and pathologic conditions, the understanding of its regulation is of outstanding importance.
82. Paavilainen, Tanja: Characterization of human iPSC-derived reactive astrocyte phenotype (Poster)
tanja.paavilainen@uta.fi, BioMediTech

List of all authors
Tanja Paavilainen 1, Sanna Hagman 1, Anssi Pelkonen 1, Susanna Narkilahti 1

Astrocytes have a central role in supporting nervous system physiology. However, in neurodegenerative diseases, such as Multiple Sclerosis (MS), astrocytes undergo activation into reactive inflammatory phenotype. Reactive astrocytes may affect disease progression by inducing axonal damage and limiting neuronal repair and remyelination. The aim of this study was to induce reactive astrocyte phenotype and characterize their inflammatory nature. To establish reactive phenotype, we treated human induced pluripotent stem cell (hiPSC)-derived astrocytes with inflammatory cytokines TNF-$\alpha$ and IL-1$\beta$. Treated astrocytes exhibited morphological change from highly filamentous shape to flattened polygonal appearance. Reactive phenotype was characterized by quantifying GFAP staining intensity and expression of inflammatory molecules in the mRNA and protein levels. Increased gene expression of known inflammatory factors were detected in reactive astrocytes as compared to controls. Moreover, astrocyte activation induced widespread protein secretion profiles of inflammatory mediators. The protocol for inducing reactive astrocytes was promising and astrocytes transformed to inflammatory phenotype. These produced reactive astrocytes can be used in co-culture models together with neurons to study their inflammatory mechanism of axonal damage as well as possible strategies for promoting regeneration.
83. Partinen, Jenni: Knock-down of Tousled-like kinase triggers a strong blood cell activation phenotype in Drosophila melanogaster (Poster)
Partinen.Jenni.S@student.uta.fi, University of Tampere

List of all authors
Jenni Partinen(1), Susanna Valanne(1), Leena-Maija Vanha-Aho(1), Kaisa Oksanen(1), Henna Myllymäki(1), Mika Rämet(1,2)

Immune defence of the fruit fly Drosophila melanogaster against pathogens is based on humoral and cellular responses. Humoral responses include expression of antimicrobial peptides whereas diverse blood cell types are responsible for cell-mediated defence. Tousled-like kinases (TLKs) are members of serine/threonine kinase family. In mammals, TLKs are responsible for chromatin organization and cell division regulation. Drosophila melanogaster genome has one gene of the TLK family, tlk, which has been shown to be essential for nuclear division and cell vitality. The aim of our study was to determine the role of Tlk in Drosophila immune defence using both in vitro and in vivo methods. S2 cell based in vitro methods showed that when tlk expression was knocked down using RNA interference, expression of Imd pathway target genes was increased. This indicates that Tlk functions as a negative regulator of Imd pathway. Strikingly, we observed that with tlk knock-down in hemocytes in vivo, melanized blood-cell aggregates emerged in larvae and in adult flies. This phenotype is normally seen in larvae after parasitoid wasp infection, but not in uninfected larvae. Furthermore, flow cytometry experiments revealed that tlk knock-down in hemocytes induces plasmatocytes to differentiate into lamellocytes in larvae even without wasp infection. To test whether this tlk hemocyte phenotype is generated via the known hemocyte-activating pathways, namely Jak/STAT, Toll, JNK and Mekk1 pathways, we generated double knock-down Drosophila lines. Our on-going results indicate that tlk phenotype and lamellocyte production are dependent on JNK and Mekk1 pathways and independent of Toll pathway activation.
Dermatitis herpetiformis (DH) is a cutaneous manifestation of coeliac disease. Increased bone fracture risk is known to be associated with coeliac disease, but fracture risk in DH is only scantily studied. In this study, self-reported fractures and associating factors were investigated in DH and compared with coeliac disease. Data were gathered from medical records, and disease specific questionnaire, Gastrointestinal Symptom Rating Scale and Psychological General Well-Being questionnaires were mailed to 413 DH and 222 coeliac disease patients diagnosed at the Tampere University Hospital. Altogether 222 DH patients were enrolled as study patients and 129 coeliac disease patients as controls. Sociodemographic and disease specific data, self-reported fractures, current lifestyle characteristics, use of medication, gastrointestinal symptoms and quality of life were assessed. We found that 20% of DH patients and 27% of coeliac disease controls had experienced at least one fracture (P=0.140). The cumulative lifetime fracture incidence did not differ between DH patients and coeliac disease controls. The cumulative incidence of fractures after diagnosis was statistically significantly greater in female coeliac disease controls compared with the female DH patients. In DH, but not in coeliac disease, the self-reported fractures were associated to a decreased quality of life. The DH and coeliac disease patients with fractures suffered from more severe reflux and were users of proton-pump inhibitor medication more often than patients without fractures. We conclude that self-reported bone fracture risk in DH is equal to that in coeliac disease, which is known to be associated with an increased risk of fractures.
85. Pekki, Henna: Long-term Follow-up in Patients with Celiac Disease: Predictors and Effect on Health Outcomes (Poster)
pekki.henna.a@student.uta.fi, University of Tampere

List of all authors
Henna Pekki, Kalle Kurppa, Markku Mäki, Heini Huhtala, Katri Kaukinen

Introduction: Current guidelines recommend regular follow-up in celiac disease, but the actual implementation and effect of this to long-term health outcomes remains unclear. We explored this issue by inviting 677 adults with celiac disease to a follow-up study.

Methods: Previous medical data were gathered through interviews and patient records. Current symptoms and quality of life were assessed by validated SF-36, PGWB and GSRS questionnaires and blood samples were drawn for serology. All results were compared between patients with and without long-term (>2 years) follow-up.

Results: Only 15% had long-term follow-up, the median duration being 10 years. Predictors for the follow-up were immunological (35% vs 24%, p=0.020) and circulatory (20% vs 12%, p=0.010) comorbidities, whereas it was less common in subjects with musculoskeletal (23% vs. 34%, p=0.045) comorbidity and those in not-at-risk group for celiac disease (16% vs. 27%, p=0.025). Demographic data, site of diagnosis, baseline clinical and histological presentation and smoking had no effect. Patients with or without follow-up were comparable in gender, age at diagnosis and at present, adherence and capability to manage gluten-free diet, and current celiac antibody positivity. Questionnaire scores were also similar, but those without follow-up reported more self-experienced overall symptoms (16% vs. 26%, p=0.043). Over 80% of patients in both groups wished regular follow-up.

Conclusion: Only a minority of patients had regular long-term follow-up. Although the groups were comparable in most health outcomes, those without follow-up reported more overall symptoms. There is an unmet need for more systematic follow-up policies in celiac disease.
Microelectrode array (MEA) measurements of human pluripotent stem cell (hPSC)-derived neuronal networks are a pivotal part of modelling brain function in vitro. However, the percentage of electrodes detecting neuronal activity (i.e. active electrodes) in these measurements tends to be low (often <20 %), and the development of measurable activity can take several weeks or even months. In previous studies, polydimethylsiloxane (PDMS) devices have been used to guide the measured network to grow directly on the measuring electrodes. Furthermore, long and narrow PDMS tunnels can amplify the electrical signals measured by the MEA, but most previously tested tunnels are too small to encompass entire neuronal networks. The aim here was to develop tunnel devices that are compatible with a commercially available MEA, are able to house entire hPSC-derived neuronal networks, and amplify the extracellular electrical signals on MEA. Therefore, we created 8 different PDMS tunnels with different heights and widths. The hPSC-derived neurons were grown in the tunnel devices on MEAs for 5 weeks. Although the tunnel devices failed to amplify the electrical signals in a relevant manner, they increased the percentage of active electrodes (52-100 %) compared with the standard MEA controls (27 %). The networks in tunnel devices produced also significantly higher spike and burst counts, and the maximal percentage of active electrodes was reached in only 3 weeks. The results suggest that tunnel devices encompassing the entire neuronal network can increase the measured spontaneous activity in hPSC-derived neuronal networks on MEAs.
Arterial wall properties affect the propagation of heart-beat induced percussion wave and its reflections. Peripheral arterial disease (PAD) is mainly affecting the lower limbs and may be present as stiffening, stenoses or occlusions. We recorded arterial pulse wave (PW) data from 27 subjects undergoing percutaneous transluminal angioplasty (PTA) for the superficial femoral artery (SFA) in immediate pre-, peri-, and post-operative phases of the PTA as well as in the follow-up visit within one month. The control group consisted of 31 same aged healthy subjects having normal ankle-to-brachial pressure index and no history of atherosclerotic changes. The data was recorded as photoplethysmographic (PPG) volume PWs from index finger and 2nd toe as well as dynamic pressure PWs from radial artery (wrist) with EMFi sensor. The data were analyzed by computing the ratios of areas under amplitude normalized PWs, i.e. Aw/At and Af/At where Aw, At and Af are the areas under wrist, toe and finger PWs, respectively. The distributions of pre-operative values of PAD patients were 0.801 (0.776...0.890) (median (IQR)) and 0.914 (0.872...0.975) for Aw/At and Af/At, respectively. For the control group, the corresponding values were 1.06 (0.994...1.16) and for 1.11 (1.04...1.19) for Aw/At and Af/At, respectively. The differences between the study groups were tested by implementing two-tailed Mann-Whitney U-tests. For both area ratios, p-values p<0.0001 were found. Based on the presented results, the ratios of areas under the PWs could be an additional tool for finding the PAD lesions.
Surgical specimens from the aorta are removed for aortic aneurysm or dissection, which are consequences of a wide spectrum of diseases, syndromes, or aging processes. Across this spectrum, there is an overlapping collection of histopathologic changes to the aorta. Over the years, the meaning of histopathologic terms used to describe medial degeneration has become confused and often misused.

The new consensus document was designed to cover three overarching themes: 1. unify nomenclature to the histopathologic findings of the noninflammatory degenerative aorta, 2. provide a new grading scheme to better and more consistently classify aortic lesions, 3. briefly catalog the primary medial degenerative diseases of the aorta along with current knowledge regarding mutated genes and known associated histologic findings.

Due to the very recent publication of the consensus statement, very little information is available on the consequences and implications of its usage in both clinical and research context.

In the presented study, we analysed and scored all aortic samples processed during 2016 at Department of Pathology, Fimlab Laboratories, Tampere, Finland. A total number of 81 samples were analysed and scored by two senior pathologists and one medical student, using both analogue and digital microscopy methods. All samples were scored with the extended version of the scoring table.

Preliminary results of overall degeneration score are as follows: mild degeneration in 19 cases, moderate degeneration in 36 cases and severe degeneration in 25 cases. Some interobserver variations have been noted and are currently reviewed and adjusted by consensus.
During the course of osteoarthritis (OA), chondrocytes display both protective and harmful changes in their gene expression profile. Glucocorticoids are assumed to counteract many of these changes, and intra-articular glucocorticoids are widely used in the treatment of OA. Here, we carried out genome wide expression analysis to further understand their effects on OA chondrocytes.

Chondrocytes were isolated from the knee cartilages of OA patients undergoing joint replacement surgery, and cultured with the glucocorticoid dexamethasone. Total mRNA was sequenced using a next generation sequencing (NGS) platform. Differential gene expression levels were determined, and functional analysis was performed against the GO (Gene Ontology) database.

In dexamethasone-treated chondrocytes, 896 genes were significantly downregulated and 685 upregulated. In the GO analysis, genes involved in inflammation, collagen metabolism, cell proliferation, and lipid and glucose metabolism were enriched among the significantly affected genes. Of note was the downregulation of several matrix metalloproteinases and pro-inflammatory factors, but also cartilage-specific collagens. Conversely, several genes associated with limiting inflammation and protection from oxidative stress were upregulated. Notably, 11 the 53 genes linked to OA in a recent GWAS study were significantly downregulated by dexamethasone.

Glucocorticoids were found to regulate the expression of a wide range of genes in OA chondrocytes. In addition to suppressing inflammation and catabolism, glucocorticoids seem to have a significant effect on genes governing carbohydrate metabolism and collagen biosynthesis. These changes most likely modulate not only symptoms but also structural changes in OA cartilage, probably in disease stage dependent manner.
Acquisition of respiratory rate (RR) constitutes a vital task in physiological monitoring, the parameter being among the first to respond to decline in patient condition. Accordingly, the prospects of introducing novel combinations of RR extraction protocols to non-obtrusive instrumentation designs, as when assessing photoplethysmography (PPG) signals, has been followed by myriad of propositions in the field of algorithm development. We have studied a combination of recursive, Bayesian tracking according to particle filtering and time-frequency spectral reassignment method called synchrosqueezing, as well as the interconnection between their properties for estimating the RR from transmittance mode PPG signal measured from a finger.

PPG signal is known to obscure various types of signal modulations, illustrated as amplitude and frequency variations, known to originate from respiratory dynamics. The tracking algorithm, a reallocative particle filter, being studied, provides a specialized filter response, which we have studied for uncovering RR components from the modulation variabilities. Synchrosqueezing methods provide a spectral reassignment means to define the appropriate frequency components at higher precision and rate than by conventional time-frequency estimation methods. Essentially, the proposed combination relieves ridge extraction limitations imposed by mathematical theory of intrinsic signal modes. When applying the algorithm to a publicly available dataset VORTAL, constituting of 39 healthy, young subjects at rest, the combination of filter and spectral outputs provide advantages in the assessment of the true PPG respiratory component; moreover, particle filtering provides stability over transients. Additionally, the variability properties of the signal modulation appear to provide a sensible relation between inherent signal components and corresponding respiratory behavior.
Aims: Mutations in the cardiac ryanodine receptor (RYR2) are the leading cause for catecholaminergic polymorphic ventricular tachycardia (CPVT). In this study, we evaluated the antiarrhythmic efficacy of carvedilol and flecainide in CPVT patient-specific induced pluripotent stem cell (iPSC) -derived cardiomyocytes carrying different mutations in RYR2.

Methods: iPSC-derived cardiomyocytes were generated from skin biopsies of three CPVT patients carrying exon 3 deletion, L4115 or V4653F mutation in RYR2 and from a healthy individual. Ca2+ kinetics and drug effects were studied with Fluo-4 AM Ca2+ indicator.

Results: Carvedilol abolished Ca2+ abnormalities in 31% of L4115F, 36% of V4653F and 46% of exon 3 deletion carrying CPVT cardiomyocytes and flecainide 33%, 30% and 52%, respectively. Both drugs lowered the intracellular Ca2+ level and beating rate of the cardiomyocytes significantly. Moreover, in healthy control cells, flecainide caused abnormal Ca2+ transient prolongation in 61% whereas abnormal transients were present in only 26% when treated with carvedilol.

Conclusions: Carvedilol and flecainide were equally effective in treating arrhythmias in CPVT specific iPSC-derived cardiomyocytes. However, the proarrhythmic risk of flecainide should be recognized as it induced arrhythmias in control cells. Even though the CPVT cardiomyocytes carrying the exon 3 deletion had the most severe Ca2+ abnormalities, they had the best response to drug therapies. Both carvedilol and flecainide are used in the clinics for the treatment of CPVT. However, according to this study, the arrhythmia abolishing effect of neither of them is optimal. iPSC-derived cardiomyocytes provide a unique platform for testing new potential drugs for CPVT.
There is increasing evidence that systemic inflammation is an etiological factor underlying the major depressive disease (MDD). Kynurenine pathway, the primary route for tryptophan catabolism, is considered to be involved in the pathophysiological mechanisms of inflammation induced depression. According to studies, high cytokine levels induce depression in some patients, which has been associated with abnormally activated kynurenine pathway. However, it is still unclear why some people develop depression as a consequence of an inflammation. Our hypothesis is that genetic factors could explain why chronic systemic inflammation causes abnormal concentrations of certain kynurenine pathway metabolites in some persons, thus inducing depression. The purpose of our study is to identify genetic polymorphisms underlying the inflammation and the MDD, focusing on 6 kynurenine pathway related genes. As markers of systemic inflammation, elevated concentrations of interleukin 6 (IL-6) and high sensitivity C-reactive protein (hs-CRP) were used. The analyzed data included 200 patients with MDD or MDD and alcohol use disorder. For each patient, we calculated genetic risk scores, a variable that summarizes the effect of multiple SNPs, and conducted linear and logistic regression analyses to investigate their association with elevated serum inflammation markers. In these analyses, we did not find an association between SNPs in the studied kynurenine pathway related genes and elevated hs-CRP or IL-6 levels. However, analysis of SNPs in other kynurenine pathway related genes would be needed to better understand the role of this pathway in inflammation induced depression.
Remodeling and maintenance of the complex structural architecture of tissues is dependent on the controlled motility and contractility of the cells embedded in the ECM. In living tissues, cells continuously monitor and integrate chemical and mechanical signals from their surroundings to regulate many central cellular functions including gene expression, cytoskeletal organization, cell polarization and cell migration. Focal adhesion protein talin functions both as a structural scaffold and a signaling hub. In previous studies, talin rod domain mechanosensing has been shown to be a critical regulator of processes such as focal adhesion assembly kinetics and cell migration. In this study, we investigated the role of talin rod domain mechanosensing in the spreading and polarization of fibroblast cells. By expressing structurally modified talin forms in talin-deficient cells, we demonstrate that the presence of mechanosensory talin rod subdomains is indispensable for cell polarization. In addition, we show that mechanical activation of talin rod is required in the transition from isotropic cell spreading to cell polarization. Furthermore, by analyzing the effects of talin proteins with modified R3 subdomains, we demonstrate that the unfolding of the talin R3 domain is a critical regulator of cellular traction force generation and adhesion kinetics. These results open new insights into the early events of cell spreading and confirm that talin-mediated mechanosensing is an important trigger for cell polarization.
Recently it was shown that it is possible to define a set of clonal truncal missense mutations, i.e. mutations present in all metastases of an individual with metastatic prostate cancer. Such mutations may be targets for effective targeted drug therapy. Effective in vitro drug screening against the individual-specific truncal mutations would serve as a useful tool for guiding patient treatment selection in personalized medicine clinical trials. Our objective is to test the feasibility of using cell lines with and without individual patient’s truncal mutations to identify and prioritize drug targets and drugs for therapy of metastatic prostate cancer. CRISPR-Cas9 is used to introduce individual mutations in selected prostate cell lines followed by high throughput drug screening with approved and investigational cancer drugs.

We have tested multiple sgRNAs for several different patient-derived and control mutations. The CRISPR cutting efficiency has varied from 6 to 60 % between the guides. Significant variation in CRISPR efficiency has also been observed depending on the cell line used, as some guides have failed to produce detectable CRISPR in one cell line while working in the other. This might reflect the chromatin status of the targeted loci within the given cell line. Additionally, we have tested the capacity of our cell lines for clonal growth, as we need clonal cell lines to assess the effects of our targeted mutations. At the moment, we are working on creating enough CRISPR’d clones to actually observe HDR-mediated DSB-repair that is needed for the introduction of wanted mutations.
Introduction: Digestive diseases are major cause of anemia in children. We aimed to explore the diagnostic yield of gastrointestinal endoscopy in children presenting with anemia with or without other intestinal symptoms.

Methods: Medical records of 1146 consecutive children who underwent gastroscopy were analyzed. Only cases with a first diagnostic endoscopy and known hemoglobin status at that time were included for further analyses (n=737). All results were compared between anemic and non-anemic patients. Furthermore, the long-term prognosis of subjects without diagnosis in the primary endoscopy was inspected.

Results: Altogether 222 (30%) children had anemia. Concurrent colonoscopy was performed in 41% of anemic and 34% of non-anemic patients. Poor growth (13% vs. 6%, p=0.021) and blood in feces (22% vs. 9%, p<0.001) were more common in children with anemia, whereas they had less often abdominal pain (55% vs. 68%, p=0.002), reflux (10% vs. 17%, p=0.010) and dysphagia (1% vs. 5%, p=0.013). Final diagnosis was reached in 77% of anemic and 52% of non-anemic children (p<0.001). The most common diagnoses in the anemia group were celiac disease (28%), ulcerative colitis (19%) and Crohn’s disease (11%). In 30 children anemia was the sole indication for upper gastrointestinal endoscopy; of these 13 had celiac disease, 2 ulcerative colitis, 1 Crohn’s disease, 2 H. pylori and 1 gastrointestinal stromal tumor. From the patients who did not get a diagnosis in primary endoscopy only 5 out of the 41 children presenting with anemia and other symptoms and none of the 11 with anemia only developed any disease in a follow-up of up to 10 years.

Conclusion: Anemia increases the likelihood of organic disease at gastrointestinal endoscopy. Celiac disease is the most common single diagnostic entity in children presenting with anemia irrespective of the presence or absence of other symptoms.
Cardiac cell aggregates typically plated on a microelectrode array (MEA) are a rather heterogeneous population including various cardiac cell subtypes and other cells. To avoid such variation, which makes the interpretation of the measurement results difficult, the measurements should be done at single cell level. However, patch clamp is a too laborious and invasive method, and standard MEAs are unable to measure single cardiac cells. Our solution to the problem is a custom designed MEA whose optimized electrode layout enables non-invasive measurements at the single cell level. If the electrodes are made of transparent material, ITO, such MEA can be used simultaneously both for field potential measurements and video analysis.
We have expressed the mitochondrial alternative oxidase (AOX), a respiratory chain alternative enzyme from the sea squirt Ciona intestinalis, in the fruit fly Drosophila melanogaster. In this system, AOX is able to counteract deleterious effects of dysfunction in complexes III and/or IV of the oxidative phosphorylation (OXPHOS) system. These features suggest that AOX could potentially be used in gene therapies for human mitochondrial diseases that currently have no treatment. However, the natural lack of alternative enzymes in the mitochondria of arthropods and vertebrates suggests a possible impediment to such an approach. The focus of our work was to investigate possible disadvantages of AOX expression on the metabolism and development of D. melanogaster, under conditions that mimic ecological scenarios in which resources are usually limited. The flies were reared on restricted diets that, unlike the standard laboratory diet (rich on a variety of carbon sources), only included glucose (5-10 %) and yeast extract (1-10 %), as a source of amino acids. The results show that on restricted diets AOX-expressing flies showed lower pupal viability (10-40 %) compared to AOX-nonexpressing flies, including lines expressing other transgenes, such as GFP. The results suggest that this developmental defect is not at least directly due to calorie restriction but to lack of specific supplements or a combination of them, yet to be determined.
Enteroviruses are a group of positive strand RNA-viruses that cause a wide variety of diseases ranging from hand foot and mouth disease to dilated cardiomyopathy and possibly type 1 diabetes (T1D). New strategies are being investigated to battle these infections and a vaccine against Coxsackie B-viruses, that cause heart and central nervous system problems and are associated to the increased risk of T1D, is being developed in Tampere University in collaboration with Karolinska Institutet in Stockholm. Measuring the efficacy and safety of said vaccine is of utmost importance, as is a way to distinguish between healthy and infected vaccinated individuals. As the viral capsid used in vaccines does not contain non-structural proteins, vaccination should not raise antibodies against these proteins unless virus replication occurs, whereas antibodies against structural proteins should be present after vaccination. Therefore, measuring antibodies against non-structural proteins has been successfully used in the livestock industry to distinguish between healthy and infected vaccinated animals and a similar strategy would be useful with vaccinated humans. We have studied how the antibody response towards viral proteases and structural proteins differs in infected mice: viral proteases elicit a strong, but short-lived antibody response, whereas antibodies against structural proteins are present at higher levels after the protease antibody levels already declined. In the future we will test this assay with vaccinated mice and paired human serum samples before and after the appearance of neutralizing antibodies.
Streptococcus pneumoniae (pneumococcus) is one of the most common causes of pneumonia, septicemia, and meningitis in humans but the complex interactions between the immune system and pneumococcus are still only partly understood. In our previous studies we showed that zebrafish (Danio rerio) are valuable hosts in the study of the innate immune response against pneumococcus. Using this model organism, we carried out a forward genetic screen for 132 mutant families to identify novel innate immunity components that are important in the defense against pneumococcus. Based on the screen, we chose 18 mutant families that showed increased susceptibility to pneumococcus and the mutated genomic regions in these families were identified with targeted deep sequencing. So far, we have found 10 genes and 9 intergenic regions that potentially affect the innate immune response against pneumococcus. Currently, we are evaluating the role of these genes by silencing them in zebrafish with the CRISPR/Cas9 method. Mutant zebrafish lines for three candidate genes (sema4gb, dchs1b, grk4) have been produced and their susceptibility to pneumococcus has been tested with survival assays. However, these assays showed that the silencing of the three genes does not affect the zebrafish survival after pneumococcal challenge. Currently, more mutant lines are produced for the other candidate genes and their susceptibility to pneumococcal infection is tested in the near future. Eventually, this screen is likely to expand our understanding of the innate immune response against pneumococcus, providing new insights into the treatment of pneumococcal infections.
Here we will present the concept and plans for our acoustically-actuated droplet microfluidics project, started in September 2017. Microfluidics and the miniaturization of the sample volume are an on-going big trend in biomedical analytics. Smaller sample volumes would a) save reagents; b) give better statistics: a measurement can be repeated several times; c) enable a greater number of different tests on a master sample; and d) have more time resolution: reactions are faster.

The state-of-the-art solutions include microwell plates and dispensing robots, continuous flow microfluidics, drops-in-channels methods and droplet microfluidics. These solutions are based on either complicated devices or application-specific microfluidic chips. In this project, we will research acoustic methods to move multiple drops simultaneously and independently on an acoustically-actuated transversely-vibrating plate (Chladni plate). The drops are controlled in closed-loop by machine vision feedback. Different biochemical assays can be implemented simply by reprogramming the controller. The project seeks to answer following research questions: How to control drop motion on a Chladni plate? How to split and dispense drops? How to induce vibration to the plate from afar? What assays can be performed on the device?

The acoustic manipulation method developed in this project will enable completely reprogrammable liquid handling, but the disposable part (functionalized plate) contains only the functionalized surface and nothing else, making the disposable part very cheap. As a proof-of-principle, we plan to demonstrate homogeneous enzyme assays, heterogeneous biosensors and colorimetric immunoassays on the device. This project contributes to lab-on-chips and point-of-care diagnostics.
Personalized medicine, sometimes labeled as a "new paradigm for healthcare," refers to the tailoring of care according to patients' individual attributes, especially in relation to their genetic and molecular profile. In practice, this personalization means moving toward treatments that have been targeted to small patient groups, potentially subcategorizing the patients into ever smaller groups as understanding and technologies improve.

In order to personalize treatment according to patients' genetic profiles, patients would have to go through much closer genetic screening during the span of their treatment. For example, since the genetic profile of cancer cells changes as the disease develops, a need to constantly analyze patients' genetic profile is raised. How does this affect the traditional roles of different kinds of professionals involved? Should scientists' findings routinely influence treatment?

These scenarios challenge traditional boundaries between research and care, as typically the lines have been drawn between scientific advancement and individual health benefit. Even though participating in research may have potential to lead to individual health benefit for patient-research subjects, health benefit should not be the reason to participate in medical research. Blurring the lines between treating a patient and carrying out scientific research raises many ethical questions, for example, concern for potential hidden conflicts of interest as it may be harder for outsiders to distinct treatment goals from research interests.

As personalized medicine brings clinicians and scientists closer to each other, flexible structures may be needed to allow free flow of communication, moving between the IRBs, clinical ethics committees, patients, clinicians and scientists in cases that raise conflict or concern. We suggest that the bridging of communication and identifying ethical issues when visible, traditional lines are blurred, may be a role suited for ethics consultation services.
Dilated cardiomyopathy (DCM) is a leading cause behind heart failure and transplant. A part of familial DCM is due to mutations in the genes encoding the lamin A/C proteins. Lamin A and C are intermediate filament proteins forming the nuclear envelope. This study is focused on modeling DCM with Finnish founder mutation S143P; a nonsense mutation in the Lamin A/C gene. The model will help understand the disease pathology at cellular level using induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) derived from skin biopsies of healthy control and from DCM patients. Morphologically, confocal imaging and immunohistochemistry revealed the DCM iPSC-CMs displayed irregular and reduced distribution of Lamin A and exhibit severe sarcomeric damage. Western blot analysis revealed DCM model had a reduced Lamin A/C expression and increased expression of stress marker proteins Hif1 alpha and P53. Electrophysiologically, the DCM model displayed bradycardia and increased arrhythmias both at single cell level in calcium imaging and multi cellular level on microelectrode array (MEA). Calcium imaging also revealed altered calcium transients in the DCM model. Stimulation using beta adrenergic agonist lead to increased arrhythmia and abnormalities in DCM compared to control. Induction of stress using hypoxic conditions displayed an exaggerated effect on the DCM cardiac cells. In conclusion, our model recapitulated major phenotype characteristics as observed in DCM patients and can serve as a platform for drug screening and to study the disease mechanisms further.
Glioblastoma is the most aggressive type of brain tumor. It often develops resistance to the available drugs and other therapeutic interventions. In drug screening, image-based analysis methods are used to study the behavior of the cell population. In addition, studying the drug effects at single-cell level is vital to quantify cell-to-cell variation. Here we present the optimization protocol for multiple glioma cells segmentation for characterizing the dynamic phenotypic responses of heterogeneous cell populations in response to multiple treatment conditions. The main objective is to quantify the state of the populations in different treatments. The glioma cell lines, SNB19 and LN229 were treated with Temozolomide and novel synthetic phenol derivative. The stained DNA content of the cells was captured under the confocal microscope. To quantify the cell cycle heterogeneity, the segmentation algorithm needs to be optimized to get the precise measurement of the DNA content since the cells differ in size and shape. The optimized protocol was also successfully tested using an image analysis software. After segmentation, the data is analyzed with a computing software by plotting cell cycle distribution histograms and classifying the cells into different stages (G0, S, G2, M and G1) based on the integrated intensity values. Our findings indicate novel phenol synthetic derivative could be considered as glioma cell cycle inhibitor and promising drug for brain tumor treatment.
Plant based cellulose nanofibrils (CNFs) have potential applications as cell culturing substrates due to their non-animal or non-human origin, abundant resources, biocompatibility, possibility for controlled enzymatic degradation, as well as various surface modification and alignment possibilities. Surface modification of CNF has an effect on its properties and on cell behavior. The objective was to investigate the cell growth and cell orientation on different CNF surfaces with or without fibronectin coating. Bleached and never-dried cellulose kraft pulps were used for the production of three different CNF grades. Four different CNF surfaces were characterized using atomic force microscope (AFM), helium ion microscope (HIM) and/or optical microscope (OM). In addition, the degree of CNF orientation was analysed from AFM images using CytoSpectre software. Cell growth on different CNF and control surfaces was investigated. Cell morphology, proliferation, metabolic activity and the degree of orientation was analysed using time-lapse images, cell numbers after plating and after 48 h cultivation, AlamarBlue reagent and CytoSpectre software, respectively. Fibronectin coating slightly enhanced cell proliferation and metabolic activity on studied surfaces. However, the cell growth on CNF surfaces without a fibronectin coating was comparable to that on control surface. The degree of cell alignment was significantly higher on aligned CNF surfaces than on less oriented CNF and control surfaces.
105. Sutinen, Maiju Reetta: Identifying breast tumors and healthy breast tissue by differential ion mobility (DMS) spectrometry analysis of diathermy smoke (Talk)
sutinen.maiju.r@student.uta.fi, University of Tampere

List of all authors
Maiju Sutinen1, Anton Kontunen2, Markus Karjalainen2, Juha Kiiski3, Jill Hannus4, Jukka Lekkala2, Teemu Tolonen4, Niku Oksala5, Antti Roine6

**Background:** Breast conserving surgery combined with postoperative radiotherapy is a safe treatment for breast cancer (BC). Due to inadequate clearance margins after breast conserving surgery reoperations remain a problem with estimated rates varying from 17 % to 68 %. Furthermore, the risk of local recurrence is higher in patients who need re-excision to achieve clear margins. Currently, no method for real-time margin assessment is in use for clinical practice. Materials and methods: An automatic RoboNose system based on differential ion mobility spectrometry (DMS) connected to a sampling unit was developed and tested for identifying BC from healthy breast tissues by analyzing diathermy smoke ex vivo. The system was tested with samples of seven patients who underwent BC surgery in Tampere University Hospital. A pathologist collected the samples during dissection. The specimens were placed in a custom-made well plate and each specimen was incised by the diathermy blade once to produce the smoke for analysis. Linear discriminant analysis (LDA) was used for statistical analysis.

**Results:** Total number of tissue specimens was 124 including malignant breast tissue (n= 45), benign breast tissue (n= 39) and adipose tissue (n= 40). The RoboNose classified correctly 87.90 % of tissue samples. The specificity was 91.11 % and sensitivity was 93.67 % for the identification of malignant tissue from benign tissues.

**Conclusions:** Surgical smoke from malignant breast tumors and benign breast tissues have distinct DMS profiles which can be identified ex vivo. Further development of the system aims to the immediate classification of tissues during operation.
Introduction: Mitochondrial respiratory disruption is a common cause for disorders through the excess production of reactive oxygen species (ROS). Conversely, ROS production is an indispensable signal for physiologic response mechanisms. We used alternative oxidase (AOX) to study the role of mitochondrial ROS in disease models.

Material & Methods: AOX-expressing mice were generated by site-directed genomic integration of AOX. Hypoxic pulmonary vasoconstriction (HPV) was studied during acute hypoxic ventilation in isolated lungs of AOX mice and in isolated pulmonary artery smooth muscle cell (PASMCs). Myopathy was assessed in AOX mice with a skeletal muscle-specific ablation of a complex IV subunit.

Results: AOX prevents HPV during acute hypoxic ventilation in isolated perfused and ventilated lungs. This lack of response is associated with inhibition of the hypoxia-induced ROS increase in PASMCs. A defect in complex IV activity evokes a process of muscle regeneration that is affected by AOX in a ROS sensitive manner.

Conclusion: Our results suggest a physiological role for mitochondria in oxygen sensing and myopathy-related remodeling. ROS production constitutes a crucial signal that is modified by AOX. Thus, AOX can be used as a tool to decipher disease mechanisms and may potentially become a therapeutic.
Bioactive glasses are promising material utilized in tissue engineering, mainly in bone-related applications. Borosilicate glasses are especially interesting, as they have been shown to have faster conversion to hydroxyapatite (HA) in physiological conditions compared to the traditional silica-based bioactive glasses, while also possessing thermal properties enabling e.g. 3-D scaffolds processing. However, borosilicate glasses are known to lead to a decrease in the cells activity.

In this study, we investigated new borosilicate glasses with molar composition of 47.1 SiO2 - 6.7 B2O3 - 22.7 Na2O – (21.8-x-y) CaO - 1.7 P2O5 – x MgO – y SrO, where x,y varied from 0 to 10 mol-%. The objective was to assess if a decreased Ca release and presence of Sr and/or Mg can enhance the cellular activity on these materials.

The in vitro dissolution of the glasses was studied in simulated body fluid. Change in dissolution rate evidenced by pH and ICP-OES measurement are correlated to changes in the glass structure. Additionally, cell activity at the surface of the glass discs was tested using mesenchymal stem cells. It was found that the glass dissolution rate decreased with increasing Ca substitution. The formation of HA layer appeared quite rapidly on all of the studied glasses, while Mg and Sr were suspected to be introduced in the precipitated HA layer. With mesenchymal stem cells, Mg and/or Sr containing glasses exhibited enhanced cell viability and proliferation.
Here, we overview our research line: Signal analysis methods for extracellular electrophysiological microelectrode array (MEA) measurements from in vitro neuronal cultures. The topics include:

1. Neuronal action potential (NAP) detection
2. NAP burst detection
3. NAP burst analysis
4. Neuronal network connectivity/synchronization analysis
5. Real-time closed-loop analysis and stimulation
6. Analysis of microelectrode constellations

NAP detection is the basis of all analysis methods operating on spikes. We developed an objective thresholding based NAP detection method. NAP bursts indicate network activity. To detect bursts in a wide range of NAP signals, burst detection needs to be based on the signal itself; such a method was developed. To extract more information from the internal dynamics of network bursts, a method to jointly analyze bursts and NAP waveforms was developed. One way to assess MEA signals is through their complexity; for this spectral entropy was employed and demonstrated beneficial. Finally, measures for network synchronization and functional connectivity were considered, and the correlated spectral entropy method (CorSE) was developed.

Currently, we are aiming at real-time MEA signal analysis and electrical stimulation in a close-loop system consisting of a neuronal network on a MEA and a digital signal processor embedded in the MEA measurement system.

On a related note, a lead field theoretical method for the analysis and design of MEA electrode constellations for cellular applications was proposed.

Much of the work was done in collaboration with NeuroGroup, University of Tampere. Please, visit the poster for the list of references indicating also the contributors.
Teppo, Susanna: Molecular profiling of diagnostic and drug resistant clones in ETV6-RUNX1-positive pediatric leukemia (Poster)
susanna.teppo@uta.fi, University of Tampere

List of all authors
Susanna Teppo 1, Juha Mehtonen 2, Riina Kaukonen 3, Tapio Lönnberg 3, Olli Lohi 1, Merja Heinäniemi 2

Despite the relatively good prognosis of many childhood leukemia subgroups, some patients still relapse. Understanding of the molecular features of chemoresistant clones could aid in development of effective targeted treatments. In preB-ALL, the most common type of pediatric leukemia, minimal residual disease (MRD) after 29 days of treatment predicts higher risk for relapse. Improvement in single cells methodologies has enabled studying these features in a more detailed manner.

In this study, 2000-8000 single cells from diagnostic or MRD pre-B-ALL bone marrow (BM) samples were isolated and their expressional landscape inspected using Chromium system (scRNA-seq). So far, four diagnostic ETV6-RUNX1-positive (E/R) pre-B-ALL containing 65-95% of leukemic blasts, and two normal BM samples, have been studied. Uncommon drug resistant clones in one of the patient after 15 and 29 days of treatment (10% and 0.08% respectively) were further inspected. Direct targets and markers for E/R positive cells were explored using an E/R cell line model. The analysis was accompanied with GRO-seq data from 20 patients. Single cell sequencing data was analyzed using R/Seurat and Scater packages.

Preliminary analysis revealed useful genes for improved segregation between HSC, pro- and pre-B-cells, which enabled comparison of pre-B-leukemic blasts with relevant reference populations. The surviving MRD clone had stem-like features and its clone of origin was deduced. Some of the differentially expressed genes were linked to differentiation and directly regulated by E/R. Further analyses are underway for better characterization of the disease.
Mechanical forces between cells play an important part in epithelial functions affecting the epithelial development, healing, remodeling, and barrier properties. Due to the mechanical coupling between the cell junctions and the nucleus by the cytoskeleton, the intercellular forces also affect the shape of the nucleus and thus the cell behavior. In addition, during wound healing and morphogenesis mechanical stress waves propagate in the epithelium through the cell junctions and cytoskeletons and guide the cell behavior. However, it is not completely understood how the intercellular forces in the scale of the epithelium affect the nuclei and how the stress waves propagate in the epithelia as well as what is the role of the cytoskeletal connection between the cell junctions and the nucleus in these waves.

To study this, we are constructing a computational model of epithelium. The model is done using immersed-boundary method in which the cell and nuclear membranes are described by closed polygons and cytoskeleton as springs between the points of the polygon.

The model will enable us to observe what kind of forces the cell junctions and the nucleus are subjected during e.g. during normal epithelial development. Further, the model can be used to study how forces propagate in the epithelium in the stress waves and what is the role of the cytoskeletal connection between the junctions and the nucleus in these waves.
111. Turunen, Sanna: Direct Laser Writing of Microtowers for 3D Culture of hPSC Derived Neuronal Cells (Poster)
sanna.turunen@tut.fi, Tampere University of Technology

List of all authors
Sanna Turunen (1), Tiina Joki (2), Susanna Narkilahti (2), Minna Kellomäki (1, 3)

Stem cell-derived neuronal cells have given new hope to the research of neural functions in health and disease; however, the development of three-dimensional in vitro cell culture models is still quite challenging. The complexity of the in vivo cellular microenvironment can be mimicked by creating 3D microstructures via CAD-based additive manufacturing technologies, such as direct laser writing by two-photon polymerization (2PP-DLW). Thus, in this study, we present the design and 2PP-DLW fabrication process of a novel 3D neuronal cell culture platform based on tubular microtowers. In particular, microtower designs both with or without intraluminal guidance cues and/or openings in the tower wall were designed and successfully fabricated from Ormocomp polymer-ceramic hybrid material. Their ability to support the adhesion, migration and orientation of neuronal cells derived from human pluripotent stem cells (hPSC) was investigated. The results showed that the platform facilitates efficient long-term 3D culturing of human neuronal cells and supports neurite orientation and 3D network formation. Thus, the proposed culture platform offers a promising concept for future 3D cultures in the field of neuroscience.
Dispenser printing provides a method to produce 2D and 3D patterns from water and other soluble biomaterials, such as nanocellulose [1]. An advantage of dispensing technique over conventional printing techniques here is avoidance of complicated ink formulation, which generally requires hazardous organic solvents that may be harmful to biological objects. Nanocellulose aerogels [2] are potential lightweight materials for example for biomedical applications. High surface area of entangled networks of cellulose nanofibrils (CNF) and permanent dipole moment of cellulose nanocrystals (CNC) makes nanocellulose interesting material also for electronic applications, such as supercapacitors [3] and piezoelectric sensors [4, 5]. By combining the dispensing printing of liquid phase materials and 3D printing of solid materials, complex structures with new functional properties can be fabricated, which is very challenging using conventional manufacturing techniques.

mPGES-1 is located down-stream of cyclo-oxygenases in the eicosanoid synthesis pathway and it catalyzes the production of prostaglandin E2 (PGE2), the most abundant prostanoid in inflammation and pain. Inhibition of mPGES-1 is presumed as a more selective and safer target for decreasing excessive PGE2 production than NSAIDS. MAP kinase phosphatase-1 (MKP-1) is an endogenous enzyme limiting inflammation. It also mediates the effects of some anti-inflammatory drugs, such as glucocorticoids. Here we investigated the role of MKP-1 in the expression of mPGES-1 in chondrocytes.

Chondrocytes from articular cartilage of MKP-1 knock-out (KO) and wild type (WT) mice were isolated and the expression of mPGES-1 and MKP-1 was investigated in primary cultures.

The expression of mPGES-1 in chondrocytes from MKP-1 KO mice was elevated as compared to the WT controls. mPGES-1 levels were enhanced when the cells were stimulated with the arthritis-related cytokine interleukin-1β. Dexamethasone significantly inhibited the expression of mPGES-1 in chondrocytes from WT mice but not in cells from MKP-1 KO mice. Moreover, dexamethasone enhanced MKP-1 expression in chondrocytes from WT mice.

The results suggest that the expression of mPGES-1 in primary chondrocytes is regulated through MKP-1. Furthermore, the inhibitory effect of dexamethasone on the expression of mPGES-1 was abolished in chondrocytes from MKP-1 KO mice indicating that this drug effect is dependent of MKP-1. Dexamethasone also increased the expression of MKP-1, which supports our hypothesis. These results increase our knowledge on the regulatory mechanisms of mPGES-1 expression in arthritis and underline the role of MKP-1 as a drug target.
CRISPR-Cas9 mutagenesis has become a valuable tool for targeted mutagenesis in model organisms and cell lines. Recently, the efficacy of the prokaryotic CRISPR-Cas9 system has been shown to be affected by eukaryotic chromatin structures. Accordingly, we have noticed in our research that genes which are more readily mutated with CRISPR-Cas9 are highly expressed in early development. In contrast, many genes which we have not been able to mutate are lowly expressed. Using open access datasets generated from early developmental stages of zebrafish, and guide RNAs (gRNAs) selected from the CRISPRz database (Varshney et al. 2015), we show that there is a significant difference in expression of genes with higher mutagenesis activity gRNAs compared to genes with lower mutagenesis activity gRNAs (p=0.04, Mann-Whitney U-test) (ArrayExpress E-GEOD-45706). Despite this general association with gene expression, we found no direct correlation between the onset of gene expression and the rate of mutagenesis in vivo. In order to further inspect the effect of chromatin, we analysed the in vitro function of our gRNAs, and found that gRNAs with low or no in vivo cutting efficiency can present over 90 % efficiency in vitro. A closer analysis on chromatin accessibility, using open access ATAC-seq data from zebrafish embryos (E-GEOD-74231), showed a significant correlation between chromatin openness and CRISPR-Cas9 mutagenesis efficiency. These results support recent evidence indicating CRISPR-Cas9 mutagenesis is also influenced by chromatin accessibility in vivo.
Negative regulation of immunity is essential in order to avoid autoinflammation. Using a Drosophila RNAi in vitro screen in S2 cells we identified the chromatin remodeling Brahma complex as negatively regulating the Toll pathway. The role of the Brahma complex in immunity in vivo was investigated using RNAi fly lines, where the expression of two of the complex members brahma and osa was silenced in the Drosophila fat body with C564-GAL4 driver. In osa and brahma knock-down flies the Toll pathway-mediated antimicrobial response and survival were enhanced. A transcriptome analysis indicated that Osa regulates the expression of immune genes by modifying the expression of the Drosophila NF-kappaB factors Relish and Dif. In addition, a long non-coding RNA gene, named Induced by Infection (lincRNA-IBIN), was identified, whose expression is induced by infection in both the fatbody and in hemocytes, and dependent on osa and NF-κB signaling. Overexpression of lincRNA-IBIN in hemocytes led to increased plasmatocyte numbers. In the fatbody, lincRNA-IBIN enhanced gene expression and survival mediated by the Toll pathway. Our data provide information about the negative regulation of immunity, and identify a novel immune-responsive non-coding RNA, which enhances the immune response.
A dual convolutional neural network (dCNN) architecture was implemented for extracting multi-scale features from histological tissue images for the purpose of automated characterization of tissue in digital pathology. The dual structure consists of two identical convolutional neural networks applied to input images with different scales, that are merged together and stacked with two fully connected layers. It has been acknowledged that deep networks can be used to extract higher-order features, and therefore, the network output at final fully connected layer was used as a deep dCNN feature vector. Further, engineered features, shown in previous studies to capture important characteristics of tissue structure and morphology, were integrated to the feature extractor module. Combined with machine learning, the acquired quantitative feature representation can be further utilized to train a discriminative model for classifying tissue types. Machine learning based methods for detection of regions of interest, or tissue type classification will advance the transition to decision support systems and computer aided diagnosis in digital pathology. The proposed feature-augmented dCNN method was applied with supervised learning in detecting cancerous tissue from whole slide images. The extracted quantitative representation of tissue histology was used to train a logistic regression model with elastic net regularization. The model was able to accurately discriminate cancerous tissue from normal tissue, resulting in blockwise AUC=0.97, where the total number of analysed tissue blocks was approximately 8.3 million that constitute the test set of 75 whole slide images.
Differentiation of corneal limbal epithelial stem cells (LESCs) from human pluripotent stem cells (hPSC) provides new tools for studying cornea development in humans. Our group has previously established a xeno- and feeder cell -free differentiation protocol that yields high amounts of clinically relevant p63a/p40-positive LESCs. Here, we aim at gaining better understanding of the marker expression changes during 24-day differentiation period, in relation to the current knowledge regarding cornea development.

Two genetically distinct cell lines were used for differentiation and their protein expression profiles for selected markers were analyzed at 2-4 day intervals and 8 subsequent time points. Qualitative immunolabeling analysis showed a distinct decrease in pluripotent markers and ascending LESC marker expression during the process. Expression of OCT3/4, ABCG2, CK15, CK14 and p63a/p40 was quantified from day 24 hESC-LESC samples. Putative LESC marker ABCG2 was transiently expressed at the early stage of differentiation, while other suggested limbal markers p63a/p40, CK15 and CK14 were expressed in later time points. Based on current literature in humans, our observations indicate that at day 24 majority of the cell population may represent transient amplifying cells (TAC), which in vivo migrate from limbal area towards central cornea. We are currently analyzing additional marker combinations for identifying hPSC-LESCs with a clonal capacity and exploring modifications to culture conditions in order to promote growth and maintenance of the clonal LESC population. These results provide important insight into the differentiation process of hPSCs towards LESCs, and further through TAC phase into stratified corneal epithelium.
Metabolomics is a field of science, which aims at the comprehensive identification and quantitative analysis of all the metabolites in any biological system or a specific physiological state. Quantifying a broad range of metabolites in a sample provides the “functional readout” of a cellular phenotype. We have developed and validated high throughput quantitative methods for targeted metabolite profiling of over hundred metabolites in low and high concentrations across 18 different biological classes. Metabolites are extracted using HAMILTON liquid handling system and separated using appropriate chromatographic techniques, and analysed using WATERS Acquity UPLC-MS-MS coupled to XEVO-TQ-S QQQ mass spectrometry instrument. Metabolites are analysed in both positive and negative polarities and multiple reaction monitoring method was employed. We can also target and quantify over 1000 lipid molecular species including free fatty acids in a single method using Lipidyzer platform from SCIEX QTRAP 5500 mass spectrometer. We have optimised the developed methods using different biological sample types i.e., biofluids, tissues, dried blood spots, stool and cells (including isolated organelle) in human and animal models, bacteria, yeast, drosophila (larvae and whole flies) and in C.elegans. We have implemented the methods to different research projects and published over 20 articles including population cohorts analysis, and showed high potential for the discovery of metabolic biomarkers for the disease risk, dietary intervention studies, and clinical trials. Website: https://www.fimm.fi/en/services/technology-centre/metabolomics
Drosophila larvae are infected by endoparasitoid wasps, which lay their eggs into the larvae. Hemocytes (blood cells) play a key role in fighting the parasites. Healthy larvae have only two types of hemocytes; the phagocytic plasmatocytes and crystal cells. Immune response against the wasp requires the differentiation of a specific hemocyte type, the lamellocyte. It is not fully understood how the innate immune response in Drosophila is controlled and how lamellocyte differentiation is induced. Ectopic expression of Anaplastic lymphoma kinase (Alk) in hemocytes leads to formation of melanotic nodules, which resemble the melanotic capsule formed around the wasp egg. We found that the transcription factor foxo in hemocytes reduces the melanotic nodules. Furthermore, the encapsulation success against Leptopilina boulardi wasps was reduced when foxo was knocked down in hemocytes, and increased when foxo was overexpressed. Also, overexpression of foxo led to differentiation of lamellocytes in the absence of infection. Foxo is controlled by the insulin signaling pathway, which promotes phosphorylation and inactivation of Foxo. However, we found that suppression of the insulin signaling pathway also led to suppression of the melanotic nodules and the wasp response, and that altering sugar content of the fly food did not change the effects brought upon by foxo manipulation. These data suggest that Foxo is involved in immune cell differentiation and acts independently of the insulin signaling in hemocytes.
Retinal pigment epithelium (RPE) is located in the back of eye and is critical for the maintenance and welfare of the retina. Like most vertebrate cells, RPE also has sensory cilia, primary cilia (PC), in their apical membrane. These organelles work as signaling hubs in many cell types and are associated with several human diseases. However, very little is known about their function in RPE. In this study, our aim was to investigate the prevalence and the length of the PC, and in addition, the localization of some important RPE proteins in the PC during maturation of the RPE. We discovered that the PC were assembled and present in most cells already at an early maturation stage of the RPE. However, the prevalence and the length of the PC seemed to decrease during maturation. Some proteins including a tight junction protein claudin19, localized in the PC at the early development stage with a shift in localization to tight junctions during maturation of the cells. Due to the possible role of the PC in the maturation of the cells, the initial longer length of the PC could be a physical effect to fit larger number of RPE maturation related proteins in the PC. The PC also are known to form connections between other PCs so the initial longer length could be a result from the capability to physically communicate between the environment. The results reveal new insight on the importance and the role of the PC to RPE and its maturation.
Introduction: In nonresponsive celiac disease (NRCD) the symptoms and duodenal damage persists despite gluten-free diet (GFD). We have previously shown association between dysbiotic microbiota and persistent symptoms. Furthermore, antibodies to Saccharomyces cerevisiae (ASCA), Pseudomonas fluorescens-associated sequence (I2) and Bacteroides caccae TonB-linked outer membrane protein (OmpW) are gluten-sensitive and present at early stage CD. We hypothesized that seroreactivity to microbial antibodies is associated with NRCD.

Methods: ASCA, I2 and OmpW were measured in 20 NRCD patients. Fifty-eight GFD responsive patients served as CD controls (55 samples on GFD) and 80 blood donors as non-CD controls.

Results: At least one microbial marker was positive in 80% of NRCD patients, 97% of untreated and 87% of treated CD patients and 44% of controls. NRCD patients had the highest frequency of ASCA positivity (64% vs 52%, 20% and 0%, respectively) and also significantly higher ASCA IgA (median 14.5 U/ml) and IgG (32.5 U/ml) titers than treated CD patients (7.0 U/ml, 13.0 U/ml) and non-CD controls (4.5 U/ml, 5.8 U/ml). ASCA did not differ between NRCD and untreated CD. The frequencies of I2 and OmpW were lower in NRCD than in untreated CD (65% and 45% vs 86% and 59%, respectively), and I2 titers were higher in NRCD (median absorbance 0.76) and untreated (1.0) and treated (0.83) CD than controls (0.32). OmpW was elevated in untreated (1.1) and treated (0.94) CD patients compared with controls (0.79).

Conclusions: Seropositivity and high titers of ASCA were associated with NRCD and might serve as follow-up tool in CD.
Background: Micro-RNAs (miRNAs) are small molecules that regulate gene expression at the post-transcriptional level. Because of their key role in the immune system regulation, miRNAs are associated with several autoimmune diseases including multiple sclerosis (MS). In MS, dysregulation of miRNAs is mostly studied in cell populations, with little attention paid to circulating miRNAs that however own strong biomarker potential due to their exceptional stability in body fluids.

Objective: The aim was to define circulating miRNA profiles in MS subtypes and their association to disability accumulation and disease activity. Previously we studied miRNA profiles in primary (PPMS) and secondary progressive MS. Now we aimed to validate findings on cohort of patients with relapsing-remitting MS (RRMS), to see how specific those miRNA are to MS.

Methods: miR-24-3p, miR-191-5p, miR-376c-3p and miR-128-3p were analyzed in serum samples of 53RRMS, 20PPMS and 27 healthy controls.

Results: In comparison to controls, miR-191-5p and miR-24-3p were overexpressed in both RRMS and PPMS, with no differences between the subtypes. In turn, miR-128-3p showed tendency towards the predominant expression in PPMS. MiR-128 positively correlated with annual relapse rate in RRMS and miR-24-3p with the progression index in the whole MS group.

Conclusion: The increased expression of miR-128-3p seems to be associated with PPMS. In addition, its correlation with an average relapse rate suggests its association with inflammatory events. The association of miR-191-5p and miR-24-3p with all MS subtypes suggests their important role in MS pathology and might be considered as indicator of immune response or neurodegenerative events.
This abstract summarizes the implementation of computational modeling methods with experiments to study a vital mechanosensor, talin. Such unique combination of diverse methods allows us to observe talin on sub-molecular level and assists further experimental planning as well as interpretation of the results.

In combination with smAFM studies, we discovered that the whole talin rod is sensitive to a range of small mechanical forces and hence functions as a fine cellular mechanosensor. Furthermore, applying such methodology we study the fundamental principles of protein unfolding under mechanical force.

In combination with cell experiments, we utilize the atom level computational modeling to design functional mutations in the talin structure with an effect on the cellular behavior, fitness or survival. Recently, we reported on a set of destabilizing mutations with significant effect on the cell polarization, spreading and overall health.

Even though the structures of the individual talin rod bundles have been solved, the final organization of these bundles is yet unknown. In the combination with SAXS structural analysis we are studying the quaternary structure of the talin molecule. Such information is vital in the correct assessment of disease causing mutations.

Finally, we utilize protein modeling, molecular dynamics and steered molecular dynamics methods to study possible impacts of reported pathogenic functional mutations on the protein conformation, mechanical stability and function in the context of disease.

Despite the limitations of computational modeling methods, they provide a unique insight to the atomic and molecular level protein chemistry. These computational tools have indeed helped in research planning as well as in resource optimization.
IL-6 is a proinflammatory cytokine contributing to the pathogenesis of rheumatoid arthritis. We investigated the potential role of IL-6 in osteoarthritis (OA), by assessing levels of IL-6 in plasma and synovial fluid (SF) and associations of IL-6 with matrix metalloproteinases (MMPs), YKL-40 and radiographic severity of OA.

Plasma and SF samples together with clinical data were collected from 100 OA patients [BMI 29.7(8.3)kg/m2, age 72(14) years, median (IQR); 62 females] undergoing knee replacement surgery in Coxa Hospital for Joint Replacement. Preoperative radiographs were evaluated using Ahlbäck classification. Primary chondrocytes were isolated from human OA cartilage. IL-6, MMPs and YKL-40 were measured by immunoassays.

IL-6 levels in SF [119.8(193.5) pg/ml, median (IQR)] were higher and did not correlate with those in plasma [3.1(2.7) pg/ml], suggesting that IL-6 is produced within the joint. SF-IL-6 correlated with MMP-1 (r=0.446, p<0.001), MMP-3 (r=0.486, p<0.001), and YKL-40 (r=0.353, p=0.013). Furthermore, SF-IL-6 levels were higher in patients with most advanced OA [Ahlbäck 4-5, 234.1(264.7) pg/ml, n=74] than in those with less severe disease [Ahlbäck 1-3, 94.6(183.0) pg/ml, n=17; p=0.027]. Primary OA chondrocytes produced IL-6 and its expression was enhanced by the arthritis associated cytokine IL-1β (100 pg/ml). In addition, when chondrocytes were exposed to exogenous IL-6, YKL-40 and MMP-3 production was enhanced.

In conclusion, IL-6 is produced within OA joints, and it is associated with increased levels of MMPs and YKL-40 and with the radiographic severity of OA. These findings propose a role for IL-6 in the pathogenesis of OA and as a target for disease-modifying drugs for the treatment of OA.
There is a growing demand for bone substitutes due to lack of grafts and in relation to ageing population and traumas. Human adipose stem cells (hASCs) are an available and accessible source of adult stem cells for bone constructs. 3D hydrogel culture simulates better cellular microenvironment compared to traditional biomaterials. This study presents osteogenic induction of hASCs encapsulated in 3D hydrogels.

The gellan gum (GG) and collagen type I (COL1) hydrogels were tested for their ability to support hASC viability, cell proliferation, gene expression and mineralization in 3D hydrogel culture. Osteogenic marker genes ALP, OSX, DLX5, and RUNX2A were analyzed to determine early osteogenic differentiation of hASCs. Late osteogenic differentiation of hASCs was analyzed based on the mineralization of the hASC ECM measured by hydroxyapatite formation and by immunofluorescence staining of bone marker protein osteocalcin. Different types of mineral residues in different hydrogels were analyzed by Raman spectroscopy. Optical projection tomography (OPT) was applied to image hASCs encapsulated in 3D hydrogel.

The hASCs were well viable in 3D hydrogel culture, where GG encapsulated hASCs had tight and round cell morphology, whereas in COL1 hydrogel hASC morphology was spread out. COL1 hydrogel showed significantly higher osteogenic marker gene expression. All the hydrogel conditions supported the formation of mineralized hydroxyapatite residues, whereas strong osteocalcin staining was visible only in COL1 hydrogel.

Our results showed the suitability of the 3D hydrogel culture methods for osteogenic differentiation of the hASCs and for development of tissue engineered bone constructs.
In tissue engineering of neuronal field good scaffold materials are intensively looked for neuronal cells as well as in vitro purposes as for regenerative medicine. Nevertheless, optimal material should fulfill several requirements. It is cytocompatible, in most cases it supports neuronal outgrowth and network formation, and it mimics neuronal ECM. Here, we aimed to develop dual mesh hydrogel for neuronal cell scaffold. The aim was to combine good properties of both used hydrogels, collagen type 1 (Col1) and hydrazone crosslinked hyaluronan-poly(vinyl alcohol) (HA-PVA) composite hydrogel.

Dual mesh hydrogels were compared to mere HA-PVA and Col1 hydrogels. Human pluripotent stem cell derived neuronal cells were cultured on top of the hydrogels for 2 weeks, and encapsulated inside the hydrogels for 4 weeks. At the end point, cells were characterized with immunocytochemically and with qPCR.

Our results show that dual-mesh hydrogels combining Col1 and HA-PVA supports the neuronal cell growth almost as well as Col1, and give better structural support compared to Col1. So, we can conclude that dual-mesh hydrogel Col1-HA-PVA takes the advantages from both component and seem to be promising scaffold material for neuronal cells.
Glioblastoma is a type of highly malignant brain tumor that originates from glial cells, which surmount all available treatment regimens. The current work aims at testing newly synthesized long-chain diol derivatives for potential inhibition of glioblastoma – incurable grade IV astrocytoma and the most common malignant brain tumor. Recent discoveries on cancer cell metabolism brought advanced improvement in the treatment of glioblastoma. However, development of new anti-glioma drugs is a constant urge, since this tumor is resistant to irradiation and chemotherapy. Based on the previous reports on the potential of diol derivatives against cancer, we study 11 novel C10 - 1,2 - diol derivatives for anticancer properties on glioblastoma cell lines. Several assays including cytotoxicity, inhibitor kinetic study, cell migration, Annexin-V/PI double staining, reactive oxygen species (ROS) and caspase 3/7 assays are conducted on this cell line. Preliminary results demonstrate that, among 11 novel compounds, compound 2 shows high cytotoxic activity with determined IC50 value of 52 µM and also inhibit cell migration effectively in a dose and time dependent manner. Analysis of the cell death pathway will be presented as well. We suggests that the novel long-chain diol derivatives is promising pharmaceutical candidate for glioma therapy.